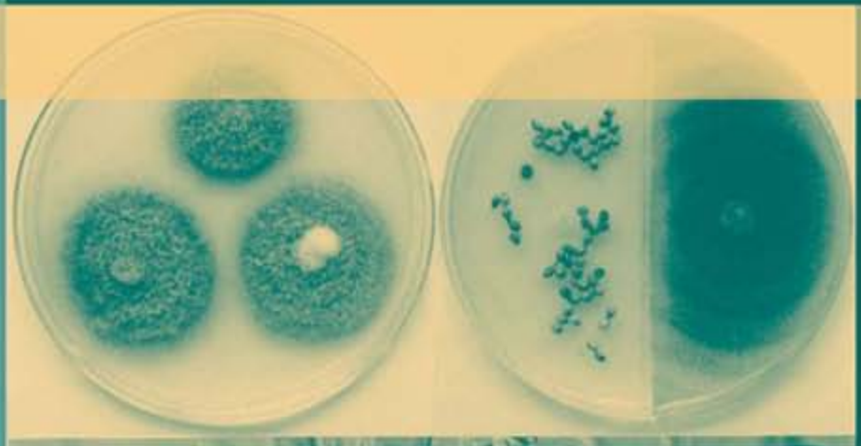
THIRD EDITION

John I. Pitt
Ailsa D. Hocking

Fungi and Food Spoilage



Springer

Fungi and Food Spoilage

John I. Pitt · Ailsa D. Hocking

Fungi and Food Spoilage

Springer

John I. Pitt
Honorary Research Fellow
CSIRO Food and Nutritional Sciences
North Ryde, NSW 2113
Australia
John.Pitt@csiro.au

Ailsa D. Hocking
Honorary Research Fellow
CSIRO Food and Nutritional Sciences
North Ryde, NSW 2113
Australia
Ailsa.Hocking@csiro.au

ISBN 978-0-387-92206-5 e-ISBN 978-0-387-92207-2
DOI 10.1007/978-0-387-92207-2
Springer Dordrecht Heidelberg London New York

Library of Congress Control Number: 2009920217

© Springer Science+Business Media, LLC 2009
All rights reserved. This work may not be translated or copied in whole or in part without the written permission of the publisher (Springer Science+Business Media, LLC, 233 Spring Street, New York, NY 10013, USA), except for brief excerpts in connection with reviews or scholarly analysis. Use in connection with any form of information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed is forbidden. The use in this publication of trade names, trademarks, service marks, and similar terms, even if they are not identified as such, is not to be taken as an expression of opinion as to whether or not they are subject to proprietary rights.

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface to the Third Edition

In contrast to the second edition, the third edition of "Fungi and Food Spoilage" is evolutionary rather than revolutionary. The second edition was intended to cover almost all of the species likely to be encountered in mainstream food supplies, and only a few additional species have been included in this new edition. The third edition represents primarily an updating – of taxonomy, physiology, mycotoxin production and ecology. Changes in taxonomy reflect the impact that molecular methods have had on our understanding of classification but, it must be said, have not radically altered the overall picture. The improvements in the understanding of the physiology of food spoilage fungi have been relatively small, reflecting perhaps the lack of emphasis on physiology in modern microbiological science. Much remains to be understood about the specificity of particular fungi for particular substrates, of the influence of water activity on the growth of many of the species treated, and even on such basic parameters as cardinal temperatures for growth and the influence of pH and preservatives. Since 1997, a great deal has been learnt about the specificity of mycotoxin production and in which commodities and products-specific mycotoxins are likely to occur. Changes in our understanding of the ecology of the included species are also in most cases evolutionary. A great number of papers have been published on the ecology of foodborne fungi in the past few years, but with few exceptions the basic ecology of the included species remains.

Recent changes in our understanding of foodborne fungi include the realisation that *Aspergillus carbonarius* is a major source of ochratoxin A in the world food supply, that *A. westerdijkiae* and not *A. ochraceus* is the other common *Aspergillus* species making this toxin and that these species are responsible for ochratoxin A in foods outside the cool temperate regions, where *Penicillium verrucosum* is the important species. In recent years a number of new species have been found to be capable of producing aflatoxin, but the fact remains that most aflatoxin in the global food supply is produced by *A. flavus* and *A. parasiticus*. The taxonomy of *Fusarium* species is still undergoing major revision. However, the renaming of *Fusarium moniliforme* as *F. verticillioides* is the only change of importance here. Recent publications have improved our understanding of species – mycotoxin relationships within *Fusarium*.

Among the colleagues who helped us to prepare this edition, we wish to particularly thank Dr Anne-Laure Markovina, now of the University of Sydney, who assisted in literature searches and some cultural and photographic work, and Mr N.J. Charley who has continued his excellent work of curating the FRR culture collection, on which so much of the descriptive work in this book is based.

Preface to the First Edition

This book is designed as a laboratory guide for the food microbiologist to assist in the isolation and identification of common foodborne fungi. We emphasise the fungi which cause food spoilage, but also devote space to the fungi commonly encountered in foods at harvest, and in the food factory. As far as possible, we have kept the text simple, although the need for clarity in the descriptions has necessitated the use of some specialised mycological terms.

The identification keys have been designed for use by microbiologist with little or no prior knowledge of mycology. For identification to genus level, they are based primarily on the cultural and physiological characteristics of fungi grown under a standard set of conditions. The microscopic features of the various fungi become more important when identifying isolates at the species level. Nearly all of the species treated have been illustrated with colony photographs, together with photomicrographs or line drawings. The photomicrographs were taken using a Zeiss WL microscope fitted with Nomarski interference contrast optics. We are indebted to Mr W. Rushton and Ms L. Burton, who printed the many hundreds of photographs used to make up the figures in this book.

We also wish to express our appreciation to Dr D.L. Hawksworth, Dr A.H.S. Onions and Dr B.C. Sutton of the Commonwealth Mycological Institute, Kew, Surrey, UK, Professor P.E. Nelson and the staff of the Fusarium Research Center, University of Pennsylvania, USA and Dr L.W. Burgess of the University of Sydney, who generously provided facilities, cultures and advice on some of the genera studied.

Preface to the Second Edition

In planning for the second edition of "Fungi and Food Spoilage", we decided that the book would benefit from a larger format, which would permit improved illustrations, and from some expansion of the text, in both numbers of species treated and overall scope. These aims have been realised. The Crown Quarto size has allowed us to include substantially larger, clearer illustrations. Many new photographs and photomicrographs have been added, the latter taken using a Zeiss Axioscop microscope fitted with Nomarski differential interference contrast optics. We have taken the opportunity to include more than 40 additional species descriptions, to add a new section on mycotoxin production for each species and to update and upgrade all of the text.

Since the first edition, changes in the climate for stabilising fungal nomenclature have resulted in development of a list of "Names in Current Use" for some important genera, including *Aspergillus* and *Penicillium*. Names of species used in the second edition are taken from that list, which was given special status by the International Botanical Congress, Tokyo, 1994. Names used in this edition have priority over any other names for a particular species. Publication of a list of "Authors of Fungal Names" (P.M. Kirk and A.E. Ansell, Index of Fungi, Supplement: 1–95, 1992) has also stabilised names of authorities for all fungal species. Abbreviations of authors' names used in this edition conform to those recommended by Kirk and Ansell. Some progress in standardisation of methods and media has also been made, primarily through the efforts of the International Commission on Food Mycology.

The first edition included some 400 references. When we began revisionary work, we felt that the number of references in the area of food mycology had probably doubled or increased by perhaps 150% during the intervening years. In fact, this second edition includes over 1900 references, almost a five-fold increase over the 1985 edition! This provides a clear indication that interest in, and study of, food mycology has greatly increased in recent years. Modern referencing systems have enabled us to expand information from tropical sources, especially in Asia and Africa, but we are conscious of the fact that treatment of fungi found in foods on a worldwide basis remains rather incomplete.

We gratefully acknowledge support and assistance from colleagues who have contributed to this new edition. Ms J.C. Eyles formatted and printed the camera

ready copy, Ms C. Heenan collated, arranged and formatted the illustrations and Mr N.J. Charley looked after the culture collection, culture growth and colony photography. Without this level of support, the book would not have been completed.

Contents

Chapter 1
Introduction

From the time when primitive man began to cultivate crops and store food, spoilage fungi have demanded their tithe. Fuzzes, powders and slimes of white or black, green, orange, red and brown have silently invaded – acidifying, fermenting, discolouring and disintegrating, rendering nutritious commodities unpalatable or unsafe.

Until recently, fungi have generally been regarded as causing only unaesthetic spoilage of food, despite the fact that *Claviceps purpurea* was linked to human disease more than 200 years ago, and the acute toxicity of macrofungi has long been known. Japanese scientists recognised the toxic nature of yellow rice 100 years ago, but 70 years elapsed before its fungal cause was confirmed. Alimentary toxic aleukia killed many thousands of people in the USSR in 1944–1947; although fungal toxicity was suspected by 1950, the causal agent, T-2 toxin, was not clearly recognised for another 25 years.

Forgacs and Carll (1952), in a prophetic article, warned of the danger from common spoilage fungi, but it was not until 1960, when the famous "Turkey X" disease killed 100,000 turkey poults in Great Britain, and various other disasters followed in rapid succession, that the Western world became aware that common spoilage moulds could produce significant toxins. Since 1960 a seemingly endless stream of toxigenic fungi and potentially toxic compounds has been discovered. On these grounds alone, the statement "It's only a mould" is no longer acceptable to food microbiologist, health inspector or consumer. The demand for accurate identification and characterisation of food spoilage fungi has become urgent.

In the flurry of research into mycotoxins, however, it must not be forgotten that food spoilage as such remains an enormous problem throughout the world. Figures are difficult to obtain. However, even given a dry climate and advanced technology, losses of food to fungal spoilage in Australia must be in excess of $10,000,000 per annum: losses in humid tropical climates and countries with less highly developed technology remain staggering. An estimate of 5–10% of all food production is not unrealistic. Research into fungal food spoilage and its prevention is clearly an urgent necessity: lacking in spectacular appeal, it is, however, often neglected. A further point, of the highest significance, needs emphasis here. Research on the fungi which cause food spoilage, and the mycotoxins they produce, can only be carried out effectively if based on accurate identification of the microorganisms responsible. Taxonomy and nomenclature (systematics) make up the vital root system of all the trees of biological science.

The prevention of fungal food spoilage as an art is old, but as a discipline, young. Drying, the oldest method of food preservation, has been practiced for millennia and is still the most common, effective and cheap technique for preserving food. Only recently have we been able to identify with certainty the fungal species which cause spoilage of dried foods. Prediction of their responses to a given environment, specified by physico-chemical parameters such as water activity, temperature, pH and oxygen tension, even now is often uncertain. Within historic times, newer methods of food preservation have been introduced – salting, curing, canning, refrigeration, freezing, the use of preservatives, irradiation and most recently, high hydrostatic pressure.

Freezing excepted, each new technique has selected for one or more fungal species resistant to

J.I. Pitt, A.D. Hocking, *Fungi and Food Spoilage*, DOI 10.1007/978-0-387-92207-2_1,
© Springer Science+Business Media, LLC 2009

the process applied. As examples we can take *Polypaecilum pisce* on salt fish, *Xeromyces bisporus* on fruit cake, *Cladosporium herbarum* on refrigerated meat, *Zygosaccharomyces bailii* in preserved juices, *Z. rouxii* in jams and fruit concentrates, *Aspergillus flavus* on peanuts, *Eurotium chevalieri* on hazel nuts, *Penicillium roqueforti* on cheeses, *Byssochlamys fulva* in acid canned foods . . . the list of quite specific food – fungus associations is extensive. The study of such associations is one of the more important branches of the young discipline, food mycology.

This book sets out to document current knowledge on the interaction of foods and fungi, in the context of spoilage and toxicity, not food production or biotechnology. Four aspects are examined. First, ecology: what factors in foods select for particular kinds of fungi? A chapter is devoted to the physical and chemical parameters which influence the growth of fungi in foods. Second, methodology: how do we isolated fungi from foods? What are the best media to use? How do we go about identifying food spoilage fungi? Third, the commodity: what fungi are usually associated with a particular food? Here ecological factors interact to produce a more or less specific habitat. Major classes of foods and their associated spoilage fungi are described. Finally, the fungus: what fungus is that? In a series of chapters, the main food spoilage moulds and yeasts are described and keyed, together with others commonly associated with food but not noted for spoilage. Where possible, further information is given on known habitats and sources, physiology, heat resistance, etc., together with a selective bibliography. Accurate information on mycotoxin production is also included.

As far as possible, the precise terminology for fungal structures used by the pure mycologist and indeed most necessary for him has been avoided in these chapters. Some concepts and terms are of course essential: these have been introduced as needed and are listed in a glossary.

The taxonomic sections of this book are designed to facilitate identification of food spoilage and common food contaminant fungi. A standardised plating regimen is used, originally developed for the identification of *Penicillium* species (Pitt, 1979b) and extended here to other genera relevant to the food industry. Under this regimen, cultures are incubated for 1 week at 5, 25 and 37°C on a single standard medium and at 25°C on two others. In conjunction with the appropriate keys, this system will enable identification of most foodborne fungi to species level in just 7 days. For a few kinds of fungi, notably yeasts and xerophiles, subsequent growth under other more specialised conditions will be necessary.

Finally, this book is dedicated to the general food microbiologist. May it help to restore equilibrium and assist in continued employment, when the quality assurance manager demands: "What is it?" . . . "How did it get in?" . . . "What does it do?" . . . "How do we get rid of it?" . . . and, worst of all . . . "Is it toxic?"

Chapter 2
The Ecology of Fungal Food Spoilage

Food is not commonly regarded as an ecosystem, perhaps on the basis that it is not a "natural" system. Nevertheless an ecosystem it is and an important one, because food plants and the fungi that colonise their fruiting parts (seeds and fruit) have been co-evolving for millennia. The seed and nut caches of rodents have provided a niche for the development of storage fungi. Fallen fruit, as they go through the cycle of decay and desiccation, have provided substrate for a range of fungi. Humans have aided and abetted the development of food spoilage fungi through their vast and varied food stores. It can be argued, indeed, some rapidly evolving organisms, such as haploid asexual fungi, are moving into niches created by man's exploitation of certain plants as food.

Food by its very nature is expected to be nutritious: therefore, food is a rich habitat for microorganisms, in contrast with the great natural systems, soil, water and plants. Given the right physico-chemical conditions, only the most fastidious microorganisms are incapable of growth in foods, so that factors other than nutrients usually select for particular types of microbial populations.

Perhaps the most important of these factors relates to the biological state of the food. Living foods, particularly fresh fruits, vegetables, and also grains and nuts before harvest, possess powerful defence mechanisms against microbial invasion. The study of the spoilage of such fresh foods is more properly a branch of plant pathology than food microbiology. The overriding factor determining spoilage of a fresh, living food is the ability of specific microorganisms to overcome defence mechanisms. Generally speaking, then, spoilage of fresh foods is limited to particular species. Such specific relationships between fresh food and fungus are discussed in Chapter 11 and under particular species.

Other kinds of foods are moribund, dormant or nonliving, and the factors which govern spoilage are physical and chemical. There are eight principal factors:

(l) water activity;
(2) hydrogen ion concentration;
(3) temperature – of both processing and storage;
(4) gas tension, specifically of oxygen and carbon dioxide;
(5) consistency;
(6) nutrient status;
(7) specific solute effects; and
(8) preservatives.

Each will be discussed in turn below.

2.1 Water Activity

Water availability in foods is most readily measured as water activity. Water activity (a_w), is a physico-chemical concept, introduced to microbiologists by Scott (1957), who showed that a_w effectively quantified the relationship between moisture in foods and the ability of microorganisms to grow on them.

Water activity is defined as a ratio:

$$a_w = p/p_o,$$

where p is the partial pressure of water vapour in the test material and p_o is the saturation vapour pressure of pure water under the same conditions.

J.I. Pitt, A.D. Hocking, *Fungi and Food Spoilage*, DOI 10.1007/978-0-387-92207-2_2,
© Springer Science+Business Media, LLC 2009

Water activity is numerically equal to equilibrium relative humidity (ERH) expressed as a decimal. If a sample of food is held at constant temperature in a sealed enclosure until the water in the sample equilibrates with the water vapour in the enclosed air space (Fig. 2.1a), then

$$a_w\,(\text{food}) = \text{ERH}\,(\text{air})/100.$$

Conversely, if the ERH of the air is controlled in a suitable way, as by a saturated salt solution, at equilibrium the a_w of the food will be numerically equal to the generated ERH (Fig. 2.1b). In this way, a_w can be experimentally controlled, and the relation of a_w to moisture (the sorption isotherm) can be studied. For further information on water activity, its measurement and significance in foods see Duckworth (1975); Pitt (1975); Troller and Christian (1978); Rockland and Beuchat (1987).

In many practical situations, a_w is the dominant environmental factor governing food stability or spoilage. A knowledge of fungal water relations will then enable prediction both of the shelf life of foods and of potential spoilage fungi. Although the water relations of many fungi will be considered individually in later chapters, it is pertinent here to provide an overview.

Like all other organisms, fungi are profoundly affected by the availability of water. On the a_w scale, life as we know it exists over the range 0.9999+ to 0.60 (Table 2.1). Growth of animals is virtually confined to 1.0–0.99 a_w; the permanent wilt point of mesophytic plants is near 0.98 a_w; and most microorganisms cannot grow below 0.95 a_w. A few halophilic algae and bacteria can grow in saturated sodium chloride (0.75 a_w), but are confined to salty environments. Ascomycetous fungi and conidial fungi of ascomycetous origin comprise most of the organisms capable of growth below 0.9 a_w. Fungi capable of growth at low a_w, in the presence of extraordinarily high solute concentrations both inside and out, must be ranked as among the most highly evolved organisms on earth. Even among the fungi, this evolutionary path must have been of the utmost complexity: the ability to grow at low a_w is confined to only a handful of genera (Pitt, 1975).

The degree of tolerance to low a_w is most simply expressed in terms of the minimum a_w at which germination and growth can occur. Fungi able to grow at low a_w are termed xerophiles: one widely used definition is that a xerophile is a fungus able to grow below 0.85 a_w under at least one set of environmental conditions (Pitt, 1975). Xerophilic fungi will be discussed in detail in Chapter 9.

Information about the water relations of many fungi remains fragmentary, but where it is known it has been included in later chapters.

2.2 Hydrogen Ion Concentration

At high water activities, fungi compete with bacteria as food spoilers. Here pH plays the decisive role. Bacteria flourish near neutral pH and fungi cannot compete unless some other factor, such as low water

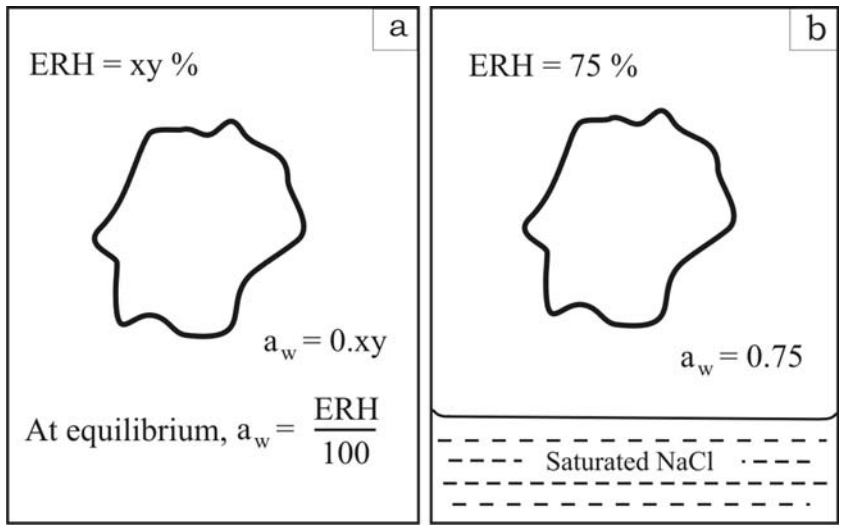


Fig. 2.1 The concept of water activity (a_w) **(a)** the relationship between a_w and equilibrium relative humidity (ERH); **(b)** one method of controlling a_w by means of a saturated salt solution, which generates a specific ERH at a specific constant temperature

Table 2.1 Water activity and microbial water relations in perspective[a]

a_w	Perspective	Foods	Moulds	Yeasts
1.00	Blood, plant wilt point, seawater	Vegetables meat, milk fruit		
0.95	Most bacteria	Bread	Basidiomycetes Most soil fungi	Basidiomycetes
0.90		Ham	Mucorales *Fusarium*	Most ascomycetes
0.85	*Staphylococcus aureus*	Dry salami	*Rhizopus, Cladosporium*	*Zygosaccharomyces rouxii* (salt)
0.80			*Aspergillus flavus* Xerophilic Penicillia	*Zygosaccharomyces bailii*
0.75	Salt lake Halophiles	Jams Salt fish Fruit cake	Xerophilic Aspergilli *Wallemia* *Eurotium*	*Debaryomyces hansenii*
0.70		Confectionery Dried fruit Dry grains	*Chrysosporium* *Eurotium halophilicum*	
0.65			*Xeromyces bisporus*	*Zygosaccharomyces rouxii* (sugar)
0.60	DNA disordered			

[a] Modified from data of J.I. Pitt as reported by Brown (1974). Water activities shown for microorganisms approximate minima for growth reported in the literature.

activity or a preservative, renders the environment hostile to the bacteria. As pH is reduced below about 5, growth of bacteria becomes progressively less likely. Lactic acid bacteria are exceptional, as they remain competitive with fungi in some foods down to about pH 3.5. Most fungi are little affected by pH over a broad range, commonly 3–8 (Wheeler et al., 1991). Some conidial fungi are capable of growth down to pH 2, and yeasts down to pH 1.5. However, as pH moves away from the optimum, usually about pH 5, the effect of other growth limiting factors may become apparent when superimposed on pH. Figure 2.2 is an impression of the combined influence of pH and a_w on microbial growth: few accurate data points exist and the diagram is schematic.

For heat-processed foods, pH 4.5 is of course critical: heat processing to destroy the spores of *Clostridium botulinum* also destroys all fungal spores. In acid packs, below pH 4.5, less severe processes may permit survival of heat-resistant fungal spores (Section 2.3).

2.3 Temperature

The influence of temperature in food preservation and spoilage has two separate facets: temperatures during processing and those existing during storage.

As noted above, heat-resistant fungal spores may survive pasteurising processes given to acid foods. Apart from a few important species, little information exists on the heat resistance of fungi. Much of the information that does exist must be interpreted with care, as heating menstrua and conditions can vary markedly, and these may profoundly affect heat resistance. High levels of sugars are generally protective (Beuchat and Toledo, 1977). Low pH and preservatives increase the effect of heat (Beuchat, 1981a, b; Rajashekhara et al., 2000) and also hinder resuscitation of damaged cells (Beuchat and Jones, 1978).

Ascospores of filamentous fungi are more heat resistant than conidia (Pitt and Christian, 1970; Table 2.2). Although not strictly comparable, data of Put et al. (1976) indicate that the heat resistance of yeast ascospores and vegetative cells is of the same order as that of fungal conidia.

Among the ascomycetous fungi, *Byssochlamys* species are notorious for spoiling heat processed fruit products (Olliver and Rendle, 1934; Richardson, 1965). The heat resistance of *B. fulva* ascospores varies markedly with isolate and heating conditions (Beuchat and Rice, 1979): a D value between 1 and 12 min at 90°C (Bayne and Michener, 1979) and a z value of 6–7C° (King et al., 1969) are practical working figures. The heat resistance of

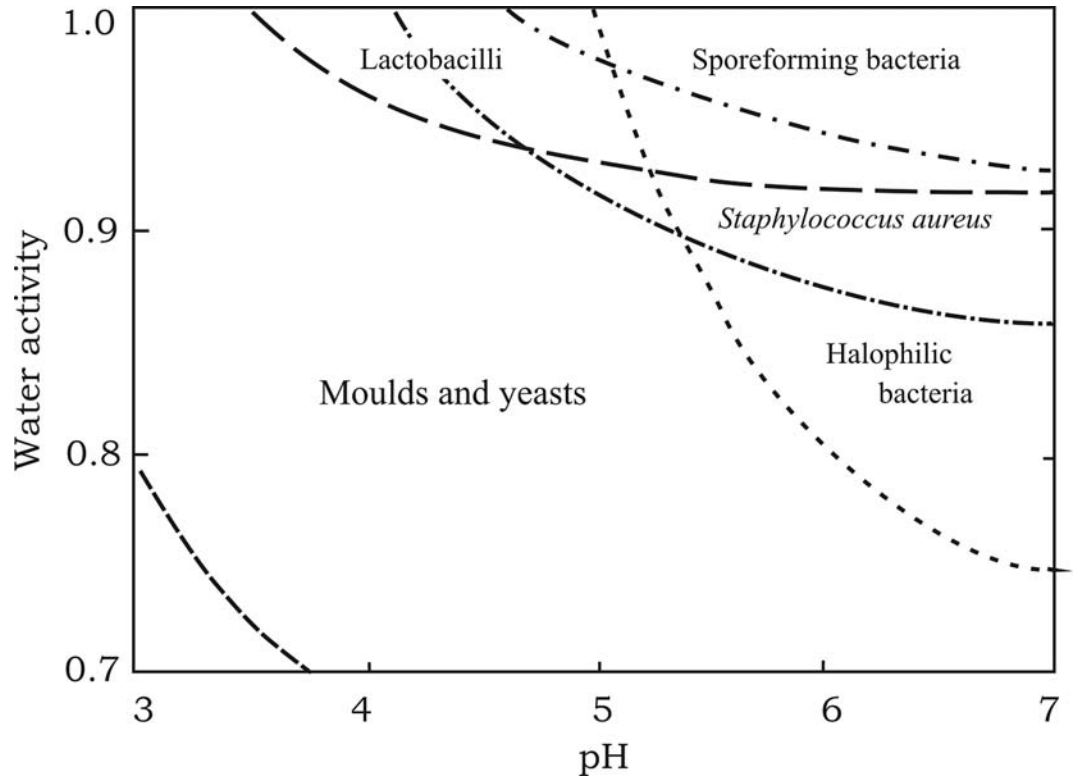


Fig. 2.2 A schematic diagram showing the combined influence of water activity and pH on microbial growth

B. nivea ascospores is marginally lower (Beuchat and Rice, 1979; Kotzekidou, 1997a).

Ascospores of *Neosartorya fischeri* have a similar heat resistance to those of *Byssochlamys fulva*, but have been reported less frequently as a cause of food spoilage. Heat resistant fungi are discussed further in Chapter 4.

Food products may be stored at ambient temperatures, in which case prevention of spoilage relies on other parameters, or under refrigeration, where temperature is expected to play a preservative role. Food frozen to –10°C or below appears to be microbiologically stable, despite some reports of fungal growth at lower temperatures. The lowest temperatures for fungal growth are in the range –7 to 0°C, for species of *Fusarium*, *Cladosporium*, *Penicillium* and *Thamnidium* (Pitt and Hocking, 1997). Nonsterile food stored at ca. 5°C in domestic refrigerators, where conditions of high humidity prevail, will eventually be spoiled by fungi of these genera. At high a_w and neutral pH, psychrophilic bacteria may also be important (mostly *Pseudomonas* species).

Table 2.2 Comparative heat resistance of ascospores and conidia[a]

			Survivors (%)		
Fungus	Spore type	Initial viable count/ml	50°C	60°C	70°C
Eurotium amstelodami	Ascospores	5.0×10^2	93	85	3
	Conidia	7.3×10^2	107	0.3	0
Eurotium chevalieri	Ascospores	1.0×10^3	103	62	21
	Conidia	8.9×10^2	128	0.1	0
Xeromyces bisporus	Ascospores	1.0×10^3	93	30	0.3
Aspergillus candidus	Conidia	3.8×10^2	102	0	0
Wallemia sebi	Conidia	7.1×10^2	42	0	0

[a] Heated at temperatures shown for 10 min. Data from Pitt and Christian (1970).

Thermophilic fungi, i.e. those which grow only at high temperatures, are rarely of significance in food spoilage. If overheating of commodities occurs, however, in situations such as damp grain, thermophiles can be a very serious problem.

Thermotolerant fungi, i.e. species able to grow at both moderate and high temperatures, are of much greater significance. *Aspergillus flavus* and *A. niger*, able to grow between ca. 8 and 45°C, are among the most destructive moulds known.

2.4 Gas Tension

Food spoilage moulds, like almost all other filamentous fungi, have an absolute requirement for oxygen. However, many species appear to be efficient oxygen scavengers, so that the total amount of oxygen available, rather than the oxygen tension, determines growth. The concentration of oxygen dissolved in the substrate has a much greater influence on fungal growth than atmospheric oxygen tension (Miller and Golding, 1949). For example, *Penicillium expansum* grows virtually normally in 2.1% oxygen over its entire temperature range (Golding, 1945), and many other common food spoilage fungi are inhibited only slightly when grown in nitrogen atmospheres containing approximately 1.0% oxygen (Hocking, 1990). *Paecilomyces variotii* produced normal colonies at 25°C under 650 mm of vacuum (Pitt, unpublished).

Most food spoilage moulds appear to be sensitive to high levels of carbon dioxide, although there are notable exceptions. When maintained in an atmosphere of 80% carbon dioxide and 4.2% oxygen, *Penicillium roqueforti* still grew at 30% of the rate in air (Golding, 1945), provided that the temperature was above 20°C. In 40% CO_2 and 1% O_2, *P. roqueforti* grew at almost 90% of the rate in air (Taniwaki et al., 2001a). *Xeromyces bisporus* has been reported to grow in similar levels of carbon dioxide (Dallyn and Everton, 1969).

Byssochlamys species appear to be particularly tolerant of conditions of reduced oxygen and/or elevated carbon dioxide. Growth of *Byssochlamys nivea* was little affected by replacement of nitrogen in air by carbon dioxide, and growth in carbon dioxide–air mixtures was proportional only to oxygen concentration, at least up to 90% carbon dioxide (Yates et al., 1967). Both *Byssochlamys nivea* and *B. fulva* were capable of growth in atmospheres containing 20, 40 or 60% carbon dioxide with less than 0.5% oxygen, but inhibition increased with increasing carbon dioxide concentration (Taniwaki et al., 2001a). *Byssochlamys fulva* is capable of growth in 0.27% oxygen, but not in its total absence (King et al., 1969). It is also capable of fermentation in fruit products, but presumably only if some oxygen is present.

At least some species of *Mucor, Rhizopus* and *Fusarium* are able to grow and ferment in bottled liquid products and sometimes cause fermentative spoilage. Growth under these conditions may be yeast-like. Species of *Mucor, Rhizopus* and *Amylomyces* used as starter cultures in Asian fermented foods can grow under anaerobic conditions, demonstrated by growth in an anaerobe jar with a hydrogen and carbon dioxide generator (Hesseltine et al., 1985). Other authors have reported growth under anaerobic conditions of such fungi as *Mucor* species, *Absidia spinosa, Geotrichum candidum*, *Fusarium oxysporum* and *F. solani* (Stotzky and Goos, 1965; Curtis, 1969; Taniwaki, 1995). The yeast-like fungus *Moniliella acetoabutans* can cause fermentative spoilage under totally anaerobic conditions (Stolk and Dakin, 1966).

As a generalisation, however, it is still correct to state that most food spoilage problems due to filamentous fungi occur under aerobic conditions, or at least where oxygen tension is appreciable, due to leakage or diffusion through packaging.

In contrast, *Saccharomyces* species, *Zygosaccharomyces* species and other fermentative yeasts are capable of growth in the complete absence of oxygen. Indeed, *S. cerevisiae* and *Z. bailii* can continue fermentation under several atmospheres pressure of carbon dioxide. This property of *S. cerevisiae* has been harnessed by mankind for his own purposes, in the manufacture of bread and many kinds of fermented beverages. *Z. bailii*, on the other hand, is notorious for its ability to continue fermenting at reduced water activities in the presence of high levels of preservatives. Fermentation of juices and fruit concentrates may continue until carbon dioxide pressure causes container distortion or explosion. The closely related species *Zygosaccharomyces rouxii* is a xerophile and causes

spoilage of low-moisture liquid or packaged products such as fruit concentrates, jams and dried fruit. The difference in oxygen requirements between moulds and fermentative yeasts is one of the main factors determining the kind of spoilage a particular commodity will undergo.

2.5 Consistency

Consistency, like gas tension, exerts considerable influence over the kind of spoilage to which a food is susceptible. Generally speaking, yeasts cause more obvious spoilage in liquid products, because single celled microorganisms are able to disperse more readily in liquids. Moreover, a liquid substrate is more likely to give rise to anaerobic conditions and fermentation is more readily seen in liquids. In contrast, filamentous fungi are assisted by a firm substrate, and ready access to oxygen.

The foregoing is not intended to suggest that yeasts cannot spoil solid products nor moulds liquids: merely that all other factors being equal, fermentative yeasts have a competitive advantage in liquids and cause more obvious spoilage under these conditions.

2.6 Nutrient Status

As noted in the preamble to this chapter, the nutrient status of most foods is adequate for the growth of any spoilage microorganism. Generally speaking, however, it appears that fungal metabolism is best suited to substrates high in carbohydrates, whereas bacteria are more likely to spoil proteinaceous foods. Lactobacilli are an exception.

Most common mould species appear to be able to assimilate any food-derived carbon source with the exception of hydrocarbons and highly condensed polymers such as cellulose and lignin. Most moulds are equally indifferent to nitrogen source, using nitrate, ammonium ions or organic nitrogen sources with equal ease. Some species achieve only limited growth if amino acids or proteins must provide both carbon and nitrogen. A few isolates classified in *Penicillium* subgen. *Biverticillium* are unable to utilise nitrate (Pitt, 1979b).

Some xerophilic fungi are known to be more demanding. Ormerod (1967) showed that growth of *Wallemia sebi* was strongly stimulated by proline. Xerophilic *Chrysosporium* species and *Xeromyces bisporus* also require complex nutrients, but the factors involved have not been defined (Pitt, 1975).

Yeasts are often fastidious. Many are unable to assimilate nitrate or complex carbohydrates; a few, *Zygosaccharomyces bailii* being an example, cannot grow with sucrose as a sole source of carbon. Some require vitamins. These factors limit to some extent the kinds of foods susceptible to spoilage by yeasts.

A further point on nutrients in foods is worth making here. Certain foods (or nonfoods) lack nutrients essential for the growth of spoilage fungi. Addition of nutrient, for whatever reason, can turn a safe product into a costly failure.

Two cases from our own experience illustrate this point, both involving spoilage by the preservative-resistant yeast *Zygosaccharomyces bailii*. In the first, a highly acceptable (and nutritious) carbonated beverage containing 25% fruit juice was eventually forced from the Australian market because it was impractical to prepare it free of occasional *Z. bailii* cells. Effective levels of preservative could not be added legally and pasteurisation damaged its flavour. Substitution of the fruit juice with artificial flavour and colour removed the nitrogen source for the yeast. A spoilage free product resulted, at the cost of any nutritional value and a great reduction in consumer acceptance.

The other case concerned a popular water-ice confection, designed for home freezing. This confection contained sucrose as a sweetener and a preservative effective against yeasts utilising sucrose. One production season the manufacturer decided, for consumer appeal, to add glucose to the formulation. The glucose provided a carbon source for *Zygosaccharomyces bailii*, and as a result several months production, valued at hundreds of thousands of dollars, was lost due to fermentative spoilage.

2.7 Specific Solute Effects

As stated earlier, microbial growth under conditions of reduced water availability is most satisfactorily described in terms of a_w. However,

the particular solutes present in foods can exert additional effects on the growth of fungi. Scott (1957) reported that *Eurotium* (*Aspergillus*) *amstelodami* grew 50% faster at its optimal a_w (0.96) when a_w was controlled by glucose rather than magnesium chloride, sodium chloride or glycerol. Pitt and Hocking (1977) showed a similar effect for *Eurotium chevalieri* and reported that the extreme xerophiles *Chrysosporium fastidium* and *Xeromyces bisporus* grew poorly if at all in media containing sodium chloride as the major solute. In contrast Pitt and Hocking (1977) and Hocking and Pitt (1979) showed that germination and growth of several species of *Aspergillus* and *Penicillium* was little affected when medium a_w was controlled with glucose–fructose, glycerol or sodium chloride.

Zygosaccharomyces rouxii, the second most xerophilic organism known, has been reported to grow down to 0.62 a_w in fructose (von Schelhorn, 1950). Its minimum a_w for growth in sodium chloride is reportedly much higher, 0.85 a_w (Onishi, 1963).

Some fungi are halophilic, being well adapted to salty environments such as salted fish. *Basipetospora halophila* and *Polypaecilum pisce* grow more rapidly in media containing NaCl as controlling solute (Andrews and Pitt, 1987; Wheeler et al., 1988c). Such fungi have been called halophilic xerophiles to distinguish them from obligately halophilic bacteria.

2.8 Preservatives

Obviously, preservatives for use in foods must be safe for human consumption. Under this constraint, food technologists in most countries are limited to the use of weak acid preservatives: benzoic, sorbic, nitrous, sulphurous, acetic and propionic acids – or, less commonly, their esters. In the concentrations permitted by most food laws, these acids are useful only at pH levels up to their pK_a plus one pH unit, because to be effective they must be present as the undissociated acid. For studies of the mechanism of action of weak acid preservatives see Warth (1977, 1991); Brul and Coote (1999); Stratford and Anslow (1998) and Stratford and Lambert (1999).

The use of chemical preservatives in foods is limited by law in most countries to relatively low levels and to specific foods. A few fungal species possess mechanisms of resistance to weak acid preservatives, the most notable being *Zygosaccharomyces bailii*. This yeast is capable of growth and fermentation in fruit-based cordials of pH 2.9–3, of 45°C Brix and containing 800 mg/L of benzoic acid (Pitt and Hocking, 1997). The yeast-like fungus *Moniliella acetoabutans* can grow in the presence of 4% acetic acid and survive in 10% (Pitt and Hocking, 1997).

Of the filamentous fungi, *Penicillium roqueforti* appears to be especially resistant to weak acid preservatives and this property has been suggested as a useful aid to isolation and identification (Engel and Teuber, 1978).

2.9 Conclusions: Food Preservation

It is evident from the above discussion that the growth of fungi in a particular food is governed largely by a series of physical and chemical parameters, and definition of these can assist greatly in assessing the food's stability. The situation in practice is made more complex by the fact that such factors frequently do not act independently, but synergistically. If two or more of the factors outlined above act simultaneously, the food may be safer than expected. This has been described by Leistner and Rödel (1976) as the "hurdle concept". This concept has been evaluated carefully for some commodities such as fermented sausages and is now widely exploited in the production of shelf stable bakery goods and acid sauces.

For most fungi, knowledge remains meagre about the influence of the eight parameters discussed here on germination and growth. However, sufficient information is now available that some rationale for spoilage of specific commodities by certain fungi can be attempted, especially where one or two parameters are of overriding importance. This topic is considered in later chapters devoted to particular commodities.

Chapter 3
Naming and Classifying Fungi

As with other living organisms, the name applied to any fungus is a binomial, a capitalised genus name followed by a lower case species name, both written in italics or underlined. The classification of organisms in genera and species was a concept introduced by Linneaus in 1753 and it is the keystone of biological science. It is as fundamental to the biologist as Arabic decimal numeration is to the mathematician. Here the analogy ends: the concept of "base 10" is rigorous; the concept of a species, fundamental as it is, is subjective and dependent on the knowledge and concepts of the biologist who described it.

3.1 Taxonomy and Nomenclature: Biosystematics

Once biologists began to describe species and to assemble them into genera, questions about their relationships began to arise: is species x described by Jones in 1883 the same as species y described by Smith in 1942? Does species z, clearly distinct from x and y in some characters, belong to the same genus? The study of these relationships is termed *taxonomy*. Modern taxonomy is based on sound scientific principles, but still involves subjective judgment.

When the decision is made that species x and species y are the same, however, the taxonomist must follow clearly established procedures in deciding which name must be used ("has priority"). The application of these procedures is termed *nomenclature* and, for fungi, plants and algae, is governed by the International Code of Botanical Nomenclature (ICBN).

The ICBN is a relatively complex document of about 70 Articles dealing with all aspects of correctly naming plants, algae and fungi. It is amended every 6 years by special sessions at each International Botanical Congress and is republished thereafter. The 17th version of the ICBN (the Vienna code) is the most recently published (McNeill et al., 2006). The ICBN impinges only indirectly on the work of the practicing mycologist or microbiologist. It is nevertheless of vital importance to the orderly naming of all plant life; to ignore the ICBN is to invite chaos.

Where confusion arises over the correct name for a botanical species – a constant source of irritation to the nontaxonomist – it stems usually from one of three causes: indecision by, or disagreement among, taxonomists on what constitutes a particular species; incorrect application of the provisions of the ICBN; or ignorance of earlier literature.

To return to our example, when species x and species y are seen to be the same, x has priority because it was published earlier; y becomes a *synonym* of x. Important synonyms are often listed after a name to aid the user of a taxonomy, and this procedure has been followed here.

Through ignorance, the same species name may be used more than once, for example, *Penicillium thomii* Maire 1915 and *P. thomii* K.M. Zalessky 1927. The name *P. thomii* has been given to two quite different fungi. Clearly *P. thomii* Maire has priority; the later name is not *valid*. To avoid ambiguity, correct practice in scientific publication is to cite the author of a species at first mention, and before any formal description.

The ICBN provides rules to govern change of genus name also. In our example, if species z is transferred to the genus to which species x and y belong, it retains its species name but takes the new

J.I. Pitt, A.D. Hocking, *Fungi and Food Spoilage*, DOI 10.1007/978-0-387-92207-2_3,
© Springer Science+Business Media, LLC 2009

genus name. The original author of the name z is placed in brackets after the species name, followed by the name of the author who transferred it to the correct genus. For example, *Citromyces glaber* Wehmer 1893 became *Penicillium glabrum* (Wehmer) Westling 1893 on transfer to *Penicillium* by Westling in 1911. Note the use of Latinised names: *glaber* (masculine) became *glabrum* (neuter) to agree with the gender of the genus to which it was transferred.

Further points on the use of the ICBN arise from this example. *P. glabrum* retains its date of original publication, and therefore takes priority over *P. frequentans* Westling 1911 if the two species are combined. When Raper and Thom (1949) combined the two species, a taxonomically correct decision, they retained the name *P. frequentans*, which was nomenclaturally incorrect, causing confusion when Subramanian (1971) and Pitt (1979b) took up the correct name. It is worth pointing out that the confusion in this and similar situations arose from Raper and Thom's action in ignoring the provisions of the ICBN, not from that of later taxonomists who correctly interpreted it.

3.2 Hierarchical Naming

A given biological entity, or *taxon* in modern terminology, can be given a hierarchy of names: a cluster of related species is grouped in a genus, of related genera in families, of families in orders, orders in classes, and classes in subkingdoms. Similarly a species can be divided into smaller entities: subspecies, varieties and *formae speciales* (a term usually reserved for plant pathogens).

In most modern classifications, the fungi are ranked, like plants and animals, as a separate kingdom. Traditionally, fungi have been divided into several subkingdoms, based on spore type and some environmental considerations. Modern molecular methods have revolutionised this. Fungi have been shown to be more closely related to animals than plants, where traditional taxonomy has always placed them. Some of the so-called "lower fungi" have been shown not to be fungi of all (though mycologists will no doubt continue to study them).

The most important change from the point of view of the food mycologist is the demise of the subkingdom Deuteromycotina, and its absorption (almost entirely) into the Ascomycotina. The connection between the Ascomycetes, the fungi that produce sexual spores in sacks, and the Deuteromycetes, where spores are always asexual, has been known for a long time. However, molecular taxonomy has provided the fundamental assurance needed to make this change. From the point of view of the food mycologist, this is a mixed blessing. The demands of the molecular systematists may yet make the taxonomy of foodborne fungi even more complicated. The taxonomic system used here is believed to be both practical and in line with the current "best practice" of the nomenclaturalists.

The hierarchical subdivisions in Kingdom Fungi of interest in the present context are shown below, using as examples three genera and species important in food spoilage:

Kingdom	Fungi	Fungi	Fungi
Subkingdom	Zygomycotina	Ascomycotina	Basidiomycotina
Class	Zygomycetes	Plectomycetes	Wallemiomycetes
Order	Mucorales	Eurotiales	Wallemiales
Family	Mucoraceae	Trichocomaceae	Wallemiaceae
Genus	*Rhizopus*	*Eurotium*	*Wallemia*
Species	*stolonifer*	*chevalieri*	*sebi*
Variety		*intermedius*	

Note that names of genera, species and varieties are italicised or underlined, while higher taxonomic ranks are not.

Three subkingdoms of the kingdom Fungi include genera of significance in food spoilage. As indicated in the examples above, these are Zygomycotina, Ascomycotina and (much less commonly) Basidiomycotina. Fungi from each of these subkingdoms have quite distinct properties, shared with other genera and species from the same subkingdom. Unlike other texts, this book will not rely on initial recognition of a correct subkingdom before identification of genus and species can be undertaken. Nevertheless, identification of the subkingdom can provide valuable information about a fungus, so the principal properties of these three subkingdoms are described below.

3.3 Zygomycotina

Most fungi within the subkingdom Zygomycotina belong to the class Zygomycetes. Fungi in this class possess three distinctive properties:

1. **Rapid growth.** Most isolates grow very rapidly, often filling a Petri dish of malt extract agar with loose mycelium in 2–4 days.
2. **Nonseptate mycelium.** Actively growing mycelia are without septa (cross walls) and are essentially unobstructed. This allows rapid movement of cell contents, termed "protoplasmic streaming", which can be seen readily by transmitted light under the binocular microscope. In wet mounts the absence of septa is usually obvious (Fig. 3.1a).
3. **Reproduction by sporangiospores.** The reproductive structure characteristic of Zygomycetes is the *sporangiospore*, an asexually produced spore which in genera of interest here is usually produced inside a sac, the *sporangium*, on the end of a long specialised hypha. Sporangiospores are produced very rapidly.

From the food spoilage point of view, the outstanding properties of Zygomycetes are very rapid growth, especially in fresh foods of high water activity; inability to grow at low water activities (no Zygomycetes are xerophiles); and lack of resistance to heat and chemical treatments. From the food safety point of view, Zygomycetes have rarely been reported to produce mycotoxins.

3.4 Ascomycotina

The subkingdom Ascomycotina is distinguished from Zygomycotina by a number of fundamental characters, the most conspicuous being the production of septate mycelium (Fig. 3.1b). Consequent on this, growth of fungi in this subkingdom is usually slower than that of Zygomycetes, although there are some exceptions.

Fungi in the subkingdom Ascomycotina, loosely called "ascomycetes", characteristically produce their reproductive structures, *ascospores*, within a sac called the *ascus* (plural, *asci*, Fig. 3.2a, b). In most fungi, nuclei normally exist in the haploid state. At one point in the ascomycete life cycle, diploid nuclei are produced by nuclear fusion, which may or may not be preceded by fusion of two mycelia. These nuclei undergo meiosis within the ascus, followed by a single mitotic division and then differentiation into eight haploid ascospores. In most genera relevant to this work, asci can be recognised in stained wet mounts by their shape, which is spherical to ellipsoidal and smoothly rounded; size, which is generally 8–15 μm in diameter; and the presence when maturity approaches of eight ascospores tightly packed within their walls. At maturity asci often rupture to release the ascospores, which are thick walled, highly refractile, and often strikingly ornamented (Fig. 3.2c, d).

Two other characteristics of asci are significant: generally they mature slowly, after incubation for 10 days or more at 25°C, and they are usually borne within a larger, macroscopic body, the general term for which is *ascocarp*. Genera of interest here usually produce asci and ascospores within a spherical, smooth-walled body, the *cleistothecium* (Fig. 3.3a), or a body with hyphal walls, the *gymnothecium* (Fig. 3.3b).

Ascospores are highly condensed, refractile spores, which are often resistant to heat, pressure

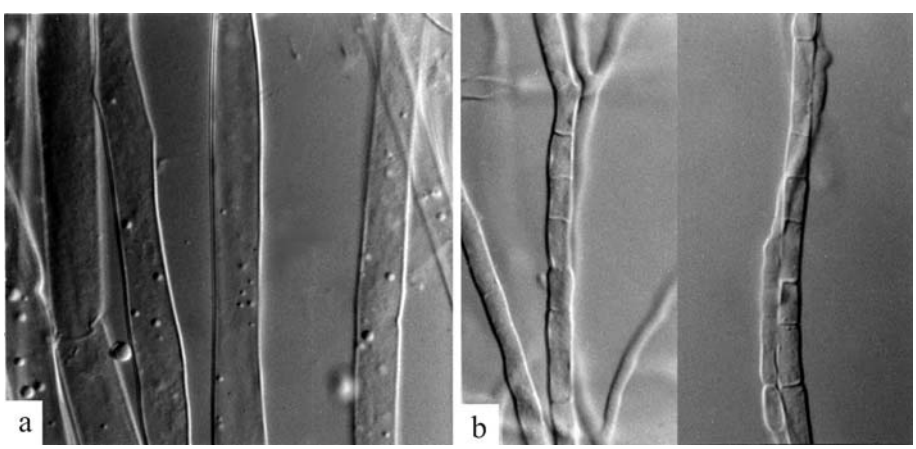


Fig. 3.1 **(a)** Nonseptate mycelium of *Syncephalastrum racemosum*; **(b)** septate mycelium of *Fusarium equiseti*

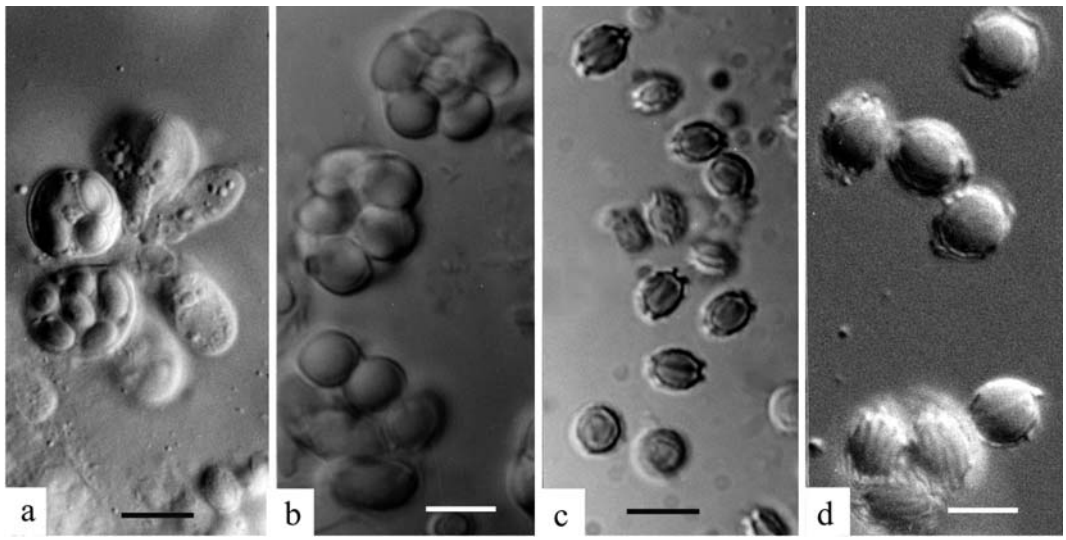


Fig. 3.2 Asci and ascocarps: **(a)** asci of *Talaromyces* species; **(b)** asci of *Byssochlamys fulva*; **(c)** ascospores of *Eupenicillium alutaceum*; **(d)** ascospores of *Neosartorya quadricincta*. Bars = 5 μm

and chemicals. Almost all xerophilic fungi are ascomycetes.

Besides their sexual spores, ascospores, ascomycetes commonly produce asexual spores. Formed after mitotic nuclear division, these spores are borne singly or in chains, in most genera of interest here from more or less specialised hyphal structures. The general term for this type of spore is *conidium* (plural, *conidia*), but other more specialised terms exist for specific kinds of conidia. Along the evolutionary process, some Ascomycetes with well-developed asexual stages lost the ability to produce ascospores, and rely entirely on conidia for dispersal.

Conidia, and the specialised hyphae from which they are borne, are astonishingly diverse in appearance. The size, shape and ornamentation of conidia and the complexity of the structures producing them

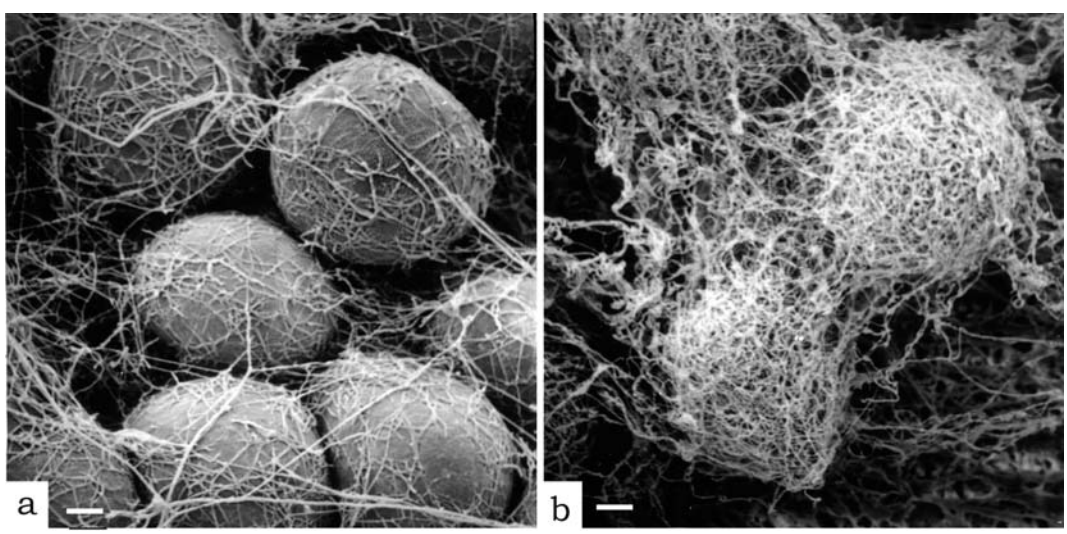


Fig. 3.3 **(a)** Cleistothecia of *Eupenicillium*; **(b)** gymnothecia of *Talaromyces*. SEM. Bars = 50 μm

provide the basis for classification of Ascomycetes that no longer produce the ascosporic (sexual) stage.

Lacking ascospores, conidial fungi are not usually heat resistant, but conidia may be quite resistant to chemicals. Some conidial fungi are xerophilic.

3.5 Basidiomycotina

The Subkingdom Basidiomycotina includes mushrooms, puffballs and the plant pathogenic rusts and smuts. Until recently it was not considered of any interest to the food mycologist. However, molecular studies indicate that the small brown species *Wallemia sebi*, long a curiosity because of its lack of resemblance to any other fungus, is a basidiomycete. It has no obvious phylogenetic affinity with any other genus and has now been classified in its own order, Wallemiales (Zalar et al., 2005). Only one other species of foodborne fungi, *Trichosporonoides nigrescens* Hocking and Pitt (1981), has a known affinity with this subkingdom.

3.6 The Ascomycete – Conidial Fungus Connection

It was established more than a century ago that many fungal species carry the genetic information to produce both ascospores and conidia. These two kinds of spores are produced by different mechanisms and have different functions, so they are not always formed simultaneously. Not surprisingly, mycologists sometimes have given different generic and species names to a single fungus producing both an ascosporic and a conidial state. The usage of these names under the ICBN depends on the circumstances under which they were originally given. Some of these circumstances are discussed briefly below.

The ascomycete state, now usually referred to as the *teleomorph*, is regarded by nomenclaturalists as the more important reproductive state, and the name applied to the teleomorph should be used when the ascomycete state is present. If the conidial state is also in evidence, the fungus is now a *holomorph* and is still correctly known by the teleomorph name. If the conidial state, known as the *anamorph*, has a separate name, this strictly speaking applies to the conidial state. It should be used only when the ascomycete state is absent, or to refer specifically to the conidial state if the ascomycete is present. However, the reader is warned that some anamorphic names are, and will continue to be, in common use for holomorphic fungi.

Under the Articles of the ICBN, a generic name originally given to an anamorphic or conidial fungus cannot be used for a teleomorphic or ascomycetous fungus. For example, the name *Penicillium*, originally given to an anamorphic fungus with no known teleomorph, cannot be used for the teleomorphs later found to be produced by other *Penicillium* species. Such teleomorphs are classified in the genera *Eupenicillium* or *Talaromyces*, depending on whether ascospores are produced in cleistothecia or gymnothecia.

Correct species names for the ascomycetous and conidial states of a single holomorphic fungus may or may not be the same, depending both on the circumstance in which the names were originally given, and on later synonymy. For example, *Eupenicillium ochrosalmoneum* Scott and Stolk and *Penicillium ochrosalmoneum* Udagawa refer to the teleomorph and anamorph of a single fungus. Udagawa (1959) described the anamorph; the teleomorph was later found, in the same isolate, by Scott and Stolk (1967).

On the other hand, the anamorph of *Eupenicillium cinnamopurpureum* Scott and Stolk (1967) is *Penicillium phoeniceum* van Beyma (1933), with *P. cinnamopurpureum* Abe ex Udagawa (1959) as a synonym. Scott and Stolk (1967) found a teleomorph in Udagawa's *P. cinnamopurpureum*; Pitt (1979b) later showed that this species was a synonym of the earlier *P. phoeniceum*. *E. cinnamopurpureum*, the first name applied to the teleomorph, is unaffected by this change in the anamorph name. In passing, note that "Abe ex Udagawa" indicates invalid (incomplete) publication of this species by Abe, with validation later by Udagawa. The species dates from the year of validation.

3.7 Dual Nomenclature

An important point here is that some isolates of *Penicillium phoeniceum* regularly produce the teleomorphic state *Eupenicillium cinnamopurpureum*, while

others, taxonomically indistinguishable, fail to produce a teleomorph at all. Because of this, it is essential to have a separate name for teleomorph and anamorph. The system of two names for a single fungus, known as dual nomenclature, has a place in the classification of fungi despite its apparent complexity. In the descriptions in later chapters, fungi for which both teleomorphs and anamorphs are known have both names listed. As noted above, if both states are found in a particular isolate, the teleomorph name is the more appropriate: to use that given to the anamorph is not incorrect, but this name is more sensibly applied to the conidial state only.

Dual nomenclature would be relatively simple if the relationship between anamorph and teleomorph was always one to one. This is not the case. As has already been mentioned, species classified in *Penicillium* may produce teleomorphs in two genera, *Eupenicillium* and *Talaromyces*. On the other hand *Talaromyces* produces anamorphs in two genera, *Penicillium* and *Paecilomyces*. *Aspergillus* is the anamorph of eight or ten teleomorphic genera. Most teleomorph–anamorph relationships encountered in food mycology belong to the genera mentioned here. These relationships will be described where necessary under these particular genera.

3.8 Practical Classification of Fungi

Fungi are classified in a vast array of orders, families, genera and species. Among natural organisms, the numbers of taxa of fungi are rivalled only by those of the flowering plants and insects. Estimates of fungal species range as high as 1.5 million; only 5% of this number have so far been described (Hawksworth, 1991).

Many fungi are highly specialised. Some will grow only in particular environments such as soil or water; many are obligate parasites and require a specific host, such as a particular plant species, and will not grow in artificial culture; many grow only in association with plant roots. From the point of view of the food microbiologist, these kinds of fungi are irrelevant. In one sense, most fungi which spoil foods are also highly specialised, their speciality being the ability to obtain nutrients from, and hence grow on, dead, dormant or moribund plant material more or less regardless of source. The principal factors influencing food spoilage by fungi are physico-chemical and have already been outlined in Chapter 2. The point being made here is that food spoilage fungi are classified in just a few orders and a relative handful of genera. For this reason there is much to be said for food mycologists avoiding the use of a traditional, hierarchical classification as outlined above and employing a less formal approach to the identification of the fungi of interest to them.

In the present work, this pragmatic approach has been followed as far as possible:

- The use of specialised terms has been kept to a minimum, while being cognisant of the need for clarity of expression.
- Hierarchical classification has been avoided as far as possible, consistent with retaining a logical approach to the presentation of fungi which are related or of similar appearance.
- Identification procedures used have been designed to be simple and comprehensible, avoiding the use of specialised equipment or procedures unavailable in the routine laboratory. To this end, identification of nearly all species included in this work is based entirely on inoculation of a single series of Petri dishes, incubation under carefully standardised conditions and examination by traditional light microscopy.
- A standard plating regimen has been used for the initial examination of all isolates (except yeasts), so that identification procedures can be carried out without foreknowledge of genus or even subkingdom.
- Cultural characters, which can be broadly defined as the application of microbiological techniques to mycology, have been used throughout.

The use of cultural characters has long been implicit in the study of fungi in pure culture on artificial substrates, especially in such genera as *Aspergillus* and *Penicillium*, genera of paramount importance in food spoilage. In *Penicillium*, cultural characters have been used as taxonomic criteria since the turn of the 20th century, but have assumed greater importance through the work of Pitt (1973, 1979b), who used the measurement of colony diameters, following incubation under standardised conditions, as a taxonomic criterion. The use of

pure culture techniques and growth data in fungal taxonomy is now widespread.

Food microbiologists, the primary audience for this book, are familiar with cultural techniques and the use of a wide range of media and varied incubation conditions, so the authors make no apology for the taxonomic approach used in the present work. This approach is a logical extension of the system used by the first author in *Penicillium* taxonomy and which has been found to have a much broader applicability.

In the field of mycology, different genera have been studied by many different people of varied backgrounds and for different reasons. Consequently, keys and descriptions have been based on a wide variety of media, often traditional formulations incorporating all sorts of natural products. This heterogeneity makes comparisons difficult and adds unnecessary complexity to the task of the nonspecialist confronted with a range of fungal genera.

The approach used here has been to examine every isolate (excluding yeasts) by a single system: inoculation onto a standard set of Petri dishes and examination of them culturally and microscopically after 7 days incubation. Most of the genera and species included in this book can be identified immediately, at that point. Only in exceptional cases has it been found necessary to reinoculate isolates onto a further set of media in order to complete identification. The exceptional fungi are first the xerophiles, many of which grow poorly if at all on the standard media, and, second, genera such as *Fusarium* and *Trichoderma*, in which some species cannot readily be differentiated on the standard regimen.

Details of the techniques used are given in Chapter 4.

Chapter 4
Methods for Isolation, Enumeration and Identification

This chapter describes techniques and media suitable for the enumeration, isolation and identification of fungi from foods. Some techniques are similar to those used in food bacteriology; others have been developed to meet the particular needs of food mycology. Most of the media have been specifically formulated for foodborne fungi. The approach taken here is designed to provide a systematic basis for the study of food mycology.

In 1984 a group of about 30 of the world's foremost scientists in food mycology met in Boston, Massachusetts, USA, to hear and discuss a wide range of presentations that explored many aspects of methodology in food mycology. Agreement was reached on broad issues and areas requiring further work pinpointed. The proceedings were published as "Methods for the Mycological Examination of Food" (King et al., 1986). At a second workshop, held in Baarn, the Netherlands, in 1990, results of a number of collaborative studies on media and methods were presented and some standardised protocols developed. The proceedings, published as "Modern Methods in Food Mycology" (Samson et al., 1992), provided a comprehensive overview of current thinking in this field.

The working group which organised those two workshops was then formalised as the International Commission on Food Mycology (ICFM), a commission under the auspices of the Mycology Division of the International Union of Microbiological Societies (IUMS). ICFM is dedicated to international standardisation of methods in food mycology. Subsequent ICFM workshops were held in Copenhagen, Denmark (1994), Uppsala, Sweden (1998), Samsø, Denmark (2003) and Key West, Florida (2007). Papers from the third and fourth workshops were published in the *International Journal of Food Microbiology* and the proceedings of the fifth (Samsø) workshop were published as "Advances in Food Mycology" (Hocking et al., 2006a).

The methodology described below is based on recommendations from ICFM and represents current thinking within the food mycology community. However, no formal endorsement from ICFM is implied.

4.1 Sampling

It must be emphasised at the outset that results from mycological assays of foods are only as good as the samples used. However, sampling is beyond the scope of this text. Excellent treatises on sampling plans for food bacteriological purposes have been produced by the International Commission on Microbiological Specifications for Foods (ICMSF, 1986, 2002) and are generally applicable to food mycology.

4.2 Enumeration Techniques

Quantification of the growth of filamentous fungi is more difficult than for bacteria or yeasts. Vegetative growth consists of hyphae, which are not readily detached from the substrate and which survive blending poorly. When sporulation occurs,

J.I. Pitt, A.D. Hocking, *Fungi and Food Spoilage*, DOI 10.1007/978-0-387-92207-2_4,
© Springer Science+Business Media, LLC 2009

very high numbers of spores may be produced, causing sharp rises in viable counts, often without any great increase in biomass.

The estimation of fungal growth or biomass is not easy, because no primary standard exists (such as cell numbers used for yeasts and bacteria). Although techniques for quantifying biomass have improved in recent years, most food laboratories continue to rely on viable counting (dilution plating) for detecting and quantifying fungal growth in foods.

As well as dilution plating, a second standard method, known as direct plating, has been developed for estimating fungal numbers and growth in foods. Both methods are described in detail below. Techniques for biomass estimation will be discussed later.

4.2.1 Direct Plating

Contributions at the international workshops mentioned above emphasised the use of direct plating as the preferred method for detecting, enumerating and isolating fungi from particulate foods such as grains and nuts. In direct plating, food particles are placed directly on solidified agar media. In most situations, particles should be surface disinfected before plating, as this removes the inevitable surface contamination arising from dust and other sources and permits recovery of the fungi actually growing in the particles. This process provides an effective measure of inherent mycological quality and permits assessment of the potential presence of mycotoxins as well.

Surface disinfection should be omitted only where surface contaminants become part of the downstream mycoflora, for example, in grain intended for flour manufacture. Even here, surface disinfection before direct plating provides the most realistic appraisal of actual grain quality.

Results from direct plating analyses are expressed as percentage infection of particles. The technique provides no direct indication of the *extent* of fungal invasion in individual particles. However, it is reasonable to assume that a high percentage infection is correlated with extensive invasion in the particles and a higher risk of mycotoxin occurrence.

The standard protocol recommended by the ICFM (Hocking et al., 2006a, p. 344) is given below, with amplification where necessary.

Surface disinfection. Surface disinfection is carried out by immersing particles in a chlorine solution. Household chlorine bleach, nominally 4–5% active chlorine, is effective. Dilute the chlorine 1 to 10 with water before use, to provide an approximately 0.4% solution. Immerse particles for 2 min, stirring occasionally, then drain the chlorine. Chlorine solutions are rapidly denatured by organic matter, so it is important to use a surplus of chlorine solution (10 times the volume of the particles) and to use the solution only once.

This process is conveniently carried out in 250–500 ml beakers. Place 50 or more particles in the beaker and add chlorine. To dislodge air bubbles, immediately stir with a pair of forceps, leaving them in the solution, and preferably cover the beaker with a watch glass. The watch glass simplifies decanting of the chlorine, and the forceps, disinfected by the chlorine, may be used to plate the particles.

Studies in our laboratory have shown that the treatment outlined here may be inadequate under some conditions. In commodities such as peanuts or maize where high levels of *Aspergillus flavus* or *Penicillium* species may be present, surface disinfection may be difficult. Here 2 min immersion in 70% ethanol followed by 2 min in 0.4% chlorine is recommended.

Rinsing. After the chlorine is poured off, particles may be rinsed once with sterile water. Use a 1 min treatment, with stirring, then pour the water off. Again a watch glass should cover the beaker during this period, and the sterile forceps should be used for stirring. It is not clear whether rinsing fulfils any essential function. Early direct plating regimens used agents such as mercuric chloride for disinfection, and rinsing was essential to remove such toxic materials before they penetrated the particles too deeply. However, chlorine is effectively denatured by the particles and is believed to penetrate very little. In our opinion, the rinsing step can be omitted without loss of efficacy of the treatment, with savings in time and materials, and reduced risk of recontamination from the air.

Plating. After disinfection and the optional rinse, particles should be plated onto solidified agar, at the rate of 6–20 particles per plate, depending on

particle size. Use the disinfected forceps. Plating should be carried out immediately: keep the watch glass on the beaker if this is not possible.

Incubation. Incubate plates upright, under normal circumstances for 5 days at 25°C. See more detailed notes below.

Examination. After incubation, examine plates visually, count the numbers of infected particles and express results as a percentage. Differential counting of various genera is often possible. Correct choice of media, a stereomicroscope and experience will all assist in this process.

4.2.2 Dilution Plating

Dilution plating is the appropriate method for mycological analysis of liquid or powdered foods. It is also suitable for grains intended for flour manufacture and other situations where total fungal contamination is relevant.

Sample preparation. The two most common methods of sample preparation for dilution plating are stomaching and blending: stomaching is recommended by ICFM (King et al., 1986). The Colworth Stomacher (Sharpe and Jackson, 1972), or equivalent equipment (e.g. BagMixer®, Interscience, Saint Nom La Bretèche, France), is a very effective device for dispersing and separating fungi from finely divided materials such as flour and spices, and soft foods such as cheeses and meats, and its use is strongly recommended. Treatment time in the stomacher should be 2 min. Harder or particulate foods such as grains, nuts or dried foods, like dried vegetables, should be soaked before stomaching. Soaking times from 30 to 60 min are generally sufficient, but for extremely hard particles such as dried legumes, soaking for up to 3 h may be required. Comminution in a Waring Blender or similar machine is a suitable alternative for these types of samples and may give a more satisfactory homogenate. Blending times should not exceed 60 sec, as longer treatments may fragment mycelium into lengths too short to be viable or overheat the homogenate.

The sample size used should be as large as possible. If a stomacher or BagMixer 400 is used, a sample size of 10–40 g is suitable.

Diluents. The recommended diluent is aqueous 0.1% peptone (Hocking et al., 2006a, p. 344), suitable for both filamentous fungi and yeasts. Saline solutions, phosphate buffer or distilled water are no longer recommended by ICFM as they may be deleterious to yeasts (Mian et al., 1997). The addition of a wetting agent such as polysorbate 80 (Tween 80) may be desirable for some products, but the natural wetting ability of the peptone is usually adequate.

Special diluents may be necessary in some circumstances. If yeasts are to be enumerated from dried products or juice concentrates, the diluent should also contain 20–30% sucrose, glucose or glycerol, as the cells may be injured or be susceptible to osmotic shock.

Dilution. Serial dilutions of fungi are carried out by the same procedures as those used in bacteriology, and the recommended dilution rate is 1:10 (= 1 + 9). Fungal spores sediment more quickly than bacteria, so it is important to draw aliquots for dilution or plating as soon as possible, preferably within 1 min (Beuchat, 1992).

Plating. Spread (surface) plating is recommended. When pour plates are used, fungi develop more slowly from beneath the agar surface and may be obscured by faster growing colonies from surface spores. Hence spread plating allows more uniform colony development, improves the accuracy of enumeration of the colonies and makes subsequent isolation of pure cultures easier.

The optimum inoculum for surface plating is 0.1 ml. Best results will be obtained if plates are dried slightly before use. After adding the inoculum, spread it evenly over the agar surface with a sterile bent glass rod ("hockey stick"). Sterilise the rod by flaming it with ethanol before use. A plate spinner is a useful accessory. It is usually possible to enumerate plates with up to 150 colonies, but if a high proportion of rapidly growing fungi are present, the maximum number which can be distinguished with any accuracy will be lower. Because of this restriction on maximum numbers, it may be necessary on occasion to accept counts from plates with as few as 10–15 colonies. Clearly, such limitations on numbers per plate and the overgrowth of slow colonies will result in higher counting errors than are usually achieved with bacteria or yeasts.

Enumerating yeasts is less difficult. In the absence of filamentous fungi, from 30 to 300 colonies per

plate can be counted and errors will be comparable with those to be expected in bacterial enumeration.

Incubation. The standard incubation conditions are 25°C for 5 days. However, other conditions may be more suitable in some circumstances (see notes below).

Reporting results. As in bacteriology, results from dilution plating are expressed as viable counts per gram of sample. Note that such results are not directly comparable with those obtained from direct plating and may not offer a direct indication of the extent of fungal growth.

4.2.3 Incubation Conditions

As noted above, the standard incubation conditions specified by ICFM are 25°C for 5 days (Hocking et al., 2006a). Undoubtedly, 25°C is the most suitable temperature for routine work in temperate to subtropical environments. Few if any common fungi are sensitive to this temperature, even those which grow readily under refrigeration. Higher temperatures are unacceptable in the temperate zone: 30°C is close to the upper limit for growth of some important *Penicillium* species. In tropical regions, incubation at 30°C is recommended as a more realistic temperature for enumerating fungi from commodities stored at ambient temperatures. In cool temperate regions such as Europe, 22°C may be a more suitable incubation temperature.

When used for fungi, Petri dishes should be stored upright. The principal reason is that some common fungi can shed large numbers of spores during handling, which in an inverted dish will be transferred to the lid. Reinversion of the Petri dish for inspection or removal of the lid may liberate spores into the air or onto benches and cause serious contamination problems.

4.3 Sampling Surfaces

Methods are outlined below for directly sampling the mycoflora of surfaces of commodities such as fruits, meats, cheeses, salamis and dried fish and also packaging materials, machinery and walls. The techniques are based on those described by Langvad (1980) for studying the fungal flora of leaves.

If samples are particulate, or can be cut up, sterile forceps can be used to press pieces of a suitable size (up to about 10 mm^2) onto a suitable medium in a Petri dish. The sample is then removed, leaving an impression, and any spores or mycelium transferred will form colonies within a few days. This technique is known as press plating.

For packaging materials such as cardboard, an alternative method is to cut a piece which will fit in a standard Petri dish. The dish is prepared by adding a sterile filter paper moistened with 10% glycerol and then placing a bent glass rod on it as a separator. After adding the sample, a thin layer of an appropriate agar medium is poured over its surface. To reduce evaporation, the dish should be sealed with Parafilm or a similar material or placed in a polyethylene bag before incubation at 25°C for a few days. If contamination levels are not too heavy, the number and types of moulds present can be effectively estimated by this method. Colonies may be subcultured for identification.

For sampling walls or other surfaces, or for non-destructive sampling, impressions may be taken using adhesive tape. Carefully handled, tape coming from the roll will be virtually sterile. Press a short length of tape firmly onto the surface to be sampled, adhesive side down, then transfer it, still with the same side down, onto a suitable growth medium. After 1–2 days incubation at 25°C, the tape may be removed to allow development and sporulation of colonies.

Surface sampling techniques for assessment of sanitation in food production and processing areas are discussed by Evancho et al. (2001). Surfaces may be sampled by using sterile swabs or by agar contact methods. The swab method involves rubbing a moistened sterile cotton swab over the test surface and placing the swab in a dilution bottle to be subsequently diluted and plated on appropriate media. Agar contact plates, also known as RODAC (replicate organism direct agar contact) plates, are restricted to use on smooth or semi-smooth flat surfaces. An alternative to agar contact plates is the agar slice technique, where agar is filled into a syringe-like apparatus or into an artificial sausage casing. The solidified agar can be pushed out onto

the surface to be sampled, then the portion making contact sliced off with a sterile scalpel or wire and placed in a Petri dish for incubation.

4.4 Air Sampling

Air sampling in the food processing environment is discussed by Evancho et al. (2001). The simplest method of air sampling is by sedimentation or settle plates. A Petri dish containing an appropriate agar medium is exposed to the atmosphere for a fixed period of time (usually 15–60 min), then closed and incubated at 25°C (Samson et al., 2004a). This method can be useful in food production areas, as it gives a direct indication of the number and types of fungi likely to come into contact with exposed product. However, the settle plate technique lacks precision, and volumetric air sampling is a much more reliable indicator of air quality.

A number of air sampling devices are commercially available. Of these, the Anderson sampler (either the two-stage or six-stage model; Anderson Instruments Inc, Atlanta, GA, USA) is probably the best, giving accurate and consistent results (Buttner and Stetzenbach, 1993). However, the Anderson sampler is expensive and requires mains power or a large battery for operation. In factory situations, the dry cell battery-operated Biotest Hycon RCS and RCS High Flow centrifugal air samplers (Biotest, Solihull, UK) and the MAS-100 air sampler (Merck, Darmstadt, Germany) are more convenient, as they are small and readily portable. The Biotest RCS Plus sampler was reported to give comparable results to larger and more sophisticated machines, but its sampling efficiency gradually decreased for particle sizes below 4 μm (Benbough et al., 1993).

4.5 Isolation Techniques

The term "isolation" is used here in its strict sense: the preparation of a pure culture, free from any contamination and ready for identification.

4.5.1 Yeasts

Streaking techniques commonly used for bacterial purification are equally suitable for the isolation of yeasts (Fig. 4.1). A method widely used by yeast specialists is to disperse a portion of a colony in 2–3 ml of sterile water, then streak a single loop of this suspension over the whole surface of a plate, moving the loop slowly down from top to bottom while simultaneously moving it rapidly across the plate

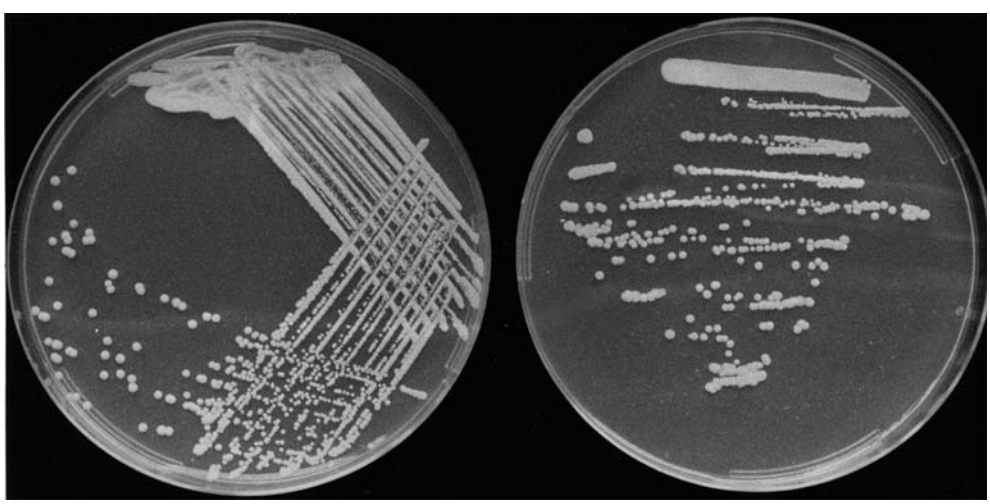


Fig. 4.1 Petri dishes of yeasts growing on malt extract agar, showing a streaking method suitable for producing isolated colonies

from side to side. After suitable incubation, well-separated single colonies should appear in the lower half of the plate (Fig. 4.1).

If all of these single colonies appear to be of similar size and appearance (taking into account the effect of crowding), the culture may be judged to be pure. Microscopic checks of some single colonies are also desirable. Disperse a needle point of cells from a colony in a drop of water, add a cover slip and examine by bright field illumination at about 400×. Cell outlines will be clearly visible. Note that, unlike those of bacteria, yeast cell sizes often vary considerably in a pure preparation. Purity is indicated not so much by uniformity of cell size within a preparation as by similarity in microscopic cell appearance from colony to colony. When a culture is considered to be pure, streak it onto an appropriate slant.

4.5.2 Moulds

Streaking techniques are ineffective for filamentous fungi and are not recommended. Isolation depends on picking a small sample of hyphae or spores – judged to be pure by eye, by hand lens or preferably under the stereomicroscope – and placing this sample on a fresh plate as a point inoculum. Purity is subsequently judged by uniformity in appearance of the colony which forms after incubation. The appearance of a mixed culture depends on the growth rates of the fungi present. If rates are diverse, a mixed culture is often indicated by a clump of dense hyphae at the inoculum point, surrounded by looser wefts of spreading hyphae. With fungi of approximately equal growth rates, mixtures are often indicated by colonies with sectoring growth: sectors will show differences in mycelial, spore or reverse colours or in radial growth rates.

The simplest starting place for isolating fungi is an enumeration plate with well-separated colonies. Use a needle of platinum or nichrome, preferably cut to a chisel point with a pair of pliers, or a steel sewing needle. Sterilise it by heating, then plunge the tip into cold agar and leave until cool – with nichrome or steel this will require several seconds. With the tip of the cold, wet needle pick off a few spores or a tuft of mycelium – just enough to be visible – and inoculate a single point on a plate or slant.

The same procedure can be applied to mixed cultures arising from direct plating or surface sampling techniques. It is advisable to keep notes on the appearance of the colony area sampled, as this will give an indication of whether the culture which grows up is the same as that intended to be isolated.

It is generally easy to isolate rapidly growing fungi from those which grow more slowly. The outermost hyphal tips are usually free of contamination. The reverse process is often much more difficult. The isolation of slowly growing fungi in the presence of rapidly growing "weeds" often requires skill, patience and ingenuity. It is desirable to watch the point inocula daily over several days at least, because a particular stage in the life cycle may give some advantage. The slow colony may germinate more rapidly, or have a sector accessible to a needle, or spore more freely. The use of higher or lower incubation temperatures, or media of low a_w or low nutrient status, or the addition of dichloran or rose bengal (see below) may all be of value in this process.

Freeing fungi from bacteria, long considered to be a very difficult procedure, has been greatly simplified in recent years with the advent of media containing antibiotics. With use of the media recommended in the next section, bacterial contamination of cultures should be a rare event.

When a pure colony is obtained, it should be inoculated onto a slant of an appropriate medium and incubated until ready for identification. Again, moulds should always be inoculated at a single point, preferably near the centre of the slant. This permits the best colony development and sporulation in most fungi.

4.5.3 Short Term Storage

For short to medium-term storage, fungi are usually stored on slants, most commonly in McCartney or Universal bottles which have the advantages of being free standing and having caps that can be sealed to retard drying during storage. During incubation and until colonies are fully mature, however, caps must be kept loose on slants in bottles. Moulds

require free access to oxygen for typical growth and sporulation. Oxygen starvation during growth will at best lead to retarded sporulation or at worst death of the culture.

Long-term preservation of fungi is dealt with later in this chapter.

4.6 Choosing a Suitable Medium

Food laboratories often rely on a single all-purpose medium to produce a "yeast and mould" count in everything from raw material to finished product. But just as the food bacteriologist uses selective media for particular purposes, so too food mycologists are developing a range of media suited to specific applications. It is plainly unrealistic to expect a single medium to answer all questions about mould and yeast contamination in all foods. The fungi that spoil meats or fresh vegetables are not the same as those that grow on dried fish. Although often used for this purpose, very dilute media such as potato dextrose agar are of little or no value for enumerating fungi from dried foods.

The most important division in types of enumeration media lies between those suitable for high water activity foods, such as eggs, meat, vegetables and dairy products, and those suited to the enumeration of fungi in dried foods. The most suitable media for dried foods depend on the type of food, the major categories being foods low in soluble solids such as cereals, high sugar foods such as confectionery and dried fruit, and salt foods. A second consideration lies in whether the primary interest is in moulds, or yeasts, or both; and a third concerns the presence or absence of preservatives. Finally, media are available for specific mycotoxigenic fungi, notably *Aspergillus flavus* and related species and *Penicillium verrucosum* plus *P. viridicatum*.

An overview of media considered most suitable for particular purposes is given in Table 4.1. The table is derived from Pitt and Hocking (1997), together with recent recommendations from ICFM (Samson et al., 1992; Hocking et al., 2006a).

Table 4.1 Recommended media for fungal detection, enumeration and isolation[a]

Type of food	Selecting for	Medium	Remarks
Fresh foods: milk and milk products, fruit, cheese, sea foods	Moulds	DRBC	Blend (where necessary) and dilution plate
	Yeasts	TGY, MEA, OGY	
	General	DRBC	
Freshly harvested grains, nuts	General	DRBC	Direct plate
	Dematiaceous Hyphomycetes	DRBC, CZID	Direct plate
	Fusarium	CZID	Direct plate
	Yeasts	TGY, MEA, OGY	Dilution plate
Fruit juices, fresh	Yeasts	TGY, MEA, OGY	Dilution plate
Fruit juices, preserved	Preservative resistant yeasts	TGYA, malt acetic agar	Dilution plate
Fruit juices, to be pasteurised, or pasteurised products	Heat resistant moulds	PDA, MEA	Special protocol
Fruit juice concentrates	Xerophilic yeasts	MY50G	Special diluents
Dried foods in general	General	DG18	Direct plate
Stored cereals, nuts	General	DG18	Direct plate
	Dematiaceous Hyphomycetes	DRBC, CZID	Direct plate
	Fusarium	CZID	Direct plate

Table 4.1 Recommended media for fungal detection, enumeration and isolation[a] (continued)

Type of food	Selecting for	Medium	Remarks
Grain for milling into flour	General	DG18	Stomach or blend and dilution plate
Dried fruit, confectionery, chocolate, etc.	Xerophilic moulds and yeasts	MY50G	Direct plate
	Fastidious xerophiles	MY50G	Direct plate
	– in presence of *Eurotium* spp.	MY70GF	Direct plate
Salt foods, e.g. salt fish	General	DG18	Direct plate or press plate
	Halophilic xerophiles	MY5-12, MY10-12	Direct plate or press plate
General	Fungi producing aflatoxins	AFPA	Direct or dilution plate
General	Fungi producing ochratoxins	DRYS	Direct or dilution plate

[a] For medium acronyms, see Section 4.6.

4.6.1 General Purpose Enumeration Media

To be effective, a general purpose enumeration medium must fulfil several requirements (Pitt, 1986). As these are sometimes overlooked, they are listed here:

- to inhibit bacterial growth completely, without affecting growth of foodborne fungi (filamentous or yeasts);
- to be nutritionally adequate and support the growth of fastidious fungi;
- to suppress the growth of rapidly spreading fungi, especially the Mucorales, but not to prevent their growth entirely, so they too can be enumerated;
- to slow radial growth of all fungi, to permit counting of a reasonable number of colonies per plate, without inhibiting spore germination;
- to promote growth of relevant fungi; and
- to suppress growth of soil fungi or others generally irrelevant in food spoilage.

Fulfilling the above requirements necessitates the use of potent inhibitory compounds, and there is sometimes a fine line between inhibition of undesirable microorganisms and suppression of growth of those being sought. Modern fungal enumeration media rely on the use of antibiotics at neutral pH for the inhibition of bacteria. Such media allow better recovery of moribund and sensitive fungi than the acidified media commonly used in the past. For many years rose bengal has been added to media to slow colony spread, while more recently 2,6-dichloro-4-nitroaniline (dichloran) has been added to inhibit rapidly spreading moulds. Many common spoilage fungi, *Aspergillus* and *Penicillium* species in particular, develop better on media with adequate nutrients. Low nutrient media of very high a_w, such as potato dextrose agar, have lost favour because they are selective against some species in these genera.

The media described below are considered to be the most satisfactory general purpose enumeration media available at this time (Hocking et al., 2006a). Formulations are given in the Media Appendix.

Dichloran rose bengal chloramphenicol (DRBC) agar. DRBC (King et al., 1979, Pitt and Hocking, 1997) is recommended for both moulds and yeasts. It is particularly suited to fresh and high a_w foods (Hocking et al., 1992). This medium contains both rose bengal (25 mg/kg) and dichloran (2 mg/kg), which restrict colony spreading without affecting spore germination unduly. Compact colonies allow crowded plates to be counted more accurately. This combination of inhibitors also effectively restricts the rampant growth of most of the common mucoraceous fungi such as *Rhizopus* and

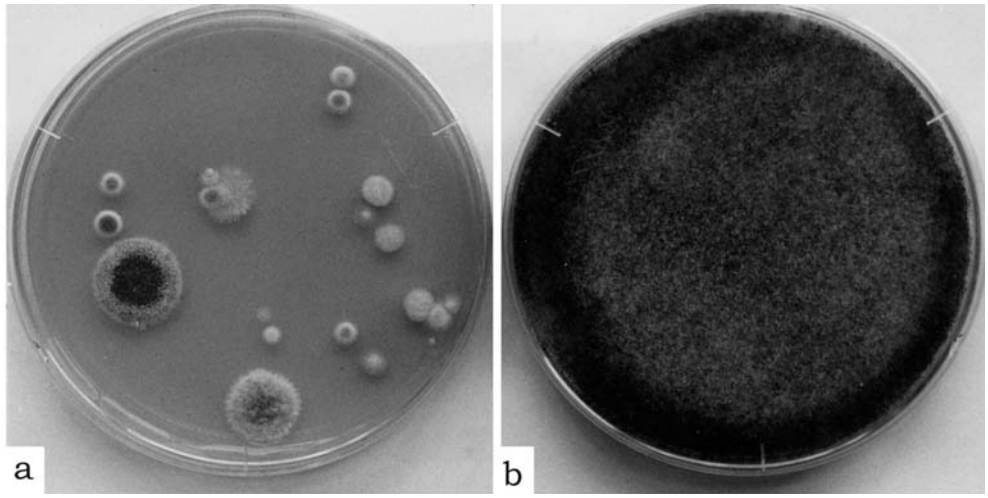


Fig. 4.2 Petri dishes of **(a)** DRBC and **(b)** RBC showing effective control of *Rhizopus* growth by rose bengal and dichloran in DRBC

Mucor (Fig. 4.2), although it does not completely control some other troublesome genera such as *Trichoderma*.

In routine use, it is recommended that DRBC plates be incubated away from light at 25°C for 5 days.

Dichloran 18% glycerol (DG18) agar. Hocking and Pitt (1980) developed DG18 to be selective for xerophilic fungi from low moisture foods such as stored grains, nuts, flour and spices. DG18 was designed for enumeration of a range of nonfastidious xerophilic fungi and yeasts. However, it has been shown since that it supports growth of the common *Aspergillus*, *Penicillium* and *Fusarium* species, as well as most yeasts, and many other common foodborne fungi.

DG18 can now be described as a general purpose medium with emphasis on enumeration of fungi from dried foods. DG18 is also recognised to be a very useful medium for enumeration of airborne fungi (Wu et al., 2000; Samson et al., 2004a). It is an effective inhibitor of Mucoraceous fungi, and bacteria are totally suppressed. However, growth of *Eurotium* species (previously known as "the *Aspergillus glaucus* group") is still somewhat too rapid, and colonies may have diffuse margins. The addition of detergent to DG18 has been reported to be an improvement in this respect (Beuchat and de Daza, 1992).

Although DG18 is a satisfactory isolation medium for *Eurotium* species, it is not suitable for their identification. *Eurotium* species are usually identified on Czapek yeast extract agar with 20% sucrose (CY20S), described later in this chapter.

Other general purpose media. Under circumstances where rapidly spreading moulds do not cause problems, two alternative general purpose enumeration media are satisfactory. These are rose bengal chloramphenicol agar (RBC; Jarvis, 1973), from which DRBC was developed, and oxytetracycline glucose yeast extract agar (OGY; Mossel et al., 1970). OGY has been found to be very suitable for yeasts in the absence of moulds (Andrews, 1992a).

Most of the media discussed above are available in ready to use dehydrated form from media suppliers such as Oxoid, Difco, BBL, etc.

4.6.2 Selective Isolation Media

Although considerable progress has been made in the past 20 years, the formulation of selective media for foodborne fungi still requires a great deal of research. The availability of effective media can greatly simplify the isolation and identification of significant food spoilage and mycotoxigenic fungi. Most attention has been paid so far to the requirements of xerophilic fungi because of their failure to develop on standard high a_w media. For mycotoxigenic fungi, satisfactory media exist only for

Aspergillus flavus and closely related species, *Penicillium verrucosum* and *P. viridicatum*, and the genus *Fusarium*. These media are considered below.

Media for *Aspergillus flavus* and related species. Bothast and Fennell (1974) developed Aspergillus differential medium after finding that *Aspergillus flavus* and *A. parasiticus* produced conspicuous, diagnostic orange yellow colours in the colony reverses in the presence of an appropriate nitrogen source and ferric salts. Few other fungi produced a similar colouration. It was later shown that these species produce aspergillic acid or noraspergillic acid, which react with ferric ammonium citrate to form the colour complex (Assante et al., 1981). However, development of the orange yellow reverse pigment is not indicative of aflatoxin production.

This medium was reformulated by Pitt et al. (1983) to produce Aspergillus flavus and parasiticus agar (AFPA). As well as more effective concentrations of the active ingredients, AFPA contains dichloran and chloramphenicol to inhibit spreading fungi and bacteria, respectively.

When incubated at 30°C for 42–48 h, colonies of *Aspergillus flavus*, *A. parasiticus* and *A. nomius* are distinguished by bright orange yellow reverse colours. Only *A. niger* can be a source of error: it grows as rapidly as *A. flavus* and sometimes produces a yellow, but not orange, reverse. After 48 h *A. niger* colonies begin production of their diagnostic black or dark brown heads, which provide a ready distinction from *A. flavus*. Prolonged incubation of AFPA, beyond 4 days, is not recommended because *A. ochraceus* and closely related species may also produce a yellow reverse after this time. AFPA is recommended for the detection and enumeration of potentially aflatoxigenic fungi in nuts, maize, spices and other commodities. Its advantages include rapidity, as 48 h incubation is usually sufficient, specificity, and simplicity, as little skill is required in interpreting results. In consequence, it can be a simple, routine guide to possible aflatoxin contamination (Pitt, 1984). This medium is also very effective for enumerating *A. flavus* in soils, where levels down to 5 spores per gram can be detected. Where soils carry a heavy bacterial load, doubling the quantity of chloramphenicol or adding other antibiotics may be of value.

Detection of aflatoxin production using Coconut Cream Agar. Dyer and McCammon (1994) developed Coconut Cream agar (CCA) to detect aflatoxin production in *Aspergillus flavus* and *A. parasiticus*. CCA can be made using any brand of commercial canned coconut cream (available from Asian food stores in many places). Dilute 50:50 with water, add agar (1.5%) and autoclave. Inoculate solidified plates with up to four colonies (picked with a wet needle) and incubate at 30°C for 5–7 days. Examine, reverse upmost, under long wave length UV light. Colonies producing aflatoxins will fluoresce bluish white or white, especially in the centres. Ignore fluorescence from the Petri dish itself. Use an uninoculated coconut cream agar plate as a control (Dyer and McCammon, 1994). Plates inoculated with known nontoxigenic and toxigenic strains are also useful controls. *A. parasiticus* isolates almost always produce aflatoxins.

Media for fungi producing ochratoxin A. Although ochratoxin A was first described from *Aspergillus ochraceus*, recent molecular studies indicate that *A. westerdijkiae* is the major ochratoxin A producing species in *Aspergillus* Section *Circumdati* (Frisvad et al., 2004). *Aspergillus carbonarius* is also an important source of ochratoxin A, particularly in grape products (Abarca et al., 2004; Leong et al., 2006a and references therein). However, there are no selective or indicative media for these fungi. When DG18 is used as the isolation medium, selection of colonies with light brown (ochre) or black sporulation is a good starting point for detecting *A. westerdjikiae* or *A. carbonarius* respectively. Coconut cream agar (Dyer and McCammon, 1994) can also be used to screen for ochratoxin production in *A. carbonarius* and species in *Aspergillus* Section *Circumdati* (Heenan et al., 1998). Plates are best incubated at 25°C rather than 30°C for potentially ochratoxigenic species of *Aspergillus*.

Penicillium verrucosum is the most important ochratoxin a producer in the genus *Penicillium* (Pitt, 1987), although *P. nordicum* can also produce this mycotoxin (Frisvad and Samson, 2004). Frisvad (1983) developed Dichloran rose bengal yeast extract sucrose agar (DRYS) for selective enumeration of *P. verrucosum*. *P. nordicum, P. viridicatum* and *P. aurantiogriseum* are also selected by DRYS. *P. verrucosum* and *P. nordicum* produce ochratoxin A, *P. verrucosum* also produces citrinin, while the latter two species produce xanthomegnin and viomellein. According to Frisvad (1983), *P. verrucosum* colonies on DRYS have a violet brown reverse, and

the latter two species produce yellow colonies with a yellow reverse. The incubation regimen recommended by Frisvad (1983) is 7–8 days at 20°C. Subsequently, Frisvad et al. (1992) developed dichloran yeast extract sucrose 18% glycerol agar (DYSG) on which *P. verrucosum* produces a red brown reverse. Because of its lower a_w, DYSG inhibits rapidly growing fungi such as *Rhizopus* and *Mucor* more effectively than DRYES. In a study of 76 samples of wheat, rye and barley, Lund and Frisvad (2003) found that DYSG was more effective than DRYES for screening grain samples for the presence of *P. verrucosum* and potential ochratoxin A contamination.

Media for *Fusarium* species. Dichloran chloramphenicol peptone agar (DCPA; Andrews and Pitt, 1986) can be used to isolate *Fusarium* species from grains and other substrates. The medium was developed from Nash-Snyder medium (Nash and Snyder, 1962) a medium for enumeration of *Fusarium* species from soils. DCPA uses a low level of dichloran as a substitute for the high level of pentachloronitrobenzene (PCNB), and chloramphenicol rather than the antibiotic mixture used by Nash and Snyder (1962).

When *Fusarium* species are dominant, DCPA is effective for their isolation from grains, animal feeds and soil. However, DCPA has been found to be less effective in mixed populations. DCPA is a very useful medium for the identification of *Fusarium* species because it often induces the abundant formation of macroconidia (Hocking and Andrews, 1987).

A much more stringent medium, effective for isolation of most *Fusarium* species occurring in foods, is Czapek iprodione dichloran agar (CZID; Abildgren et al., 1987). As well as dichloran, this medium contains the fungicide iprodione. CZID is highly selective for *Fusarium* species, and is probably the best medium for *Fusarium* enumeration and isolation. CZID is suitable for isolation of *Fusarium* species by direct plating of surface disinfected grains and other commodities, or dilution plating of homogeneous samples such as flour. Some questions remain concerning whether CZID may be too selective and not support growth of all foodborne *Fusarium* species. However, the common species all grow well.

Castellá et al. (1997) developed a *Fusarium* selective medium (MGA 2.5) using 2.5 μg/L malachite green as the selective agent. They reported that MGA 2.5 was more selective than Nash-Snyder medium as it did not allow development of colonies of other fungal genera. Bragulat et al. (2004) compared the efficacy of MGA 2.5 medium with DCPA, CZID and several other *Fusarium* media, using pure cultures of twelve *Fusarium* species commonly found in foods as well as naturally contaminated samples. They reported that there was no statistically significant difference in colony counts of the *Fusarium* spp. tested, but that colonies on MGA 2.5 were smaller than the other media. MGA 2.5 did not allow growth of other fungi such as Zygomycetes and yeasts from naturally contaminated samples, thus providing better selectivity than the other media.

Media for dematiaceous Hyphomycetes. Although designed primarily for *Fusarium* isolation, CZID has been found in our laboratory to be very useful for isolating dematiaceous Hyphomycetes, provided that iprodione is added at half the usual concentration, as full strength iprodione tends to restrict colony diameters too severely. *Alternaria, Bipolaris, Curvularia, Stemphylium* and *Ulocladium* species will grow and sporulate well when incubated for 5 days at 25°C with a 12 h photoperiod. *Drechslera* species will grow but will not sporulate on this medium.

DRBC is also of value for isolating these fungi, but some species do not sporulate readily on it.

DCPA, originally developed for isolation of *Fusarium,* may also be used as an isolation medium for the dematiaceous Hyphomycete genera mentioned above. *Alternaria, Curvularia* and related genera usually grow rapidly and sporulate well on DCPA. However, fungi should not be maintained or stored on DCPA for more than two weeks, as ammonia is produced by aging cultures.

Media for xerophilic fungi. Xerophilic fungi are of great importance in food spoilage, and hence media and techniques for their enumeration and isolation have received much attention. Xerophiles range from those which grow readily on normal media and which are only marginally xerophilic, such as many *Aspergillus* and *Penicillium* species, to those, such as *Xeromyces bisporus*, which will not grow at all on normal (high a_w) media. It is not surprising that no single medium is suitable for quantitative estimation of all xerophilic fungi found causing food spoilage. As noted earlier, DG18 was developed as a general medium for xerophiles, and remains the medium of choice for this purpose. DG18 should be used in any general examination of the mycoflora of dried foods.

Media for extreme xerophiles. Fungi discussed here include *Xeromyces bisporus*, xerophilic

Chrysosporium species, *Eremascus* species, *Eurotium halophilicum* and the halophilic xerophiles *Polypaecilum pisce* and *Basipetospora halophila*. These extreme xerophiles grow slowly if at all on DG18 and are quickly overgrown by rapidly spreading xerophiles such as common *Eurotium* species. Such species require special media and techniques.

Since the early 1950s, studies on extremely xerophilic fungi have been carried out in our laboratory (Scott, 1957). A very wide variety of media have been used for their cultivation. The most effective of these, suitable for all except the halophilic xerophiles, is malt extract yeast extract 50% glucose agar (MY50G; Pitt and Hocking, 1997).

Isolation techniques for extreme xerophiles. As well as special media, extreme xerophiles require special techniques for isolation. If a low a_w commodity such as ground spice, dried fruit, fruit cake, confectionery or dried fish shows signs of white mould growth, it is likely that the fungus responsible is an extreme xerophile. Fungi of this kind are usually sensitive to diluents of high a_w and often cannot be isolated by dilution plating. For direct plating, a convenient technique is to place small pieces of sample, without surface disinfection, onto a rich, low a_w medium, such as MY50G. Alternatively, examine the food under the stereomicroscope, which will often provide useful information in any case, and use an inoculating needle to pick off pieces of mycelium or spores from the surface of the spoiled food. Place these pieces (three to six per plate) directly onto MY50G. Colonies should develop after 1–3 weeks incubation at 25°C. Quite frequently extreme xerophiles will be present in pure culture and can be readily isolated. Examination with the stereomicroscope will usually indicate whether this is the case. Selection of growth which appears to cover the range of types seen will assist isolation of the principal fungi present.

Direct sampling by the press plating technique described earlier in this chapter can be useful for isolating xerophiles.

Sometimes extreme xerophiles are accompanied by *Eurotium* species, also capable of growth at very low a_w. The latter are identifiable under the stereomicroscope by their *Aspergillus* heads. Under these circumstances, isolation of extreme xerophiles is more difficult. A medium of sufficiently low a_w to slow the growth of *Eurotium* species is necessary. The most satisfactory medium is malt extract yeast extract 70% glucose fructose agar (MY70GF).

MY70GF is of similar composition to MY50G, except that it contains equal parts of glucose and fructose to prevent crystallisation of the medium at the concentration used (70% w/w). It is made in a similar manner to MY50G.

Growth of even the extreme xerophiles on MY70GF is extremely slow, and plates should be incubated for at least 4 weeks at 25°C. Once growth is apparent, pick off small portions of colonies and transfer them to MY50G, to allow more rapid growth and sporulation.

Media for halophilic xerophiles. Some xerophilic fungi from salted foods such as salt fish grow more rapidly on media containing NaCl and hence are correctly termed halophilic xerophiles. Malt extract yeast extract 5% salt 12% glucose agar (MY5-12) and malt extract yeast extract 10% salt 12% glucose agar (MY10-12) are suitable for these fungi. Techniques for enumeration and isolation are similar to those described above for other extreme xerophiles.

4.6.3 Techniques for Yeasts

The simplest enumeration and growth medium for most food spoilage yeasts is malt extract agar (MEA). Although originally introduced as a growth medium for moulds, its rich nutritional status makes it very suitable for yeasts, and its relatively low pH (usually near 5.0) reduces the possibility of bacterial contamination.

More recently, tryptone glucose yeast extract agar (TGY) has been recommended for enumeration of yeasts (Hocking et al., 1992, 2006a). Due to a higher glucose concentration (10%) and higher pH (5.5–6.0), this medium is more effective in recovery of stressed yeast cells, and colony development is usually faster than on MEA. However, its higher pH means that an antibiotic should be incorporated for enumeration of yeasts from food samples which may contain bacteria. Recommended antibiotics are chloramphenicol or oxytetracycline at the concentrations used in DRBC and OGY.

Both MEA and TGY are suitable for enumeration of yeasts in products such as fruit juices, fruit purees and yoghurt, where moulds are usually present only in low numbers.

If large numbers of moulds are present, often the case with solid products such as cheese, a general purpose enumeration medium such as DRBC or DG18 should be used.

For circumstances where yeast enumeration remains difficult on media such as DRBC, a valuable technique is that of de Jong and Put (1980), which involves a 3-day anaerobic incubation. They recommended using pour plates or streak plates of mycophil agar (BBL Microbiological Systems, Cockeysville, Maryland, USA), but malt extract agar should be equally suitable. Antibiotics may be added if necessary. Plates are incubated at 25°C for 3 days in an anaerobic jar, then taken out and incubated aerobically for another 2 days at 25°C. As many spoilage yeasts are able to grow anaerobically, colonies will commence development without the possibility of overgrowth by moulds. This technique is unlikely to be suited to the enumeration of yeasts on surfaces of fresh fruit or vegetables, as yeasts which colonise these surfaces are often strict aerobes.

Enrichment for yeasts in liquid products. In liquid food products, low numbers of yeasts may be difficult to detect, but may have a serious potential to cause spoilage. Enrichment techniques are the only satisfactory way of monitoring products in these circumstances.

For products or raw materials free from suspended solids and of low viscosity, standard membrane filtration techniques are a satisfactory method for detecting low numbers of yeasts. The filter can be placed directly onto a suitable medium such as MEA or TGY and staining can be carried out subsequently. Centrifugation may also be used, but has the disadvantage that only relatively small volumes of product can be screened.

If products or raw materials are viscous, of low a_w, or contain pulps and cannot be filtered efficiently, other enrichment techniques are needed. In many cases, the best enrichment medium is the product itself, diluted 1:1 with sterile water. A 1:1 dilution increases the a_w of juice concentrates or honey to a level which will allow growth of potential spoilage yeasts without causing a lethal osmotic shock to the cells. If the product contains preservative, dilution will lower the concentration and permit cells to grow.

To detect low numbers of spoilage yeasts in cordials, fruit juice concentrates and similar materials, simply decant half the product from the container and replace it with sterile water. Leave the cap loose, incubate at room temperature or 30°C and watch for evidence of fermentation. Shaking the container daily will help to detect gases resulting from fermentation.

Classical enrichment techniques used in bacteriology can also be used for yeasts. TGY broth has been used very successfully in our laboratory for enrichment of low numbers of yeasts in liquid products. Add 10 ml of product to 90 ml TGY broth and incubate at 25°C for 3–4 days, or 30°C for 2–3 days. Look for signs of fermentation and streak out onto TGY agar.

Detection of preservative resistant yeasts. A few species of yeasts are able to grow in products containing preservatives such as sorbic, benzoic and acetic acids or sulphur dioxide. The most important of these is *Zygosaccharomyces bailii*. The simplest and most effective way to screen for preservative resistant yeasts is to spread or streak product onto plates of malt acetic agar (MAA), which is MEA with 0.5% acetic acid added (Pitt and Richardson, 1973) or TGY with 0.5% acetic acid added (TGYA, Hocking, 1996).

MAA and TGYA are made by adding glacial (16 N) acetic acid to melted and tempered basal medium to give a final concentration of 0.5%. Mix and pour immediately. These media cannot be held molten for long periods or remelted because of their low pH (approximately 3.2 and 3.8 for MAA and TGYA, respectively). The acetic acid does not need sterilisation before use.

MAA and TGYA are suitable media for monitoring raw materials, process lines and products containing preservatives for resistant yeasts. They are also effective for testing previously isolated yeasts for preservative resistance.

Erickson (1993) developed a selective medium for *Zygosaccharomyces bailii*. Zygosaccharomyces bailii medium (ZBM) is based on Sabouraud dextrose agar amended with fructose, NaCl, tryptone, yeast extract and trypan blue dye, then acetic acid (0.5%) and potassium sorbate (0.01%) are added to make the medium selective. It is designed as a plating medium for detection of *Z. bailii* in acidified ingredients in conjunction with hydrophobic grid membrane filtration (Erickson, 1993).

When compared with MAA and TGYA in an interlaboratory study, ZBM was found to be highly selective for *Zygosaccharomyces bailii*, to the exclusion of other important preservative resistant yeasts such as *Schizosaccharomyces pombe* and *Pichia*

membranaefaciens. In addition, recovery of *Z. bailii* cells sublethally injured by lyophilisation was significantly lower on ZBM than on TGYA or MAA (Hocking, 1996). Its high selectivity and complex formulation make ZBM unsuitable for routine laboratory use as a medium for detection of preservative resistant yeasts.

Enrichment of preservative resistant yeasts. A technique capable of detecting yeast numbers as low as 1 cfu/ml within 4 days has been developed in our laboratory (Hocking et al., 1996). This method is particularly suitable for the detection of *Zygosaccharomyces bailii* in raw materials or finished product, but can also be used for the detection of *Schizosaccharomyces pombe*, *Pichia membranaefaciens* and other species of preservative resistant yeasts. The method involves a 2- to 3-day enrichment step followed by a plating step with a further 2 days of incubation. Triplicate 20 ml tryptone glucose yeast extract (TGY) broths containing 0.5% acetic acid (TGYA) are each inoculated with 1 g or 1 ml of product and the broths incubated at 30°C. After incubation for 48 and 72 h, 0.1 ml from each broth is spread plated onto TGY agar containing 0.5% acetic acid and the plates incubated at 30°C.

The detection time of the method is shortened by incubating broths and plates at 30°C rather than the traditional temperature of 25°C, as the optimum growth temperature for *Zygosaccharomyces bailii* is 30–32°C (Jermini and Schmidt-Lorenz, 1987b). The sensitivity of the method is greatly increased by using triplicate broths instead of single or duplicate broths and by spread plating 0.1 ml from each broth instead of streaking a loopful onto TGYA agar.

This method has been used to detect low numbers of cells of *Zygosaccharomyces bailii* in experimentally inoculated cordial syrup, mayonnaise, salad dressing and barbecue sauce and other preservative-resistant yeasts such as *Schizosaccharomyces pombe, Pichia membranaefaciens* and some preservative resistant strains of *Saccharomyces cerevisiae*. Yeasts of intermediate preservative resistance (e.g. *Debaryomyces hansenii*, *Candida krusei* and *Torulaspora delbrueckii*) can also be detected by this method. Better recoveries were obtained using TGY than a malt extract agar and broth system, possibly due to the fact that the pH of TGY broth + 0.5% acetic acid is 3.8, compared with pH 3.2 for MEA + 0.5% acetic acid, and there is a lower concentration of glucose (2%) in the ME system compared with 10% in TGY. Yeasts which are unable to grow in the presence of acetic acid or other weak acid preservatives (sorbic or benzoic acids and their salts) are not detected by this method.

4.6.4 Techniques for Heat-Resistant Fungi

Heat resistant spoilage fungi, such as *Byssochlamys*, *Talaromyces*, *Neosartorya* and *Eupenicillium* species can be selectively isolated from fruit juices, pulps and concentrates by laboratory pasteurisation using various methods (Beuchat and Rice, 1979; Hocking and Pitt, 1984; Beuchat and Pitt, 2001; Houbraken and Samson, 2006). Three methods are described here: a plating method based on that of Murdock and Hatcher (1978), a direct incubation method and a filtration method for liquid samples such as liquid sugar.

Plating method. If the sample to be tested is more concentrated than 35°Brix, it should first be diluted 1:1 with 0.1% peptone or similar diluent. For very acid juices such as passionfruit, normally about pH 2.0, the pH should be adjusted to 3.5–4.0. Two 50 ml samples are taken for examination. Erlenmeyer flasks (250 ml) or polyethylene Stomacher bags may be used as heat penetration into these containers will be rapid. If using Stomacher bags, the tops should be heat sealed. If a heat sealer is not available, the tops may be folded over and secured with a clip and should not be fully immersed. The two samples are heated in a closed water bath at 80°C for 30 min, then rapidly cooled. Each 50 ml sample is then mixed with an equal volume of double strength MEA distributed over four 150 mm Petri dishes. The Petri dishes are loosely sealed in a plastic bag to prevent drying and incubated at 30°C for up to 30 days. Plates are examined weekly for growth. Most moulds will produce visible colonies within 10 days, but incubation for up to 30 days will allow for the possible presence of badly heat damaged spores, which may germinate very slowly. This long incubation time also allows most moulds to mature and sporulate, aiding their identification.

The main problem associated with this technique is the possibility of aerial contamination of the plates with common mould spores, which will give false positive results. The growth of green *Penicillium*

colonies, or colonies of common *Aspergillus* species such as *A. flavus* and *A. niger*, is a clear indication of contamination as these fungi are not heat resistant. To minimise this problem, plates should be poured in clean, still air or a Class 2 biohazard cabinet if possible. If a product contains large numbers of heat resistant bacterial spores (e.g. *Bacillus* species), antibiotics can be added to the agar. The addition of chloramphenicol (100 mg/l of medium) will prevent the growth of these bacteria.

Direct incubation method. A more direct method used for screening fruit pulps and other semisolid materials avoids the problems of aerial contamination. Place approximately 30 ml of pulp in each of three or more flat bottles such as 100 ml medicine flats. Heat the bottles in the upright position for 30 min at 80°C and cool, as described previously. The bottles of pulp can then be incubated directly, without opening and without the addition of agar. They should be incubated flat, allowing as large a surface area as possible, for up to 30 days at 30°C. Any mould colonies which develop will need to be subcultured onto a suitable medium for identification. If containers such as Roux bottles are available, larger samples can be examined by this technique, but heating times must be increased. Bottle contents should reach at least 75°C for 20 min when checked by a thermometer suspended near the centre of the pulp.

For further details of the above methods see Hocking and Pitt (1984) or Beuchat and Pitt (2001).

Filtration method for liquid sugars. This method permits the detection of very low numbers of cells in clear liquids such as liquid sugar. Sample size should be at least 100 g, taken after vigorously shaking the container from which the sample is drawn. Add 100 ml diluent (0.1% aqueous peptone) to 2 × 50 g samples and mix well to dissolve. Filter both samples sequentially through the same sterile 0.45 μm membrane filter. After both samples have passed through the filter, rinse the interior of the funnel with 3 × 20–30 ml volumes of sterile diluent. Remove the filter from the filter holder using sterile forceps and place it in a sterile bottle or Stomacher bag. Add 10 ml diluent to the bottle or bag containing the filter and place in a water bath at 75°C for 30 min. Ensure the sample is submerged in the water bath (weigh down if necessary). Cool rapidly to room temperature, shake well, then divide the 10 ml of diluent between three Petri dishes. Add a generous portion of MEA with antibiotics to each plate, mix the agar and sample well, then let the plates solidify. Incubate at 30°C for up to 30 days, examining weekly. Count colonies and report count per 100 g. This method was developed by BCN Research Laboratories, Rockford, Tennessee, USA.

4.6.5 Other Plating Techniques

Three other techniques which were developed for counting bacteria have been applied to fungal enumeration.

Spiral plate count. Zipkes et al. (1981) evaluated the application of the spiral plate procedure to the enumeration of yeasts and moulds. They compared this procedure with the traditional pour plate and streak plate methods and found that spiral plating gave a higher overall recovery and lower replicate plating errors than the other two methods. The medium they used was potato dextrose agar, but the technique should be no less efficient using the media recommended here. Automation of spiral plate counting was studied by Manninen et al. (1991). They compared counts of pure bacterial, yeast and mould cultures using standard plating methods and spiral plate counts determined both manually and with a laser colony detector. They concluded that counts were not significantly different except where large colonies (10–15 mm diameter) of *Rhizopus oligosporus* were enumerated. Other *Rhizopus* and *Mucor* species are also likely to interfere with this method unless a suitable plating medium (such as DRBC) is used. Alonso-Calleja et al. (2002) found that the spiral plate technique compared well with standard plate counting on OGYE agar for enumeration of yeasts and moulds in goat's milk cheeses; however, García-Armesto et al. (2002) found the method unsuitable for enumeration of yeasts in raw ewe's milk.

Hydrophobic grid membrane filters. Membrane filters overprinted with a square hydrophobic grid have been developed for rapid enumeration of bacteria. The hydrophobic grid membrane filter (HGMF) system is marketed as ISO-Grid™ by Neogen (Lansing, MI, USA). The HGMF "count" is determined by a most probable number (MPN) calculation. Brodsky et al. (1982) applied the HGMF technique to counting yeasts and moulds in foods. They compared it with spread plating on

potato dextrose agar amended with antibiotics and found that the HGMF technique produced higher counts in 2 days than the traditional method did after 5 days. The HGMF method could find use for evaluating the quality of raw materials before incorporation into a product. For example, Erickson (1993) has proposed a method using HGMF in conjunction with a selective medium for detection of the preservative resistant yeast *Zygosaccharomyces bailii* in acidified ingredients. The HGMF method was collaboratively trialled by 20 laboratories using 6 naturally contaminated foods. Although there were some differences between counts obtained by HGMF and the reference method (5 day pour plate), the differences were not significant, and the HGMF method was adopted by the AOAC International (Entis, 1996). Spangenberg and Ingham (2000) found that the HGMF method gave equivalent results to DRBC for enumeration of yeasts and moulds in grated low moisture Mozzarella cheese.

Use of HGMF requires special holders for the square membrane filters, and an automated counting system is necessary to take full advantage of the method. The number of colonies on an HGMF can be counted visually, but it is a relatively slow process.

Petrifilm ™ Yeast and Mould. Petrifilm YM (3 M Company, St. Paul, MN, USA) is a proprietary system for enumerating fungi on a layer of medium enclosed in a plastic film, which eliminates the use of Petri dishes. A collaborative study carried out by the AOAC (Knight et al., 1997) concluded that Petrifilm YM gave comparable results with the BAM method (USFDA, 1992) for yeast and mould counts in five different food types inoculated with a cocktail of several species of *Aspergillus* and *Penicillium* and two species of yeast. Consequently, Petrifilm YM was adopted first action by AOAC International. Other workers (Beuchat et al., 1990, 2007; Taniwaki et al., 2001b; Ferrati et al., 2005) have shown that Petrifilm YM performed satisfactorily compared with conventional cultural methods when tested on a range of foods. However, Petrifilm was not particularly effective in inhibiting the growth of spreading moulds such as *Rhizopus* and *Mucor*. In addition, subculturing colonies for identification was more difficult from Petrifilm YM than from traditional Petri dishes. Petrifilm YM has a high a_w and we do not recommend it for analysis of foods of less than 0.95 a_w.

4.7 Estimation of Fungal Biomass

A deficiency in all of the enumeration techniques which rely on culturing fungi is that the result is at best poorly correlated with growth or *biomass*. Biomass is usually regarded as the fundamental measure of fungal growth in biotechnology, but it is not easy to quantify under the conditions existing in foods. Mycelial dry weight is most commonly used as a biomass estimate, but its relationship to mycelial wet weight and to metabolism varies widely in foods, due to the great influence of a_w on both of the latter parameters. Fungi growing at reduced a_w can be expected to be more dense than at high a_w due to increased concentrations of internal solutes, though this is exceptionally difficult to measure experimentally. The question of a satisfactory fundamental measure of fungal biomass remains unanswered.

Despite these basic problems, several chemical and biochemical techniques are available to estimate the extent of fungal growth in commodities. These techniques rely either on some unique component of the fungus that is not found in other microorganisms or foods or on immunological or molecular techniques. Some are still in the developmental phase: the most important ones are described briefly here.

4.7.1 Chitin

Chitin is a polymer of *N*-acetyl-D-glucosamine and is a major constituent of the walls of fungal spores and mycelium. It also occurs in the exoskeleton of insects but is not present in bacteria or in foods. Hence the chitin content of a food or raw material can provide an estimate of fungal contamination.

Chitin is most effectively assayed by the method of Ride and Drysdale (1972). Alkaline hydrolysis of the food sample at 130°C causes partial depolymerisation of chitin to produce chitosan. Treatment with nitrous acid then causes partial solubilisation and deamination of glucosamine residues to produce 2,5-anhydromannose, which is estimated colorimetrically using 3-methyl-2-benzothiazolone hydrazone hydrochloride as the principal reagent. Alkaline hydrolysis is more readily accomplished at

121°C in an autoclave (Jarvis, 1977). Improved assay sensitivity was achieved by derivatisation of glucosamine and other products with *o*-phthalaldehyde, separation by high performance liquid chromatography and detection of fluorescent compounds with a spectrofluorimeter (Lin and Cousin, 1985). Ekblad and Nasholm (1996) also described an HPLC method which measured fluorescence of a 9-fluorenylmethylchloroformate derivative of glucosamine. The chitin assay remains rather complex and slow, usually requiring about 5 h.

A number of studies have indicated that the chitin assay is a valuable technique for estimating the extent of fungal invasion in foods such as maize and soybeans (Donald and Mirocha, 1977), wheat (Nandi, 1978) and barley (Whipps and Lewis, 1980) to estimate mycorrhizal fungi and fungal pathogens in plant material and soil (Ekblad and Nasholm, 1996; Ekblad et al., 1998; Penman et al., 2000; Singh, 2005) and measure wood rotting fungi (Nilsson and Bjurman, 1998). Particular attention has been paid to the possibility of developing the chitin assay as a replacement for the Howard mould count for tomato products (Jarvis, 1977; Bishop et al., 1982; Cousin et al., 1984).

The chitin assay has some shortcomings and has been severely criticised by some authors (e.g. Sharma et al., 1977). The relationship between dry weight and chitin content varies at least twofold for different food spoilage fungi (Cousin et al., 1984; Lin and Cousin, 1985; Cousin, 1996). Some foods contain naturally occurring amino sugars such as glucosamine and galactosamine, which should be removed by acetone extraction prior to hydrolysis (Whipps and Lewis, 1980). Products from rot-free tomatoes give positive glucosamine assays even after acetone extraction (Cousin et al., 1984) and chitin content does not increase proportionally with fungal growth (Sharma et al., 1977). Insect contamination of grain samples has been reported to produce grossly misleading results (Sharma et al., 1977), but the presence of fruit flies in tomato-based products was less serious (Lin and Cousin, 1985). Materials such as stored grains frequently contain insect fragments and need to be checked before chitin assays are attempted. Because of these difficulties, the use of chitin as a chemical assay for fungi in foods has largely been superseded by the ergosterol assay.

4.7.2 Ergosterol

Ergosterol is the major sterol produced by fungi, but at most is a minor component of plant sterols (Weete, 1974). Ergosterol occurs as a component of fungal cell membranes, so is inherently likely to be correlated with hyphal growth and biomass. It is therefore a good candidate as a chemical for measuring fungal growth in foods and raw materials. Methodology for estimating ergosterol in cereals was developed by Seitz et al. (1977, 1979). Samples are blended with methanol, saponified with strong alkali, extracted with petroleum ether and fractionated by high pressure liquid chromatography. Ergosterol is detected by ultraviolet absorption, optimally at 282 nm, a wavelength at which other sterols exhibit little or no absorbance. For refinements to this methodology, see Newell et al. (1988). Liquid chromatography or gas chromatography with mass spectrometric detection (LC-MS; GC-MS) have also been used to estimate ergosterol (Headley et al., 2002; Dong et al., 2006; Varga et al., 2006). GC-MS in combination with non-discriminating flash pyrolysis (Py-GC-MS) provides the advantages of little sample preparation and small sample size (Parsi and Gorecki, 2006).

The ergosterol assay provides a useful method for quantifying fungal growth, so numerous studies have assessed the relationship between fungal growth and ergosterol production, using both liquid and solid substrates.

Using liquid cultures, Zill et al. (1988) showed a correlation between ergosterol production, mycelial wet weight and mycelial protein in *Fusarium graminearum*. Matcham et al. (1985) reported that ergosterol correlated better with mycelial dry weight than chitin or laccase, a polyphenol oxidase. Variation exists, however. Torres et al. (1992) reported that *Aspergillus ochraceus* grown in liquid culture showed a threefold increase in ergosterol concentration in relation to mycelial dry weight as the culture aged from 2 to 26 days. Other reports (Matcham et al., 1985; Newell et al., 1987; Seitz et al., 1979) have indicated smaller variations, only one to twofold. Marfleet et al. (1991) showed that fungal biomass and ergosterol levels were correlated for three representative fungal species over a range of a_w on solid substrates but not in liquid media. Nout et al. (1987) showed that the ergosterol content of *Rhizopus oligosporus* varied widely, from 2 to 24 μg/mg

mycelial mass, and varied with substrate, aeration and growth phase. The ergosterol content was low during the rapid growth phase but tended to increase, at times sharply, as growth slowed. Taniwaki (1995) demonstrated that in fungi growing in atmospheres low in O_2 and with elevated CO_2 levels, the ergosterol content of hyphae was significantly reduced. In atmospheres containing 60% CO_2, ergosterol content per unit of hyphal length was up to six times less than in air. Growth medium also affected ergosterol concentrations: on average, seven foodborne fungal species grown on PDA produced more than twice as much ergosterol per unit hyphal length as when grown on CYA (Taniwaki, 1995; Taniwaki et al., 2006). However, there was a reasonable correlation between ergosterol and mycelium dry weight for seven of the eight species tested. *Eurotium chevalieri* was the exception: this species produced little ergosterol and appeared to produce several other sterols (Taniwaki et al., 2006). Marín et al. (2005) examined 16 species of food spoilage fungi and concluded that ergosterol content and colony diameters were better correlated to fungal biomass than fungal counts were. Marín et al. (2008) showed that, for 14 common food spoilage fungi, correlation coefficients between ergosterol and colony diameters were sufficiently significant over a range of a_w values (0.95–0.85), pHs (5–7) and potassium sorbate concentrations (0.5–1.5%) for both parameters to be useful in growth modelling.

Quantifying ergosterol production in foods has proved more difficult. Seitz et al. (1977) showed a good correlation between damage in rice grains and their ergosterol content, between ergosterol in wheat and rainfall during the growing season and between ergosterol content and fungal invasion in several sorghum hybrids. Matcham et al. (1985) reported good correlations between linear extension of *Agaricus bisporus* grown on rice grains and chitin, ergosterol and laccase production. Ergosterol content correlated with colony counts of fungi on wheat grains at 0.95 a_w but not at 0.85 a_w (Tothill et al., 1992). Using a stereomicroscope for visual examination, they concluded that sound grain contained up to 6 μg/g ergosterol, microscopically mouldy grain 7.5–10 μg/g and visibly mouldy grain more than 10 μg/g ergosterol. From studies on ergosterol levels, colony counts and mould growth in a variety of grain samples, Schnürer and Jonsson (1992) concluded that ergosterol correlated with colony counts better on DG18 ($r = 0.77$) than on MEA ($r = 0.69$). Ergosterol levels of food grade wheat ranged from 2.4 to 2.8 μg/g dry weight, samples from field trials (of unspecified quality) from 3.0 to 5.6 μg/g and feed grains from 8 to 15 μg/g dry weight.

After an extensive survey of ergosterol levels in Danish crops, Hansen and Pedersen (1991) concluded that the normal levels of ergosterol in barley were 7.6 ± 2.8, wheat for bread making 5.0 ± 1.5, rye for bread making 6.8 ± 2.2, peas 2.2 ± 2.7 and rapeseed 2.4 ± 1.3 μg/g dry weight. Ochratoxin A in barley correlated well with ergosterol content and reached significant levels when ergosterol increased to 25 μg/g dry weight. However, aflatoxin B_1 became detectable in cottonseed meal when ergosterol reached only 4 μg/g. "Burned" rapeseed, a measure of quality, became significant when ergosterol reached 1.4 μg/g dry weight. Lamper et al. (2000) found that ergosterol content correlated well ($r = 0.87$) with deoxynivalenol levels in wheat inoculated with *Fusarium graminearum* or *F. culmorum*. Moraes et al. (2003) found that there was good correlation between mould counts and ergosterol content of Brazilian maize ($r = 0.94$) but a poor correlation ($r = 0.4$) between ergosterol and aflatoxin content. Pietri et al. (2004) found significant correlation between ergosterol content of Italian maize and the major mycotoxins, fumonisin B_1 (1995 crop) or zearalenone and deoxynivalenol (1996 crop). Ergosterol content correlated strongly with fat acidity values and germination ability of stored canola (Pronyk et al., 2006). These authors also noted that *Penicillium* and *Aspergillus* species contributed more to ergosterol than *Eurotium* species. Ergosterol levels in sound canola were between 1.46 and 1.67 μg/g, whereas levels above 2 μg/g indicated significant levels of spoilage (Pronyk et al., 2006).

Karaca and Nas (2006) examined ergosterol content of dried figs and found good correlation ($r = 0.92$) between aflatoxin and ergosterol in reject figs which were fluorescent, but no significant correlation with patulin content. Kadakal et al. (2005) found good correlation ($r = 0.98$) between ergosterol and patulin in apple juice and that both patulin ($r = 0.99$) and ergosterol ($r = 0.99$) were linearly related to the proportion of decayed apples used to make the juice. Ergosterol has been used to assess mould growth in cheese with variable results (Pecchini, 1997; Taniwaki et al., 2001a).

Ergosterol content has also been investigated as an indicator of the mycological status of tomato products. Battilani et al. (1996) found a significant correlation between ergosterol, Howard mould count (HMC) and fungal growth, but with a high level of uncertainty. Kadakal et al. (2004) found a linear relationship between degree of decay in tomato pulp and HMC ($r = 0.97$) and ergosterol ($r = 0.96$) and concluded that ergosterol has the potential to be used in quality assessment of tomatoes. Sio et al. (2000) described an improved method for extraction of ergosterol from tomato products.

Other applications of ergosterol as a measurement of fungal biomass include estimation of mould spores in indoor air and aerosols (Miller and Young, 1997; Robine et al., 2005; Lau et al., 2006), estimation of wood decay by fungi (Eikenes et al., 2005) and estimation of fungi in soil and wetlands (e.g. Headley et al., 2002; Zhao et al., 2005).

The ergosterol assay is reported to have a high sensitivity and, in contrast to the chitin assay, requires only 1 h for completion (Seitz et al., 1979). Despite its limitations, it appears to be a useful indicator of fungal invasion of foods and to hold promise as a routine technique for quality control purposes.

4.7.3 Impedimetry and Conductimetry

Metabolites produced by growth of microorganisms in liquid media alter the medium's impedance and conductance. The use of changes in these properties as a measure of bacterial growth was suggested by Hadley and Senyk (1975) and was first applied to yeasts by Evans (1982) and to moulds by Jarvis et al. (1983). Most of the subsequent work on fungi has been carried out with yeasts, but the methodology is often applicable to moulds also.

During the 1980s there were a number of studies aimed at optimising media for inducing detectable and reproducible changes in either conductance or capacitance during fungal growth (see Pitt and Hocking, 1997). Watson-Craik et al. (1989, 1990) studied 27 mould species on a wide range of both commercially available and specially prepared media and concluded that conductance and capacitance were both medium and species specific. The use of media high in ammonium ions and glucose, with added yeast extract and peptone, decreased the influence of product variability and induced higher conductance changes (Owens et al., 1992). An impedimetric method for detection of heat resistant fungi in fruit juices was described by Nielsen (1992). The detection limit in artificially contaminated juices was one *Neosartorya* ascospore per millilitre, detectable in 100 h. Huang et al. (2003) reported an impedance method for detection of bacteria and fungi in bottled water which shortened the detection time from 5 days to 27.1 h for fungi and from 48 to 11.3 h for bacteria.

Although impedimetry and conductimetry promised to be effective rapid methods when used under well-defined conditions for a specific purpose with a particular kind of food, the methodology does not appear to have been broadly taken up for food mycology applications.

4.7.4 Adenosine Triphosphate (ATP)

ATP has also been suggested as a measure of microbial biomass because bioluminescence techniques provide a very sensitive assay (Jarvis et al., 1983). Provided that background levels of ATP in plant or other cells are very low, or that microorganisms can be effectively separated from such other materials, the method has some potential as a microbial assay. A good correlation was shown between ATP production and viable counts of six species of psychrotrophic yeasts grown in pure culture (Patel and Williams, 1985). The effective detection of low levels of yeasts in carbonated beverages by ATP has also been reported (LaRocco et al., 1985). However, living plant cells contain high levels of ATP and fungi are often very difficult to separate from food materials. Moreover, extraction of molecules from fungal cells is notoriously difficult, so this potential may be difficult to realise in food mycology. The most widespread application of ATP bioluminescence in the food industry is for monitoring hygiene of surfaces in food production facilities (Easter, 2007).

4.7.5 Fungal Volatiles

Methods for detection and characterisation of fungal volatiles are finding increasing applications in

food mycology, for the detection of fungal deterioration of grain and other food products, for the identification of particular fungi and for assessing potential mycotoxin contamination. Over the last two decades, there has been rapid growth in the development of gas sensor arrays. Gas sensors are chemical sensors that produce an electronic signal which is used as input into a pattern recognition system in order to recognise different volatiles and odours. This integrated system of gas sensor and pattern recognition is often called an "electronic nose or e-nose" (Gardner and Bartlett, 1994; Schaller et al., 1998). Arrays comprising equipment to collect headspace volatiles, analysis by GC-MS or other sensitive analytical methods, coupled with computer analysis are also commonly used.

Deterioration of stored grain results from a combination of chemical and biological changes, with changes due to fungal growth often predominant. Deterioration is marked by off-odour development, loss of germinability, caking, rancidity and sometimes mycotoxin development (Abramson et al., 1980). Fungi produce volatile chemicals during growth and particular chemicals may be associated with grain deterioration (Kaminski et al., 1974, 1975). Sinha et al. (1988) monitored production of 3-methyl-1-butanol, 1-octen-3-ol and 3-octanone in stored grain and found that the presence of these volatiles usually correlated with seed infection by *Alternaria alternata*, *Eurotium repens*, *Aspergillus versicolor* and several *Penicillium* species. Octenol and octanone in particular seemed to be associated with deterioration in grain due to fungal growth. Adamek et al. (1992) identified methylfuran, 2-methylpropanol and 3-methylbutanol as the most important metabolites from *Eurotium amstelodami*, *Aspergillus flavus*, *Penicillium cyclopium* and *Fusarium culmorum* growing on wheat. The volatiles produced by several *Penicillium* species were also studied. Börjesson et al. (1989) collected volatiles produced by several food spoilage fungi grown in pure culture on wheat grain and found that some compounds, especially 3-methyl-1-butanol, were produced early in fungal growth and could be used as an early warning of potential deterioration, before fungal growth became visible. The production of volatile metabolites by *Penicillium* species in grain correlated well with carbon dioxide production and ergosterol formation (Börjesson et al., 1990, 1992). The use of volatile compound production as an indicator of mould deterioration in grains has been extensively assessed and reviewed (Kaminski and Wasowicz, 1991; Schnürer et al., 1999; Magan and Evans, 2000; Paolesse et al., 2006; Balasubramanian et al., 2007).

Fungal volatiles can be used to detect potential mycotoxin contamination, to discriminate between fungal species (Sunesson et al., 1995; Keshri et al., 1998) and even between toxigenic and non toxigenic strains of particular fungi (Sahgal et al., 2007). Karlshøj et al. (2007a) used an electronic nose to differentiate between closely related *Penicillium* species (*P. camemberti*, *P. nordicum*, *P. paneum*, *P. carneum*, *P. roqueforti* and *P. expansum*) from cheese. Volatile profiles can be used to predict mould spoilage in bakery products (Vinaixa et al., 2004; Marín et al., 2007a) and to detect and differentiate between toxigenic and non toxigenic *P. verrucosum* strains in bakery products (Needham and Magan, 2003). Volatile profiles have also been used to differentiate between toxigenic and non toxigenic *Fusarium* strains (Keshri and Magan, 2000; Demyttenaere et al., 2004), to identify mycotoxins (aflatoxins, ochratoxin A and deoxynivalenol) in durum wheat (Tognon et al., 2005) and to detect and quantify ochratoxin A and deoxynivalenol in barley (Olsson et al., 2002). This technology has also been applied to predict the presence of *P. expansum* and patulin in apple products (Karlshøj et al., 2007b) and to detect and discriminate diseases of potato tubers (Kushalappa et al., 2002) and stem-end rot and anthracnose in mangoes (Moalemiyan et al., 2006). Electronic nose technology has also been used for early detection of moulds in libraries and archives (Pinzari et al., 2004).

4.7.6 Immunological Techniques

Cell wall proteins of fungi produce antigens, which can be detected by immunological methods. Some antigens are derived from components common to a wide range of fungi, and hence are indicative of general fungal growth, while others are genus or even species specific. A variety of methods have been developed to take advantage of these fungal antigens, although none is in widespread commercial use.

Enzyme-linked immunosorbent assay (ELISA). The preparation of antigens from three common foodborne fungi (*Penicillium aurantiogriseum*, *Mucor racemosus* and *Fusarium oxysporum*) was described by Notermans and Heuvelman (1985). Preparation of immunoglobulin antibodies against these antigens was followed by development of an ELISA assay. Fungi were detected in both unheated and heat processed foods by this method. Antigens were relatively genus specific: the *M. racemosus* antigens reacted with other *Mucor* and *Rhizopus* species, and the *Penicillium* antigen reacted with the *Aspergillus* species tested. It was subsequently shown that the *Penicillium* antigen reacted with 43 of 45 *Penicillium* species tested and that antigen production correlated with mycelial weight and was unaffected by culture conditions, medium, temperature and a_w (Notermans et al., 1986). The *Penicillium* antigen also reacted with *Aspergillus flavus* and the level of antigen correlated with aflatoxin production (Notermans et al., 1986).

ELISA techniques have also been studied as a potential replacement for the Howard mould count. Antigens from tomato moulds (*Alternaria alternata*, *Geotrichum candidum* and *Rhizopus stolonifer*) were used to produce an ELISA test sensitive to 1 μg/g of mould in tomato. A correlation was observed between antigen formation and mould added to tomato puree, while background interference was very low (Lin et al., 1986). The method was tested against a broader range of foods, with encouraging results (Lin and Cousin, 1987). Robertson and Patel (1989) improved the sensitivity of the method for tomato paste by using a polyclonal antibody against *Botrytis cinerea*, *Mucor piriformis* and *Fusarium solani* in addition to the three species used by Lin et al. (1986).

ELISA based methodology has been reported for the detection of *Fusarium* species in corn (Meirelles et al., 2006), cornmeal (Iyer and Cousin, 2003) and grain (Rohde and Rabenstein, 2005). Correlations with other measures of fungal growth (ergosterol, colony count, mycotoxin levels) were variable.

Aspergillus species have also been examined as targets for immunological detection because of their importance in mycotoxin contamination. ELISA detection of *A. ochraceus* in wheat (Lu et al., 1995), coffee powder, chilli powders and poultry feed (Anand and Rati, 2006) has been investigated, with reasonable correction with other parameters (amount of ochratoxin A, $r = 0.93$; percentage infection $r = 0.89$) reported by Lu et al. (1995) but poorer correlation with colony forming units ($r = 68$) and glucosamine content ($r = 0.64$). Anand and Rati (2006) did not attempt to correlate their ELISA with external measurements of fungal biomass, but used an internal comparison method using freeze dried mycelium. Immunological detection of aflatoxigenic fungi has also been the subject of several studies, using antibodies raised to *A. flavus* or *A. parasiticus* (Candlish et al., 1997; Tsai and Yu, 1997, 1999; Yong and Cousin, 2001). Tsai and Yu (1999) reported good correlation between viable count and ELISA measurements, depending on substrate, with correlation coefficients varying from 0.96 for wheat to 0.86 for peanuts in artificially inoculated samples.

ELISA tests for *Botrytis* and *Monascus* in foods (Cousin et al., 1990) and for the detection in rice of *Penicillium islandicum* (Dewey et al., 1990) and *Humicola lanuginosa* (Dewey et al., 1992) have been described. ELISA tests have also been applied to detection of yeasts in dairy products (Garcia et al., 2004) and fruit juice (Yoshida et al., 1996), moulds in paprika powder (Kisko et al., 1998) and moulds in agricultural commodities (Park et al., 2003) with varying success. Linfield et al. (1995) reported that polyclonal antibodies raised against *Botrytis allii* were useful in early detection of neck rot of onions caused by this pathogen.

Immunochemical detection of fungi in food and feeds has been reviewed by Li et al. (2000).

Latex agglutination. A different approach to the immunological detection of fungi in foods has been the coating of latex beads with antibodies and detection of agglutination of the beads in the presence of antigens (Kamphuis et al., 1989; Notermans and Kamphuis, 1992; Stynen et al., 1992). It was found that 0.8 μm latex beads coated with antibodies from the extracellular polysaccharide produced by *Penicillium digitatum* specifically detected *Aspergillus* and *Penicillium* species (Kamphuis et al., 1989). Detection limits were as low as 5–10 ng/ml of the purified antigen. Commercially produced latex agglutination tests were of value for screening the mycological quality of grains and processed foods (Braendlin and Cox, 1992; van der Horst et al., 1992), although one kit performed poorly in detection of mould in tomato products (van der Horst et al., 1992).

Schwabe et al. (1992) compared the latex agglutination assay with ergosterol production for detection of *Penicillium*, *Aspergillus* and *Fusarium* species in pure culture. They concluded that the two methods were comparable for *Penicillium* and *Aspergillus* but that ergosterol was more sensitive for *Fusarium*. In food samples, both the latex agglutination test and ergosterol were effective means of detecting mould growth, but no clear correlation existed in values obtained by the two methods. Kesari et al. (2004) used a latex agglutination test to detect teliospores of Karnal bunt (*Tilletia indica*) in single grains of wheat and were able to detect a few as 750 teliospores, which they reported as suitable for single seed analysis.

Fluorescent antibody techniques. Fluorescent antibody techniques have also been used directly for the detection of mould in foods. Warnock (1971) detected *Penicillium aurantiogriseum* in barley by this method, while Robertson et al. (1988) used antisera from five fungi to visualise moulds and simplify their detection in the Howard mould count technique.

4.7.7 Molecular Methods

The first techniques for detecting DNA sequences using specific probes were devised more than 30 years ago (Southern, 1975). With the development of the polymerase chain reaction (PCR), gene cloning and oligonucleotide synthesis, DNA sequences can now be prepared in large quantities for use in probes. Depending on its role in the genome, DNA may be specific at almost any taxonomic level. Molecular methods can be used in both detection and identification of fungi, and real-time PCR methods even allow quantitative detection. This field of research continues to develop very rapidly, and significant advances may be expected.

Producing a broadly based or even strain specific DNA probe is theoretically possible for any organism. Full genome sequences are available for a number of important foodborne fungi, including several *Aspergillus* species (*A. nidulans, A. fumigatus, A. oryzae, A. flavus, A. niger, A. terreus, A. clavatus* and *Neosartorya fischeri*), *Botrytis cinerea, Chaetomium globosum, Fusarium graminearum, F. oxysporum, F. verticillioides, Rhizopus oryzae* and several yeast species (*Saccharomyces cerevisiae, Candida albicans, C. guilliermondii, C. lusitaniae, C. tropicalis, Schizosaccharomyces japonicus* and *Sch. pombe*) (Broad Institute, 2008). *Penicillium* species are noticeably absent from the list, although the genome of the pathogenic species *P. marneffei* has recently been sequenced (JCVI, 2008).

There have been rapid advances in molecular methods for the detection of mycotoxigenic fungi. Probes have been developed that target genes in the mycotoxin biosynthetic or regulatory pathways for many species of *Aspergillus, Penicillium* and *Fusarium,* enabling these fungi to be detected and/or quantified in grains, grapes, apples, coffee and other food matrices (Edwards et al., 2002; Paterson, 2006; Geisen, 2007; Niessen, 2007a, b; Rossi et al., 2007).

Molecular methods have also been developed for the detection of spoilage fungi, particularly yeasts, e.g. *Brettanomyces*/*Dekkera* in grapes and wine (Phister and Mills, 2003; Agnolucci et al., 2007; Hayashi et al., 2007), *Hanseniaspora* in wine (Phister et al., 2007); *Zygosaccharomyces bailii* in wine and fruit juices (Rawsthorne and Phister, 2006) and *Kluyveromyces marxianus* in yoghurt (Mayoral et al., 2006). More general techniques have been described for detection of yeasts in juices (Casey and Dobson, 2004; Ros-Chumillas et al., 2007), milk, yoghurt and cheese (Cocolin et al., 2002; Bleve et al., 2003; García et al., 2004; Lopandic et al., 2006; Gente et al., 2007), sourdough fermentation (Meroth et al., 2003), table olives (Arroyo-Lopez et al., 2006) and vacuum packaged ham (Sanz et al., 2005). PCR based methods have also been described for detection of moulds in orange juice (Wan et al., 2006), *Alternaria* in cereal grains (Zur et al., 2002) and *Botrytis* in onion seeds (Walcott et al., 2004).

As gene sequencing has become more readily available and cheaper, along with freely accessible databases such as GenBank (http://www.ncbi.nlm.nih.gov/Genbank/), sequence analysis and comparison is becoming a routine method for identification of yeasts and filamentous fungi. For yeasts, the most common target region is the D1/D2 domain of the 26S rDNA (Kurtzman et al., 2003), but the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) may also be used (van der Vossen et al., 2003; Pulvirenti and Giudici, 2003; Solieri et al., 2006; Villa-Carvajal et al., 2006).

Sequencing of the ITS region along with "housekeeping genes" such as calmodulin, β-tubulin and elongation factor 1-α is now commonly applied for purposes of identification and phylogenetic analysis of important food spoilage and mycotoxigenic fungi in the genera *Aspergillus* (Varga, 2006; Geiser et al., 2007; Peterson, 2008), *Penicillium* (Peterson, 2004, 2006; Samson et al., 2004b; Wang and Zhuang, 2007; Serra et al., 2008) and *Fusarium* (O'Donnell et al., 2004; Scott and Chakraborty, 2006; Leslie et al., 2007).

Despite the apparent power of molecular techniques, they need to be applied with some caution, particularly when comparing DNA sequences with those in the publicly available databases to identify yeast or mould isolates. Correct identification relies on the database sequences having the correct name attached to them by the depositor, which is not always the case. If the identification makes sense, the percent homology is 98% or greater and the number of base pairs on which the homology is scored is high, then the answer is probably correct.

4.8 Identification Media and Methods

4.8.1 Standard Methodology

The identification keys in this book are based primarily on the standardised procedure described for the identification of *Penicillium* species by Pitt (1979b). Cultures are grown for 7 days on three standard media at 25°C, and on one of these at 5 and 37°C also. The three Media are Czapek yeast extract agar (CYA; Pitt, 1973) used at all three temperatures; malt extract agar (MEA; Raper and Thom, 1949) and 25% glycerol nitrate agar (G25N; Pitt, 1973). Their formulae are given in the Media Appendix. Preparation time of CYA and G25N is reduced by the use of Czapek concentrate (Pitt, 1973), which is added to the media at the rate of 1% of the aqueous portion.

As media ingredients have become more purified in recent years, difficulties with extent and colour of sporulation on CYA have been encountered, especially with some *Penicillium* species. To overcome this problem, Czapek concentrate has been reformulated (Pitt, 2000) by the inclusion of traces of zinc and copper (Smith, 1949) (see Media Appendix).

4.8.2 Plating Regimen

As noted above, cultures for identification are grown on three media and at three temperatures. Maximum efficiency in time, incubator space and materials is achieved by inoculating two cultures on a single Petri dish of G25N, and at 5 and 37°C, as shown in Fig. 4.3. Cultures are rarely mutually inhibitory under these conditions, although overgrowth of one culture by another is occasionally a problem at 37°C. Standard sized Petri dishes (90–100 mm) are used, except that at 5°C, smaller plates (50–60 mm in diameter) can be an advantage. The smaller size is easier to examine under the low power microscope. All plates are incubated for a standard time of 7 days.

Plates incubated at 37°C should be enclosed in polyethylene bags to prevent evaporation and drying of the medium. Unless the humidity is very low, plates at 25°C will not dry excessively in 7 days. If no 5°C incubator is available, use a polyethylene food container or insulated box in a household refrigerator. The box should be equipped with a thermometer, and its location moved by trial and error until a place with a 5°C average temperature is located. Temperatures at 5 and 37°C should ideally be ±0.5°C and be checked frequently; at 25°C, ±2°C control is adequate (Okuda, 1994).

4.8.3 Inoculation

As shown in Fig. 4.3, Petri dishes of CYA and MEA for incubation at 25°C are inoculated with a single culture at three points, equidistant from the centre and the edge of the plate and from each other. Plates of the other media are inoculated with two points per culture, as illustrated.

With some fungi, especially *Penicillium* and *Aspergillus*, it is important to minimise colonies from stray spores. The most satisfactory technique is to inoculate plates with spores suspended in semi-solid agar (Pitt, 1979b). Dispense 0.2–0.4 ml of melted agar (0.2%) and detergent (0.05%), such as polysorbitan 80 (Tween 80), in small vials and sterilise. To use, add a needle point of spores and mycelium to a vial and mix slightly. Then, before flaming the needle, use it to stab inoculate the 5°C plate;

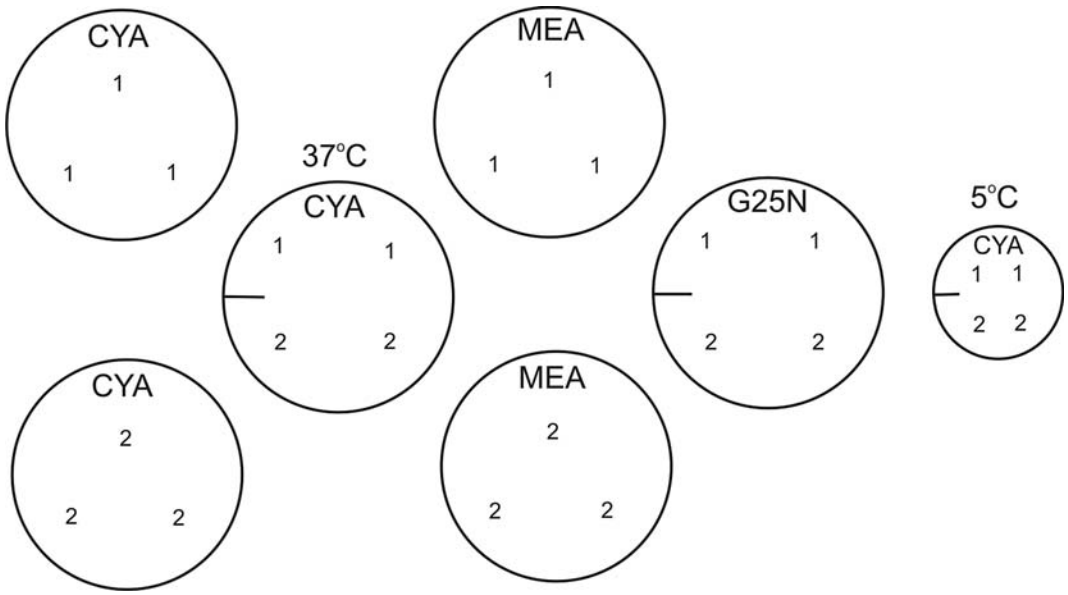


Fig. 4.3 Schematic of regimen used for culturing fungal isolates for identification

residual spores on the needle make a good inoculum. Next, take a sterile loop, mix the vial contents thoroughly and inoculate the standard plates. Used vials can be sterilised by steaming and reused several times before being washed or discarded.

4.8.4 Additional Media and Methods

The plating regimen outlined above can be used to identify most of the fungi described in subsequent chapters of this book. Some exceptions exist, because certain genera either grow poorly or fail to sporulate on the standard media. As noted earlier in this chapter, fastidious xerophiles are identified on MY50G agar. *Eurotium* species, traditionally identified on Czapek agar with 20% sucrose, are identified here on Czapek yeast extract agar with 20% sucrose (CY20S; see Appendix). *Trichoderma* species are best identified on potato dextrose agar (PDA) after a relatively short incubation time (3–4 days), as the structures tend to autolyse as cultures mature.

Penicillium* subgenus *Penicillium. Many species classified in subgenus *Penicillium* are morphologically similar, and identification using traditional morphological techniques remains difficult. These Penicillia are very common in foods, and many produce mycotoxins, so correct identification is often critical. Species within subgenus *Penicillium* fall into two groups: those with an affinity for proteinaceous foods and those which grow more vigorously in foods high in carbohydrate. Frisvad (1981, 1985) introduced creatine sucrose agar (CREA) to permit differentiation between these two groups. Creatine (as the sole nitrogen source) permitted growth of the former group while inhibiting the latter. Incorporation of bromocresol purple enabled visualisation of pH changes, either acid or alkaline, depending on the creatine or sucrose metabolism of a particular species. However, discrimination between positive and negative responses was not always clear, and the main tabulation of species reactions to CREA (Table 1 in Frisvad, 1985) was difficult to interpret. Frisvad (1993) subsequently produced a number of variations of CREA, including acid and neutral pH formulations and substitution of sucrose with fructose or lactose. Although the merits of each formulation were discussed, no firm recommendation resulted from this exercise.

Pitt (1993a) modified Frisvad's strongly alkaline CREA medium by studying several sucrose and creatine concentrations over a wide pH range. The result was neutral creatine sucrose agar (CSN), a medium producing eight different reactions among

the 20 *Penicillium* subgenus *Penicillium* species tested. When included in the normal plating regimen for identification of *Penicillium* cultures from the subgenus *Penicillium*, CSN provides a very useful aid to distinguishing between these difficult and closely related species. See Chapter 7 for details of use of CSN, its reactions and interpretation. Its formulation is given in the Media Appendix.

Dematiaceous Hyphomycetes. The natural habitats for many dematiaceous Hyphomycetes are plants or plant material, so finding suitable laboratory media and conditions to induce typical sporulation can be difficult. *Alternaria, Curvularia, Stemphylium* and *Ulocladium* species are best identified from dichloran chloramphenicol malt extract agar (DCMA; Andrews, 1992b) plates incubated for 7 days at 25°C under lights. Conidial characteristics of *Bipolaris* species vary with type of medium. Species described here are best identified from tap water agar (TWA) containing a natural substrate such as sterilised wheat or millet seeds, or wheat straw. *Drechslera* species will grow well on DCMA but sporulation is poor. The best sporulation is achieved with V-8 juice (V-8 J) agar or TWA with one of the natural substrates mentioned above, incubated at 25°C for 7–10 days under lights with a 12 h photoperiod. Light/dark periodicity is important as *Drechslera* cultures require light to produce conidiophores and darkness to produce conidia, possibly due to light inactivation of flavine necessary for conidial formation (Knan, 1971; Platt et al., 1977).

For the identification of *Trichoderma* and *Fusarium* species, potato dextrose agar (PDA) is used. *Fusarium* species also require additional methods and media as outlined below.

4.8.5 *Identification of* Fusarium *Species*

Fusarium isolates exhibit unusually high variability in colony morphology and also may deteriorate rapidly in culture. Thus, they should be identified as soon as possible after initial isolation with a minimum of subculturing to avoid deterioration. It is common practice to prepare cultures of Fusaria from single spores for growth on identification media, as this reduces both of these problems.

Single sporing. The technique for preparing single spore cultures is as follows (Nelson et al., 1983; Leslie and Summerell, 2006). Pour about 10 ml of 2% water agar into unscratched glass or plastic Petri dishes and allow to dry, either by holding the plates at room temperature for several days or by placing them inverted in an oven at 37–45°C for about 30 min. Prepare a suspension of conidia in a 10 ml sterile water blank so that it contains 1–10 spores per low-power (10×) microscope field when a drop from a 3 mm loop is examined on a slide. With experience, this concentration can be gauged simply by observing the turbidity of the suspension. Pour the suspension of spores onto a dried water agar plate, drain off the excess liquid and incubate in an inclined position at 20–25°C for 18–20 h.

After incubation, open the Petri dish, shake off any accumulated moisture droplets and examine under a stereomicroscope using transmitted light. The germinating conidia should be visible under 25× magnification. A dissecting needle with a flattened end and sharpened edges is used to cut out small squares of agar containing single, germinating conidia. These single conidia are then transferred on the agar blocks to the desired growth medium.

If the original culture is contaminated with bacteria, a drop of 25% lactic acid may be added to the water blank. Allow this acidified spore suspension to stand for 10 min before pouring onto a water agar plate. Germination of acid-treated *Fusarium* conidia may be delayed by 24 h or more.

Media. Two media have been used in this book for the identification of *Fusarium* isolates: potato dextrose agar (PDA) for colony characteristics and colour and dichloran chloramphenicol peptone agar (DCPA) for the development of diagnostic macro-, micro- and chlamydoconidia.

A third medium, carnation leaf agar (CLA), is recommended by some *Fusarium* specialists for *Fusarium* cultivation and identification (Nelson et al., 1983; Leslie and Summerell, 2006). CLA is an excellent medium, on which most *Fusarium* species readily produce their diagnostic macroconidia. Production of macroconidia on DCPA is usually comparable with that on CLA, but microconidia and chlamydoconidia are often more plentiful on CLA due to greater production of aerial hyphae. DCPA is used in the present work rather than CLA,

however, because dried, gamma-irradiated carnation leaves are difficult to obtain in many localities.

Inoculation and incubation. For identification by the methods used in this book, single spore cultures of *Fusarium* isolates should be prepared on agar blocks as outlined above, inoculated, one per plate, onto two plates each of PDA and DCPA and incubated at 25°C for 7 days. Individual plates may be used for each medium, or alternatively divided plates may be used, with one medium on each half of the plate. Illumination during incubation is essential for the production of macroconidia. The light source may be diffuse daylight (not direct sunlight) or light from a bank of fluorescent tubes. A photoperiod of 12 h per day is normally used. Alternating temperatures of 20 and 25°C have been recommended (Nelson et al., 1983; Leslie and Summerell, 2006) but are not essential.

A simple light bank may be constructed from a standard 40 watt fluorescent fixture with two cool white tubes, suspended 0.5–1 m above the laboratory bench or shelf supporting the cultures. The addition of a black light tube (e.g. Philips TL 40 W/80 RS F40BLB) is also desirable and in some cases essential to induce macroconidial or chlamydoconidial production.

4.8.6 Yeasts

Yeast identification systems. Identification of foodborne yeasts remains a difficult task, as colony characteristics and microscopic morphology are of limited value. Generally it has been necessary to use biochemical and physiological tests such as fermentation of carbohydrates, assimilation patterns for a range of carbon and nitrogen sources and growth at various temperatures. Details of these methods and media may be found in Kurtzman and Fell (1998), Kurtzman et al. (2003) or Barnett et al. (2000). Molecular methods (see below) are being used increasingly in yeast identification, replacing time consuming biochemical testing.

Identification using systems based on biochemical and physiological testing is complex and time consuming. However, a number of attempts have been made to assist those who wish to persevere with yeast identification. Several simplified systems have been published in the literature, and both automated and manual yeast identification systems are now commercially available.

Deák and Beuchat (1987) published a simplified identification key (SIK) which included 215 species of foodborne yeasts. They subsequently modified their system, restricting it to the 76 species most frequently occurring in foods (SIM), and reported that it was much more successful than the API 20C system (BioMérieux, Marcy-l'Etoile, France) for identification (Deák, 1992). The SIM uses only two Petri dishes and three test tubes to examine each strain for ability to assimilate 10 carbon sources, fermentation of glucose, assimilation of nitrate and splitting of urea. These biochemical tests are supplemented by morphological observations. SIM separates the yeasts into six groups by a dichotomous key utilising the results of five key tests. Further tests are used to differentiate the yeasts in these six groups using secondary dichotomous keys.

A dichotomous key to 25 common species of foodborne yeasts was published by Smith and Yarrow (1995), who used 17 biochemical and physiological tests to distinguish the species.

Of the commercial systems available, the most widely used for foodborne yeasts are the Biolog, which is an automated system, and the BioMérieux ID32C yeast identification strips which can be read manually or automatically using the ATB system. BioMérieux also markets the fully automated VITEK 2 system, based on a card containing 64 tests. However, the database comprises only 46 clinically important species (Aubertine et al., 2006) and so has limited application to foodborne yeasts.

The Biolog (Biolog Inc., Hayward, CA, USA; http://www.biolog.com/microID.html) utilises a yeast identification test panel (YT MicroPlate™) consisting of a matrix of 8 × 12 wells. The first three rows contain 35 carbon source oxidation tests using tetrazolium violet as an indicator of oxidation. The next five rows contain carbon assimilation tests which are scored turbidimetrically against a negative control panel containing only water. The last row contains two carbon sources and tests for the co-utilisation of various carbon sources with D-xylose. The hardware (Biolog MicroStation Reader) consists of an automated plate reader coupled with a computer, which interprets the

results and compares them with the resident database which currently includes 267 species. Manual interpretation of the Biolog plates is not recommended. This system has been designed with the food industry in mind, and the database contains all the common foodborne yeasts, unlike other systems which are usually aimed at the clinical market.

The ATB ID32C system (BioMérieux, Marcy-l'Etoile, France) is an automated system utilising the BioMérieux ID32C yeast identification strips. These strips contain 30 assimilation tests plus a positive (glucose) and a negative control well, all of which are inoculated with a yeast suspension of specified density. The strips are incubated at 30°C for 48–72 h. As with the Biolog, the ATB automated system consists of a plate reader attached to a computer. The database associated with the ATB system contains 63 yeast species.

The BioMérieux ID32C strips can be read manually, and the results enable identification of yeasts using published keys or computer identification programmes such as that of Barnett et al. (1996). This system may be used with reasonable success, particularly when the test results from the ID32C strips are supplemented with extra tests (glucose fermentation, urease production, nitrate utilisation, growth in 0.5 and 1% acetic acid and in 50 and 60% glucose, growth at 37°C, production of pseudohyphae, ascospore formation and morphological observations) to give a more comprehensive base for the identification. Growth in 0.5 and 1% acetic acid indicates preservative resistance, and growth in 50 and 60% glucose gives an indication of ability to grow at reduced a_w. Both these parameters are important in determining a yeast's ability to cause spoilage in particular products.

Identification using DNA sequencing is increasingly becoming the method of choice, as extensive databases such as GenBank are freely available for identification purposes. The 600–650 nucleotide D1/D2 region of the large subunit (26S) ribosomal DNA is the most widely targeted section of the genome, and sometimes the ITS region may also be used (Kurtzman et al., 2003). Sequencing of the D1/D2 region, along with some supplementary physiological and biochemical tests, is the identification method currently used in our laboratory.

Even using the available systems, identification of yeasts still requires some specialist knowledge and interpretation and remains time consuming.

Our experience indicates that no more than 12 species of spoilage yeasts are of real concern in foods. It is possible to differentiate these few species by relatively simple techniques, i.e. colony and microscopic morphology, growth on the standard media used for filamentous fungi, growth on other media which test for preservative resistance, ability to use nitrate as a nitrogen source and adaptation to high NaCl concentrations. Details of these techniques are given in Chapter 10.

4.9 Examination of Cultures

As noted above, all cultures for identification should first be grown on the standard regimen described earlier. After 7 days incubation, the following examination should be carried out and then the general key to fungi in Chapter 5 should be used. That key will be of assistance even in the event of cultures failing to grow under one or more of the standard conditions.

4.9.1 Colony Diameters

Measure the diameters of macroscopic colonies in millimetres from the reverse side (Fig. 4.4). Microscopic growth or germination at 5°C is assessed by low power microscopy (60–100×), by putting the 5°C Petri dish on the microscope stage and examining by bright field transmitted light. Growth at 37°C is assessed macroscopically only; germination of spores at 37°C is an unreliable character.

4.9.2 Colony Characters

Colony appearance can be judged by eye or with a hand lens, but examination is more effective if a stereomicroscope is used. Magnifications in the range of 5–25× are the most useful. Characters such as type and location of sporing structures and

Fig. 4.4 Technique for measuring colony diameters by transmitted light

extent of sporulation are best gauged with the stereomicroscope. Reflected light is usually more effective than transmitted light.

To determine colony colours, examine colonies by daylight or by daylight-type fluorescent light. In some genera, reference to a colour dictionary is helpful. The Methuen "Handbook of Colour" (Kornerup and Wanscher, 1978) has been used in this work and is highly recommended.

4.9.3 Preparation of Wet Mounts for Microscopy

Fungi should always be examined microscopically as wet mounts rather than fixed and stained like bacteria. To prepare a wet mount, use an inoculating needle to cut out a small portion of the colony which includes sporing structures. Examination with the stereomicroscope can be an invaluable aid here. For freely sporing fungi with little mycelium, cut a piece of colony near the edge, where fruiting structures are young and spore numbers not excessive. Take structures which may enclose spores, i.e. cleistothecia, from near colony centres, where the probability of mature spores is highest. If the only differentiated parts of the colony appear to be buried in the agar, e.g. pycnidia, take a sample of these with a small piece of the agar. Float the cut colony sample from the needle onto a slide with the aid of a drop of 70% ethanol. It may be necessary to tease out the specimen with the needle and the corner of a cover slip (square cover slips are best). Fungal specimens may be highly hydrophobic: the ethanol helps to wet the preparation, minimising the amount of entrapped air. When most of the ethanol has evaporated, add a drop of lactic acid (for phase or interference contrast optics) or lactofuchsin stain (see below) for bright field. Add a cover slip; if necessary remove excess liquid from the preparation by gently blotting with facial tissue or similar absorbent paper. The preparation is now ready for examination.

4.9.4 Staining

A wide variety of stains are in use for mycological work. However, most are time consuming to prepare, or to use, or are slow to act, because fungal walls and spores are highly resistant to stains. By far the most effective stain for use in food mycology is lactofuchsin (Carmichael, 1955), which suffers from none of these faults. It consists of 0.1% acid fuchsin

dissolved in lactic acid of 85% or higher purity. Young actively growing fungal structures are preferentially coloured bright pink, so sporing structures can usually be readily distinguished against a background of older mycelium. Cleistothecial initials, developing asci and maturing ascospores are also seen more readily in preparations stained with lactofuchsin.

Like most other mycological stains, lactofuchsin is corrosive. Take care to clean it off microscope parts or skin! Be especially careful of the objective faces, because lactic acid will slowly corrode the relatively soft glass used in lenses.

4.9.5 Microscopes and Microscopy

Choice of microscope. Fungal identifications inevitably involve microscopy. A high quality, binocular compound microscope is essential for serious mycological work. Bright field, phase contrast and interference contrast optics are all suitable. If bright field optics are used, preparations should be stained. Phase contrast optics avoid the need for staining, although as explained above, staining has merit for certain structures in any case. Resolution under phase contrast sometimes suffers from excessive halo effects because fungal structures are highly refractile. Experience suggests that bright field optics are more satisfactory than phase contrast for examining most fungi. Interference contrast is superior to both bright field and phase contrast and is strongly recommended. The very thin optical section cut by this system provides higher resolution of dense structures and the relatively low contrast is restful to use.

The microscope should be equipped with at least four objectives: 6× for examining Petri dishes under low power (for germination, etc.); 10× or 16× for searching fields for sporing structures; 40× for examining structural details and measuring stipes, fruiting structures and large conidia; and 100× oil immersion for observation of details of spore attachment, surface texture, ornamentation of hyphae and spores and fine measurements. Oculars should be 10× or 12.5× and may with advantage be wide field and have a high eye point suitable for use with spectacles. One should be a focusing ocular, equipped with an eyepiece micrometer, which is essential for measuring dimensions of spores and sporogenous structures.

In the examination of fungal mounts, it is stressed that it is most important to use low power optics before succumbing to the temptation to use oil immersion. The principal reason is that fungal preparations usually remain as small clumps and do not disperse as bacteria do. Only under low power is the search for the optimal area of the slide for the observation of fruiting structures likely to be rewarded. Once a suitable area is located under the 10× or 16× objective, move to the 40×. This should be the lens most used; microscope optics are such that only the finest details of ornamentation can be observed more effectively under oil immersion than at this magnification.

Aligning the microscope. Correct alignment of the microscope is essential, so that its resolution is as high as possible and it can be used for long periods without discomfort. An incorrectly aligned microscope will lead to poor observation, discomfort, fatigue, headache and eye strain. A person of normal visual acuity should be able to use a correctly aligned instrument throughout a whole working day without discomfort. The steps to correctly align a microscope are given below. They should be read in conjunction with the microscope manufacturer's instructions.

1. Mount a slide on the stage and bring it into approximate focus. If a prepared slide is not available, a slide marked with a marking pen or ink line is a satisfactory substitute.
2. Close the microscope's field diaphragm (the one at the microscope base nearer the light source). The image of the diaphragm opening should now be visible in the microscope field. If it is, first focus it with the condenser focusing knob and then centre it in the field with the condenser centring screws. If the diaphragm opening cannot be seen, first rack the condenser up and down and watch to see if the opening becomes visible; if it does not, rack the condenser to its highest position and then slowly open the field diaphragm until the opening comes into view. Centre the diaphragm approximately and proceed as above.
3. For bright field optics, the condenser diaphragm should be adjusted each time the objective power is changed. Remove one ocular; close the

condenser diaphragm so that the field seen down the open tube is about two-thirds its maximum size. With phase contrast and interference contrast systems, this adjustment is less critical.

The preceding steps align the microscope itself and should be checked frequently. If optimal illumination is desired, each step should be carried out for each new slide and each objective change. As a routine habit, the whole process should take only a few seconds.

If the available microscope is not equipped with a built-in light source, a field diaphragm and a fully centring condenser, it is unlikely to be satisfactory for the identification of small spored fungi such as *Penicillium* species.

The following steps are designed to align the observer with the microscope, compensating for individual differences in sight. Provided settings on the microscope are remembered, these steps need be carried out only occasionally, to check that visual acuity has not altered. Different settings will be needed for an individual with and without spectacles or contact lenses.

4. Assuming the microscope is binocular, pull the oculars out to their greatest distance apart and then, while watching a focused field, move them gradually together until a single circular field is seen without strain or head movement. Note the distance on the scale between the oculars; this is the individual's interpupillary distance. Repeat this operation two or three times until satisfied that the correct distance has been found. This distance should always remain the same and be similar on any microscope.
5. Under the 40× or 100× objective, locate a tiny, readily recognised point on the slide and focus on it. Take a piece of white card and place it between the focusing ocular and the corresponding eye. Leave the eye open. Now focus the tiny point with the other eye, carefully, with the microscope fine focus. Next, transfer the white card to the other ocular and, using the focusing collar beneath the ocular, refocus the tiny point. Remove the card and note the setting on the scale. Repeat until satisfied the correct setting has been found.
6. On some microscopes, the eyepiece micrometer can be focused independently. Use the focusing system on the ocular itself to focus the micrometer. Note the setting on the scale on the side of the ocular.

Always check the settings on the microscope before use and after making measurements with the micrometer. It is very easy to upset the ocular alignment when measuring.

4.10 Preservation of Fungi

Many fungi are stable in culture and can be subcultured repeatedly without apparent change or deterioration. Others, especially *Fusarium* species and other plant pathogens and some mycotoxigenic species, will degenerate rapidly after only a few transfers.

For stable isolates that are used routinely in the laboratory, storage on agar slopes is satisfactory. Many freely sporing fungi will survive for several months, and sometimes much longer, when stored at 1–4°C on a medium such as CYA. One of the hazards of storage at these temperatures is contamination by psychrophilic *Penicillium* or *Cladosporium* species. Storage at freezer temperatures (–18 to –20°C) prevents such contamination, but some fungi do not survive well under these conditions.

Storage at room temperatures, at 10°C or above, for long periods is not advisable because of the likelihood of invasion by culture mites (see below).

4.10.1 Lyophilisation

For unstable cultures, and indeed for the long term storage of any food spoilage fungi, freeze drying or *lyophilisation* is probably the best method of preservation. Many commercial systems are now available for carrying out this process.

A satisfactory menstruum for lyophilisation of most fungi is 1.5× normal strength reconstituted nonfat milk powder (15% in distilled water). For fungi with hydrophobic conidia, such as *Aspergillus* and *Penicillium*, a small amount of detergent (0.05%) such as polysorbitan 80 (Tween 80) should be added to the milk. Dispense the milk in 10 ml lots in small tubes or 12.5 ml (0.5 oz) McCartney bottles, and sterilise by steaming for 20 min on three successive days (the Tyndallisation process). The milk must be stored at 20°C or above between steamings, to permit bacterial spores to germinate.

Occasionally bacterial spores will survive this process: it is advisable to store the milk at room temperature for some days after Tyndallisation. Any bottles which show clotting or other breakdown should be autoclaved and discarded. Autoclaving at 121°C, even for 10 min or less, is not recommended, as browning will occur and browning compounds are known to be inhibitory to microorganisms.

Most common spoilage fungi survive lyophilisation well. However, in our experience and that of others (Mikata et al., 1983; Smith and Onions, 1994), some yeasts, plant pathogens and xerophiles do not. Storage of lyophilised ampoules at refrigeration temperatures (0–4°C) is recommended, but room temperature is probably satisfactory provided sunlight is avoided and temperatures do not exceed 30°C. Some laboratories routinely store lyophilised cultures at –18 to –20°C.

Strains being maintained for a particular trait or utilised for a specific purpose such as system testing or metabolite production should always be lyophilised. Continued subculturing often leads to deterioration or loss of the desired character. The ability of a strain to produce a particular mycotoxin, for example, may decrease with each transfer. Isolates should be lyophilised as soon as possible after primary isolation to prevent degeneration.

4.10.2 Other Storage Techniques

A variety of systems other than lyophilisation have been proposed for long term storage of fungi. Of these, liquid nitrogen storage has found most acceptance with major culture collections. This type of storage appears to be superior to any other for plant pathogens and fungi which will not sporulate in pure culture. However, liquid nitrogen systems are expensive to establish and maintain and are only suitable for large collections.

Freezer units which run at very low temperatures (–80°C or below) are available and are well suited to the needs of culture collections. In our laboratory we routinely store cultures at –80°C, using glycerol (60–80%) as a cryoprotectant. Spore suspensions are prepared by taking conidia or ascospores from a freely sporulating sector of the colony, dispersing them in the glycerol then freezing in screw capped cryovials. An 80% solution of glycerol remains viscous at –80°C, which enables cultures to be removed from the freezer for subculture without the need for defrosting.

For smaller laboratories that do not have access to lyophilisation, liquid nitrogen or ultra-low temperature storage, some simple techniques exist that can be used to maintain fungal cultures over relatively long periods (e.g. 1–10 years) without the need for subculturing.

Water storage. A simple and inexpensive method of fungal culture preservation is storage of agar blocks in water (Smith and Onions, 1994). Small agar blocks (7–10 mm^2) are cut from the growing margin of a young fungal colony and placed in sterile water in a bottle such as a Bijou bottle (6.25 ml or 0.25 oz McCartney bottle). The rubber lined cap is screwed down and the bottles stored in a cool room (1–10°C). Cultures may be revived by removal of a block and placing it on a suitable growth medium. Using this method, cultures have been reported to remain viable and retain their characteristics for up to 7 years (Boeswinkel, 1976; Smith and Onions, 1994).

Some yeasts may be maintained by storing as suspensions in water (Kirsop, 1984). Growth from a late logarithmic slant culture is suspended in sterile distilled water and transferred to a sterile container so that 90% of the volume is filled with the suspension. Containers are stored at room temperature. Survival of some *Candida, Saccharomyces, Cryptococcus, Rhodotorula* and *Schizosaccharomyces* species for up to 4 years has been reported (Kirsop, 1984).

Silica gel storage. Many fungal cultures may be maintained for long periods (often more than 10 years) by drying spore suspensions onto silica gel (Smith and Onions, 1983; Smith, 1984). This method is not suitable for mycelial cultures, but can be used with some success for yeasts (Kirsop, 1984). As silica gel liberates heat when moistened, the technique depends on keeping the cultures cool enough to avoid damaging the spores during preparation. Medium grain plain (non-indicating) silica gel of 6–22 mesh is placed in suitable glass bottles (Bijou or McCartney bottles) to a depth of about 1 cm and sterilised, either by dry heat at 180°C for 2–3 h or by autoclaving at 121°C for 15 min. Autoclaved silica gel must be thoroughly

dried in an oven before use. Bottles are precooled by placing in a tray of ice or refrigerating for 24 h, then transferring to an ice tray for inoculation.

Suspensions of fungal spores or yeasts are prepared in sterile skimmed milk (as for lyophilisation, above) and the suspension added to the cooled silica gel to wet three-quarters of it. The bottles and gel are allowed to dry at room temperature for 10–14 days with caps slightly loosened. Caps are then screwed down and the bottles stored at 4°C (storage at room temperature is also satisfactory) in air-tight containers over indicating silica gel to absorb any moisture.

Cultures are revived by shaking a few crystals of silica gel onto a suitable growth medium (broth culture may be better for yeasts). Survival varies according to species or even strain, but survival of yeasts for up to 5 years (Kirsop, 1984) and fungi for more than 10 years (Smith and Onions, 1983) has been reported.

4.11 Housekeeping in the Mycological Laboratory

Like any other microbiological laboratory, a mycological laboratory should be kept in a clean condition. Discard unwanted cultures regularly and dispose of them by steaming or autoclaving. Wipe bench tops regularly with ethanol (70–95%). Floors should be wet mopped or polished only with machines equipped with efficient vacuum cleaners and dust filters. Where possible store food and plant materials away from the laboratory. Open Petri dishes carefully. Use small inocula on wet needles. Transport Petri dishes to the stereomicroscope stage before removing lids. Do not bump cultures during transport.

Contrary to popular belief, a well-run mycological laboratory is not a source of contamination to bacteriological laboratories. The air in a mycological laboratory should not carry a significant population of fungal spores. The reverse problem can occur, however, because bacteria multiply more rapidly than do fungi. Bacterial spores are often present in food laboratories, readily infect fungal plates and can rapidly outgrow and inhibit fungal mycelia, especially at 37°C.

If for any reason fungal spore concentrations do build up in a laboratory and cause an unacceptable level of contamination, the air should be purified. The simplest technique is to spray the air throughout the laboratory with an aerosol before it is closed in the evening. Any aerosol spray, such as a room deodoriser or air freshener, is effective. Aerosol droplets entrain fungal spores very efficiently and carry them to the floor.

A more drastic and effective treatment in cases of severe contamination is to spray a solution of 2% thymol in ethanol around the room and close it for a weekend. The spray is rather pungent, and while not harmful to humans, it effectively kills fungal spores and mites (see below). Do not leave cultures on benches before fumigation.

4.11.1 Culture Mites

A major hazard in growing and maintaining fungal cultures is the culture mite. Many species of mites live on fungal hyphae as their main or sole diet in nature and find culture collections an idyllic environment. Mites crawl from culture to culture, contaminating them with fungi and bacteria as they go or, given long enough, may eat them out entirely.

Mites are very small (0.05–0.15 mm long), usually just visible to the observant naked eye. They are arachnoids, related to spiders, and hermaphroditic. Each mite leaves a trail of eggs about half adult size as it goes. Eggs hatch within 24 h and reach adulthood within 2 or 3 days. The damage an unchecked mite plague can do to a fungal culture collection has to be experienced to be believed.

The most common sources of mites are plant material, soil, contaminated fungal cultures and mouldy foodstuffs. Mites can also be carried on large dust particles. Building work near a laboratory almost always induces a mite infestation.

The avoidance of losses due to mites requires constant vigilance. Always watch for telltale signs, such as contaminants growing around the edges of a Petri dish, a “moth-eaten” appearance to colonies or “tracks” of bacterial colonies across agar. Examination of suspect material or cultures under the stereomicroscope will readily reveal the presence of mites and mite eggs.

Adult mites are rapidly killed by freezing, and mite eggs will only survive 48–72 h at –20°C. Cultures contaminated by mites can often be recovered by freezing for 48 h, then subculturing from uninfected portions of the culture with the aid of the stereomicroscope. Suspect food and other samples being brought into the laboratory should also be frozen for 24 h to destroy mites before enumeration or subculturing is carried out.

Infestation by mites can be minimised by good housekeeping, i.e. by avoiding accumulation of dust or old cultures in the laboratory. It is also good practice to handle and store food and plant samples well away from areas where fungi are inoculated and incubated.

To control a mite infestation, remove all contaminated material, including cultures. Freeze Petri dishes and culture tubes which must be recovered; autoclave, steam or add alcohol to all others. Clean benches thoroughly with sodium hypochlorite (household bleach) or 70% ethanol. Incubators can be disinfested with aerosol insecticides.

4.11.2 Problem Fungi

There are three fungal invaders which should be watched for carefully in a food mycology laboratory: *Aspergillus fumigatus*, *Rhizopus stolonifer* and *Chrysonilia sitophila*. The first is a human pathogen; the others can cause a contamination chain which is difficult to break.

Aspergillus fumigatus may cause invasive aspergillosis in the lungs or serious allergenic responses in some individuals. It is sound practice to immediately kill and discard cultures of this fungus as soon as it is recognised. On no account should it be used for experimental studies in food spoilage or biodeterioration without precautions to prevent dissemination of spores. The morphology of *A. fumigatus* is described in detail in Chapter 8, but it is readily recognisable in the unopened Petri dish:

- colonies are low, dull blue and broadly spreading, with a velvety texture;
- growth is very rapid at 37°C, covering a Petri dish in 2 days;
- long columns of blue conidia are readily seen under the stereomicroscope.

Rhizopus stolonifer is a ubiquitous fungus in many kinds of foods. It grows rampantly at 25°C, filling a Petri dish with sparse, dark mycelium in 2 days. It produces barely macroscopic aerial fruiting structures which are at first white, then become black. Given seven undisturbed days, it sheds dry, black spores outside the Petri dish, providing an effective inoculum for a continuous chain of future contamination. Once such an infection occurs, it is essential to carefully place the contaminated plate in a suitable container such as a plastic bag before transporting it to steamer or autoclave. Then use 70% ethanol to clean areas on which the plate had been incubated or placed. At daily intervals, carefully examine all plates inoculated subsequently, discarding any which show *Rhizopus* contamination, until infection ceases. Spraying the air with aerosols or thymol in ethanol will assist. Unlike *A. fumigatus*, *R. stolonifer* is not pathogenic.

The third problem fungus, *Chrysanilia sitophila*, is known as "the red bread mould". It used to be common, but due to changes in manufacturing practice, it is now encountered less frequently, either in the bakery or in the laboratory. Like *Rhizopus stolonifer*, it grows with great rapidity at 25°C. It forms a thin, pink mycelial growth across a Petri dish, clearly following the oxygen gradient which leads to the open air. It will force its way unerringly between dish and lid and, once outside, will produce masses of pink spores, which are quickly shed and build up around the base of the Petri dish. Decontamination relies on the same techniques as for *Rhizopus*: *C. sitophila* is probably the more difficult fungus to eradicate. Again, apart from its nuisance value in the laboratory, it is harmless.

4.11.3 Pathogens and Laboratory Safety

While it must be said that any fungus which is capable of growth at 37°C is a potential mammalian pathogen, the physiology of the healthy human is highly resistant to nearly all of the fungi encountered in the food laboratory. Nevertheless, fungi which can grow at 37°C should be treated with caution. In particular, the habit of sniffing cultures is to be avoided wherever possible. It is true that odours produced by fungi have been used quite frequently as taxonomic criteria, especially in older

publications, but their subjective and ephemeral nature makes them of little value for this purpose, and the risks involved are serious. Some laboratories regard fungal volatiles as such a serious risk that cultures such as *Penicillium* and *Aspergillus* species are handled in a biohazard cabinet. Many types of fungal spores are allergenic or carry mycotoxins. Inhalation of fungal spores should be avoided as far as possible.

Of the fungi described in this book, only the Aspergilli normally pose any direct threat to health. *Aspergillus fumigatus* has already been mentioned, and care should also be taken when handling cultures of *Neosartorya* species, which are closely related to *A. fumigatus* and also grow prolifically at 37°C. Other *Aspergillus* species, particularly *A. flavus*, *A. niger* and *A. terreus*, have been isolated from human specimens from time to time. For more details, see de Hoog et al. (2000). These species appear to be mainly opportunists and pose little threat to healthy people. Careful handling and good housekeeping are all that are required.

However, immunocompromised individuals are in a different category. It is increasingly evident that such people have little resistance to fungal infection. Persons suspected to be immunocompromised, regardless of the cause, should not work in a mycology laboratory nor indeed be permitted knowingly to enter one.

Chapter 5
Primary Keys and Miscellaneous Fungi

Principles underlying fungal classification have been outlined in Chapter 3, including a brief overview of the relevant divisions of the Kingdom Fungi and their principal methods of reproduction. Some further detailed information is necessary in this chapter to assist in the use of the keys which follow.

Ascomycetes. As discussed in Chapter 3, Ascomycetes produce ascospores in asci (Fig. 3.2). One genus, *Byssochlamys*, produces asci which are unenclosed; all other genera of relevance here produce asci in some kind of fruiting body, or ascocarp. The two kinds of ascocarp commonly seen in food spoilage fungi, the cleistothecium and the gymnothecium, have been described and illustrated in Chapter 3 (Fig. 3.3). Both types of ascocarp are usually pale or brightly coloured, not dark, and release ascospores by rupturing irregularly. Of genera relevant here, cleistothecia are produced by *Emericella, Eurotium*, *Eupenicillium, Monascus* and *Neosartorya*, and gymnothecia by *Talaromyces*.

A third class of ascocarp, less commonly encountered in foodborne fungi, is the perithecium. Perithecia have cellular walls like cleistothecia, but are distinguished by the presence of an apical pore or ostiole through which asci or ascospores are liberated; also asci are long and clavate with ascospores arranged linearly within them. In the one perithecial genus of interest here, *Chaetomium*, the perithecia are black and have stout hyphae attached to the walls (Fig. 5.10).

Conidial fungi. The strictly conidial fungi, also known as *Fungi imperfecti* or Deuteromycetes or anamorphic fungi, possess an amazing variety of ways of producing conidia. Terminology for structures bearing conidia and for conidia themselves has become astonishingly complex in recent years; fortunately most of it is not essential for the recognition of the genera discussed in this text. Terms which are important in the keys which follow are described below.

A fundamental division within the asexual fungi separates genera which form conidia aerially, grouped in the class Hyphomycetes, from those in which conidia are borne in some sort of enveloping body, the class Coelomycetes.

Hyphomycetes. Fungi have developed seemingly endless ways of extruding or cutting off conidia, solitarily or in chains, from fertile cells which themselves may be borne solitarily or aggregated into more or less ordered structures. Hyphomycete taxonomy attempts to thread a way through this maze. In general, type and degree of aggregation of the fertile cells, and type of conidium, provides the basis for generic classification, while details of these characters and of spore size, shape and ornamentation are more commonly used to distinguish species.

Features of conidia used in the keys in this work are length, septation, ornamentation and colour, particularly whether walls are light or dark. The method of conidium formation (ontogeny) is seldom emphasised here, because terminology is complex and distinctions may not be obvious. The principal point to note is the disposition of conidia: they may be borne *solitarily*, i.e. just one conidium per point of production; *singly*, i.e. successively from a single point, but unattached to each other; or *in chains*. Solitary conidia are borne on a relatively broad base and usually adhere to the fertile cell. Conidia formed in chains are usually extruded from a small cell of determinate length, often a

J.I. Pitt, A.D. Hocking, *Fungi and Food Spoilage*, DOI 10.1007/978-0-387-92207-2_5,
© Springer Science+Business Media, LLC 2009

phialide, which in most genera narrows to a distinct neck. Conidia borne singly may be extruded in this same manner, or be borne by extrusion from a pore in a hypha or fertile cell, or be cut off by hyphal fragmentation.

Phialidic Hyphomycetes. Hyphomycetes may produce phialides solitarily (the genus *Acremonium*) or in loosely ordered structures (*Trichoderma*) or highly ordered structures (*Aspergillus*, *Penicillium* and related genera). Genera of interest here with less ordered phialidic structures can mostly be differentiated by macroscopic characters, e.g. colony diameters and colours. However, differentiating genera with highly ordered phialidic structures will necessitate careful microscopic examination. Phialides in *Aspergillus*, *Penicillium* and related genera are sometimes borne directly on a stalk or *stipe* which arises from a hypha; in other species, however, the phialides are borne from supporting cells, termed *metulae* (sing. *metula*) and in some species the metulae may in turn be supported by other cells, termed branches or *rami* (sing. *ramus*). The whole structure, including the stipe, is called a *conidiophore*.

In *Aspergillus*, stipes are always robust, with thick walls and usually without septa; the stipe terminates in a more or less spherical swelling, the *vesicle*, which bears phialides, or metulae and phialides, over most of its surface. In *Aspergillus*, phialides (and metulae) are always produced simultaneously, and this feature can readily be recognised by examining young developing conidiophores (Fig. 8.1a). Similar structures, though smaller, are produced by some *Penicillium* species: these are clearly distinguished from *Aspergillus* species by stipes which are septate and by phialides which are produced over a period of time (successively; Fig. 8.2b). Most *Penicillium* species, and those of related genera, do not produce phialides on vesicles, but in a cluster directly on a stipe, or on metulae and/or rami. The fruiting structure in *Penicillium* and related genera is termed a *penicillus*, while that in *Aspergillus* (for want of a better term) is called a *head* (or more recently, an *aspergillum*, Klich, 2002).

Coelomycetes. As noted earlier, Coelomycetes produce conidia within an enveloping body, termed a *conidioma* (pl. *conidiomata*). In Petri dish culture, conidiomata are produced on or just under the agar surface and are macroscopically visible, usually being 100–500 μm in diameter. Two kinds of conidioma are important here: the *pycnidium*, a more or less spherical body with one or more pores (ostioles) through which conidia are released, and the *acervulus*, a flat body from which conidia are released by lifting or rupturing of a lid. The majority of Coelomycetes are pathogens on plants and many have not been studied in pure culture. In consequence, their taxonomy is difficult and genera and species are often poorly delimited. For a complete account of Coelomycete taxonomy see Sutton (1980).

Yeasts. Yeasts are fungi which have developed the ability to reproduce by forming single vegetative cells by budding or, in a few species, by fission, in a manner similar to bacteria. Like bacteria, and unlike fungal spores, such cells are metabolically active and may in turn reproduce by budding (or fission). Yeast cells may survive for long periods both in culture and in nature. In consequence many yeasts produce true spores rarely or not at all.

Yeasts are readily distinguished from filamentous fungi on the agar plate by their soft-textured colonies and limited growth. They are usually also readily distinguished from bacteria by their raised and often hemispherical colonies, white or pink colours and lack of "bacterial" odour. If in doubt, make a simple wet mount of a colony in water or lactofuchsin, add a cover slip and examine with the oil immersion lens. Yeast cells are larger than bacteria, measuring at least 3×2 μm and are nonuniform in size. If the culture is not too old, some cells will usually show developing buds.

Yeasts cannot be classified solely by morphological features or growth on the standard media, and so are considered in a separate chapter (Chapter 10).

5.1 The General Key

The taxonomic terms discussed above will enable the use of the general and miscellaneous keys which follow, although some other taxonomic terms may be introduced in discussions of particular genera. *It is emphasised that these keys are designed for use on isolates which have been incubated for 7 days on the standard plating regimen outlined in Chapter 4*. Colony diameters should be measured in millimetres from the reverse side by transmitted light. The general key has been designed to be as simple as possible and suitable for routine use, but it should be read in conjunction with the notes below it.

General key to food spoilage fungi

1	No growth on any standard medium in 7 days	Chapter 9 – "Xerophilic fungi"
	Growth on one or more standard media	2
2 (1)	Colonies yeasts, either recognisably so on isolation or in culture, i.e. colonies soft, not exceeding 10 mm diam on any standard medium	Chapter 10 – "Yeasts"
	Growth filamentous, exceeding 10 mm diam on one or more standard media	3
3 (2)	Growth on CYA and/or MEA faster than on G25N	4
	Growth on G25N faster than on CYA and MEA	Chapter 9 – "Xerophilic fungi"
4 (3)	Hyphae frequently and conspicuously septate	5
	Hyphae lacking septa or septa rare	Chapter 6 – "Zygomycetes"
5 (4)	No mature spores present in 7 days	6
	Mature spores present in 7 days	9
6 (5)	Immature fruiting structures of some kind present	7
	No fruiting structures (or spores) detectable by low-power microscopy or wet mounts from CYA or MEA	See section on "Miscellaneous fungi" below
7 (6)	Colonies and fruiting structures white or brightly coloured	8
	Colonies or fruiting structures dark	Continue incubation; when spores mature, refer to section on "Miscellaneous fungi" below
8 (7)	Colonies and fruiting structures white	Chapter 8 – "*Aspergillus* and its teleomorphs"
	Colonies or fruiting structures brightly coloured	Chapter 7 – "*Penicillium* and related genera"
9 (5)	Spores (conidia) less than 10 μm long, borne in chains on clustered fertile cells (phialides), on well-defined stipes	10
	Spores (conidia) of various sizes, borne singly or solitarily, or if borne in chains, then chains not in aggregates	See section on "Miscellaneous fungi" below
10 (9)	Phialides or metulae and phialides borne on more or less spherical swellings on the stipe apices	11
	Phialides borne on penicilli, i.e. on unswollen stipes with or without intervening metulae and rami	Chapter 7 – "*Penicillium* and related genera"
11 (10)	Conidia blue or green, phialides produced successively on vesicles, vesicles less than 10 μm diam, stipes usually septate	Chapter 7 – "*Penicillium* and related genera"
	Conidia variously coloured, phialides and/or metulae produced simultaneously on vesicles, vesicles larger than 10 μm diam, stipes nonseptate	Chapter 8 – "*Aspergillus* and its teleomorphs"

5.1.1 Notes on the General Key

Couplet 1. No growth on any standard medium indicates an extreme xerophile, i.e. *Xeromyces bisporus* or a *Chrysosporium* species, or a nonviable culture. Next inoculate culture onto MY50G for 7 days at 25°C. If growth occurs, enter the key in Chapter 9, "Xerophilic Fungi"; no growth on MY50G indicates a nonviable culture. *Chrysosporium* and *Xeromyces* isolates are usually white or rarely golden brown. If the original culture used as inoculum is coloured other than pure white or golden brown, it is probably nonviable.

Couplet 2. Yeasts are usually readily distinguished by slow growth, soft, easily sampled colonies, small spherical to ellipsoidal cells, often of variable size and shape and by reproduction by budding. See Chapter 10, "Yeasts" for identification procedures.

Couplet 3. The ability to grow more rapidly on G25N than on CYA or MEA indicates a xerophile (at least for keying purposes here). Check the key in Chapter 9, "Xerophilic Fungi". Some isolates can be identified from the standard plates, while others will require growth on CY20S or MY50G for identification.

Couplet 4. The absence of septa in young, growing hyphae indicates an isolate belongs to subkingdom Zygomycotina, discussed here in Chapter 6 "Zygomycetes".

Couplets 5, 6. Some isolates from a variety of fungal genera will not produce spores on the standard media in 7 days. Continue to incubate such cultures, preferably in diffuse daylight such as a laboratory window sill, at temperatures near 25°C. Also inoculate such cultures onto two or three plates of DCMA and incubate these at 25°C or thereabouts in darkness and in diffuse daylight or, if possible, under fluorescent illumination (see Chapter 4). After 1–2 weeks, check again for spores or fruiting bodies. If such structures are not seen, the isolate is unlikely to be significant in foods.

Apparently asporogenous cultures should also be checked with a stereomicroscope while scraping up a sector of the colony with a needle. Fruiting bodies submerged in the agar will sometimes become visible with this technique.

Couplet 7. Some isolates which produce white or brightly coloured fruiting bodies also produce very sparse aerial conidial structures which are easily overlooked. Check such cultures carefully with the stereomicroscope; if conidial structures are found, make a wet mount and reenter the key at Couplet 5. A finely drawn glass needle will sometimes be of assistance in making mounts from delicate conidial structures on the colony.

Nearly all dark fruiting structures encountered will mature at 25°C within 2 weeks. Light does not usually influence this process. When mature spores are formed, refer to the following section.

5.2 Miscellaneous Fungi

In this section are considered the genera which do not logically fit into some larger grouping considered elsewhere. Some are important in specific food spoilage problems, others are found in particular habitats such as cereals, while still others represent the aerially dispersed fungal flora found as ubiquitous contaminants or saprophytes. As will be seen, they are a very heterogeneous collection.

Most fungi significant in food spoilage or food contamination and not treated in other chapters are included here. It is inevitable, though, that occasional isolates from foods will not belong to the genera considered in this section. The key has not been designed to take account of this, as it would be a practical impossibility. So when an isolate appears to key out satisfactorily, it must be checked against the description to confirm the identification. Some isolates will of course belong to a recognisable genus, but not the species described; in that case the references indicated will provide further information.

The miscellaneous fungal genera are considered in alphabetical order following the key.

Key to miscellaneous genera

1	Colonies on CYA and MEA not exceeding 60 mm diam in 7 days	2
	Colonies on CYA or MEA exceeding 60 mm diam in 7 days	12
2 (1)	Conidia borne within a fruiting body on or beneath the agar surface	*Phoma*
	Conidia borne from aerial or surface hyphae	3
3 (2)	Mycelium and conidia hyaline or brightly coloured	4
	Mycelium and/or conidia dark coloured	11
4 (3)	Conidia with a single lateral septum	*Trichothecium*
	Conidia nonseptate or with more than one septum	5
5 (4)	Conidia borne from gradually tapering fertile cells (phialides)	6
	Conidia borne directly on hyphae or by budding or hyphal fragmentation	7

6 (5)	Colonies exceeding 50 mm diam on CYA	*Fusarium*
	Colonies not exceeding 50 mm diam on CYA	*Acremonium*
7 (5)	Colonies exceeding 45 mm diam on MEA; conidia borne solely by the breakup of hyphae to form arthroconidia	*Geotrichum*
	Colonies not exceeding 40 mm diam on MEA; conidia not exclusively arthroconidia	8
8 (7)	Conidia exceeding 12 μm long; developing cleistothecia, fist-like on arm-like stalks, also present	*Monascus*
	Conidia not exceeding 12 μm long; no evidence of cleistothecial development	9
9 (8)	Conidia yeast-like, borne on spicules (small projections) from hyphae, or by budding; conidia 5 μm or less long	*Endomyces*
	Some conidia yeast-like, borne by budding; hyphal fragments, arthroconidia and/or chlamydoconidia also present; conidia >5 μm long	10
10 (9)	Budding hyphal fragments (10–50 μm long) present	*Hyphopichia*
	Hyphal fragments absent; or if present, not budding	*Moniliella*
11 (3)	Colonies low, mucoid and yeast-like, becoming grey to black in both obverse and reverse	*Aureobasidium*
	Colonies dry and velutinous, obverse green, reverse olive or deep blue black	*Cladosporium*
12 (1)	Spores borne within an enclosed fruiting body on or under the agar surface	13
	Spores borne from aerial or surface hyphae	17
13 (12)	Spores consistently less than 15 μm long	14
	The larger or all spores more than 15 μm long	15
14 (13)	Fruiting bodies perithecia with stout, black hyphae attached to the walls	*Chaetomium*
	Fruiting bodies pycnidia, without attached hyphae	*Phoma*
15 (13)	Fruiting bodies roughly spherical (pycnidia)	*Lasiodiplodia*
	Fruiting bodies flat (acervuli)	16
16 (15)	Conidia hyaline or brightly coloured, nonseptate, without terminal appendages	*Colletotrichum*
	Conidia dark, with three or four septa and with spike-like, sometimes branched, terminal appendages	*Pestalotiopsis*
17 (12)	Colonies and conidia hyaline or brightly coloured	18
	Colonies and/or conidia dark coloured	22
18 (17)	Colonies with grey or green areas	19
	Colonies white, orange, pink or purple	20
19 (18)	Colonies green	*Trichoderma*
	Colonies grey	*Botrytis*
20 (18)	Colonies low and persistently white	*Geotrichum*
	Colonies floccose, white or becoming brightly coloured	21
21 (20)	Colonies predominantly orange, orange conidia shed profusely around the Petri dish rim	*Chrysonilia*
	Colonies white, pink or purple, sporulation on MEA weak or absent, better on DCPA under lights	*Fusarium*
22 (17)	Conidia consistently less than 15 μm long	23
	Conidia frequently exceeding 15 μm long	25

23 (22)	Conidiophores long, branched, apically swollen, bearing closely packed pale brown conidia	*Botrytis*
	Conidiophores short or ill-defined, dark brown or black conidia borne irregularly	24
24 (23)	Conidia dark brown, often with a lighter coloured band around the periphery	*Arthrinium*
	Conidia uniformly jet black	*Nigrospora*
25 (22)	Conidia approximately spherical	*Epicoccum*
	Conidia elongate	26
26 (25)	Conidia with transverse septa (or thick walls between cells) only	27
	Larger conidia with both transverse and longitudinal septa or irregularly septate	30
27 (26)	Conidia clavate (club shaped), often with long hyphal appendages at the apices; found almost exclusively on rice	*Trichoconiella*
	Conidia cylindroidal, ellipsoidal or an elongate "D" shape; source more general	28
28 (27)	Conidia cylindroidal, with parallel sides except at the terminal cells	*Drechslera*
	Conidia fusoid, narrowing from the central cells to the terminal cells, often bent or "D" shaped	29
29 (28)	Conidia not exceeding 40 μm long	*Curvularia*
	Conidia usually exceeding 40 μm long	*Bipolaris*
30 (26)	Conidia clavate (club shaped)	*Alternaria*
	Conidia spherical to roughly ellipsoidal or short cylindrical	31
31 (30)	Conidia often tapering towards the base and sometimes pointed, i.e. pyriform or apiculate	*Ulocladium*
	Conidia spherical to short cylindrical, not tapering from base to apex, with rounded ends	*Stemphylium*

5.3 Genus *Acremonium* Link

Commonly referred to as *Cephalosporium* Corda in pre-1970 literature, *Acremonium* is a large and varied genus characterised by the production of small, hyaline, single-celled conidia borne singly, i.e. successively but not connected to each other, from solitary phialides.

A variety of species have been recorded from foods from time to time. One species, *A. strictum*, described here, is of relatively common occurrence. In this species, the phialides gradually taper to the apices without basal thickening or formation of a distinct neck, and conidia aggregate in balls of slime. Under the stereomicroscope, the slime balls look like large single spores, but their true nature becomes evident in wet mounts. *A. strictum* appears mainly in the earlier food literature under the name *C. acremonium*. As noted by Domsch et al. (1980), this name has been used for a variety of species, so that reports on physiology and occurrence are unreliable (Fig. 5.1).

Acremonium strictum W. Gams **Fig. 5.1**

Cephalosporium acremonium (name of uncertain application; no valid authority).

Colonies on CYA 20–30 mm diam, white or orange to pink, dense to floccose or funiculose; reverse pale or with orange to pink tones. Colonies on MEA 13–20 mm diam, similar to those on CYA or of slimy texture. Colonies on G25N <5 mm diam, usually 1–2 mm, of white mycelium. Sometimes growth at 5°C. No growth at 37°C.

Conidia borne successively but singly from the apices of long, solitary, usually unbranched phialides, aggregating in a slime ball at the phialide tip, cylindrical to ellipsoidal, hyaline, 3–6 × 1–2 μm, smooth walled.

Distinctive features. See genus preamble.

Taxonomy. As noted above, *Acremonium strictum* is the correct name for the fungus commonly called *C. acremonium* in the earlier food literature (Gams, 1971).

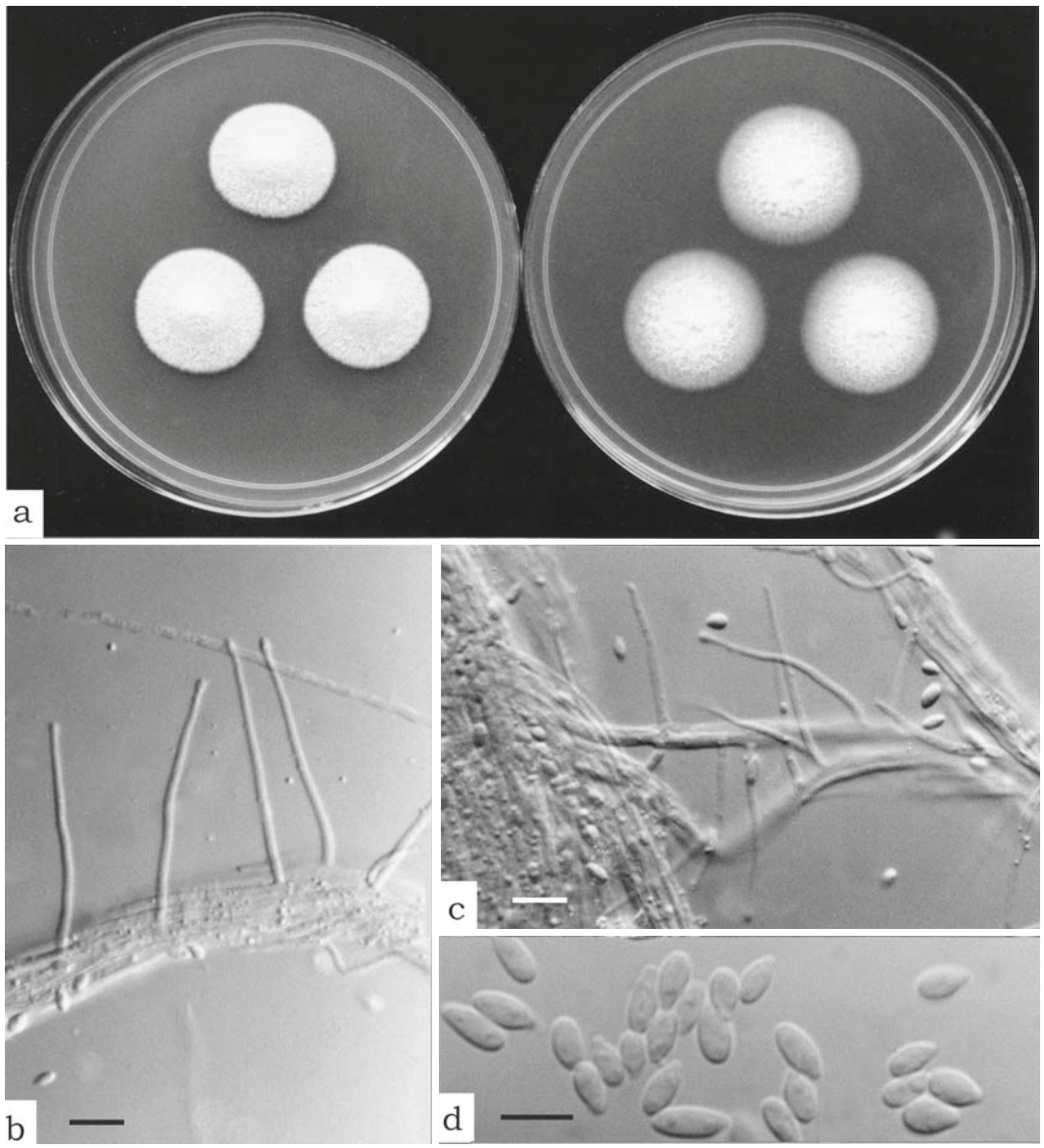


Fig. 5.1 *Acremonium strictum* **(a)** colonies on CYA and MEA, 7 d, 25°C ; **(b, c)** phialides, bar = 10 µm; **(d)** conidia, bar = 5 µm

Physiology. Mycelial growth and sporulation of *Acremonium strictum* were found to be enhanced by the addition of mannitol to MEA (Teixeira et al., 2005).

Mycotoxins. *A. strictum* and other *Acremonium* species encountered in foods are not known to produce mycotoxins.

Ecology. The main source of *Acremonium strictum* is maize (Pitt et al., 1993; Pitt and Hocking, 1997; Williams et al., 1992). It has recently been isolated from black bean seeds in Argentina (Castillo et al., 2004) and raw cork (*Quercus suber*) in Spain (Alvarez-Rodriguez et al., 2002). Other records, including those of *Acremonium* spp. or the earlier name *Cephalosporium acremonium*, include wheat, barley and rice, bananas showing crown rot, fresh vegetables, peanuts, pecans, hazelnuts and walnuts, soybeans, frozen meat, salami and biltong (Pitt and Hocking, 1997).

References. Gams (1971); Domsch et al. (1980).

5.4 Genus *Alternaria* Nees: Fr.

Alternaria produces large brown conidia with both longitudinal and transverse septa, borne from inconspicuous conidiophores, and with a distinct conical narrowing or "beak" at the apical end. These conidia are often formed in chains. Two other genera, *Stemphylium* Wallroth and *Ulocladium* Preuss, produce similarly septate conidia, but are uncommon in foods. Those of *Ulocladium* are roughly ellipsoidal, usually somewhat narrower at the base, and at most have small beaks. Both *Alternaria* and *Ulocladium* produce new conidia by lateral growth of the conidiophore from near the apex or through the beak area. *Stemphylium* conidia look like those of *Ulocladium*, but new conidia never form from a beak. New conidia are produced through the pore from which a conidium has already been produced. For a more detailed account of the distinctions among these three genera, see Simmons (1967).

Trichoconiella forms conidia similar to those of *Alternaria*, but with transverse septa only. This genus is associated with rice.

Emory Simmons has spent a lifetime working with *Alternaria* and related genera and has recently revised the taxonomy of *Alternaria* comprehensively (Simmons, 2007). The simple notions of earlier authors, i.e. that *Alternaria alternata* is everywhere and that specific plants were colonised by specific pathogens, have been painstakingly replaced by a much more robust taxonomy. For example, *Alternaria* from citrus fruits may be *Alternaria citri,* but may also be one of 20 other species.

The monograph by Simmons (2007) includes 276 species and is an essential laboratory manual for anyone who wishes to identify *Alternaria* species from a range of sources. *Alternaria* is not an easy genus for the inexperienced, however. Treatment here is confined to three common species only, with cereals as their major source.

Key to included species

1.	Conidia produced in unbranched chains on DCMA	*Alternaria tenuissima* (see *A. alternata*)
	Conidia produced in branched clusters on DCMA	2
2.	Conidia in unbroken chains, i.e. narrow hyphal elements between spores lacking; species occurring on wheat and many other substrates	*A. alternata*
	Primary conidia producing secondary conidia, i.e. conidia often separated by short hyphal lengths; species usually occurring on wheat	*A. infectoria*

Alternaria alternata (Fr.) Keissl. Fig. 5.2

Alternaria tenuis Nees

Colonies on CYA and MEA 50–70 mm diam, or covering the whole Petri dish, plane, of deeply floccose off-white to grey brown mycelium; reverse brown to nearly black. Colonies on G25N 10–15 mm diam, low and dense, olive brown or grey; reverse brown to almost black. At 5°C, at least microcolonies, often colonies up to 4 mm diam. Usually no growth at 37°C, occasionally colonies up to 10 mm diam, similar in appearance to those at 25°C, or white.

Colonies on DCMA 60–70 mm diam or covering the whole Petri dish, plane, floccose but not sparse, mycelium grey to dark grey; reverse grey to dark grey or black, less commonly bluish black.

Conidia blown out from the apices of undistinguished conidiophores as short, irregularly branched chains of up to 10 units, and then septating both laterally and longitudinally, with up to six transverse and two to three longitudinal or oblique septa, usually of clavate or pyriform shape overall, tapering towards the apices, forming a short beak, in culture usually 20–40 × 8–12 μm, with walls smooth to conspicuously roughened.

Distinctive features. *A. alternata* is the most common saprophytic *Alternaria* species, occurring on a wide variety of sources, in contrast to other species considered here. On DCMA, conidia are produced more or less uniformly above the agar surface, in quite lengthy chains. The larger conidia have both longitudinal and transverse septa.

Taxonomy. The first edition of this book noted that with acceptance of the 1821 starting point date for *Alternaria* nomenclature, *A. alternata* slowly became accepted as the correct name for this

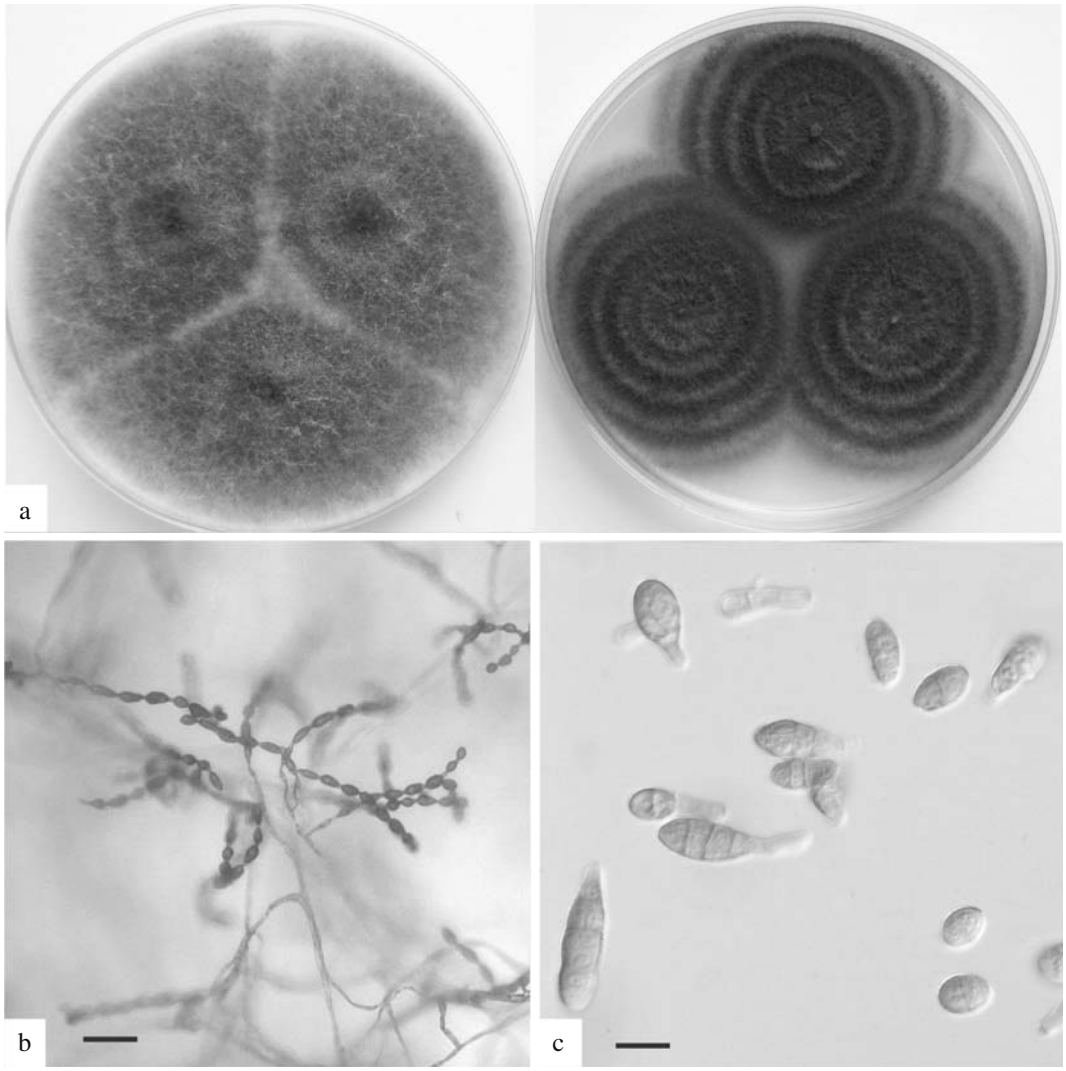


Fig. 5.2 *Alternaria alternata* **(a)** colonies on PDA and DCMA, 7 d, 25°C ; **(b)** conidia in situ, bar = 50 μm; **(c)** conidia, bar = 20 μm

species. With the reversion to be the 1753 starting point date, the correct name again became *A. tenuis*. However, publications by Fries, 1821–1832, were later given special status under the ICBN, so *A. alternata* is once more the correct name. Further changes appear unlikely.

Physiology. Optimum growth of *Alternaria alternata* is near 25°C with minima variously reported as –5 to 6.5°C and maxima near 36°C (Hasija, 1970; Domsch et al., 1980). The minimum a_w for growth at 25°C is 0.86 (Hocking et al., 1994). Optimal growth occurs at pH 4–5.4, and the pH range for growth is 2.7–8.0 (Hasija, 1970). *A. alternata* is able to grow in oxygen concentrations as low as 0.25% (v/v) in N_2, with growth rates being proportional to oxygen concentration (Follstad, 1966; Wells and Uota, 1970). The presence of the volatile compounds 3-methyl-1-butanol, 1-octen-3-ol and 3-octanone in stored wheat grains is usually correlated with infection by *A. alternata*, amongst other fungi (Sinha et al., 1988).

Mycotoxins. *Alternaria alternata* produces several toxins, of which the most important is tenuazonic acid (TA) (Logrieco et al., 2003). Tenuzoic acid is toxic to a wide range of plants and animals, particularly mice, chick embryos and chickens (Logrieco et al., 2003). Sorghum grains containing high levels of TA were associated with the human

haematological disorder known as onyalay (Bottalico and Logrieco, 1998). Less toxic compounds include alternariol (AOH) and alternariol monomethyl ether (AME), altenuene (AE) and altertoxin I, II and III (Logrieco et al., 2003). AOH and AME are usually found in combination and have some teratogenic and fetotoxic effects in pregnant mice (Bottalico and Logrieco, 1998). The production of one or more of these toxins has been reported in tomatoes (Bottalico and Logrieco, 1998), citrus fruits (Logrieco et al., 1990a), capsicum and melons (Bottalico and Logrieco, 2001), cereal grains (Logrieco et al., 1990b; Webley et al., 1997), rapeseed and olives (Logrieco et al., 2003), and apples (Robiglio and Lopez, 1995; Tournas and Stack, 2001). Maximum production of alternariol, its monomethyl ether and altenuene occurred at 25°C and 0.98 a_w (Magan et al., 1984). However, the optimum for tenuazonic acid production was reported as 0.90 a_w at 25°C (Etcheverry et al., 1994). Altertoxins I, II and III are produced by many *Alternaria* species, however, *A. alternata* is the most important producer. Altertoxins have received much attention due to their mutagenic activity, particularly that of altertoxin III, whose mutagenicity is approximately ten times lower than that of aflatoxin B_1 (Bottalico and Logrieco, 1998).

Ecology. Under the names *Alternaria alternata* and *A. tenuis*, this species has been reported from a very wide range of foods. However, the very broad species concept used by many authors is not accepted by Simmons (2007). The following reports undoubtedly include a number of other species. It is a major pathogen of fresh tomatoes (Harwig et al., 1979; Zitter and Wien, 1984; Pose et al., 2004), though other *Alternaria* species are probably involved here too. Spoilage of eggplants and peppers (Snowdon, 1991), apples (Combrink et al., 1985) and bananas stored under modified atmospheres (Wade et al., 1993) have been reported. Damage may occur also to a wide variety of fresh vegetables including beans, cauliflowers, cucumbers, melons, peas and potatoes (Webb and Mundt, 1978; Snowdon, 1991). *A. alternata* has been recorded frequently from a wide range of cereals, particularly wheat (Pitt et al., 1998b; Clear et al., 2005, Fakhrunnisa et al., 2006; Lugauskas et al., 2006 and references quoted in Pitt and Hocking, 1997), barley (Andersen et al., 1996; Fakhrunnisa et al., 2006; Medina et al., 2006 and references quoted in Pitt and Hocking, 1997), but also in rice, rapeseed, sunflower seed (Pozzi et al., 2005) and sorghum (Fakhrunnisa et al., 2006). In Australia and Southeast Asia, *A. alternata* has caused severe damage, spoilage and mycotoxin production in wheat and sorghum (Pitt et al., 1994; Webley et al., 1997 and our unpublished observations). This species has been recorded quite frequently from nuts, including peanuts, hazelnuts and pecans (see Pitt and Hocking, 1997). Other sources include soybeans, cold stored and frozen meat, biltong, bottled water and spices (Pitt and Hocking, 1997; Cabral and Fernadez Pinto, 2002). This species has occasionally been associated with gushing in beer (Niessen et al., 1992).

Closely related species. *Alternaria tenuissima* (Kunze) Wiltshire differs from *A. alternata* by the formation of longer conidia (up to 60 μm) in unbranched chains (Simmons, 2007). The metabolite profiles of *A. alternata* and *A. tenuissima* are very similar. In a recent study, it was found that most *A. tenuissima* isolates examined produced alternariol, alternariol monomethyl ether and tenuzoic acid, whereas three of the *A. alternata* isolates produced altenuene, alternariol, alternariol monomethyl ether and altertoxin I (Andersen et al, 2002). Andersen and Frisvad (2004) reported *A. tenuissima* from mouldy tomatoes. In our experience this species is of common occurrence in cereals.

References. Simmons (1967, 2007); Ellis (1971); Domsch et al. (1980).

Alternaria infectoria E.G. Simmons Fig. 5.3

Colonies on CYA and MEA 60 mm diam or more, on CYA plane, low to moderately deep, mycelium dark grey to almost black, sometimes with a paler grey floccose overlay; reverse bluish black; on MEA moderately deep to deep, dense to floccose, of dark grey mycelium sometimes overlaid with lighter grey hyphae; reverse bluish black. On G25N, colonies 8–15 mm diam, of grey or greenish grey mycelium; reverse pinkish, grey or black. At 5°C, at least germination, usually colonies of 2–5 mm diam formed. At 37°C, colonies 5–25 mm diam, low to deep, grey, reverse dark.

Colonies on DCMA 60 mm or more diam, usually with wide margins, low to subsurface,

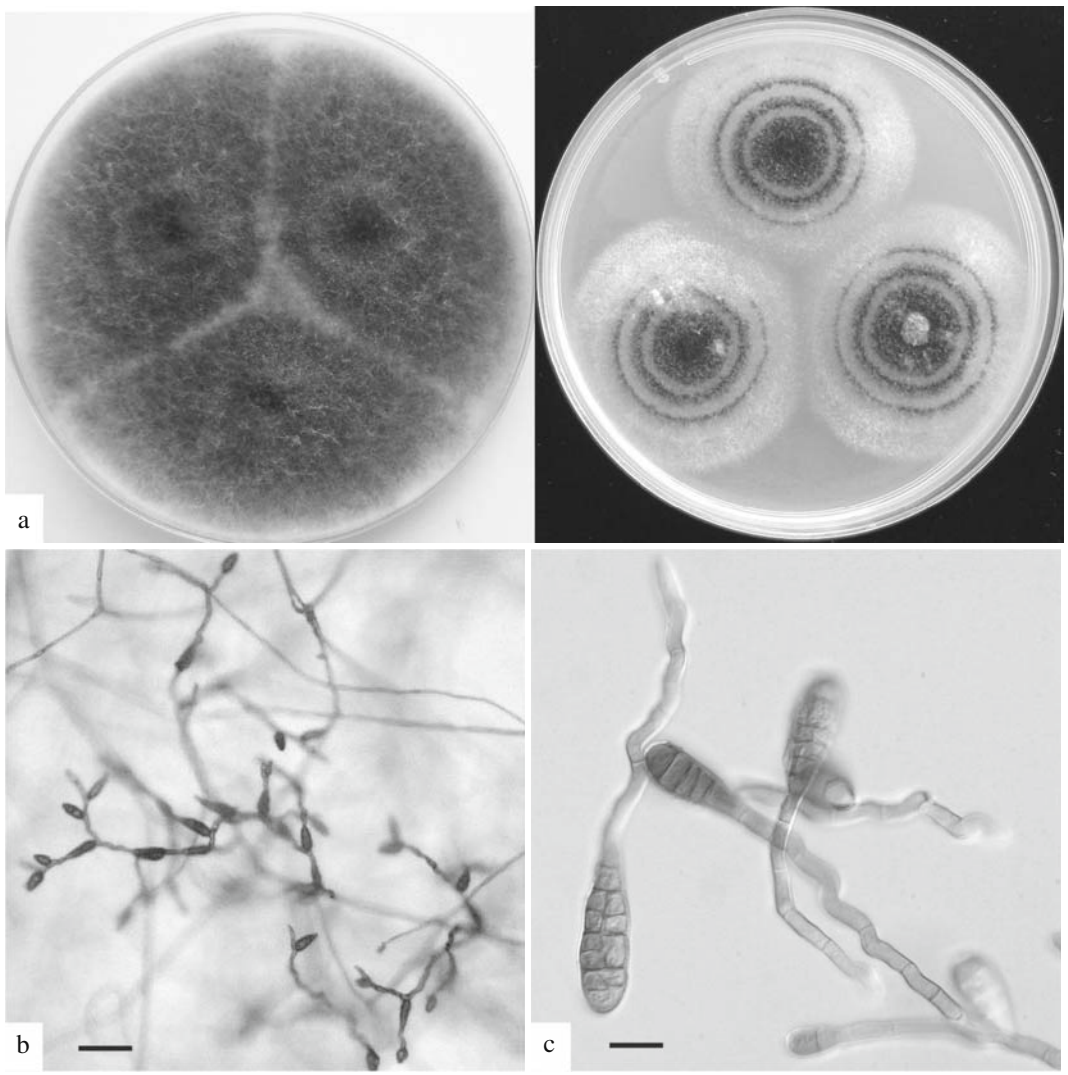


Fig. 5.3 *Alternaria infectoria* **(a)** colonies on PDA and DCMA, 7 d, 25°C ; **(b)** conidia, bar = 50 µm; (c) conidia, bar = 20 µm

centrally usually sparse and floccose, aerial mycelium white to pale olive brown; reverse pale brown, brown or olive brown, sometimes darker in annular or irregular patches.

Conidia on DCMA observed under the stereomicroscope characteristically formed in short, irregularly branched chains, forming clusters, with primary conidia borne from knobby hyphae, usually bearing secondary conidia borne from the tips, at the end of short (30–100 µm) hyphal lengths; conidia characteristically clavate (clubshaped) 20–50 × 7–14 µm, with up to 7 transverse septa and 1 or more longitudinal septa as well, or sometimes irregularly septate, walls and septa thick and dark brown, and with walls sometimes conspicuously roughened.

Distinctive features. After growth on DCMA, *Alternaria infectoria* is readily distinguished from *A. alternata* and other *Alternaria* species under the stereomicroscope. *A. infectoria* forms clusters of conidia borne in irregularly branching chains that, unlike those of *A. alternata,* have short hyphal segments between the conidia. Colonies on DCMA have low, wide margins, are less floccose than those of *A. alternata* and (apart from conidia) have paler colours.

Taxonomy. This species was commonly known as the *Alternaria* state of *Pleospora infectoria* until Simmons (1986) provided a binomial name, *A. infectoria*. He showed that its teleomorph does not belong in *Pleospora*, as had been widely accepted, and erected a new genus, *Lewia* Barr and Simmons, for it (Simmons, 1986). Simmons (1986) reported that *Alternaria* species related to *A. infectoria* have very rarely been seen to produce a teleomorph; however, on several occasions we have seen *Lewia* teleomorphs in isolates of *A. infectoria* grown on autoclaved wheat grains.

Physiology. No physiological studies on this species have been reported.

Mycotoxins. *Alternaria infectoria* is chemically very different from other *Alternaria* species (Andersen et al., 2002). *A. infectoria* does not produce any of the known *Alternaria* metabolites (Andersen and Thrane, 1996) and shares only a few metabolites with other *Alternaria* species (Andersen et al., 2002). We have confirmed the absence of any toxin production by this species (unpublished).

Ecology. *Alternaria infectoria* is most commonly associated with grains, having been reported from small grains in the United States (Bruce et al., 1984), Australian wheat (Pitt et al., 1998b), Danish barley (Andersen et al., 1996) and Norwegian grains (Kosiak et al., 2004). The lack of other reports of *A. infectoria* more likely reflects failure by most investigators to differentiate *Alternaria* species, rather than true rarity. It seems probable that this species may be confined to small grains.

References. Simmons (1986, 2007).

5.5 Genus *Arthrinium* Kunze

Arthrinium, in earlier literature more commonly known by its synonym *Papularia*, is not a common genus in foods, but has occasionally caused spoilage. *Arthrinium* produces relatively large, dark-walled (but not black) conidia borne solitarily, both terminally and laterally, on short, narrow conidiophores. Two species are treated here, *A. phaeospermum* and the *Arthrinium* state of *Apiospora montagnei*, distinguished from each other by conidial size.

Arthrinium phaeospermum (Corda) M.B. Ellis Fig. 5.4

Papularia sphaerosperma (Pers.) Höhn.

Colonies on CYA and MEA covering the whole Petri dish, mycelium low or floccose, coloured white or grey, sometimes with conspicuous areas of pink,

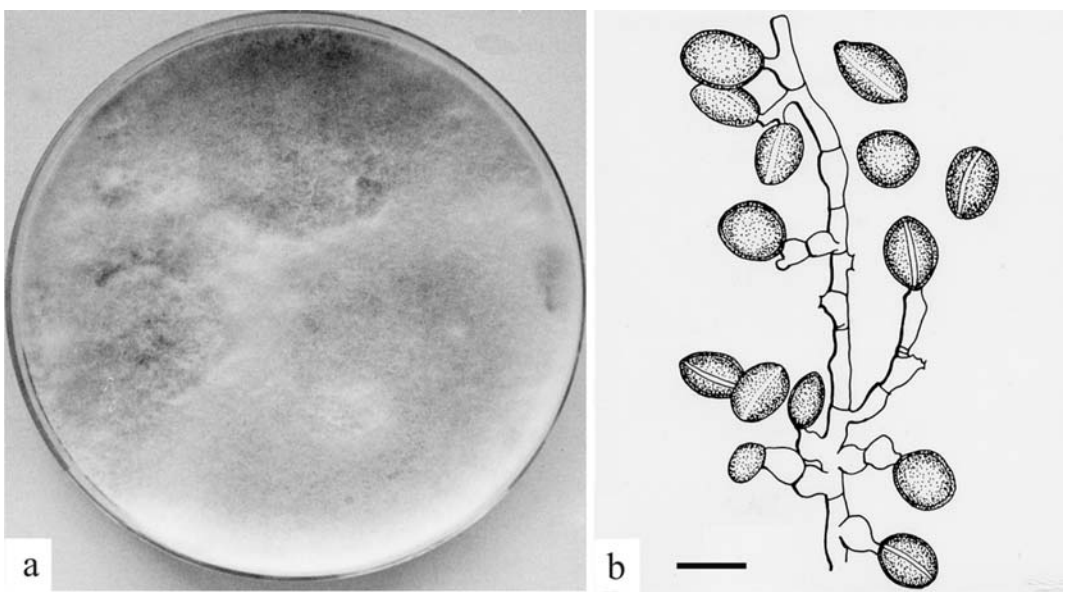


Fig. 5.4 *Arthrinium phaeospermum* **(a)** colony on CYA, 7 d, 25°C; **(b)** conidia, bar = 10 µm

darkening in age; reverse yellow or brown. Colonies on G25N 10–18 mm diam, of white mycelium. Sometimes germination at 5°C. No growth at 37°C.

Reproduction by solitary conidia, blown out from the ends of, or from denticles on the sides of, short, narrow, sometimes sinuous conidiophores, themselves borne in clusters from mother cells on natural substrates, but often singly in culture; conidia circular in plan view but elliptical from the side, 8–12 × 5–7 μm, dark brown, smooth walled, often with a narrow, hyaline band around the longest periphery.

Distinctive features. The conidium of *Arthrinium* is distinctive: solitary, dark-walled, circular in plan but elliptical from the side, and often with a hyaline peripheral band. Cultivation on DCPA and under lights may assist sporulation. In the present context, *Arthrinium* is distinguished from *Nigrospora* by the latter's production of jet black conidia entirely devoid of ornamentation.

Physiology. Conidia of *Arthrinium* appear to be highly heat resistant. In apple juice, conidia survived a pasteurising process of 88°C for 1.5 min, and in water, heating at 105°C for 2.5 min (Anon, 1967). No experimental details, i.e., numbers heated or come-up time, were reported, however, and a decimal reduction time cannot be calculated. Even so, survival of a heat treatment at 88°C indicates that *A. phaeospermum* is uncommonly heat resistant for a conidial fungus. Optimum germination of *A. phaeospermum* occurs when the conidia are suspended in sterile saline solution at 20°C for 15 min before plating onto 2% malt extract agar (Agut and Calvo, 2004). The authors also noted that the viability of *Arthrinium* conidia was low, making this genus relatively rare in the environment.

Mycotoxins. *Arthrinium* species are not known to produce mycotoxins, however, reports of secondary metabolites with antimicrobial properties have been found for some *Arthrinium* species (quoted in Agut and Calvo, 2004).

Ecology. *Arthrinium phaeospermum* (reported as *Papularia sphaerosperma*) has been recorded as a cause of spoilage of pasteurised apple juice (Anon., 1967). It has also been reported on barley, wheat flour, rice, pecans and airline meals (see Pitt and Hocking, 1997). It was isolated from Morelo cherries (Olszak, 1994) and Iranian barley (Asgari et al., 2004). *A. phaeospermum* has also been recorded as a pathogen on sweet potatoes (Ravichandran and Sullia, 1983). We isolated *A. phaeospermum* at low levels from paddy rice, mung beans, soybeans, black beans and cashews in Thailand (Pitt et al., 1993).

Closely related species. The *Arthrinium* state of *Apiospora montagnei* Sacc., also known as *Papularia arundinis* (Corda) Fr., is similar to *A. phaeospermum* in all characters examined, except for the production of smaller conidia, 6–8 μm long. Data sheets at the International Mycological Institute, Egham, Surrey, UK, record its isolation from white flour and molasses; it has also been reported from wheat (Pelhate, 1968), barley (Flannigan, 1969) and cashews (Pitt et al., 1993).

References. Ellis (1971); Domsch et al. (1980).

5.6 Genus *Aureobasidium* Viala and G. Boyer

Growth of *Aureobasidium* isolates is at first yeast-like, but, while remaining very low and mucoid, colonies spread rapidly and turn black in patches. Microscopically, hyphae as well as budding yeast-like cells are present; the latter are actually conidia. The conidia are borne from small denticles (minute projections) directly from the hyphal walls or from short lateral protrusions on the hyphae; characteristically 2–4 denticles on one cell will produce conidia synchronously. There are more than 10 species (Hermanides-Nijhof, 1977), but only one, *Aureobasidium pullulans*, is of importance in foods (Fig. 5.5).

Aureobasidium pullulans (de Bary) G. Arnaud — Fig. 5.5

Dematium pullulans de Bary
Pullularia pullulans (de Bary) Berkhout

Colonies on CYA and MEA 25–35 mm diam, low and mucoid, faintly pink, becoming grey to black in areas at 7–10 days; reverse in similar colours. Colonies on G25N 10–12 mm diam, similar to those on CYA. At 5°C, microcolonies to colonies up to 3 mm diam. No growth at 37°C.

Conidia borne on denticles directly from hyphae or sometimes small lateral protrusions; conidia yeast-like, primary ones borne from the denticles, usually measuring 10–16 × 3–6 μm, and secondary

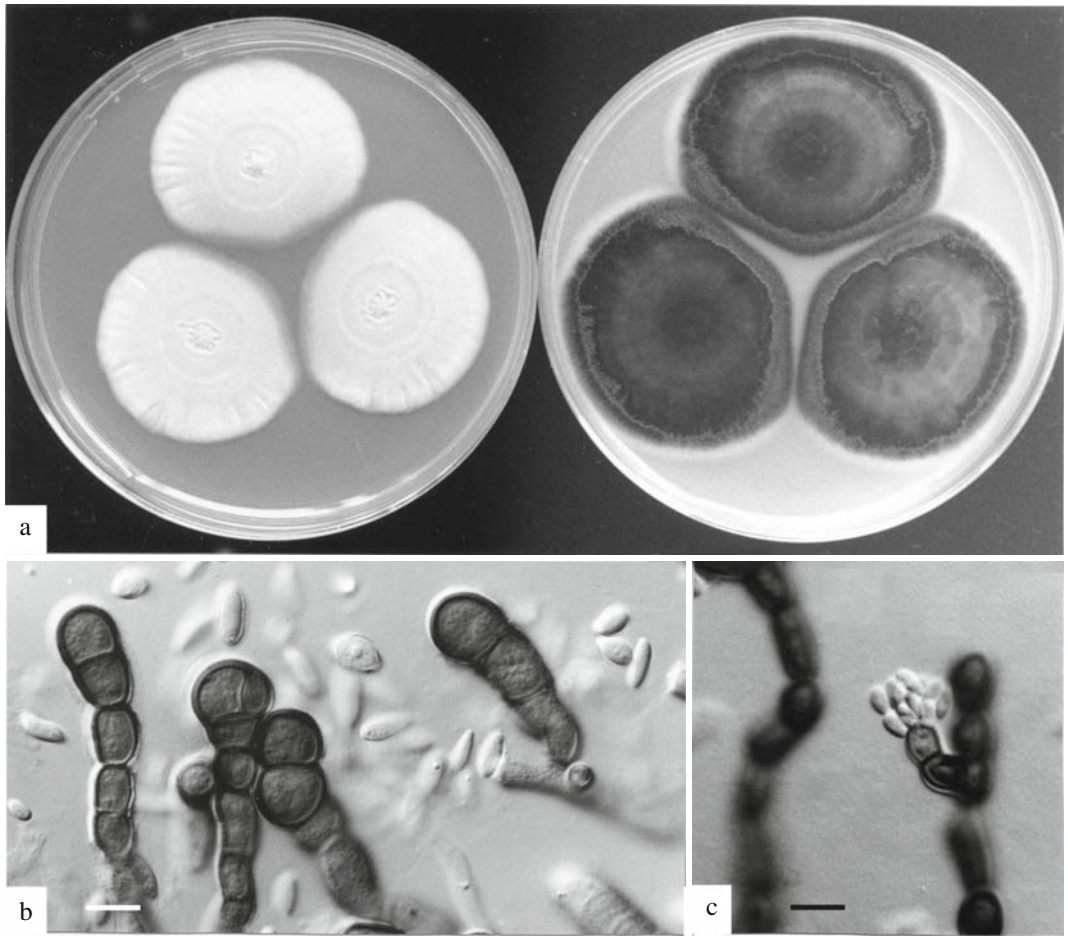


Fig. 5.5 *Aureobasidium pullulans* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** conidia, bar = 10 µm

ones by budding from the primaries, 7–10 × 3–5 µm, not adhering to each other, smooth walled.

Distinctive features. Within the present context, *Aureobasidium pullulans* is readily recognised by its distinctive low, mucoid, white then pink to black colonies and yeast-like conidia. Several other genera have a similar appearance (Hermanides-Nijhof, 1977; Beh, 2007) but they rarely occur in foods.

Taxonomy. Earlier literature discusses this species under the names *Dematium pullulans* and *Pullularia pullulans*.

Mycotoxins. Mycotoxins are not known to be produced.

Physiology. The temperature range for growth of *Aureobasidium pullulans* has been reported as 2–35°C, with an optimum of 25°C (Skou, 1969), but some earlier data, together with its abundance in low temperature habitats, suggests that some strains grow down to –5°C (Michener and Elliott, 1964). It is of common occurrence in salterns (Butinar et al., 2005a), so is likely to be a xerophile. Heat resistance is very low (Skou, 1969). The ability of *A. pullulans* to grow on sugars used in yeast taxonomy has been reported by de Hoog and Yurlova (1994) and Beh (2007).

Ecology. A ubiquitous saprophyte from all sorts of moist and decaying environments, *Aureobasidium pullulans* has been reported from a very wide range of foods, but only rarely as a cause of spoilage. Its prevalence in frozen foods is noteworthy, being the predominant mould isolated from blueberry, apple and cherry pies by Kuehn and Gunderson (1963). It was the most frequently isolated mould in Canadian icewine must, but played no role in fermentating

must (Subden et al., 2003). The surface of healthy grapes is predominately colonised by *A. pullulans* (Fleet, 2003a; Prakichaiwattana et al., 2004; Beh, 2007). *A. pullulans* has been reported in yoghurt in a survey of a Slovakian dairy plant (Pieckova et al., 2002) and has been associated with the spoilage of cold stored meat and cheese (see Pitt and Hocking, 1997). *A. pullulans* has been recorded in a variety of fresh foods and commodities including fresh vegetables, cabbage, strawberries, grapes, citrus and pasteurised orange juice (see Pitt and Hocking, 1997). Other records include shrimp, green olives, barley, wheat and flour, oats and nuts (see Pitt and Hocking, 1997).

References. Hermanides-Nijhof (1977); Domsch et al. (1980); de Hoog and Yurlova (1994).

5.7 Genus *Bipolaris* Shoemaker

In separating unrelated species from *Helminthosporium*, Shoemaker (1959) revised the genus *Drechslera* S. Ito and erected *Bipolaris*. The fact that species of *Bipolaris* (in the sense of Shoemaker, 1959) were associated with two distinct teleomorph types, and that correlated with morphological differences, led to segregation of *Exserohilum* from *Bipolaris* by Leonard and Suggs (1974). These genera produce long, large conidia with transverse septa only; the septa are thick and quite different from those in e.g. *Trichoconiella*. In *Drechslera*, conidia are cylindrical and germinate at any cell, while conidia of *Bipolaris* and *Exserohilum* are fusoid, i.e. gradually narrowing, and conidia germinate only from the end cells (bipolar germination). In *Bipolaris*, germ tubes develop roughly along the line of the conidium axis, while in *Exserohilum*, the germ tube is often offset from the axis and develops at an angle (Alcorn, 1983). *Exserohilum* species are apparently rare in foods, although *E. rostratum* (Drechsler) K.J. Leonard and Suggs has been reported from sorghum (Usha et al., 1994).

The literature on these genera is voluminous and complex. The most useful publications are by Alcorn (1983) and Sivanesan (1987). One common species of *Bipolaris*, *B. maydis*, is treated here (Fig. 5.6).

Bipolaris maydis (Nishik. and C. Miyake) Shoemaker Fig. 5.6

Colonies on CYA and MEA 45–55 mm diam, plane, deeply floccose, of light to dark grey mycelium; reverse dark brown to dark blue black. On G25N, colonies 2–6 mm diam. At 5°C, germination. At 37°C, colonies 5–15 mm diam, usually low, dense and wrinkled, of grey mycelium, reverse deep blue black.

Colonies on DCMA 30–40 mm diam, plane, of sparse to dense, floccose grey mycelium, reverse brown, grey or black.

Conidia borne singly from nodes on knobby or geniculate mid to dark brown hyphae, usually slightly curved, (40–)50–80 × 12–16 μm, with six to nine inconspicuous septa and with smooth, dark, thick walls.

Distinctive features. Large conidia, often slightly curved, of fusoid shape and with several thick transverse septa are characteristic of this genus. Conidial curvature and shape distinguish it from *Drechslera*, while conidial length and the presence of more than three, thick septa allow distinction from *Curvularia*. The species treated here is common on maize.

Taxonomy. Some species of *Bipolaris* produce teleomorphs, classified in the genus *Cochliobolus*.

Physiology. Species of *Bipolaris* sporulate significantly better when grown on substrates containing cellulose (Pratt, 2006).

Mycotoxins. No significant mycotoxins are known.

Ecology. We have isolated *Bipolaris maydis* from 1% of Thai maize kernels, and less frequently from Thai sorghum and mung bean samples (Pitt et al., 1993, 1994). It was also found at similar levels in commodities from Indonesia and the Philippines (Pitt et al., 1998a). Other records of *Bipolaris* in foods are rare. However, references to *Drechslera* species in foods often have referred to taxa correctly classified in *Bipolaris*, e.g. in rice, *B. oryzae* (Breda de Haan) Shoemaker; in barley, *B. australiensis* (M.B. Ellis) Tsuda and Uemaya, *B. maydis* and *B. setariae* (Sawada) Shoemaker; in peanuts, *B. spicifera* (Bainier) Subram.; in coriander seed, *B. bicolor* (Mitra) Shoemaker; and in avocados, *B. setariae* (see Pitt and Hocking, 1997).

References. Alcorn (1983); Sivanesan (1987).

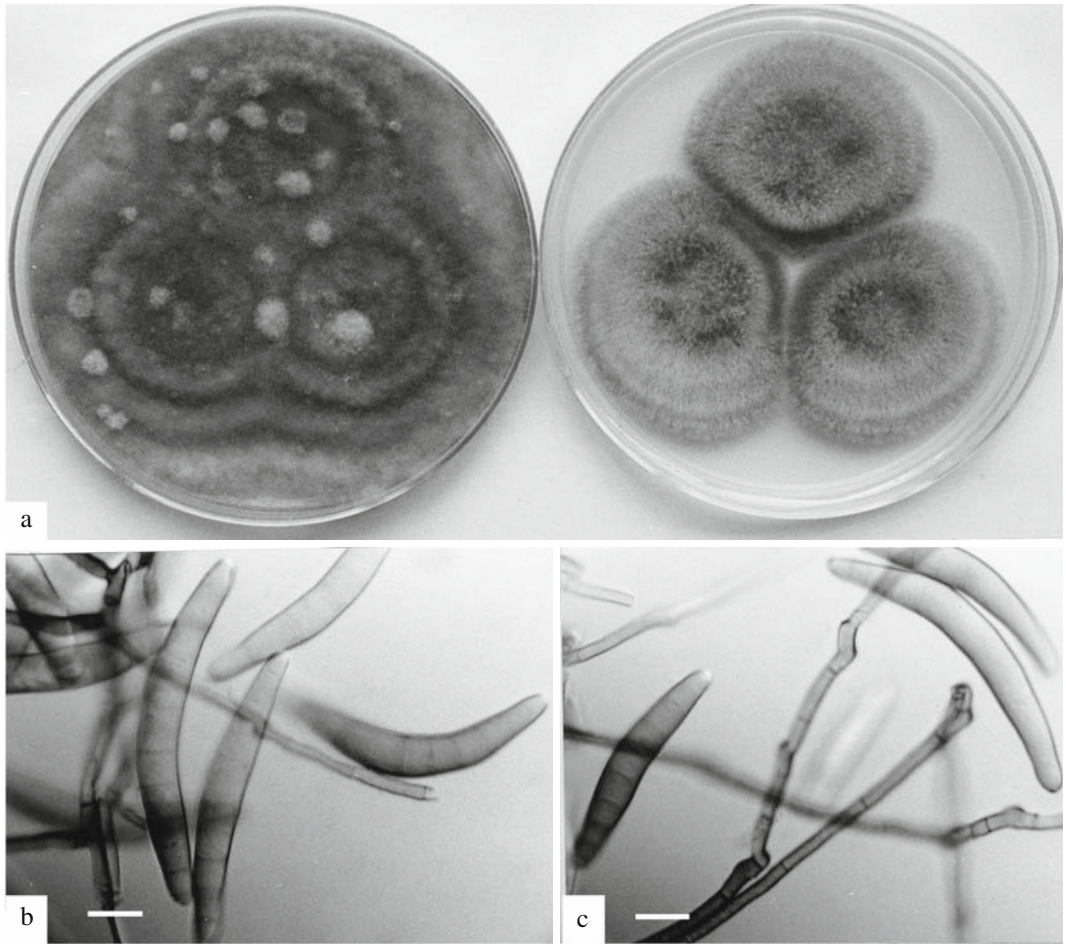


Fig. 5.6 *Bipolaris maydis* **(a)** colonies on MEA and DCMA, 7 d, 25°C; **(b, c)** conidiophores and conidia, bars = 10 µm

5.8 Genus *Botrytis* P. Micheli: Fr.

Botrytis is a common genus in the temperate zones, where it occurs mainly as a pathogen on a variety of plant crops. Vegetables and small berry fruits are particularly susceptible. Invasion may occur before maturity or postharvest, both in transport and in storage. Onions and other *Allium* species and grapes are the most susceptible crops. In the latter, it is notable that the disease is sometimes encouraged. Grapes affected by *Botrytis*, in this circumstance called "the noble rot", are used in the production of certain high quality sweet wines in France, Germany, Australia and other countries.

Botrytis species are characterised by the production of conidia on pegs from spherical swellings. The most commonly encountered species in foods is known as *Botrytis cinerea*, although it is probable that this name includes a group of related species rather than a single well defined taxon (Coley-Smith et al., 1980).

Botrytis cinerea Pers. Fig. 5.7

Colonies on CYA and MEA covering the whole Petri dish, floccose, growth sometimes patchy or irregular, mycelium white, becoming grey to dark grey as conidiogenesis proceeds; reverse pale to grey. On G25N, colonies 10–18 mm diam, irregular in outline, floccose centrally or in patches, becoming grey; reverse grey. At 5°C, colonies up to 5 mm diam produced, low and sparse. No growth at 37°C.

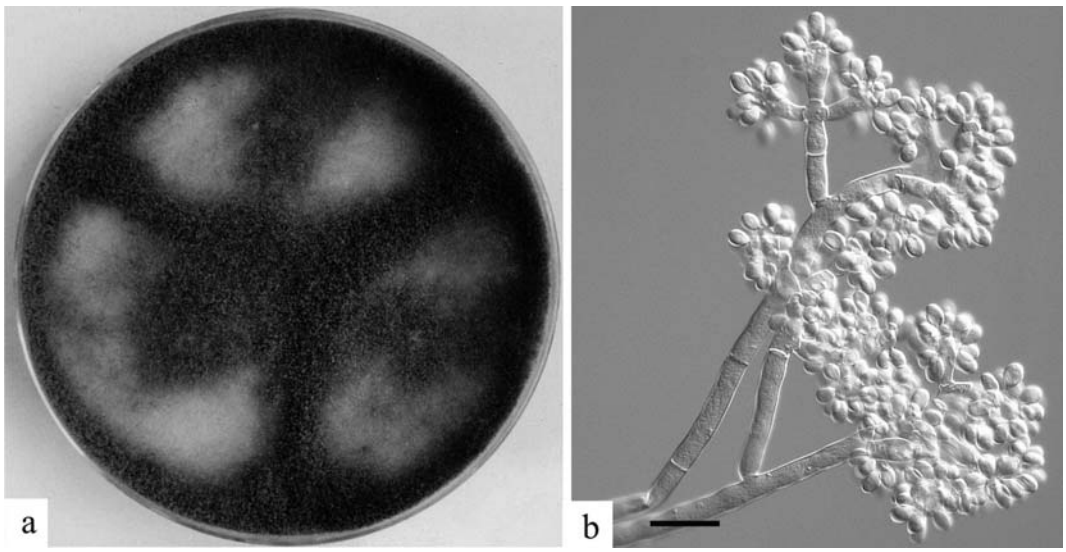


Fig. 5.7 *Botrytis cinerea* **(a)** colony on CYA, 7 d, 25°C; **(b)** conidiophores and conidia, bar = 25 μm

Conidiophores borne from aerial hyphae, stipes of indeterminate length, each bearing terminally an irregular cluster of short branches, 10–30 μm long, with swollen spherical apices, 8–10 μm diam; conidia borne singly from these apices on denticles (small pegs), ellipsoidal, 8–12 μm long, smooth walled, not released at maturity.

Distinctive features. Solitary conidia borne on denticles from terminally swollen, short branches are characteristic of *Botrytis*.

Physiology. Growth has been reported at 0.93 a_w (Snow, 1949) and at 0.90 a_w (Jarvis, 1977). Lahlali et al. (2007) observed no growth of *Botrytis cinerea* at 0.93 a_w in the presence of NaCl or at 0.89 a_w in the presence of nonionic solute. Reported growth temperatures are rather variable, with minima from –2 to 5°C or even 12°C, maxima 28–35°C, and optima of 22–25°C (Domsch et al., 1980). *B. cinerea* will grow from pH 2–8 (Jarvis, 1977) and in O_2 concentrations down to 1% (Follstad, 1966). The effect of low temperatures and controlled atmosphere storage on the growth of *B. cinerea* on susceptible fruits and vegetables has been reported (Reyes, 1990). *B. cinerea* produces pectinases (polygalacturonase) and the cell wall degrading enzymes cellulase and xylanase (El-Habbaa, 2003).

Mycotoxins. Mycotoxin production has not been reported – fortunately, in view of this species role in speciality wine production.

Ecology. *Botrytis cinerea* is a virulent pathogen, the cause of rots in many kinds of fresh fruits (Snowdon, 1990). It is the most important cause of disease in grapes, both before harvest and in storage and the most common cause of spoilage in berries such as strawberries, blueberries, raspberries and blackberries (Snowdon, 1990; Tournas and Katsoudas, 2005), and also causes large losses in apples and pears, tomatoes, stone fruits and kiwi fruit (see Pitt and Hocking, 1997). A gel-like turbidity can be caused in raspberry juice, making filtration difficult (Will et al., 1992). For a review of many aspects of occurrence, pathogenicity and control, see Elad et al. (2004).

Although vegetable spoilage is perhaps less important, the range of crops affected is very large, and includes asparagus, beans, cabbages, carrots, celery, melons and potatoes (Snowdon, 1991; Lugauskas et al., 2005; Tournas, 2005). It has also been isolated from a wide variety of dried or processed foods, but here its role is uncertain and it is probably present only as a contaminant.

Other important species. Several other species of *Botrytis* are commonly occurring pathogens. Of interest here are *B. allii* Munn, *B. byssoides* Walker and the *Botrytis* state of *Sclerotinia porri* van Beyma, all of which cause spoilage of onions and leeks (Ellis, 1971; Snowdon, 1990).

Botrytis allii grows optimally at 20–25°C, with a minimum below 5°C and a maximum near 35°C. The optimum a_w for growth is in excess of 0.99. Growth

occurs down to 0.96 a_w in media containing NaCl and 0.93 a_w in media containing KCl or sucrose, or on onion leaves (Alderman and Lacy, 1984).

References. Ellis (1971); Coley-Smith et al. (1980); Domsch et al. (1980); Elad et al. (2004).

5.9 Genus *Chaetomium* Kunze

Chaetomium is the only genus of Ascomycetes which produces black ascocarps and which is commonly encountered in foods. The ascocarps in *Chaetomium* are perithecia, similar in appearance to cleistothecia, but having an opening (ostiole) through which the ascospores are released. There is a superficial resemblance to the genus *Phoma*, which produces black conidiomata (pycnidia); ascospores in *Chaetomium* are often freed from the ascus before being discharged singly from the perithecium, and hence could be mistaken for conidia. *Chaetomium* is readily distinguished from *Phoma* by the presence of stout black or dark coloured hyphae attached to the perithecial walls, which are visible in culture under the low power microscope.

Chaetomium species are notable as producers of cellulases and so commonly occur on wood and paper products. They are relatively common in some kinds of foods. *C. globosum* is the most frequently encountered species and is described here as representative of the genus. In addition descriptions of *C. brasiliense* and *C. funicola*, both quite common in tropical commodities, are provided here.

Key to *Chaetomium* species included here

1	Colonies on CYA at 37°C much larger than at 25°C	*C. brasiliense*
	Colonies on CYA at 37°C smaller than at 25°C or absent	2
2 (1)	Colonies on CYA and MEA at 25°C less than 50 mm diam, ascospores 5–7 μm long	*C. funicola*
	Colonies on CYA and MEA at 25°C usually covering the whole Petri dish, ascospores 8–10 μm long	*C. globosum*

Chaetomium brasiliense Bat. & Pontual — Fig. 5.8

Colonies on CYA and MEA 25–35 mm diam, on CYA low to moderately deep and dense, of white to grey mycelium; reverse sometimes pale, more commonly dark grey to almost black; on MEA low and sparse, dark grey, with some paler grey aerial hyphae; reverse dark grey. No growth on G25N or germination at 5°C. Colonies at 37°C covering the whole Petri dish, 65 mm or more diam, general appearance similar to those on CYA at 25°C; reverse very dark grey to greyish black (Fig. 5.8).

Reproductive structures perithecia not observed on CYA or MEA at 25°C, but on CYA at 37°C abundant, forming a deep, contiguous layer on the agar surface, most readily observed at colony margins, especially between colonies, surrounded by radiating long, stout hyphae, straight at first but at maturity curling into long corkscrew shapes, 75–100 μm diam, maturing in 7 days at colony centres; asci not seen, ascospores subspheroidal to broadly ellipsoidal, 6–8 μm long, brown, with smooth walls. Conidia not produced.

Distinctive features. Unlike the other *Chaetomium* species considered here, *C. brasiliense* grows much more rapidly at 37°C than 25°C, and perithecia are formed in much greater numbers at the higher temperature. Perithecia are black, but under the stereomicroscope appear surrounded by grey hyphae. Ascospores are smaller than those of *C. globosum*.

Physiology. No studies on physiology are known to us. However, the description above indicates that this species has a high temperature optimum for growth.

Mycotoxins. Significant mycotoxins are not known to be produced.

Ecology. We have isolated this species not infrequently from tropical sources. From Thailand, 1% of mung beans, black beans, paddy rice grains and cashew kernels, and 7–10% of soy bean and sorghum samples, but <1% of individual seeds, contained *C. brasiliense* (Pitt et al., 1993, 1994). Similar figures were obtained from Indonesian commodities (Pitt et al., 1998a). In samples from the Philippines, this species was found in maize, peanuts, soybeans, mung beans,

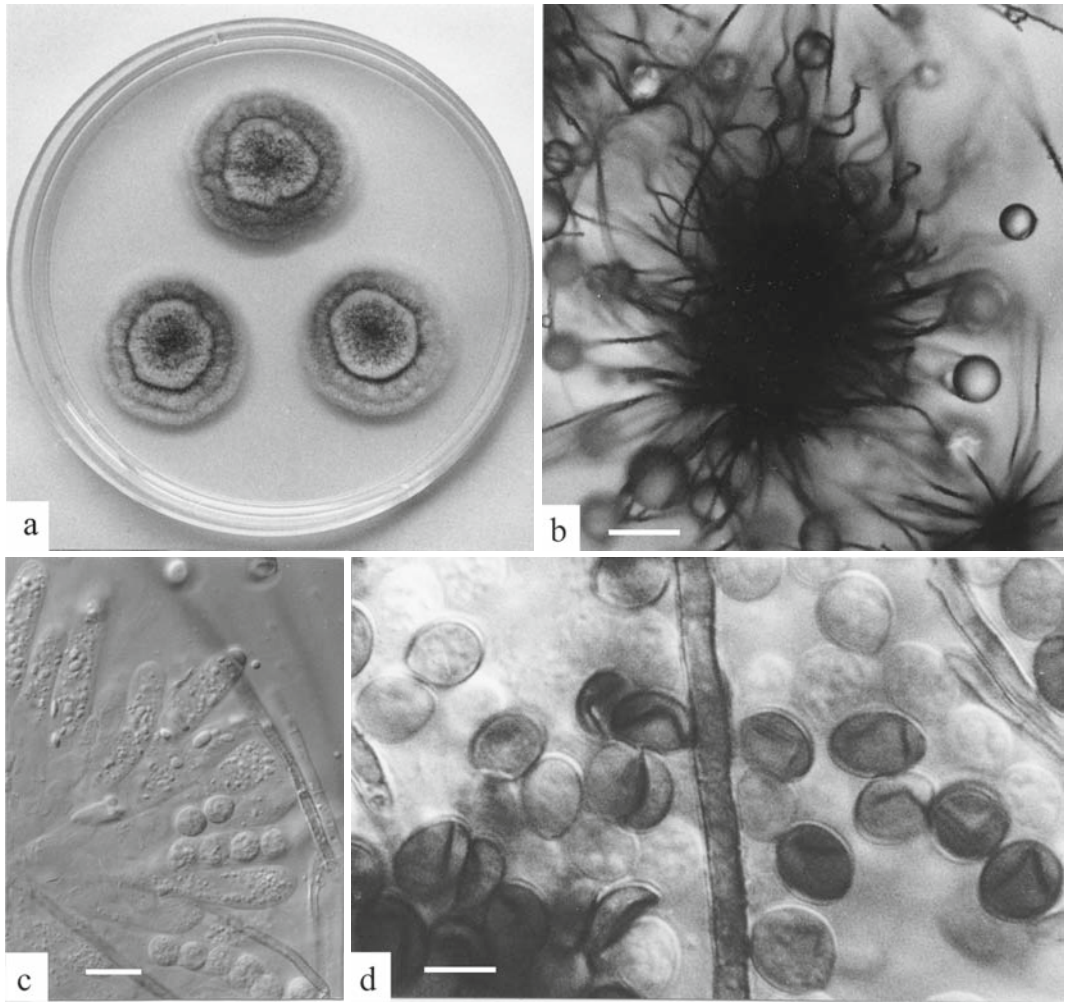


Fig. 5.8 *Chaetomium brasiliense* **(a)** colony on CYA, 7 d, 25°C; **(b)** perithecium in situ, bar = 25 µm; **(c)** asci, bar = 10 µm; **(d)** ascospores, bar = 5 µm

black pepper, paddy and milled rice, all near 1% of total particles examined (our unpublished data).

Reference. Von Arx et al. (1986).

Chaetomium funicola Cooke Fig. 5.9

Colonies on CYA 20–25 mm diam, plane, of low and sparse white mycelium, sometimes pale grey centrally, enveloping abundant developing perithecia, reverse pale or greyish brown. Colonies on MEA 35–40 mm diam, plane, sparse, low to somewhat floccose, mycelium white or grey, enveloping developing perithecia; reverse pale to greyish olive or pale brown. On G25N, usually no growth. At 5°C, no germination. At 37°C, usually no growth.

Perithecia borne in a layer on the agar surface, 120–160 mm diam, surrounded by almost straight, dark walled, rough to spiky hyphae; asci maturing in 14 days, breaking down immediately, ascospores ellipsoidal, 5–7 × 3–5 µm, grey or brown, sometimes with one or two internal oil droplets visible, often collapsing in age, smooth walled.

Distinctive features. *Chaetomium funicola* grows only slowly at 25°C and usually not at 37°C. Ascospores are smaller than those of *C. globosum*.

Physiology. No studies are known to us. Domsch et al. (1980) indicated that perithecial production is maximal at about 20°C, indicating that this species is better adapted to low than high temperatures.

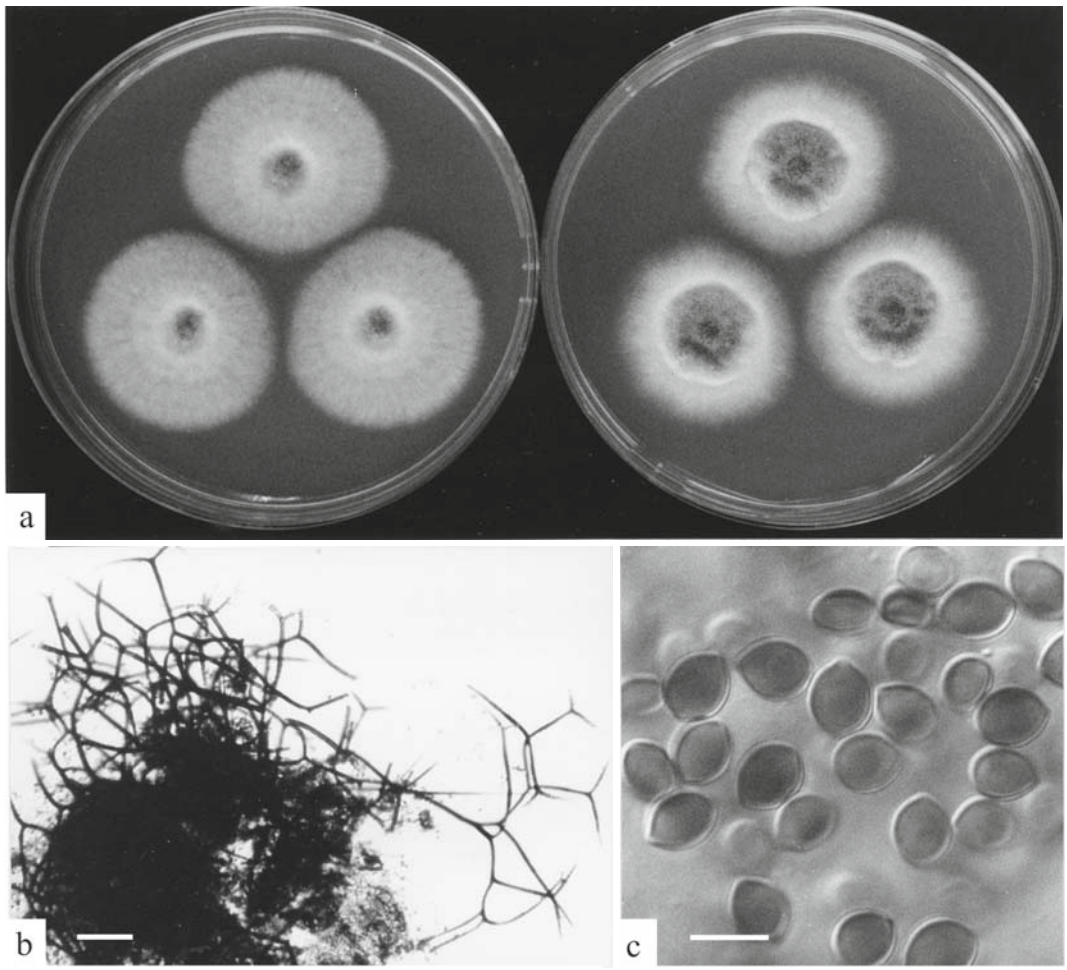


Fig. 5.9 *Chaetomium funicola* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b)** perithecium in situ, bar = 50 μm; **(c)** ascospores, bar = 5 μm

Mycotoxins. Mycotoxin production has not been reported.

Ecology. Despite its low temperature optimum, we repeatedly isolated *Chaetomium funicola* from tropical commodities. It was present in 23% of Indonesian soybean samples, ranging up to 50% infection in some samples, and in 3% of beans overall (our unpublished data). Lower levels of infection (1% or more overall) were observed in peanuts, cashews, copra, mung beans and sorghum from Thailand (Pitt et al., 1993, 1994), in mung beans and maize from Indonesia, and paddy rice from the Philippines (Pitt et al., 1998a and our unpublished data).

References. Domsch et al. (1980); von Arx et al. (1986).

Chaetomium globosum Kunze Fig. 5.10

Colonies on CYA and MEA covering the whole Petri dish, on CYA low and sparse, of scanty white mycelium and conspicuous though usually sparse black perithecia, ca. 0.2 mm diam; on MEA growth more dense but still low, coloured grey or greenish black from hyphae enveloping abundant perithecia; reverse on both media usually brown. On G25N, colonies less than 5 mm diam produced. No growth at 5°C. At 37°C, colonies usually 20–30 mm diam, of white mycelium.

Reproductive structures perithecia, black, 150–200 μm diam, with numerous stout, dark hyphae appended; ascospores produced after 1–2 weeks,

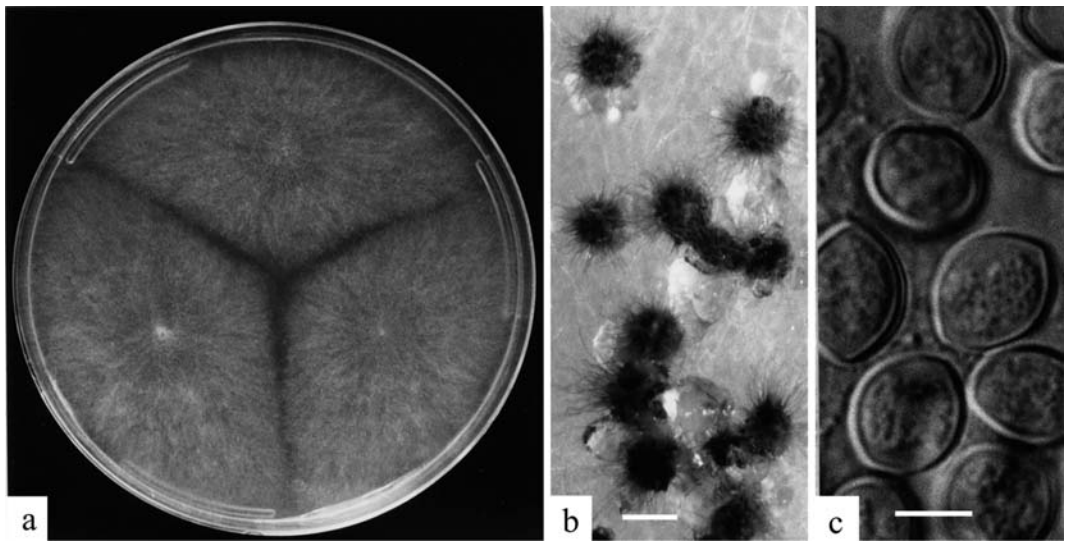


Fig. 5.10 *Chaetomium globosum* **(a)** colony on CYA, 7 d, 25°C; **(b)** perithecium in situ, bar = 100 μm; **(c)** ascospores, bar = 5 μm

spheroidal, broadly ellipsoidal or apiculate, commonly 8–10 μm long, smooth walled. Conidia not generally produced.

Distinctive features. In contrast to *Chaetomium brasiliense*, *C. globosum* grows much more rapidly at 25°C than 37°C. Ascospores are larger than those of *C. funicola*.

Physiology. Chapman and Fergus (1975) reported that *Chaetomium globosum* ascospores germinated from a minimum temperature of 4–10°C to a maximum of 38°C, and most rapidly at 24–38°C. They also reported germination over the whole pH range tested, 3.5–7. Heat resistance was low: 1% of ascospores survived 10 min at 55°C, but none survived at 57°C for a similar period. Ahammed et al. (2005) observed maximum sporulation at 28°C and pH 5.0 on a medium containing sucrose and thiamine. *C. globosum* can grow down to 0.94 a_w in soil (Kouyeas, 1964).

Mycotoxins. *Chaetomium globosum* produces chaetoglobsins A and C, moderately toxic compounds reported to be produced in building materials (Fogle et al., 2007). Their relevance to foods is unknown.

Ecology. This species has been isolated from a variety of commodities, particularly wheat, barley, rice, beans and soybeans. *Chaetomium globosum* has also been recorded in nuts (see Pitt and Hocking, 1997) and spices (Mandeel, 2005). We isolated it frequently from tropical commodities: from 12 to 17% of maize samples, 14 to 32% of mung bean samples and 33 to 35% of soybean samples from Thailand, Indonesia and the Philippines, with overall infection rates of 1–4% of all particles examined.

Relatively high infection levels were also encountered in Thai cashews, copra and paddy rice. Kemiri (candle) nuts from Indonesia were highly infected: 37% of samples, up to 40% in infected samples and 9% of nuts overall (Pitt et al., 1998a). This species has not been reported to cause food spoilage, although it has recently been implicated in causing disease in pears in Egypt (Ismail and Abdalla, 2005).

References. Domsch et al. (1980); von Arx et al. (1986).

5.10 Genus *Chrysonilia* Arx

Chrysonilia was erected by von Arx (1981a) to accommodate *Monilia sitophila*, for many years known as "the red bread mould". *Chrysonilia sitophila* is characterised by very rapid growth and the production of conidia in chains, cut off from the apices of undifferentiated hyphae. This species is readily recognisable in Petri dish culture: sparse, white to pink mycelium spreads rapidly across the dish and up the walls, then within 3 days sheds

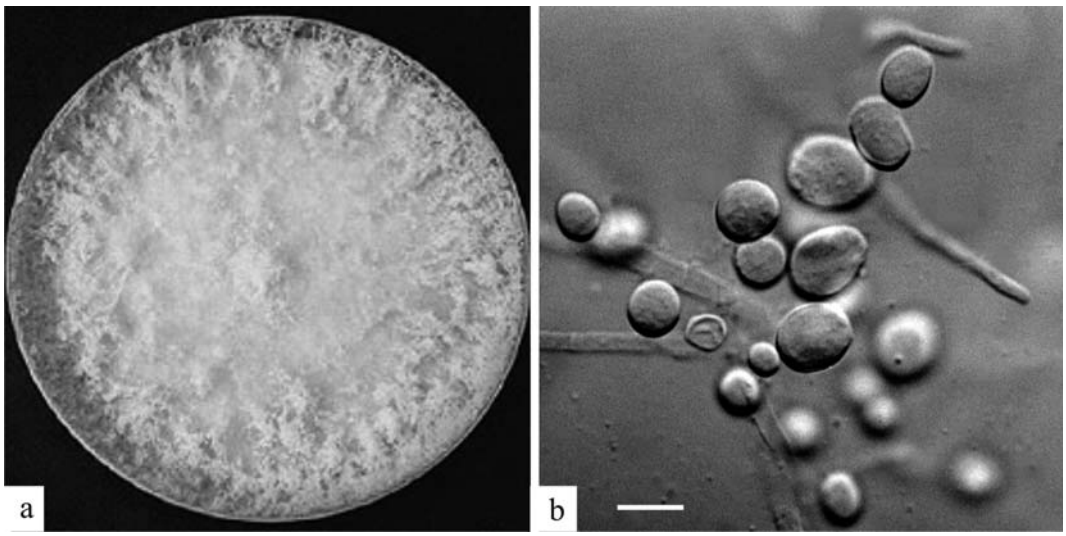


Fig. 5.11 *Chrysonilia sitophila* **(a)** colony on CYA, 7 d, 25°C; **(b)** conidia, bar = 10 μm

enormous numbers of orange conidia *outside* the dish. This attribute makes it a particularly troublesome laboratory contaminant. Cultivation in Petri dishes is therefore not recommended: observation of these characters in a culture tube is sufficient for positive identification.

The teleomorph of this species is *Neurospora sitophila*, a well-known Ascomycete which has been of great value in genetic studies. It is not found in foods, however.

Chrysonilia sitophila (Mont.) Arx Fig. 5.11

Monilia sitophila (Mont.) Sacc.

Teleomorph: *Neurospora sitophila* Shear and B.O. Dodge

Colonies in 28 ml McCartney bottles or test tubes distinguished by pale pink floccose growth, filling the entire bottle, then turning salmon, first at the bottle neck or tube plug and subsequently throughout, as sporulation occurs.

On CYA and MEA, colonies covering the whole Petri dish, mycelium pink, reaching the lid in tufts or patches and all around the rim, producing vast numbers of salmon conidia at or near the rim and sometimes for several millimetres beyond it, and shedding them profusely; reverse salmon or pink. On G25N, colonies up to 30 mm diam produced, low, dense and mucoid. No growth at 5°C. At 37°C, colonies covering the whole Petri dish, similar to at 25°C.

Reproductive structures arthroconidia, cut off in succession from the apices of branching hyphae; conidia at maturity variable in size and shape, spherical to ellipsoidal, pale orange, 6–15 μm diam, with thin smooth walls.

Distinctive features. See remarks above.

Physiology. According to Panasenko (1967), *Chrysonilia sitophila* is capable of growth down to 0.88–0.90 a_w.

Mycotoxins. No mycotoxins have been recorded for this species.

Ecology. For long a common sight in bakeries and on bread, *Chrysonilia sitophila* is less commonly encountered now, but can still be a great source of trouble as a persistent contaminant in laboratories (see Chapter 4). It has sometimes been reported from other foods: pastries, hazelnuts, beans and meat products (see Pitt and Hocking, 1997). We isolated it frequently from Thai cassava, where it infected 36% of all pieces examined, and Philippine peanuts, where it infected up to 90% of nuts in some samples, and was present in 1% of nuts overall. It was also present in 1% of all Indonesian peanuts, sorghum and maize grains examined (Pitt et al., 1998a). Infection rates in maize and peanuts ranged up to 40% in some samples (our unpublished data). We also found it at lower levels in Thai soybeans, black beans and

cashews (Pitt et al., 1994). Recently, *C. sitophila* was reported in the mycoflora of cork slabs (Oliveira et al., 2003; Pereira et al., 2006).

Reference. Von Arx (1981a).

5.11 Genus *Cladosporium* Link

Cladosporium is a very commonly isolated genus and is often dominant in studies of airborne microflora. Species occur both as saprophytes and as plant pathogens. Conidia of *Cladosporium* species are particularly well adapted to aerial dispersal, being small, dry, heavily pigmented and apparently highly resistant to sunlight. The genus is highly diverse, and includes many species not yet characterised (Crous et al., 2007).

In culture, *Cladosporium* is readily recognised. Colonies are 15–40 mm in diameter, low, dense and velvety, and coloured olive. Colony reverses on CYA are often a deep iridescent blue black, uncommon among slowly growing genera.

Reproductive structures are fragile, tree-like conidiophores, which branch irregularly by budding from the youngest cells. The structures can be seen by examination of colonies under the stereomicroscope. They disintegrate partially or totally in wet mounts, leaving masses of conidia, which may show buds or bud scars. In shape, but not colour, the conidia often resemble yeast cells; however, walls are thick, coloured olive and often roughened.

Cladosporium species occur as pathogens on fresh fruit and vegetables (Snowdon, 1990, 1991) and may cause spoilage of strawberries (Beneke et al., 1954; Dennis et al., 1979) or tomatoes (Harwig et al., 1979). On other foods, *Cladosporium* species usually occur as contaminants rather than as spoilage fungi. However, all common species grow at temperatures near 0°C, and Cladosporia have been reported to cause spoilage of chilled meats, cheese and other refrigerated commodities from time to time (see Pitt and Hocking, 1997).

A major taxonomic revision of *Cladosporium*, its teleomorphs and related genera has recently been published (Crous et al., 2007). In overall terms, the taxonomy of the common species treated here has been unaffected. The most frequently reported saprophytic species is *Cladosporium herbarum*, but *C. cladosporioides* and *C. sphaerospermum* are also ubiquitous. In addition, *C. macrocarpum* is described here.

Key to *Cladosporium* species included here

1	Single celled conidia small, less than 4 μm wide	2
	Single celled conidia often exceeding 4 μm wide	3
2 (1)	Single celled conidia ellipsoidal or apiculate (lemon-shaped)	*C. cladosporioides*
	A high proportion (greater than 40%) of single-celled conidia roughly spherical or pyriform	*C. sphaerospermum*
3 (1)	Conidia 4–6 μm wide	*C. herbarum*
	Conidia commonly exceeding 6 μm wide	*C. macrocarpum*

Cladosporium cladosporioides (Fresen.) G.A. de Vries Fig. 5.12

Colonies on CYA and MEA 25–40 mm diam, low and dense, lightly wrinkled or plane, surface velutinous or lightly floccose; conidia abundant, coloured olive; reverse bluish grey. Colonies on G25N 5–12 mm diam, plane, sometimes centrally raised, velutinous, coloured as on CYA; reverse bluish black. At 5°C, colonies usually 1–2 mm diam, occasionally only germination occurring. No growth at 37°C.

Conidiophores *in situ* dendritic (tree-like), closely packed, with stipes bearing branching structures of acropetally produced cells, all functioning as conidia at maturity and separating in liquid mounts; conidia heavy walled, pale olive brown, larger ones non- or singly septate, 10–30 × 2–5 μm, smooth walled, smaller ones nonseptate, ellipsoidal to apiculate, 3–7 × 2–4 μm, with walls smooth to finely roughened.

Distinctive features. *Cladosporium cladosporioides* produces smaller conidia than *C. herbarum* and *C. macrocarpum*; unlike *C. sphaerospermum*,

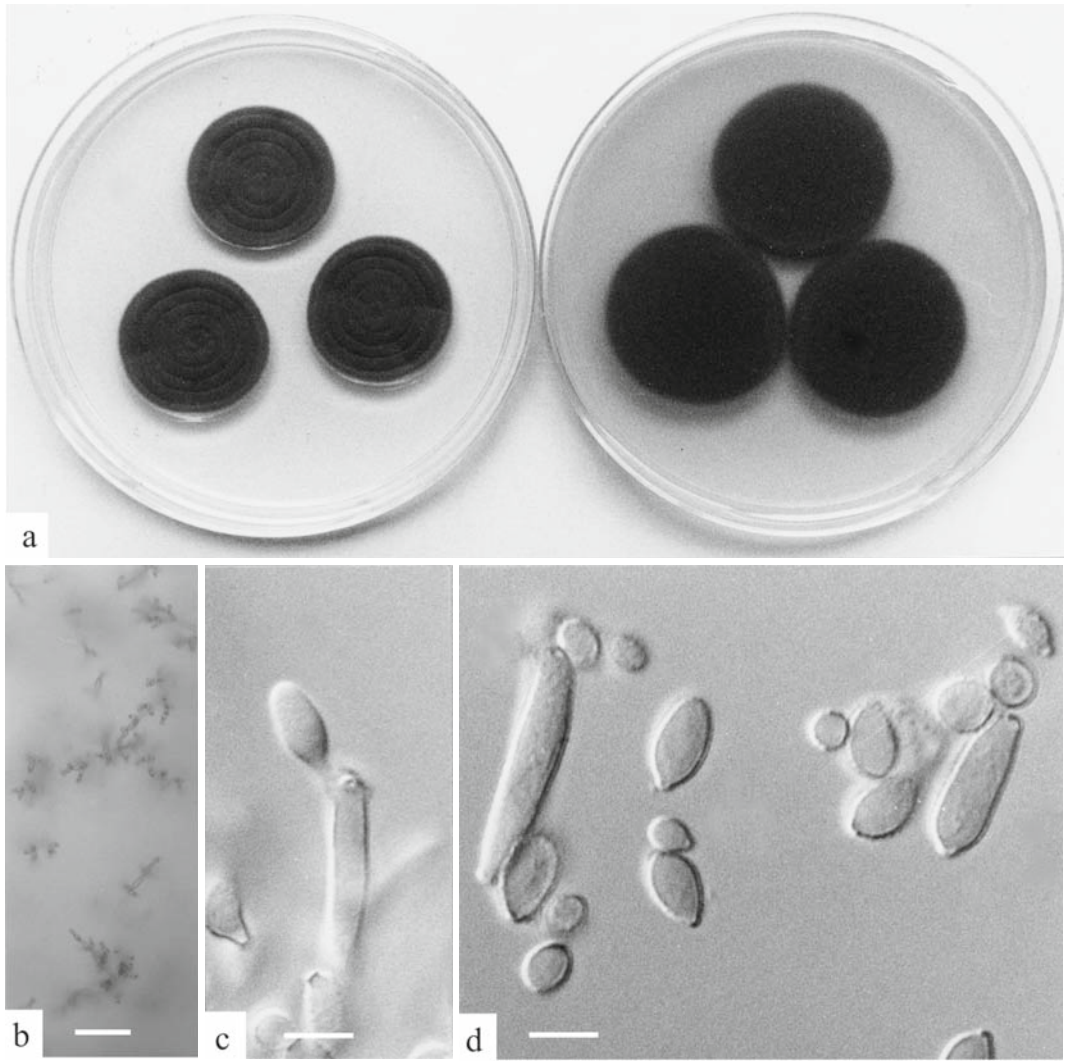


Fig. 5.12 *Cladosporium cladosporoides* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b)** fruiting structures in situ, bar = 50 µm; **(c)** conidiophores, bar = 5 µm; **(d)** conidia, bar = 5 µm

the majority of conidia are ellipsoidal. Growth on CYA and MEA is much faster than that of *C. sphaerospermum*.

Physiology. Gill and Lowry (1982) reported a minimum growth temperature of –5°C for *Cladosporium cladosporioides*; the maximum is near 32°C (Domsch et al., 1980). It is capable of growth down to 0.86 a_w at 25°C (Hocking et al., 1994) and is relatively resistant to microwave heating (Dragoni et al., 1990). The minimum inhibitory concentration of sorbic acid is 160 mg/kg at pH 5 (Skirdal and Eklund, 1993).

Mycotoxins. This species is not known to produce mycotoxins.

Ecology. *Cladosporium cladosporioides* has been isolated from a very wide variety of foods, including wheat and flour, barley, rice, sorghum, fresh and frozen meat and fresh vegetables (see Pitt and Hocking, 1997). It occurred on 16% of 74 dried fish samples from Indonesia (Wheeler et al., 1986), and was common on Egyptian walnuts and hazelnuts (Abdel-Hafez and Saber, 1993). It has also been found in peanuts (El-Magraby and El-Maraghy, 1988).

Cladosporium cladosporioides is not as common a cause of spoilage in fresh fruit as *C. herbarum*, but can cause rots of raspberries and melons and sooty discolouration of bananas (Snowdon,

1990). Because of its psychrophilic nature, *C. cladosporioides* causes spoilage of refrigerated foods such as cheese (Northolt et al., 1980) and meat (Gill et al., 1981). It was the dominant fungus causing losses in vacuum packed Australian cheese blocks (Hocking and Faedo, 1992).

Cladosporium cladosporioides is also very common in major Southeast Asian commodities: we isolated this species from 33% of mung bean, 14% of peanut, 13% of soybean and 10% of maize samples from the Philippines and 21% of soybeans and 14% of mung bean samples from Indonesia (Pitt et al., 1998a and our unpublished data).

References. De Vries (1952); Ellis (1971); Domsch et al. (1980).

Cladosporium herbarum (Pers.) Link Fig. 5.13

Colonies on CYA and MEA 18–32 mm diam, velutinous to lightly floccose, plane or slightly wrinkled, coloured olive; reverse olive grey to dark greenish grey. Colonies on G25N 5–10 mm diam, low and

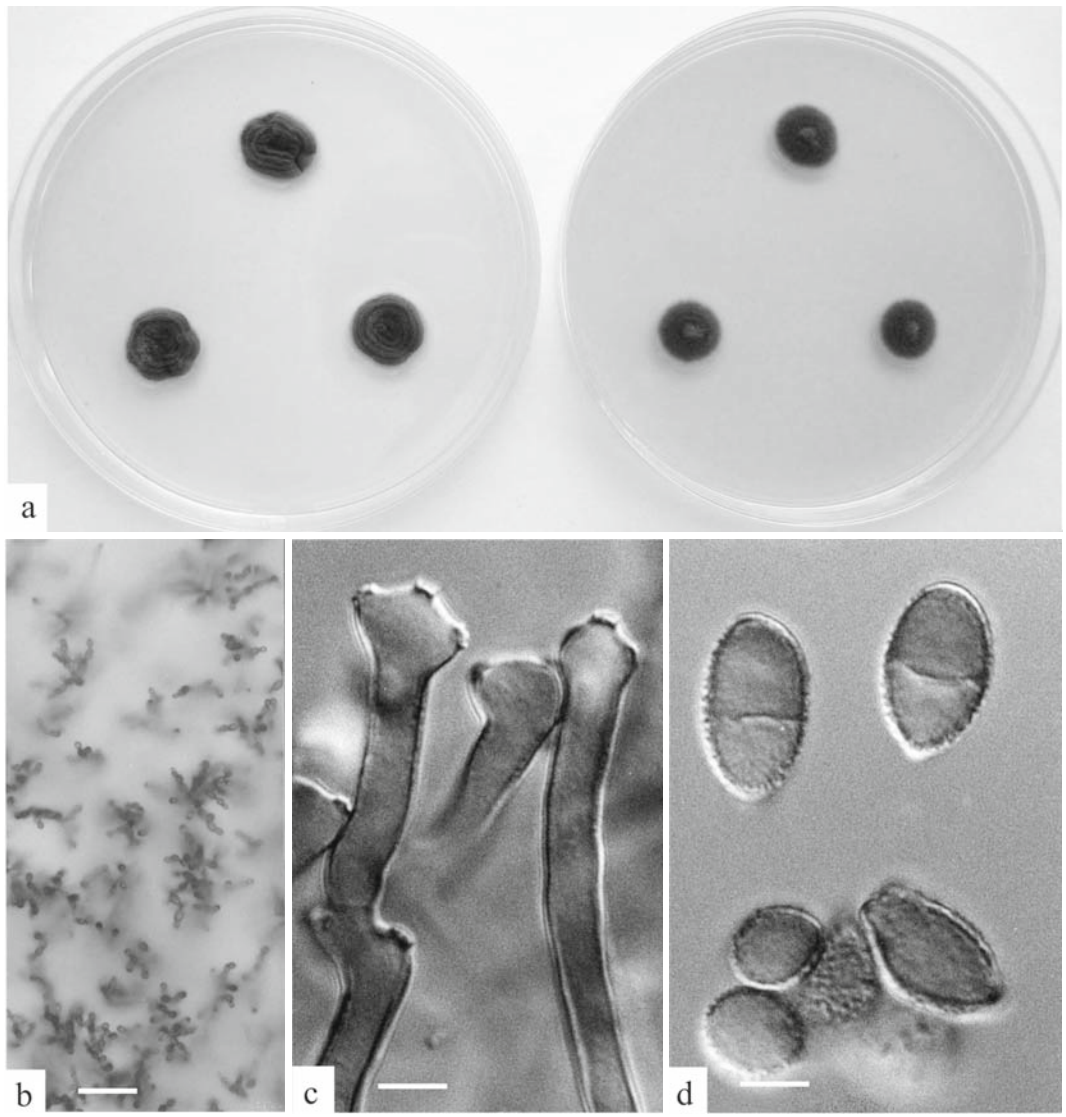


Fig. 5.13 *Cladosporium herbarum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b)** fruiting structures in situ, bar = 50 µm; **(c)** conidiophores, bar = 5 µm; **(d)** conidia, bar = 5 µm

sparse to deep and dense, coloured as on CYA or paler. At 5°C, colonies usually 1–2 mm diam; occasionally only germination. No growth at 37°C.

Conidiophores *in situ* sparsely and irregularly branched dendritic structures, borne on long, dark stipes, separating in fluid mounts; conidia ellipsoidal to cylindroidal, extremities sometimes irregular due to bud scars, usually nonseptate but larger cells with one to two septa, commonly 8–15(–20) × 4–6 μm, pale brown, with densely roughened walls.

Distinctive features. The conidia of *Cladosporium herbarum* are 4–6 μm wide, with distinctly roughened walls; conidia of *C. macrocarpum* are 6–9 μm wide and have finely roughened walls.

Taxonomy. It was concluded by Dugan and Roberts (1994) that *Cladosporium herbarum* represented part of a continuum with *C. macrocarpum*, while Ho et al. (1999) considered that *C. macrocarpum* was a variety of *C. herbarum*. However, in a major study, Schubert et al. (2007) have kept these two species. They indicated that *C. herbarum* as commonly used includes several other species, but the concept of *C. herbarum* used here is still valid. The teleomorph of *C. herbarum* is *Davidiella tassiana* (De. Not.) Crous and U. Braun (Schubert et al., 2007). This is not usually seen in pure culture.

Physiology. Growth of this species has been reported down to 0.88 a_w (Snow, 1949) and down to –10°C (Joffe, 1962). The maximum growth temperature is 28–32°C (Domsch et al., 1980). This species can grow and sporulate in an atmosphere containing 0.25% oxygen (Follstad, 1966).

Mycotoxins. No mycotoxins are known to be produced.

Ecology. Because *Cladosporium herbarum* has been the best known *Cladosporium* name for a long time, it seems likely that some reports of this species really refer to other Cladosporia, especially *C. cladosporioides,* in our experience the more common species in foods. For example, Waghray et al. (1988) reported *C. herbarum* as common on Indian rice, but according to Pitt et al. (1994, 1998a and unpublished), the common *Cladosporium* species on Southeast Asian rice is *C. cladosporioides*. In contrast to *C. cladosporioides, C. herbarum* causes spoilage of fresh fruits and vegetables: rots in fresh yams (*Dioscorea* sp.; Adeniji, 1970a), stored apples (Snowdon, 1990; Kalafatoglu and Karapinar, 1991), stone fruit, tomatoes, melons, grapes (Snowdon, 1990, 1991; Camili and Benato, 2005), passionfruit (Ribeiro and Dias, 2005) and pawpaw (Echerenwa and Umechuruba, 2004). Its common occurrence on fresh apples can lead to contamination of apple juice and high acid fruit based products. As a psychrophile, *C. herbarum* can cause "black spot" spoilage of meat in cool stores (Gill et al., 1981), of cheese during ripening (Gueguen, 1988) and in storage under vacuum (Hocking and Faedo, 1992). It has been isolated from fresh and frozen meat and processed meat products (see Pitt and Hocking, 1997). *C. herbarum* has also been isolated from eggs, peanuts, hazelnuts, walnuts, cereals, chickpeas, soybeans and frozen fruit pastries (see Pitt and Hocking, 1997; Kuehn and Gunderson, 1963; Arya, 2004). Growth on dough carrying machinery (Gradel and Müller, 1985) and on wine corks (Daly et al., 1984) has been reported.

References. Dugan and Roberts (1994); Ho et al. (1999); Schubert et al. (2007).

Cladosporium macrocarpum Preuss Fig. 5.14

Colonies on CYA 18–25 mm diam, velutinous to lightly floccose, plane or slightly wrinkled, coloured olive to dark olive; reverse bluish grey. Colonies on MEA 16–24 mm diam, low to floccose, pale olive to olive; reverse olive grey to dark olive grey. Colonies on G25N 3–8 mm diam, low and sparse to deep and dense, coloured as on MEA or paler. At 5°C, colonies usually 1–2 mm diam; occasionally germination only or no growth observed. No growth at 37°C.

Conidiophores as seen under the stereomicroscope long and dark, with small clusters of conidia borne in short, irregularly branched chains; conidia borne from irregularly located pores on geniculate ("knee-like") swellings, ellipsoidal or short cylindroidal, with rounded ends, (10–)15–20 × 6–9 μm, usually aseptate, with finely roughened brown walls.

Distinctive features. Colonies are similar to those of *Cladosporium herbarum*, but of somewhat paler colour. Conidia are considerably larger than those of *C. herbarum* or of the other *Cladosporium* species described here.

Taxonomy. From an examination of 32 isolates from cherries in Washington state, USA, Dugan and Roberts (1994) concluded that *Cladosporium macrocarpum* and *C. herbarum* represented part of a continuum and that *C. macrocarpum* was a

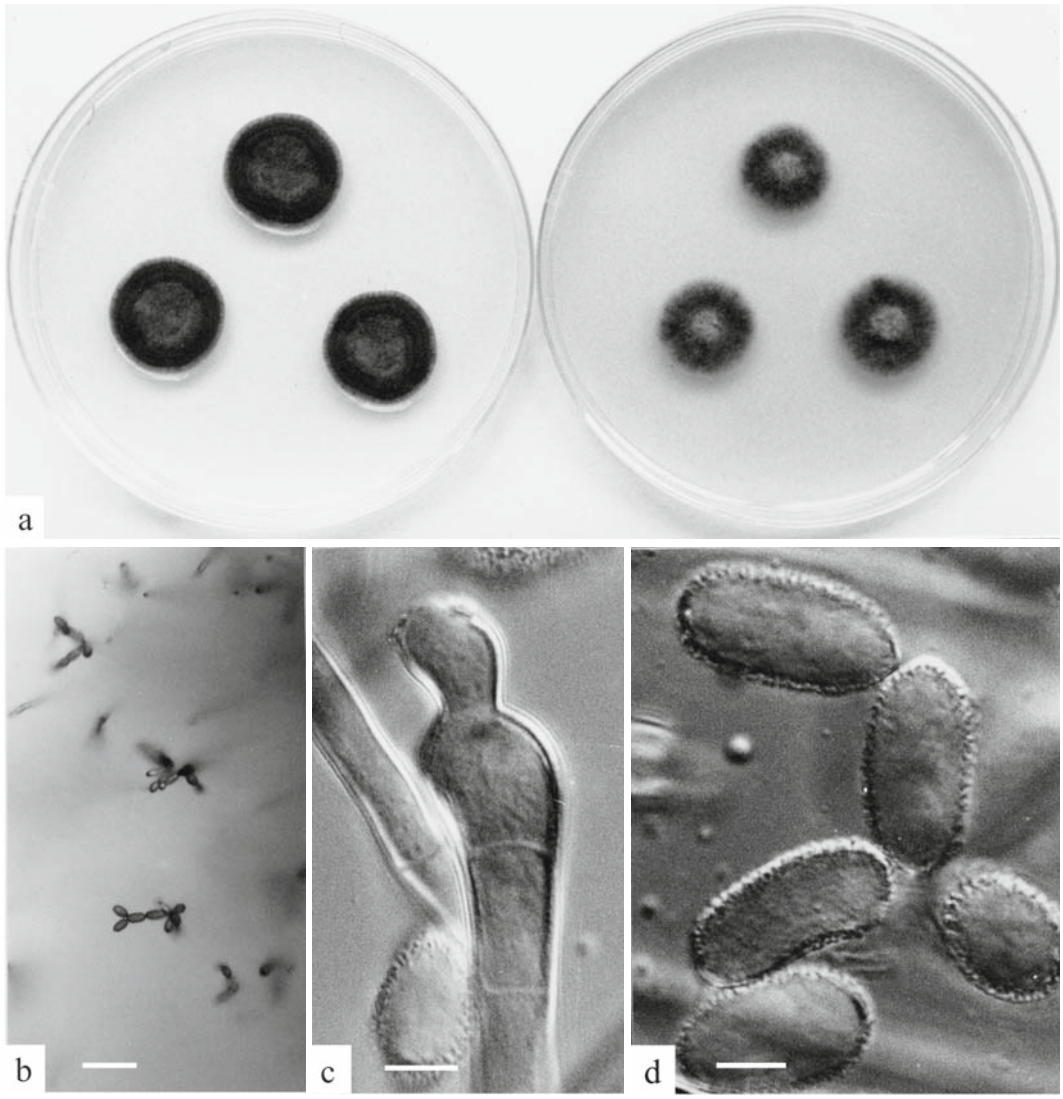


Fig. 5.14 *Cladosporium macrocarpum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b)** fruiting structures in situ, bar = 50 μm; **(c)** conidiophores, bar = 5 μm; **(d)** conidia, bar = 5 μm

synonym. Ho et al. (1999) considered that *C. macrocarpum* was a variety of *C. herbarum*. However, in a major study, Schubert et al. (2007) kept the two species separate.

Physiology. No reports on the physiology of this species have been located. Properties should be similar to those of *Cladosporium herbarum*, to which it is closely related.

Mycotoxins. No mycotoxins have been reported.

Ecology. *Cladosporium macrocarpum* is uncommon in foods. Abdel-Kader et al. (1979) and Petters et al. (1988) both reported it from barley, the latter as one of the principal fungi found in a large survey. Dugan and Roberts (1994) reported frequent occurrence on Bing cherries in the United States (*see* Taxonomy). It occurs on hazelnuts and walnuts in Egypt (Abdel-Hafez and Saber, 1993). We have isolated this species at a low frequency from cashews in Thailand (Pitt et al., 1993) and mung beans in the Philippines (our unpublished data). It was also reported on fresh asparagus (Kadau et al., 2005).

References. Dugan and Roberts (1994); Ho et al. (1999); Schubert et al. (2007).

Cladosporium sphaerospermum Penz. Fig. 5.15

Colonies on CYA 18–25 mm diam, low and dense, usually plane, surface velutinous; conidia abundant, coloured olive to dark olive; reverse bluish grey. Colonies on MEA 12–20 mm diam, of similar appearance to those on CYA, sometimes lighter in colour. Colonies on G25N 8–12 mm diam, plane or centrally raised, velutinous, coloured as on CYA; reverse almost black. At 5°C sometimes germination. No growth at 37°C.

Conidiophores under the stereomicroscope dendritic (tree-like), closely packed, with stipes bearing branching structures of acropetally produced cells, all functioning as conidia at maturity, and separating in liquid mounts; conidia heavy walled, pale olive brown, larger ones 10–30 × 2–4 µm, only occasionally septate, smooth walled, smaller ones nonseptate, subspheroidal, ogival or apiculate, 4–6(–10) × 3–4 µm, with walls smooth to definitely roughened.

Distinctive features. Similar to *Cladosporium cladosporioides* in most respects, *C. sphaerospermum* is distinguished by slower growth on CYA and MEA and by production of a high proportion (40% plus) of more or less spherical conidia.

Taxonomy. Several new species very similar to *Cladosporium sphaerospermum* were described by Zalar et al. (2007), but none was of common occur-

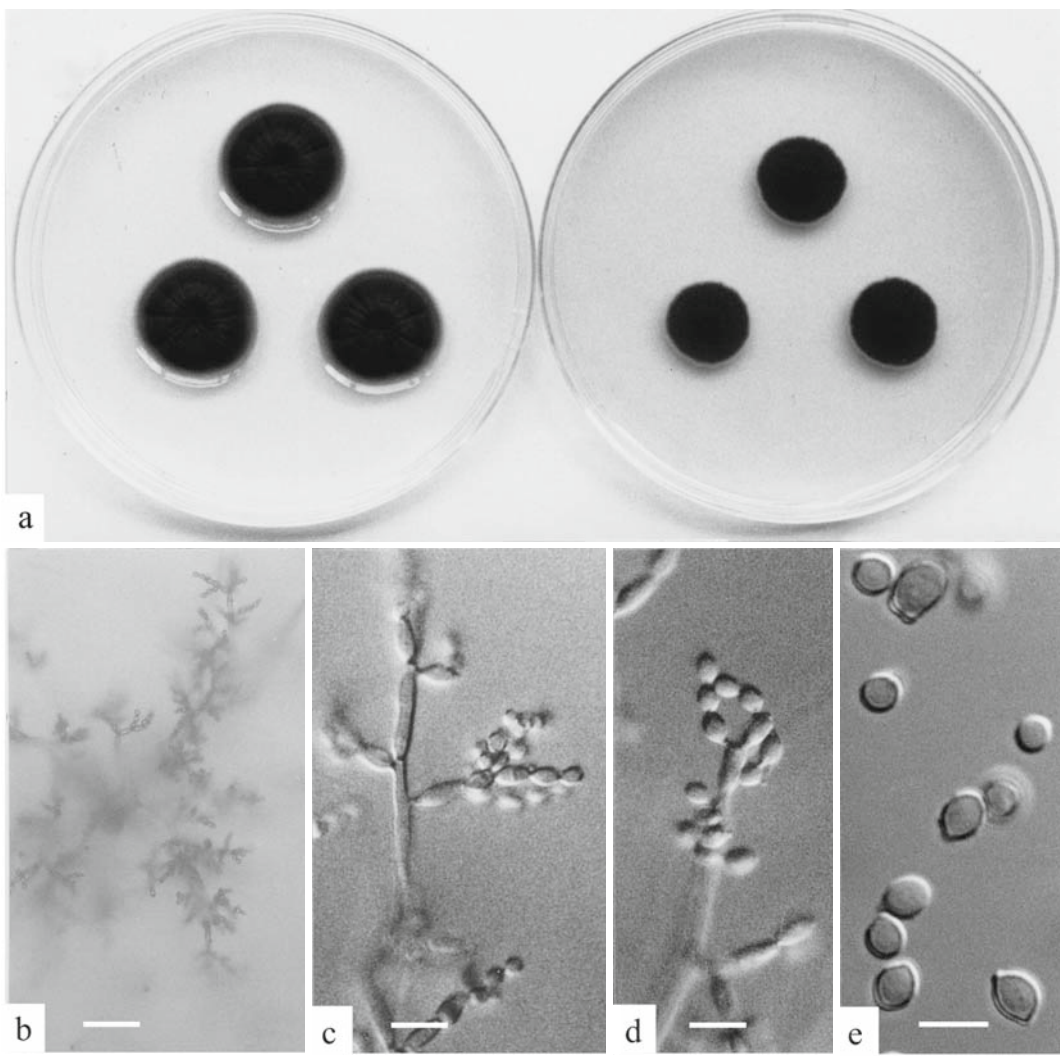


Fig. 5.15 *Cladosporium sphaerospermum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b)** fruiting structures in situ, bar = 50 µm; **(c,d)** conidiophores and conidia (slide culture), bar = 10 µm; **(e)** conidia, bar = 5 µm

rence. The concept of *C. sphaerospermum* as used here remains unchanged.

Physiology. *Cladosporium sphaerospermum* has an optimum a_w for growth near 0.97 at 25°C. This species is a xerophile, able to germinate and grow slowly at 0.815 a_w (Hocking et al., 1994). No other physiological studies have been reported.

Mycotoxins. No mycotoxins are known to be produced.

Ecology. Although less common than *Cladosporium cladosporioides, C. sphaerospermum* has been isolated from a wide range of foods, including barley and peanuts in Egypt, US pecan nuts, European meat products, spoiled cheese in Europe and Australia, stored apple fruit in Turkey (see Pitt and Hocking, 1997) and carbonated soft drinks in Nigeria (Odunfa, 1987) and Argentina (Ancasi et al., 2006) . We have isolated this species at low frequency from peanuts from Thailand (Pitt et al., 1993), rice and soybeans from the Philippines, and maize and mung beans from Indonesia (Pitt et al., 1998a).

References. Zalar et al. (2007).

5.12 Genus *Colletotrichum* Corda

Colletotrichum is a genus with many species, widespread as plant pathogens. In this genus, conidia are borne inside an *acervulus*, a structure with a more or less closed lid which eventually ruptures. Acervuli are readily seen on the agar surface with the unaided eye or by low power microscope. Conidia of *Colletotrichum* are single celled and hyaline or brightly coloured. They may be cylindrical or pointed, straight or curved.

A number of *Colletotrichum* species cause spoilage of fresh fruits and vegetables. Examples include *C. acutatum* J.H. Simmonds on strawberries, *C. circans* (Berk.) Voglino on leeks and onions, *C. coccodes* (Wallr.) S. Hughes on potatoes, tomatoes and eggplants, *C. higginsianum* Sacc. on cabbage and other cucurbits, *C. lindemuthianum* (Sacc. and H. Magn.) Broome and Cavara on beans and *C. musae* (Berk. and Curtis) Arx on bananas (Snowdon, 1990, 1991).

One species, *Colletotrichum gloeosporioides*, a common cause of spoilage of fruits, especially from the tropics, is treated here as an example of this rather ill-defined though important genus. In the opinion of Sutton (1980), *C. gloeosporioides* as currently accepted is an agglomerate of several species with a similar appearance. The species description given below is taken from a limited number of isolates and undoubtedly does not cover the full range of variation.

Colletotrichum gloeosporioidides (Penz.) Sacc. Fig. 5.16

Teleomorph: *Glomerella cingulata* (Stonemason) Spauld. & Schrenk.

On CYA and MEA, colonies 60 mm diam or more, often covering the whole Petri dish, with a dense basal layer of hyphae and conidial fruiting bodies (acervuli) overlaid by areas of floccose white, orange or grey mycelium; acervuli up to 500 μm long, pale, grey or orange; reverse with pale grey or orange areas. On G25N, colonies 2–5 mm diam, pale or black. At 5°C, no growth to germination. No growth at 37°C.

Reproductive structures flat, lidded acervuli, opening irregularly, containing a single closely packed layer of phialides, of irregular dimensions; conidia borne singly, cylindroidal, with rounded ends, nonseptate, 12–18 × 3–3.5 μm, hyaline and smooth walled.

Distinctive features. In the present context, *Colletotrichum gloeosporioides* is distinguished by producing conidia in acervuli and by pathogenicity on tropical fruits; conidia are aseptate cylinders with rounded ends, 12–18 μm long.

Taxonomy. This species as currently delimited is probably too broadly defined (Sutton, 1980). The teleomorph of *Colletotrichum gloeosporioides*, *Glomerella cingulata* (Stonemason) Spauld. and Schrenk., is not found in foods.

Physiology. *Colletotrichum gloeosporioides* was insensitive to storage in 10–13% CO_2 in the presence of 3–7% O_2 (Wade et al., 1993).

Mycotoxins. Mycotoxins are not known to be produced.

Ecology. According to Sutton (1980), the International Mycological Institute Herbarium has records of *Colletotrichum gloeosporioides* from 470 different host genera – a remarkable host range, even taking into account that several biological species may be involved.

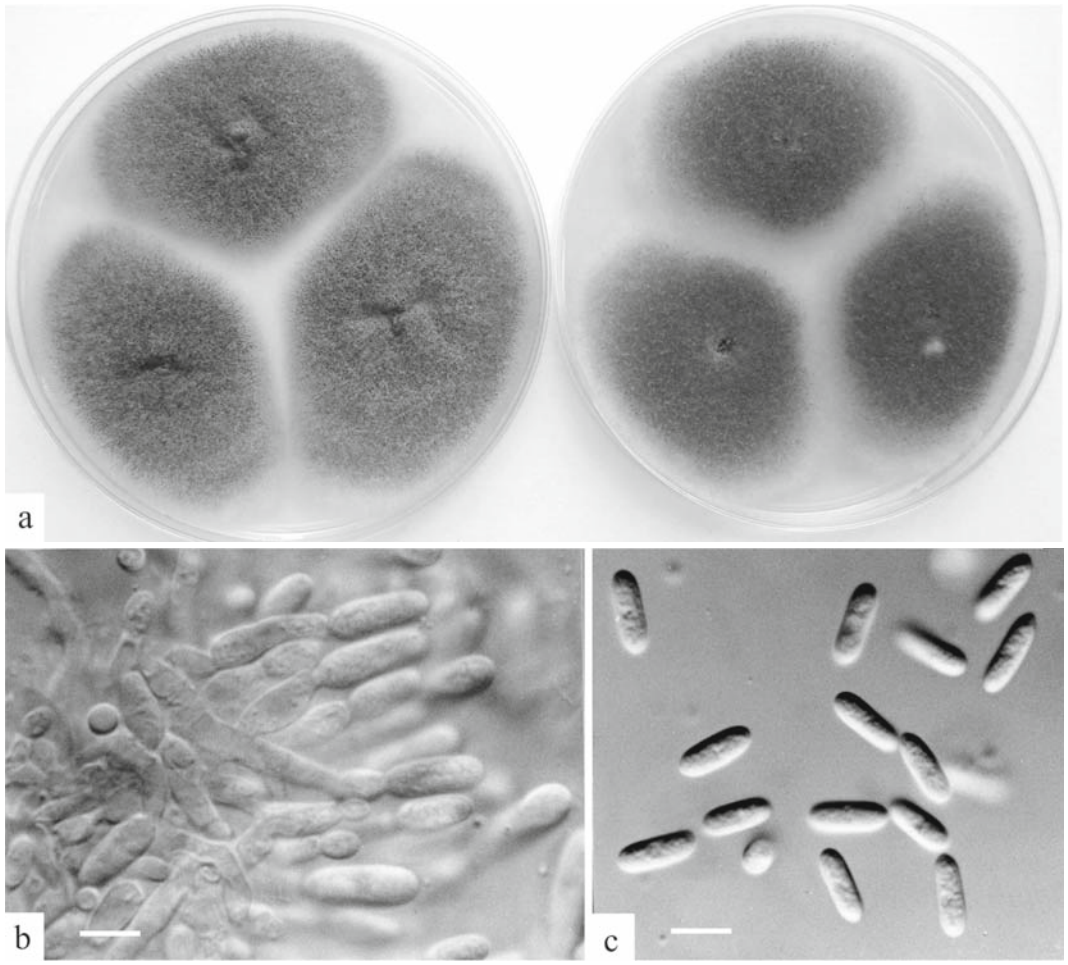


Fig. 5.16 *Colletotrichum gloeosporioides* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b)** immature conidiophores, bar = 10 µm; **(c)** conidia, bar = 5 µm

Colletotrichum gloeosporioides and closely related species cause anthracnoses of tropical fruits, including avocados, bananas, pawpaws (papayas) and mangoes, and of temperate fruits, including apples, stone fruits and grapes. *Colletotrichum* species cause damage to bananas (*C. musae*), strawberries (*C. acutatum*), grapefruit, tomatoes and other fruits (Snowdon, 1990; Pitt and Hocking, 1997).

Anthracnoses on fruit are dark, relatively dry, shrunken skin blemishes which expand rapidly as the fruit ripens. Except in advanced stages, the blemishes are only skin deep, and the fruit is edible, if unsightly. Advanced lesions may develop pinkish masses of conidia. Control in bananas is generally possible with benzimidazole or similar fungicides; in mangoes, pawpaws and avocados, hot water dips, with or without fungicide, can be beneficial (Smoot and Segall, 1963; Hall and Scott, 1977). Gamma radiation can be used to control *C. gloeosporioides* in pawpaws (papayas) (Cia et al., 2007).

Colletotrichum species were isolated from 40% of paddy rice samples examined from Thailand (Pitt et al., 1994). *C. dematium* was found at low levels in several Southeast Asian commodities, including soybeans, cowpeas, peanuts and black pepper (Pitt et al., 1998a).

References. Von Arx (1957); Mordue (1971); Sutton (1980); Gunnell and Gubler (1992).

5.13 Genus *Curvularia* Boedijn

In *Curvularia*, conidia are long and ellipsoidal, with three to four transverse septa. As the name implies, conidia are often curved due to an asymmetrically

swollen central cell. Most species are plant pathogens. The species most common in foods is *Curvularia lunata*; *Curvularia pallescens* is described here as a second foodborne species.

Key to *Curvularia* species included here

1	Mycelium on CYA and MEA grey to dark grey; reverse often blue black; conidia with central cells dark-walled	*C. lunata*
	Mycelium on CYA and MEA pale to mid grey; reverse brown; all cells in conidia with uniform wall colour	*C. pallescens*

Curvularia lunata (Wakker) Boedijn Fig. 5.17

Teleomorph: *Cochliobolus lunatus* R.R. Nelson & F.A. Hassis

On CYA and MEA, colonies at least 60 mm diam, often covering the whole Petri dish, usually deep, moderately dense and floccose, mycelium off-white to grey, often approaching black, in age sometimes developing orange or salmon coloured areas; reverse usually grey to bluish black, sometimes with areas of salmon. On G25N, colonies 5–15 mm diam, low and dense, grey to black with reverse similar. At 5°C, usually germination. At 37°C, colonies (5–)20–40 mm diam, of similar appearance to those at 25°C.

Colonies on DCMA 55–65 mm diam or covering the whole Petri dish, plane, sparse, velutinous, reverse brown or dark grey.

Conidia, best seen in mounts from growth close to the agar surface on MEA or on DCMA, borne from pores along the sides of short knobby conidiophores, elongate, smooth walled, with three septa, almost always curved at an asymmetric cell third from the base, 16–25(–30) × 8–14 µm, end cells pale brown, central cells darker.

Distinctive features. *Curvularia lunata* is the species of *Curvularia* commonly isolated from foods. It is distinguished from *C. pallescens* by darker colonies, a blue black reverse, and nonuniform pigmentation in conidial walls. Conidial production usually occurs on MEA, but culturing on DCMA and/or under lights may assist recognition if MEA plates are sterile.

Taxonomy. When grown in pairs, some isolates of this species mate to produce an ascomycetous state, *Cochliobolus lunatus* R.R. Nelson and F.A. Haasis. This state is not encountered in agar culture of single isolates.

Physiology. *Curvularia lunata* was able to germinate at 0.86 a_w, but only grew down to 0.89 a_w at 25°C (Hocking et al., 1994).

Mycotoxins. No reliable reports of mycotoxin production by this species are known to us.

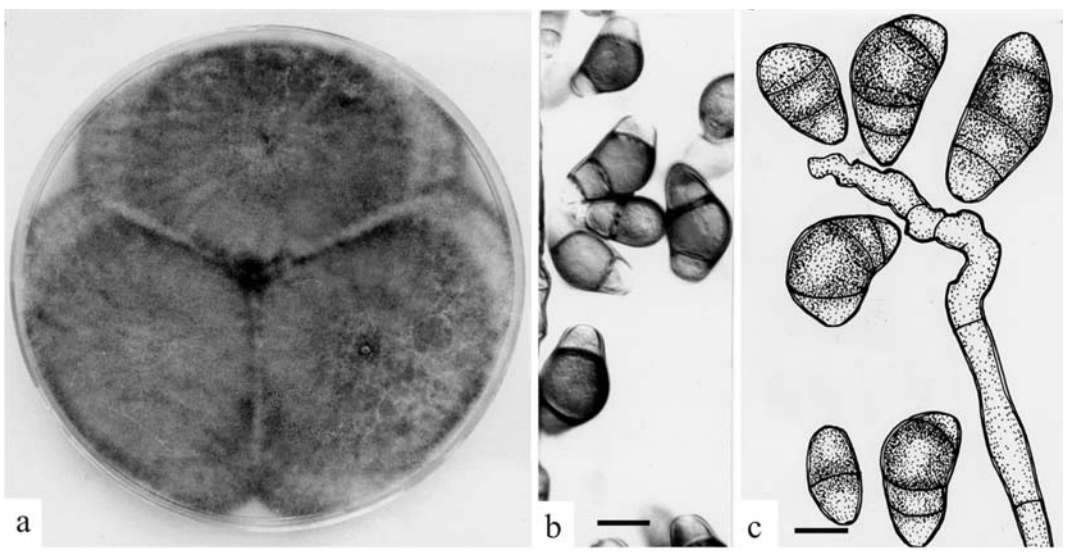


Fig. 5.17 *Curvularia lunata* **(a)** colony on CYA, 7 d, 25°C; **(b, c)** conidiophore and conidia, bar = 10 µm

Ecology. As *Curvularia lunata* is primarily an invader of monocotyledon plants (Domsch et al., 1980), the most common food sources are cereals: records include rice, barley, wheat, maize and sorghum (Pitt and Hocking, 1997; Mtisi and McLaren, 2003; Echemendia, 2005, Fakhrunnisa et al., 2006). It has been reported as one cause of spoilage of fresh tomatoes in Nigeria (Muhammad et al., 2004) and has also been found on litchi fruit (Wells et al., 1981), soybeans (Ahammed et al., 2006), hazelnuts and walnuts (Abdel-Hafez and Saber, 1993), peanuts and spices (see Pitt and Hocking, 1997).

In Thailand, *Curvularia lunata* is a major invader of sorghum: we isolated it from 56% of samples and 8% of all grains examined. It was also prevalent in paddy rice samples, where 28% of samples and 3% of all grains examined were infected (Pitt et al., 1994). It was found in 9–10% of paddy rice samples from Indonesia and the Philippines (Pitt et al., 1998a and unpublished data).

Additional variety. *Curvularia lunata* var. *aeria* (Bat.) M.B. Ellis is a floccose taxon accepted as a variety of *C. lunata*. Colonies of *C. lunata* var. *aeria* on CYA 50–65 mm diam, plane, floccose, of pale to mid grey mycelium, reverse deep blue black. Colonies on MEA covering the whole Petri dish, plane, deeply floccose, especially so at colony to colony and colony to Petri dish junctions, mycelium white to orange grey, reverse yellow brown, dark brown or blue black. Colonies on G25N 4–6 mm diam, of dark grey mycelium. No growth at 5°C. At 37°C, colonies 50–65 mm diam, similar to those on CYA at 25°C, sometimes less floccose, reverse dark blue black. Colonies on DCMA 55–65 mm diam, plane, sparse, floccose, especially at colony junctions, reverse brown or dark grey. Conidia are similar to those of *C. lunata*.

The variety is distinguished from *Curvularia lunata* var. *lunata* by a more floccose growth habit and faster growth at 37°C. It occurs in similar foods and commodities to *C. lunata*, the highest level we have encountered being 2% of all Thai rice grains and 1% of all Thai sorghum grains examined (Pitt et al., 1994).

References. Ellis (1971); Domsch et al. (1980); Sivanesan (1987).

Curvularia pallescens Boedijn Fig. 5.18

Colonies on CYA 50–65 mm diam, on MEA covering the whole Petri dish, plane, of low to floccose mycelium, pale grey to mid grey, reverse brown to dark brown. On G25N, colonies 3–6 mm diam, brown or grey. At 5°C, usually germination. At 37°C, colonies 40–50 mm diam, of floccose grey mycelium, sometimes brown soluble pigment, reverse dark brown.

On DCMA, colonies 50–65 mm diam, plane, with pale brownish grey to mid brown mycelium, reverse reddish brown to dark brown.

Conidia borne from pores in geniculate hyphae, ellipsoidal, with three septa, sometimes inconspicuous, almost straight along one side but with the eccentric swelling of the penultimate cell character-

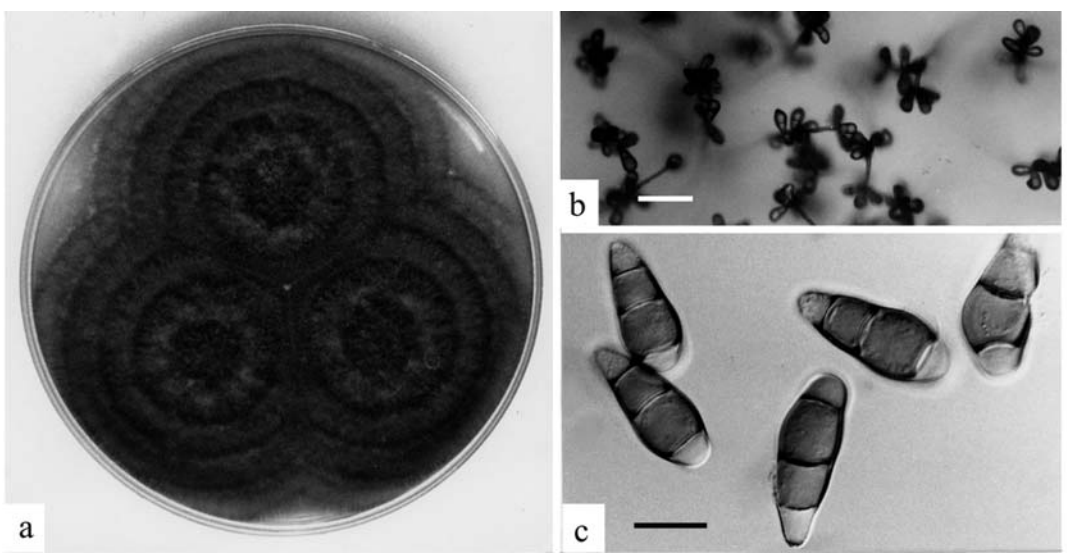


Fig. 5.18 *Curvularia pallescens* **(a)** colony on MEA, 7 d, 25°C; **(b)** fruiting structures in situ**,** bar = 50 µm; **(c)** conidia, bar = 10 µm

istic of the genus, 18–25 × 9–12 μm, with walls smooth, pale to mid brown, and with all cells in each conidium of a similar colour.

Distinctive features. This species lacks the abrupt curvature in the conidia observed in other foodborne species; conidia are relatively light in colour and characterised by an even colour in all cells. Colony reverses on CYA and MEA are brown, not blue black.

Physiology. Like *Curvularia lunata, C. pallescens* was able to germinate at 0.86 a_w, but only grew down to 0.89 a_w at 25°C (Hocking et al., 1994).

Mycotoxins. This species is not known to produce mycotoxins.

Ecology. The major known source of *Curvularia pallescens* is sorghum (Pitt et al., 1994; Ishrat et al., 2005). However, it has also been recorded on rice (Pitt et al., 1994; Gutierrez et al., 2002) and causing rots in melons (Sharma et al., 2002).

References. Ellis (1971); Sivanesan (1987).

5.14 Genus *Drechslera* S. Ito

Shoemaker (1959) revised the genus *Drechslera*, provided a clear separation from *Helminthosporium*, and erected *Bipolaris*. Leonard and Suggs (1974) segregated *Exserohilum* from *Bipolaris*. These three genera produce long, large conidia with transverse septa, which are much thicker than those in e.g. *Trichoconiella*. In *Drechslera*, conidia are cylindrical and germinate at any cell, while in *Bipolaris* and *Exserohilum*, conidia narrow gradually and germinate only from the terminal cells.

Drechslera species are serious pathogens on cereal plants, and a number of species have been recorded to occur on grains from time to time. However, they appear to have little or no role in food spoilage. Species are mostly distinguished by differences in spore size; one, *Drechslera tritici-repentis*, is treated here as an example.

Drechslera tritici-repentis (Diedicke) Shoemaker Fig. 5.19

Teleomorph: *Pyrenophora tritici-repentis* (Diedicke) Drechsler

Colonies on CYA 55–70+ mm diam, sometimes covering the Petri dish, of low to deep, floccose, pale to mid grey mycelium, sometimes lightly sporing in upper mycelial layers; exudate and soluble pigment absent; reverse dark grey to blue black. Colonies on MEA covering the Petri dish, similar to on CYA but sporulation usually absent; reverse brownish grey to bluish black. Colonies on G25N 4–8 mm diam, of sparse, pale grey mycelium. At 5°C, sometimes germination. At 37°C, no growth.

Colonies on DCMA when grown under lights 45–70+ mm diam, similar to those on CYA, sometimes lightly sporing; reverse pale to dark grey.

Conidiophores 150–250(–400) μm long, straight or sinuous, sometimes knobby terminally, bearing solitary conidia; conidia cylindroidal, commonly 70–120 × 14–17 μm, with five to seven septa, brown, smooth walled.

Distinctive features. See genus preamble.

Taxonomy. The teleomorph of this species is the Ascomycete *Pyrenophora tritici-repentis* (Diedicke) Drechsler. This is not usually seen in pure culture; sometimes immature perithecia may be formed (Sivanesan, 1987).

Physiology. No physiological studies on *Drechslera* species are known to us. In common with most other plant borne dematiaceous Hyphomycetes, growth is unlikely to occur below about 0.90 a_w. Species appear to be mesophilic.

Mycotoxins. No mycotoxins are known to be produced.

Ecology. *Drechslera tritici-repentis* is one cause of "pink tip" in wheat. We have isolated it from 56% of 344 surface disinfected North American wheat samples, where it was present at up to 27% in infected samples and 2% of all grains examined (unpublished data). Occurrence in Australian wheat was much lower (5% of samples; unpublished data) and it was not isolated from Southeast Asian commodities (Pitt et al., 1994, 1998a).

Several other *Drechslera* species have been reported from grains and seeds. However, most records examined refer to species now included in *Bipolaris* or, less commonly, *Exserohilum*. Only *D. sorokiniana* (Sacc.) Shoemaker, from German durum wheat (Nirenberg et al., 1995) relates with certainty to a *Drechslera* species.

References. Alcorn (1983); Sivanesan (1987).

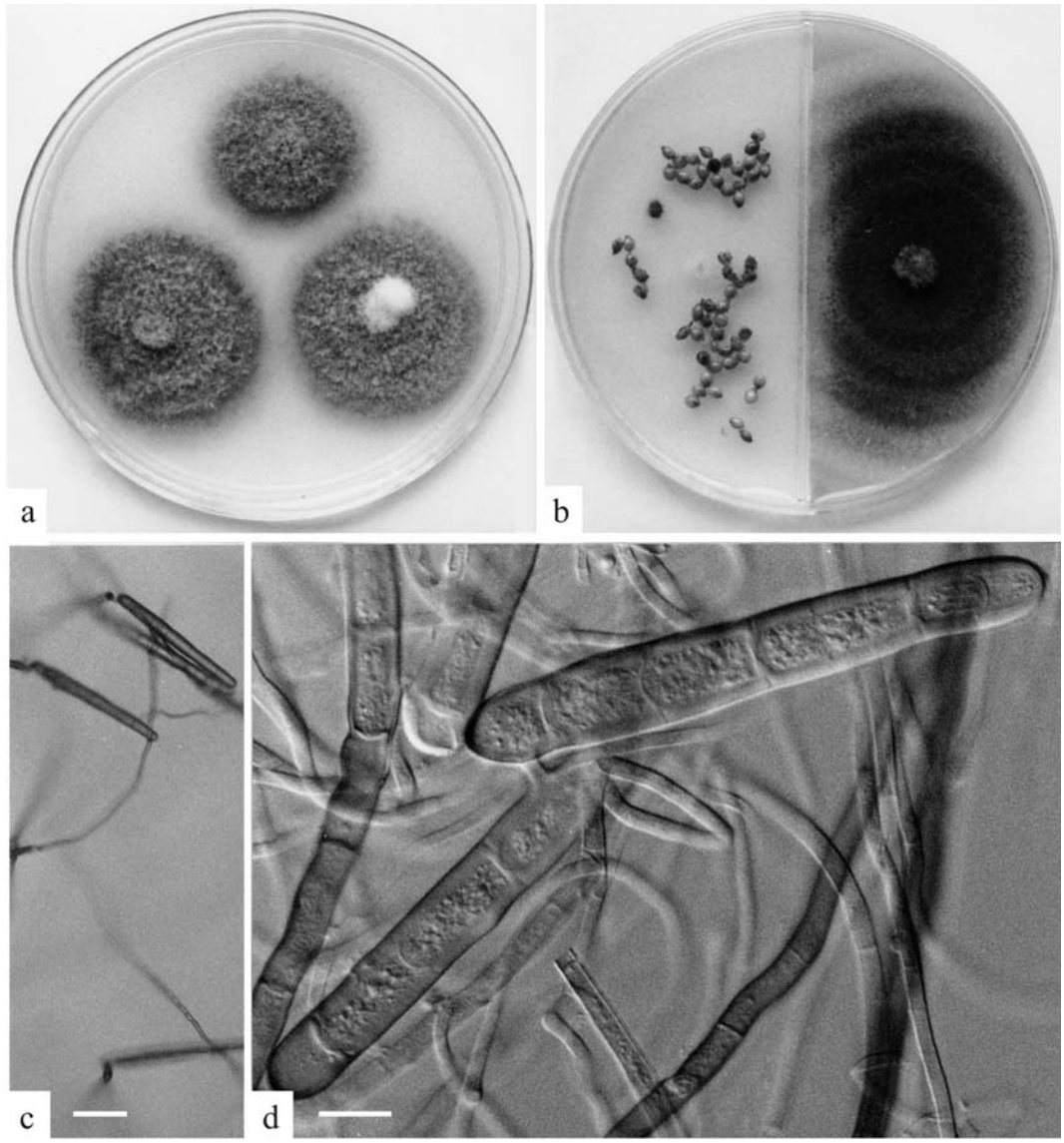


Fig. 5.19 *Drechslera tritici-repentis* **(a)** colony on MEA, 7 d, 25°C; **(b)** colonies on tap water agar plus millet seed and on V-8 juice, 7 d, 25°C; **(c)** conidia in situ**,** bar = 50 µm; **(d)** conidia, bar = 10 µm

5.15 Genus *Endomyces* Reess

Endomyces is a genus of yeast-like Ascomycetes, indeed often classified with the yeasts, in *Saccharomycopsis* (Kurtzman and Fell, 1998; Barnett et al., 2000). However, Yamada et al. (1996) considered that differences in DNA from the ITS region are sufficient to consider *Endomyces* as a genus separate from *Saccharomycopsis*. As growth is filamentous and spreading on agar plates, their decision is followed here. The conidial state consists of yeast-like cells borne on spicules or small projections. The Ascomycete state occurs sometimes in culture: the production of unenclosed, evanescent asci is suggestive of a relationship with the true yeasts. The only species common in foods is *Endomyces fibuliger*. See also *Hyphopichia*, which appears to be a closely related genus.

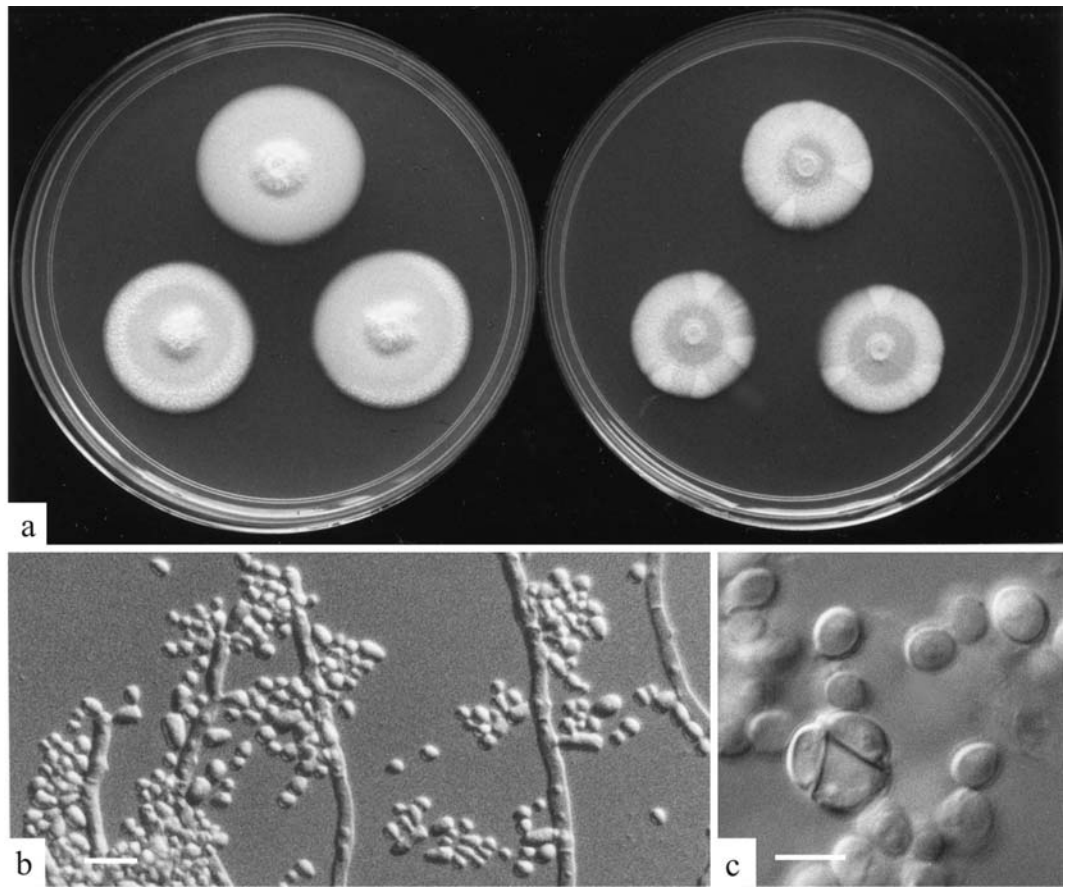


Fig. 5.20 *Endomyces fibuliger* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b)** conidiophores and conidia (slide culture), bar = 10 µm; **(c)** adherent ascospores, bar = 5 µm

Endomyces fibuliger Lindner **Fig. 5.20**

Endomycopsis fibuliger (Lindner) Dekker
Saccharomycopsis fibuligeria (Lindner) Klöcker

Colonies on CYA 18–30 mm diam and on MEA 15–25 mm diam, of low and sparse to moderately dense white to pale grey mycelium, sometimes centrally umbonate; reverse pale, off-white or very pale yellow. Colonies on G25N 8–15 mm diam, similar to those on CYA. No germination at 5°C. At 37°C, colonies usually 5–20 mm diam, similar to at 25°C; reverse off-white to dull yellow brown.

Conidia borne from spicules (small projections) along the length of undifferentiated hyphae, yeast-like, spherical, ellipsoidal or pyriform, 3–6 µm long, with thin, smooth walls. Ascospores sometimes produced in cultures on CYA or MEA, asci evanescent, ascospores observed singly or more commonly in tight clusters of 2–4, ellipsoidal, with a longitudinal flange, offset from the spore axis to give a "bowler hat" appearance, 7–8 µm long, including the flange.

Distinctive features. This species produces white, powdery, filamentous colonies which bear conidia from spicules on vegetative hyphae, and sometimes also by budding (yeast-like cells).

Taxonomy. This species has been described under both *Endomycopsis* and *Saccharomycopsis*; in the latter case the epithet is spelled "*fibuligera*". As explained above, we consider *E. fibuliger* to be the valid name. More recent taxonomies list this species under *Saccharomycopsis fibuligera* (Lindner) Klöcker (Kurtzman and Fell, 1998; Barnett et al., 2000) without taxonomic justification.

This species and *Hyphopichia burtonii* have a close resemblance in culture. Both grow at more or less the same rates and have similar habitats. The

fragmenting of fertile hyphae in *H. burtonii* is the only feature which provides a characteristic difference in morphology.

Physiology. The physiology of *Endomyces fibuliger* has not been studied in any detail. A minimum growth temperature around 5°C has been reported (Spicher, 1986b). *E. fibuliger* on wheat and rye bread was less affected by reduced O_2 levels than other spoilage moulds and its growth could not be prevented by modified atmosphere packaging (Suhr and Nielsen, 2005).

Mycotoxins. This type of fungus is unlikely to produce toxic compounds.

Ecology. This species is not uncommon in cereals and cereal products, especially packaged bread (Spicher, 1984a, 1985). In Europe, along with *Hyphopichia burtonii*, it is known as "chalky mould" of bread (Spicher, 1986b). It produces powerful amylases (Gogoi et al., 1987; Manilal et al., 1991). *Endomyces fibuliger* (*Saccharomycopsis fibuligera*) was found in fermented potato pulp used in an Indonesian starter Ragi tape (Abe et al., 2004) and in "Marcha", a traditional amylolytic starter used to produce sweet-sour alcoholic drinks in the Himalayan regions of India, Nepal, Bhutan and Tibet (Tsuyoshi et al., 2005).

References. Von Arx (1981b); Barnett et al. (1990; 2000).

5.16 Genus *Epicoccum* Link

Epicoccum is a Hyphomycete genus, a saprophyte or secondary invader of senescent plant tissue. It is characterised by the production of masses of large, spherical, stalked, irregularly septate conidia, borne on rapidly growing multicoloured colonies. *Epicoccum* is very widely distributed in the air, in soil and on decaying vegetation, one particular source being drying grass (Kilpatrick and Chilvers, 1981). Its ubiquity in the environment means it is commonly found on foods but it is an uncommon cause of spoilage. There is a single species, *Epicoccum nigrum*.

Epicoccum nigrum Link — Fig. 5.21

Epicoccum purpurascens Ehrenb. ex Schltdl.

Colonies on CYA 60 mm diam or more, often covering the whole Petri dish, low and dense or funiculose or floccose; mycelium orange brown, brown, or sometimes reddish or greenish, in fresh isolates enveloping or surmounted by brown black clusters of conidia, sometimes dominating colony appearance; clear to red brown exudate sometimes produced; reverse usually orange brown to black or with pink, red or green areas. On MEA colonies generally similar to on CYA, sometimes with a different colour combination, or occasionally with surface slimy. On G25N colonies 3–10 mm diam, low to deep, yellow to dark brown; reverse similar, sometimes with yellow soluble pigment. At 5°C, response variable, no growth to colonies up to 8 mm diam formed. No growth at 37°C.

Conidia borne solitarily on short conidiophores, usually in dense clusters, spherical with a broad, tapering, truncate base; brown, irregularly septate when mature, commonly 15–25 (–30) μm diam, with rough walls obscuring numerous septa.

According to Schol-Schwarz (1959), conidia on stems of sterile lupins measure 7–65 × 6–54 μm. As has been observed with other genera, conidia on agar media are much less variable in size and often smaller than on natural substrates.

Distinctive features. See the genus preamble.

Taxonomy. Kilpatrick and Chilvers (1981) examined the variability of 2000 isolates of *Epicoccum* and concluded that all belonged to a single, genetically variable species. A study of the 5.8S and ITS regions of DNA confirmed this (Wang and Guo, 2004). In early literature this species was usually known as *E. nigrum* Link. With the gradual acceptance of the 1821 starting date (See Chapter 3), this name was in time replaced by *E. purpurascens*. Now, with the reversion to the 1753 starting date, *E. nigrum* is again the correct name.

Physiology. According to Kilpatrick and Chilvers (1981), maximum growth rates vary widely among isolates of this species. However, optimal temperatures for growth are usually 20–25°C, with a maximum at 30–35°C and a minimum below 5°C. The optimum water potential for growth is –20 bars (0.98 a_w) and minimum –120 bars (0.91 a_w) (Kilpatrick and Chilvers, 1981).

Mycotoxins. Mycotoxin production has not been recorded.

Ecology. As noted in the genus preamble, *Epicoccum* is common in the general environment and hence readily finds its way onto foods such as cereals and nuts. It has been reported to cause spoilage of cantaloupes, in which it produces a

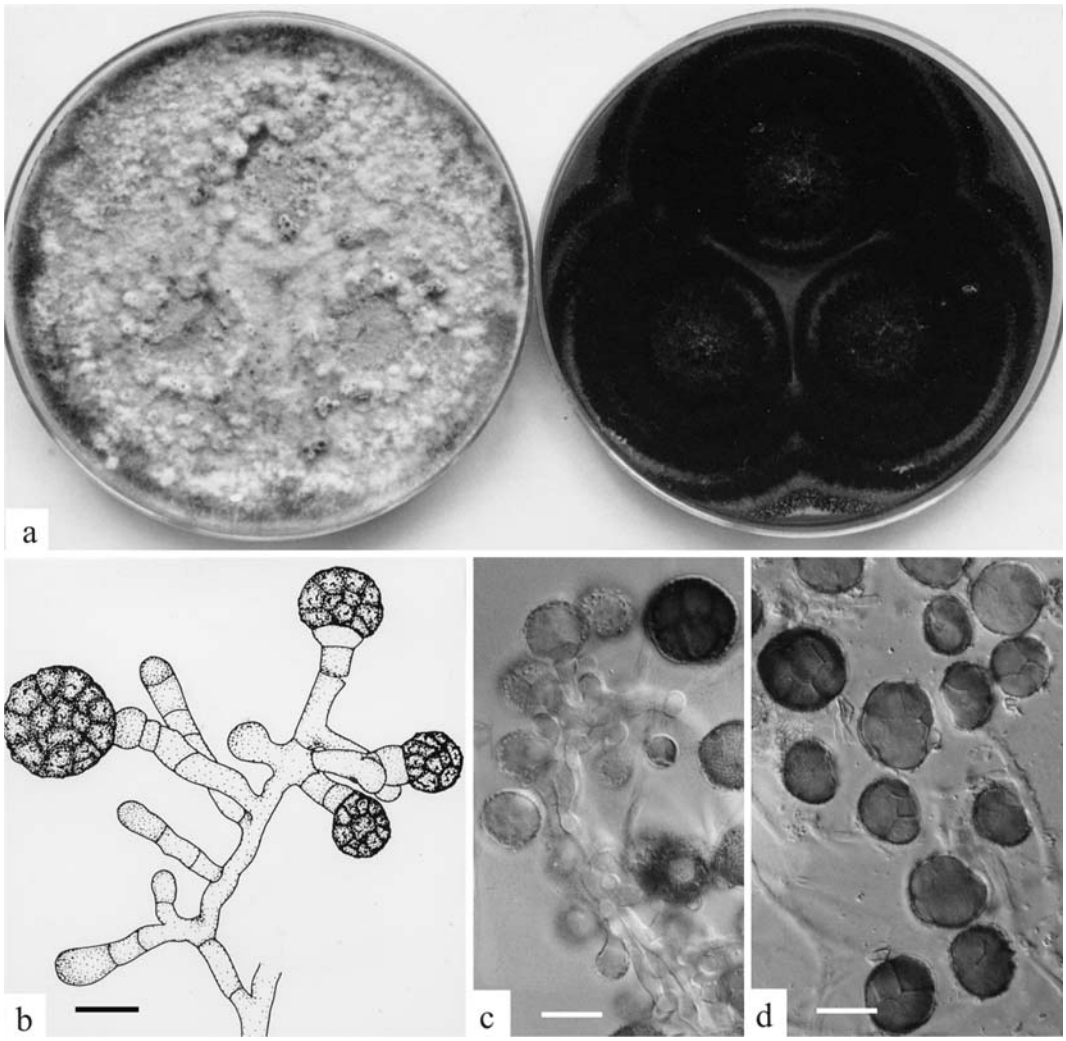


Fig. 5.21 *Epicoccum nigrum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b)** conidiophores and conidia, bar = 10 μm; **(c)** conidia, bar = 10 μm

red discolouration (Snowdon, 1991), and as a pathogen on cucumbers, tomatoes, apples and pears (Bruton et al., 1993). Among other fungi, *E. nigrum* can produce core rot of apples (Combrink et al., 1985). It has been associated with spoilage of pecans and is sometimes present in barley after harvest and during malting, but is not a cause of gushing (see Pitt and Hocking, 1997; Niessen et al., 1991; Hudec, 2007). *E. nigrum* has been reported on fresh vegetables, nuts and cereals. Other records include rice, wheat, maize and wheat; gourds and muskmelons, pecans, peanuts, frozen and cured meats and biltong (see Pitt and Hocking, 1997; Gros et al., 2003).

Epicoccum nigrum was isolated from 29% of soybean samples and 7% of cashew samples in Thailand, with up to 12% infection in infected soybean samples, 15% in cashews and 1% infection overall in both (Pitt et al., 1994).

References. Schol-Schwarz (1959); Domsch et al. (1980); Kilpatrick and Chilvers (1981).

5.17 Genus *Fusarium* Link

The character which defines the genus *Fusarium* is the production of septate, fusiform to crescent-shaped conidia, termed *macroconidia*, with a foot-

shaped basal cell and a more or less beaked apical cell. Macroconidia may be produced in discrete pustules, called *sporodochia*, or in confluent, slimy masses, known as *pionnotes*. Mounts from these areas, which are usually cream, salmon pink or orange, will reveal masses of these spores. However, they are rarely widely distributed over the colony and frequently are entirely absent. A light bank is invaluable for promoting formation of macroconidia in *Fusarium* cultures.

Many species of *Fusarium* also produce smaller one to two celled conidia, *microconidia*, of various shapes. *Chlamydoconidia*, either terminal or intercalary, are characteristic of some species also. *Fusarium* colonies are usually fast growing and consist of felty aerial mycelium which may be pale, or brightly coloured in shades of pink, red, violet or brown.

Fusarium species are renowned for their role as plant pathogens, causing a wide range of diseases such as vascular wilts, root and stem rots, pre- and post-emergence blight and many others. Leslie and Summerell (2006) include 70 species in their laboratory manual but the taxonomy of *Fusarium* is still undergoing change, with biological and phylogenetic criteria as well as morphological concepts used to delineate species (Leslie and Summerell, 2006). *Fusarium* species are widely distributed in soils, particularly cultivated soils, and are active in the decomposition of cellulosic plant materials. They are a major cause of storage rots of fruits and vegetables and are commonly associated with cereals and pulses, which they usually invade before harvest. De Nijs et al. (1996) have published a compendium of the occurrence of more than 60 *Fusarium* species in raw materials, foods and feeds but, in our experience, a relatively small number of species are important in food spoilage.

A number of *Fusarium* species have teleomorphs belonging to the genera *Nectria* Fr., *Calonectria* De Not. and *Gibberella* Sacc. These teleomorphs are perithecial Ascomycetes, and are generally not produced in culture. However, fertile strains of *F. solani* sometimes form reddish brown perithecia on PDA and *F. graminearum* usually produces dark purple perithecia on Carnation Leaf Agar. These teleomorphs are known as *Nectria haematococca* Berk. and Broome and *Gibberella zeae* (Schwein.) Petch, respectively (Booth, 1971).

Taxonomy. A bewildering array of taxonomic systems have been applied to *Fusarium*, ranging from the milestone work of Wollenweber and Reinking (1935), which distinguished 65 species, to the simplification by Snyder and Hansen, who accepted only 9 species in a series of papers in the 1940s. The interrelationships of the principal taxonomic schemes for *Fusarium* are discussed by Booth (1971), Nelson et al. (1983) and more recently by Leslie and Summerell (2006). In recent years, *Fusarium* taxonomy and nomenclature has undergone many changes, with molecular methods elucidating the existence of cryptic species within some of the broader species concepts. In addition, many new species have been described, often from natural ecosystems rather that economically important crops (see Leslie and Summerell, 2006). The nomenclature of *Fusarium* species described in this work follows that of Leslie and Summerell (2006) which is based on Nelson et al. (1983) which, in turn, is based on Wollenweber and Reinking (1935). Reference is made to the names used by Nelson et al. (1983) where they differ from those adopted by Leslie and Summerell (2006).

Mycotoxins. *Fusarium* is one of the three major fungal genera producing toxins. The most widespread *Fusarium* mycotoxins are the trichothecenes, a family of sesquiterpenes. More than 50 such compounds are known to be produced by species in this genus. Some are highly toxic: none appears to be benign. Trichothecenes are often produced as mixtures even under pure culture conditions and are very difficult to separate, so the toxicity of many compounds remains uncertain. The most important are deoxynivalenol (and sometimes nivalenol), produced by *F. graminearum, F. culmorum* and related species. Zearalenone, not a true mycotoxin, really an oestrogen analogue produced by fungi, is formed by the same species that make deoxynivalenol. T-2 toxin, produced primarily by *Fusarium sporotrichioides,* caused the deaths of many people and animals during the 20th century, but is of uncommon occurrence now. Some *Fusarium* species produce other types of toxic compounds. The most important of these are the fumonisins, produced by *F. verticillioides, F. proliferatum* and several other species. Fumonisins are sphingosine analogues, interfering with membrane function in man and animals (Miller and Trenholm, 1994). Other

less toxic or less important compounds, such as moniliformin and fusaric acid, are discussed briefly under the appropriate species.

Deoxynivalenol and nivalenol. Like all trichothecene mycotoxins, deoxynivalenol (DON) and nivalenol are inhibitors of protein synthesis (Feinberg and MacLaughlin, 1989). Nivalenol is much more toxic than DON, but is produced in much lower quantities in grains, and is considered a less significant mycotoxin (Miller et al., 2001). DON is especially toxic to pigs, where, at quite low intake levels (< 5 mg/kg in feed) it causes vomiting, feed refusal and reduced weight gain due to neurotoxic effects. Cattle and poultry are resistant to reasonable levels (> 5 mg/kg in feed) of DON (Miller et al., 2001).

In humans, high doses of DON may cause abdominal pains, dizziness, headache, nausea, vomiting and other effects. Cases of such acute poisoning are rare, but have been recorded from India (Bhat et al., 1989), Japan (Udagawa, 1988) and several outbreaks in China (JECFA, 2001). DON is also immunosuppressive, and because of its widespread occurrence in grains, is undoubtedly responsible for susceptibility to bacterial and viral diseases in man and animals (Miller et al., 2001).

The most comprehensive study of DON including occurrence, toxicity, analytical methods and intake levels is that by JECFA (2001). They concluded that DON is not significantly carcinogenic, mutagenic or teratogenic (JECFA, 2001). The Committee set a provisional tolerable daily intake of 1 μg/kg weight per day. They were unable to estimate the level of DON in foods below which any acute effect in humans would occur.

Zearalenone. Zearalonone is one of the five most significant mycotoxins (Miller, 1995). It is not a true mycotoxin, being a nonsteroidal oestrogen produced by a fungus. It is not acutely toxic and has not been associated with any fatal disorder in animals or humans. However, it has caused oestrogenic syndromes in pigs, and perhaps in human adolescents as well. Zearlaenone is produced by the same *Fusarium* species that produce deoxynivalenol, i.e. *F. graminearum* and related species, especially *F. culmorum, F. equiseti* and *F. crookwellense*. The association of these fungi with cereal crops is worldwide, as is the production of zearalenone (Marasas et al., 1984).

Pigs are especially sensitive to zearalenone. Signs of hyperoestrogenism generally appear at dietary levels > 1 mg/kg, but can occur at lower levels sometimes. In prepubertal gilts, clinical signs include vulval swelling, uterine enlargement and mammary development. Mature sows can develop ovarian atrophy, constant oestrus and pseudopregnancy (Hagler et al., 2001). Prepubertal male pigs can undergo a feminising effect with mammary development, decreased testicular size and loss of libido, but mature boars are resistant (Hagler et al., 2001).

It was once claimed that humans had shown similar signs in Puerto Rico, but an FDA investigation failed to confirm this (Goodman et al., 1987).

T-2 toxin. T-2 toxin is the cause of alimentary toxic aleukia (ATA) a devastating disease which occurred in the USSR during and after Second World War, in times of extreme food shortage resulting in consumption of overwintered cereals (Joffe, 1978; Beardall and Miller, 1994). Many people, probably hundreds of thousands, died as a result (Marasas et al., 1984). ATA was characterised by leucopoenia, bleeding from nose, throat and gums, haemorrhagic rash, exhaustion of the bone marrow and fever. Vomiting, nausea, diarrhoea and abdominal pain also usually occurred. Decrease in immunological functions led to susceptibility to bacterial and viral diseases, and often death (Joffe, 1978; Beardall and Miller, 1994). Haemorrhagic syndrome in cattle, pigs and poultry in the United States in the 1960s was also probably due to T-2 toxin (Desjardins, 2006).

T-2 toxin is produced by *F. sporotrichioides* and, less commonly, *F. poae* (Desjardins, 2006). It appears to be produced only under cold conditions, and fortunately is now uncommon. The occurrence, toxicity and biology of T-2 toxin were examined in detail by JECFA (2001). They established that T-2 (and its metabolite HT-2) were immunotoxic and haemotoxic compounds in several animal species after short-term intake. Long-term effects could not be evaluated. T-2 was at most weakly genotoxic. In the absence of long-term studies, T-2 was not classifiable as to carcinogenicity (JECFA, 2001).

Fumonisins. Fumonisins are produced by *F. verticillioides* (formerly known as *F. moniliforme*), by the closely related species *F. proliferatum,* uncommonly by *F. subglutinans* and *F. oxysporum*,

and about 10 other species not treated here. The first two named species are by far the most important. *F. proliferatum* is one cause of maize ear rot in Europe and is a pathogen on a variety of other crops. *F. verticillioides* is endemic in maize and found wherever that crop is grown. This species grows well at higher temperatures, and ear rot and fumonisin accumulation are associated with drought, insect stress and growing hybrids outside their areas of adaptation (Miller, 2001, 2008).

Fumonisins are analogues of sphingosines, essential components of cell membranes. Fumonisins have been reported to interfere specifically with dihydroceramide, an essential enzyme in the biosynthesis of sphingosine and related compounds (Wang et al., 1991). Fumonisins have been found to be biologically active in many animal species, but horses and pigs are the most affected. In horses, fumonisins are responsible for leucoencephalomalacia, a rapidly progressing disease that causes tissue liquefaction in equine brains. It has been known in the United States for more than 100 years that feeding maize to horses is a dangerous practice (Desjardins, 2006): it was not until 1988 that the reason became apparent (Gelderblom et al., 1988; Marasas et al., 1988). For horses, consumption of feed containing $>$ 10 mg/kg fumonisin B_1 in the diet (equivalent to 0.2 mg/kg body weight per day) was associated with increased risk of developing this disease (JECFA, 2001). In pigs, fumonisins cause pulmonary oedema, due to left ventricle heart failure (JECFA, 2001), while in rats the primary effect is to cause liver cancer (Gelderblom et al., 1991), but they also cause programmed cell death (apoptosis; Tolleson et al., 1996). It is unusual for a single toxin to have such diverse effects in different animal species, and the reasons for that remain unknown (Desjardins, 2006).

In humans, fumonisins and *Fusarium verticillioides* are associated with oesophageal cancer. Extensive studies in areas of low and high maize consumption in South Africa have established this connection (Rheeder et al., 1992; JECFA, 2001). This disease is also prevalent in areas of China and occurs at significantly higher levels than background also in parts of Iran, northern Italy, Kenya and a small area of the southern USA (JECFA, 2001). In all of those areas consumption of maize and maize products is very high (JECFA, 2001). There is also increasing evidence that fumonisins are associated with neural tube defects such as spinal bifida in a population along the Texas–Mexican border (Desjardins, 2006). For the human population, a provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg body weight per day was established by JECFA (2001).

Determining which particular *Fusarium* species produces which mycotoxins has not been easy. Unstable taxonomy and misidentification have combined to cloud the picture. In a major contribution, Marasas et al. (1984) examined several hundred *Fusarium* isolates, identifying them according to Nelson et al. (1983) and then assessing mycotoxin production. Sections on mycotoxins in the species descriptions which follow are based on Marasas et al. (1984), Desjardins (2006) and Leslie and Summerell (2006). Claims concerning mycotoxin production by particular species in more recent references should be treated with some caution.

Cultural instability. Many *Fusarium* species are notorious for their instability in culture. Isolates of some species will degenerate quickly, often after only one or two transfers. For this reason, it is important to identify Fusaria as soon as possible after primary isolation. Pure cultures for identification are traditionally started from a single germinated spore, as the mass transfer of Fusaria appears to increase the rate of deterioration of strains in culture.

Identification procedures. Identification of *Fusarium* isolates is often difficult, but the task can be made easier by observing a few basic rules:

- identify cultures as soon as possible after primary isolation;
- always grow cultures for identification from single germinated conidia;
- use standardised media and incubation conditions;
- use a light bank (Chapter 4) whenever possible.

Diagnostic features. The main characters used to distinguish species of *Fusarium* are (1) the size and shape of the macroconidia; (2) the presence or absence of microconidia; (3) the manner in which microconidia are produced; (4) the type of phialide on which microconidia are produced; (5) the presence or absence of chlamydoconidia; and (6) the colours and morphology of colonies on PDA.

The morphology of macroconidia is a principal diagnostic feature for *Fusarium* species. Macroconidia generally have at least three septa, with a differentiated apical cell which may be pointed, rounded, hooked or filamentous, and a basal cell which may be foot-shaped, with a distinct heel, or just slightly notched. *Fusarium* macroconidia generally exhibit some degree of curvature, the convex and concave sides being referred to as the dorsal and ventral sides, respectively. Although some macroconidia are usually produced in the aerial mycelium, the shape and size of those in sporodochia are more regular and are used for identification purposes where possible.

Microconidia are usually produced in the aerial mycelium and their shape can be very important in *Fusarium* identification. Most species which produce microconidia form only a single type, the most common shape being ellipsoidal to clavate. However, *F. poae* produces spherical to apiculate microconidia and *F. sporotrichioides* produces a variety of shapes: ellipsoidal, pyriform and spherical. The method of production of microconidia and the types of phialides on which they are borne are also useful diagnostic criteria. *F. verticillioides* produces its microconidia in long, delicate, dry chains, which are best observed by using the 10× objective of the compound microscope. Some species produce microconidia in *false heads* (small, mucoid, adherent balls of conidia) and others produce them singly. In some species, microconidia are produced on phialides with only one pore, and these are termed *monophialides,* but a few species produce phialides with more than one pore (*polyphialides*). Species which produce polyphialides usually produce monophialides as well.

Descriptions. Descriptions of the microscopic features of species in this book are based on structures formed in cultures grown on DCPA at 25°C, with a 12 h photoperiod, under a light bank consisting of two cool white fluorescent tubes and one black light tube (see Chapter 4). Cultures should be examined at 7 days, then at 10 days and 14 days if sporulation is poor. Aerial mycelium often develops better after 10 days incubation on DCPA and chlamydoconidium production is more reliable in older cultures.

Descriptions of the colony characteristics are taken from cultures grown on PDA at 25°C for 7 days, also with a 12 h photoperiod. Additional information may be gained by recording the growth rates of colonies on PDA at 25°C and 30°C after 3 days (Burgess et al., 1994). Growth rates at 30°C on PDA are particularly helpful in distinguishing isolates of *Fusarium avenaceum* from those of *Fusarium acuminatum*.

Key to *Fusarium* species included here

1	Microconidia abundant	2
	Microconidia rare or absent	9
2 (1)	Colonies on PDA with mycelium and/or reverse coloured greyish rose or burgundy	3
	Colonies on PDA in shades of cream, pale salmon or violet	5
3 (2)	Microconidia spherical to apiculate, borne singly on monophialides	*F. poae*
	Microconidia ellipsoidal, clavate, fusiform and/or pyriform, borne on polyphialides or both polyphialides and monophialides	4
4 (3)	Microconidia clavate only, produced profusely, giving colonies on PDA a powdery appearance	*F. chlamydosporum*
	Microconidia various shapes: clavate, pyriform and spindle shaped (check PDA cultures also)	*F. sporotrichioides*
5 (2)	Microconidia produced in long or short chains (some false heads may also be present)	6
	Microconidia produced singly or in false heads	7
6 (5)	Microconidia produced from monophialides only	*F. verticillioides*
	Monophialides and polyphialides both present	*F. proliferatum*

7 (5)	Colonies cream or bluish, sporodochia cream	*F. solani*
	Colonies pale salmon or violet, sporodochia salmon	8
8 (7)	Microconidia borne on short, stout monophialides; chlamydoconidia usually produced	*F. oxysporum*
	Microconidia borne on polyphialides and slender monophialides; chlamydoconidia not produced	*F. subglutinans*
9 (1)	Colonies cream, pale salmon or brown	10
	Colonies greyish rose to burgundy	11
10 (9)	Macroconidia cigar- or spindle-shaped, produced in the aerial mycelium	*F. semitectum*
	Macroconidia obviously curved, produced in sporodochia	*F. equiseti*
11 (9)	Macroconidia robust, ventral side straight; aerial mycelium tan to brown	12
	Macroconidia delicate and slender, slightly or definitely curved; aerial mycelium white or pinkish	13
12 (11)	Macroconidia short and stout, up to 7 μm wide	*F. culmorum* (See *F. graminearum*)
	Macroconidia longer and narrower, maximum width 5.5 μm	*F. graminearum*
13 (11)	Macroconidia with elongated basal cell and long, whip-like apical cell	*F. longipes*
	Macroconidia with basal and apical cells not obviously elongated	14
14 (13)	Macroconidia delicate and needle-like, with sides almost parallel	*F. avenaceum*
	Macroconidia with slight to definite curvature	*F. acuminatum*

Fusarium acuminatum Ellis & Everh. Fig. 5.22

Telemorph: *Gibberella acuminata* Wollenw.

Colonies on CYA 40–50 mm diam, of dense, felty mycelium, white to greyish rose or greyish magenta; reverse uniformly pale or with areas of greyish rose. Colonies on MEA 45–65 mm diam, yellow brown centrally, greyish rose at the margins; reverse deep brownish yellow to brownish orange, occasionally pale. Colonies on G25N 9–15 mm diam. At 5°C, colonies 7–12 mm diam. No growth at 37°C.

On PDA, colonies usually covering the whole Petri dish, of dense to floccose white to pale salmon mycelium, sometimes greyish rose at the margins; reverse dark ruby centrally, greyish ruby at the margins. On DCPA, colonies sparse, of floccose to funiculose white to pale salmon mycelium; reverse pale or with brownish red annular rings.

Macroconidia relatively slender, usually with five septa, but three and four septa not uncommon, with a long, tapering apical cell and foot-shaped basal cell, distinctly but not highly curved, with the widest point often one third of the distance from the base, giving a "bottom-heavy" appearance; microconidia produced sparsely by some isolates; chlamydoconidia produced, relatively slowly.

Distinctive features. Ruby to dark ruby reverse colours on PDA, and relatively slender, slightly curved macroconidia, usually with five septa, are the distinctive features of *Fusarium acuminatum*. However, unless chlamydoconidia are present, this species can be confused with *F. avenaceum* (see below). *F. armeniacum* is very closely related (Burgess and Summerell, 2000).

Taxonomy. Perithecia of *Gibberella acuminata* Wollenw., the teleomorph of *F. acuminatum*, are formed in the laboratory when opposite mating types are inoculated onto sterile wheat straws (Booth, 1971). Isolates of *F. acuminatum* show considerable variability in culture, and this variability, correlated with secondary metabolite production (Logrieco et al., 1992), has been shown to be due to the fact that two species were included within *F. acuminatum*. *F. armeniacum* (Forbes et al.) L.W. Burgess and Summerell, previously described as *F. acuminatum* var. *armeniacum* G.A.

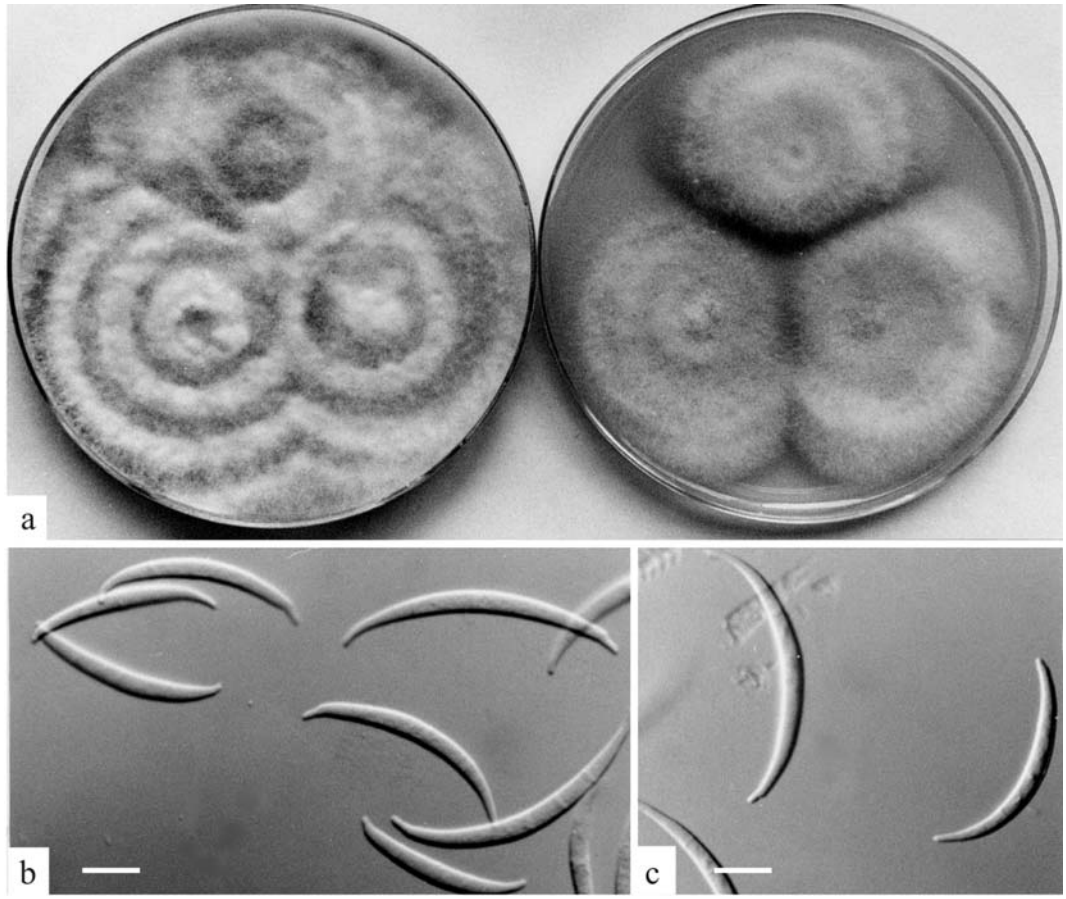


Fig. 5.22 *Fusarium acuminatum* **(a)** colonies on PDA and DCPA, 7 d, 25°C; **(b, c)** macroconidia, bar = 10 µm

Forbes et al. has been elevated to species level (Burgess and Summerell, 2000) being distinguished on morphological grounds, isoenzyme patterns, molecular markers and mycotoxin profiles. Macroconidia of *F. armeniacum* are produced in distinct apricot coloured sporodochia.

Physiology. Some isolates of *Fusarium acuminatum* have antioxidant enzyme activity (Kayali and Tarhan, 2005).

Mycotoxins. Most of the mycotoxin production reported from *Fusarium acuminatum*, particularly the production of trichothecene toxins, is probably more correctly due to *F. armeniacum* (Desjardins, 2006; Leslie and Summerell, 2006). Isolates producing type A trichothecenes including T-2 toxin, HT-2 toxin and diacetoxyscirpenol (see Pitt and Hocking, 1997) are most likely to be *F. armeniacum*. In a survey of 25 isolates of *F. acuminatum* from different sources and geographic locations, Logrieco et al. (1992) divided the isolates into three categories, (a) enniatin B and/or moniliformin producers (probably *F. acuminatum* sensu stricto), (b) T-2, HT-2 and/or neosolaniol producers (probably *F. armeniacum*) and (c) nontoxigenic. *F. acuminatum* sensu stricto produces moniliformin (Chelkowski et al., 1990; Logrieco et al., 1992) and enniatins (Logrieco et al., 1992; Kononeko et al., 1993; Desjardins, 2006; Leslie and Summerell, 2006) as well as some other minor toxins (Desjardins, 2006).

Ecology. *Fusarium acuminatum* has been isolated from a wide variety of plants throughout the world. Although some isolates may cause severe root rot in particular legume species (Leslie and Summerell, 2006), *F. acuminatum* is generally regarded as a saprophyte. It has been reported to cause rot in pumpkins (Elmer, 1996), is one cause of rot in stored potatoes (Theron and Holz, 1990) and is weakly pathogenic in

bananas (Jiménez et al., 1993). It is quite common in poor quality wheat from cool temperate zones (Mills and Wallace, 1979; Abramson et al., 1987). It has been isolated from developing peanut pods (Barnes, 1971), barley (Abdel-Kader et al., 1979) and, in our laboratory, from rain damaged sorghum and soybeans. The incidence of *F. acuminatum* in tropical commodities was low (Pitt et al., 1993, 1994).

References. Domsch et al. (1980), under *G. acuminata*; Nelson et al. (1983); Desjardins (2006); Leslie and Summerell (2006).

Fusarium avenaceum (Fr.) Sacc. Fig. 5.23

Teleomorph: *Gibberella avenacea* R.J. Cooke

Colonies on CYA covering the whole Petri dish, moderately deep to deep, of open, floccose mycelium coloured white, very pale rose or deeper greyish rose; reverse varying from pale to pale yellow or with areas of greyish rose or sometimes uniformly deep burgundy. Colonies on MEA 45–55 mm diam, low to moderately deep, of open floccose to funiculose mycelium, coloured white, pale rose or greyish rose, sometimes brown centrally; reverse brownish orange, sometimes paler centrally or at the margins. Colonies on G25N 9–15 mm diam. At 5°C, colonies 10–12 mm diam. No growth at 37°C.

On PDA, colonies moderately deep to deep, of dense mycelium coloured white, pale salmon or sometimes dark brownish red, with central masses of reddish orange sporodochia, sometimes surrounded by an outer ring of paler sporodochia; reverse greyish red, with darker annular rings, paler towards the margins. On DCPA, colonies deep, of moderately dense white to pale salmon mycelium with a central mass of orange to

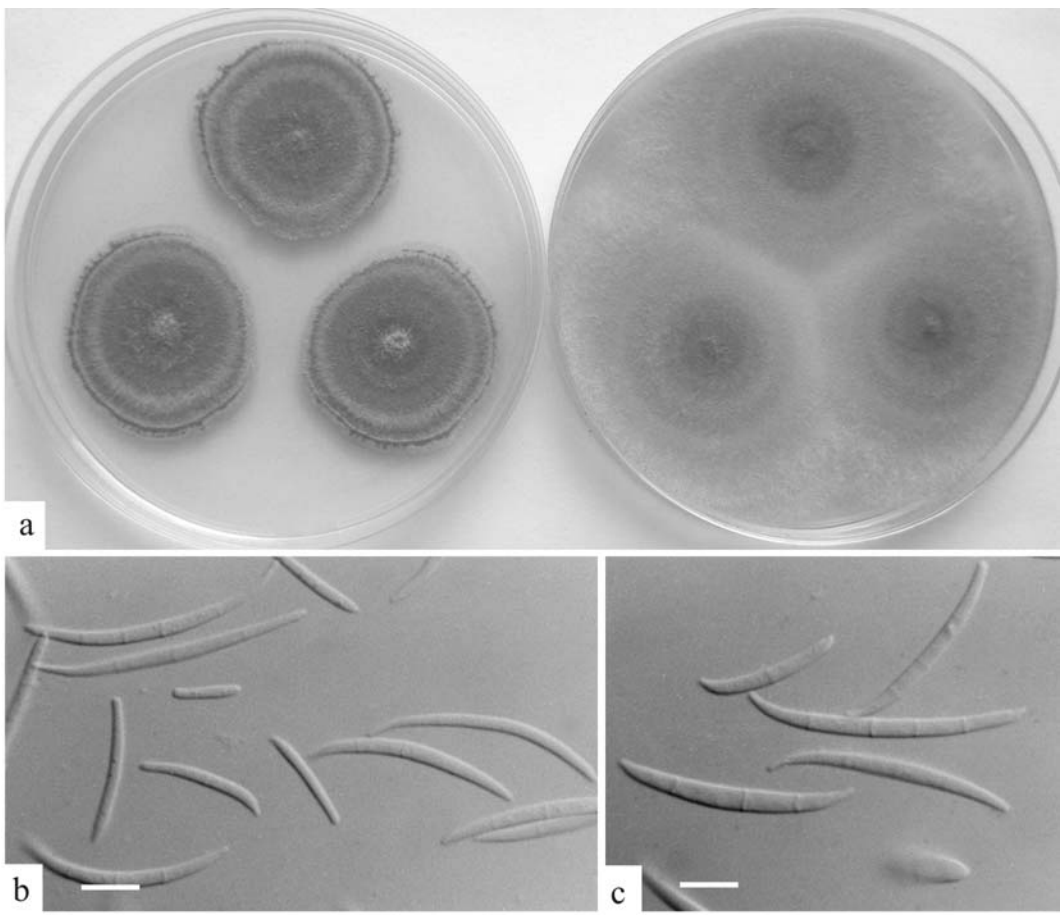


Fig. 5.23 *Fusarium avenaceum* **(a)** colonies on PDA and DCPA, 7 d, 25°C; **(b, c)** macroconidia, bar = 10 µm

salmon sporodochia, often surrounded by concentric rings of sporodochia; reverse pale.

Macroconidia long, slender, with four to seven septa, thin-walled, straight or slightly curved, with a tapering apical cell and a notched or foot-shaped basal cell; microconidia produced sparsely by some isolates; chlamydoconidia absent.

Distinctive features. *Fusarium avenaceum* is distinguished by thin walled, needle-like macroconidia and by the absence of chlamydoconidia. Despite the fact that *F. avenaceum* and *F. acuminatum* are not considered by *Fusarium* taxonomists to be closely related, these two species can be difficult to distinguish, as isolates with macroconidia of intermediate form are not uncommon. Colony diameters on PDA at 30°C after 3 days can be a useful differentiating feature: under these conditions colonies of *F. avenaceum* are usually 8–15 mm diam, whereas those of *F. acuminatum* are 15–28 mm diam (Burgess et al., 1994). Isolates of *F. avenaceum* show an unusually broad range of colours on PDA and also have a very broad host range. However, extensive genetic analysis has shown no bases from splitting the species, and pathogenicity tests on single strains have confirmed the broad host range (Nalim, 2004).

Taxonomy. The teleomorph of *Fusarium avenaceum* is *Gibberella avenacea* R.J. Cook. It is not usually seen in culture on the media used here.

Physiology. The optimum growth temperature for *Fusarium avenaceum* is 35°C, the minimum near –3°C and the maximum 31°C (Domsch et al., 1980). The minimum a_w for growth is approximately 0.90 at 25°C (Magan and Lacey, 1984c), and the pH optimum ranges between 5.4 and 6.7 (Domsch et al., 1980).

Mycotoxins. This species has been reported to produce a variety of trichothecene and other mycotoxins. However, Nelson et al. (1983) regarded only reports of moniliformin production as accurate. Later reports have confirmed this (Chelkowski et al., 1990; Abbas et al., 1991; Bosch and Mirocha, 1992). Reports of production of fusarin C (Farber and Sanders, 1986; Thrane, 1988; Leonov et al., 1993) and enniatins (Blais et al., 1992; Kononeko et al., 1993) also appear to be reliable (Desjardins, 2006; Leslie and Summerell, 2006). Production of any trichothecene toxin has not been confirmed, and *Fusarium avenaceum* does not carry the *tri5* gene which is essential for trichothecene production (Tan and Niessen, 2003).

Ecology. *Fusarium avenaceum* has a worldwide distribution wherever crops are grown, but is relatively uncommon in food commodities. It is a major component of Fusarium head blight in cereals in Europe, the US Pacific Northwest region and Canada (Desjardins, 2006). Logrieco et al. (2002) identified *F. avenaceum* as a component of Fusarium ear rot of maize in Europe. It has been reported from barley (Flannigan, 1969; Petters et al., 1988; Stenwig and Liven, 1988) where it may inhibit germination of malting grains (Hudec, 2007), but is of minor importance in gushing of beer (Niessen et al., 1992). Other reported sources are sorghum (Onyike and Nelson, 1992), peanuts (Joffe, 1969), pigeon peas (Maximay et al., 1992) and, in our laboratory, triticale. *F. avenaceum* has been reported to cause spoilage of cool-stored broccoli (Mercier et al., 1991), dry rot of stored carrots in Italy (Marziano et al., 1992) and dry rot of rutabaga (swede turnip) in Canada (Peters et al., 2007). It has occasionally caused spoilage of apples, pears, asparagus, tomatoes, eggplant and potatoes (Snowdon, 1990, 1991) and has been reported as a postharvest pathogen of stonefruit in New Zealand (Hartill and Broadhurst, 1989).

References. Domsch et al. (1980), as *G. avenacea*; Nelson et al. (1983); Leslie and Summerell (2006).

Fusarium chlamydosporum Wollenw. & Reinking Fig. 5.24

Fusarium fusarioides (Gonz. Frag. & Cif.) Booth

Colonies on CYA covering the whole Petri dish, of low to moderately deep floccose mycelium, coloured white to pale rosy pink, often with surface appearing powdery due to production of microconidia; reverse pale to greyish rose or brownish red. Colonies on MEA 55–70 mm diam, of low, moderately dense mycelium in shades of yellow brown, or greyish rose to greyish ruby, paler at the margins; reverse deep yellow brown to orange brown. Colonies on G25N 15–20 mm diam. At 5°C, colonies 1–2 mm diam. At 37°C, colonies 5–15 mm diam.

On PDA, colonies of felty mycelium, coloured pale salmon, sometimes browner, or with patches of greyish red, often with a powdery appearance from profuse microconidial production; reverse deep violet brown to dark ruby, paler at the margins. On DCPA, colonies of sparse, floccose, pale salmon mycelium, often powdery with microconidia, showing poorly defined annulations; macroconidia

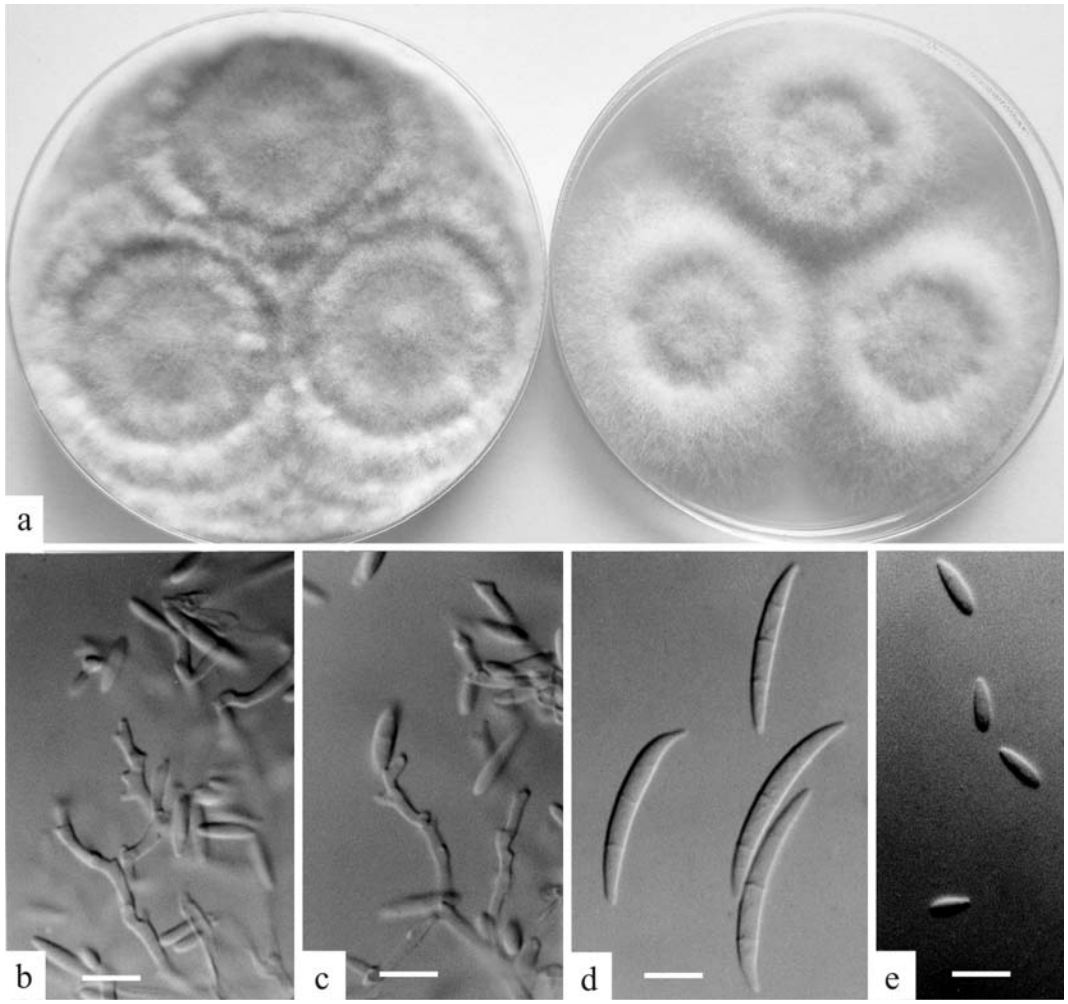


Fig. 5.24 *Fusarium chlamydosporum* **(a)** colonies on PDA and DCPA, 7 d, 25°C; **(b, c)** polyphialides, bar = 10 µm; **(d)** macroconidia, bar = 10 µm; **(e)** microconidia, bar = 10 µm

occasionally produced near the colony centres in salmon sporodochia; reverse pale.

Macroconidia often rare, relatively short and stout, usually with three to five septa, slightly curved; microconidia produced abundantly from polyphialides in the aerial mycelium, with zero to two septa, fusiform to slightly clavate. Chlamydoconidia usually abundant in older cultures, produced singly, in pairs or in clumps.

Distinctive features. The presence of abundant fusiform microconidia borne on polyphialides is the most outstanding feature of *Fusarium chlamydosporum*. Also colonies on PDA have dark violet brown to dark ruby reverse colours.

Taxonomy. *Fusarium chlamydosporum* has priority over *F. fusarioides* as the correct name for this species (Domsch et al., 1980; Nelson et al., 1983; Leslie and Summerell, 2006).

Physiology. This species has minimum, optimum and maximum temperatures for growth of 5, 27 and 37°C (Seemüller, 1968).

Mycotoxins. Production of type A trichothecenes (including T-2 toxin, HT-2 toxin, monoacetoxyscirpenol, neosolaniol and iso-neosolaniol) by *Fusarium chlamydosporum* was reported by Park and Chu (1993); however, subsequent studies have found no evidence of trichothecene production in this species (Desjardins, 2006). Moniliformin is the

major mycotoxin produced by *F. chlamydosporum* (Marasas et al., 1984; Desjardins, 2006).

Ecology. *Fusarium chlamydosporum* is mainly an inhabitant of soils in warmer climates (Domsch et al., 1980; Leslie and Summerell, 2006), and is not regarded as a plant pathogen or spoilage fungus. However, it is commonly isolated from grains in drier areas, particularly in the Middle East, southern Europe, central Asia and Australia (Leslie and Summerell, 2006), and has also been isolated from pearl millet (Wilson et al., 1993; Jurjevic et al., 2007), pecans (Huang and Hanlin, 1975) and sorghum (Rabie et al., 1975; Onyike and Nelson, 1992). A low incidence of *F. chlamydosporum* was found in peanuts from both Indonesia and the Philippines (Pitt et al., 1998a) and from mung beans and sorghum in Thailand (Pitt et al., 1994). Involvement in dry rot of potatoes has also been reported (Somani, 2004; Esfahani, 2006).

References. Domsch et al. (1980); Nelson et al. (1983); Leslie and Summerell (2006).

Fusarium culmorum (W.G. Smith) Sacc.

Fig. 5.25

Colonies on CYA covering the whole Petri dish, of dense felty mycelium, often with a floccose overlay, sometimes reaching the Petri dish rim, pale red to pastel red; reverse pastel red to deep red. Colonies on MEA 60 mm or more diam, floccose, in age often reaching the Petri dish lid, pale red to pastel red, commonly with a greyish orange to yellowish brown overlay; reverse brown to reddish brown. Colonies on G25N usually 5–10 mm diam, mycelium orange white, reverse yellow to orange. At 5°C, germination. No growth at 37°C.

On PDA, colonies covering the whole Petri dish, of dense to floccose mycelium, pale red and pale yellow brown; reverse red to deep red. On DCPA, colonies 50–65 mm diam, of sparse mycelium, orange to pinkish white, bearing abundant macroconidia in orange sporodochia; reverse dull orange brown.

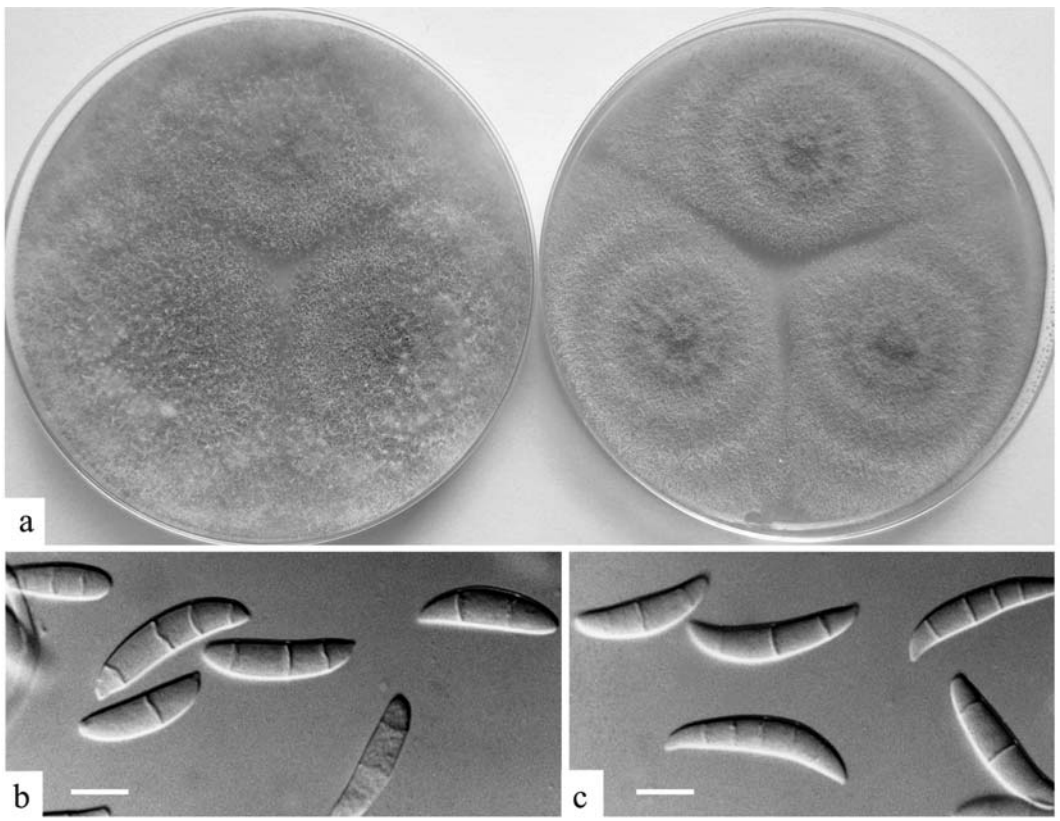


Fig. 5.25 *Fusarium culmorum* **(a)** colonies on PDA and DCPA, 7 d, 25°C; **(b, c)** macroconidia, bar = 10 µm

Macroconidia relatively short, wide and only slightly curved, with four to five septa, 30–45 μm long, with rounded or sometimes papillate apical cells; basal cells with a slight to definite notch, sometimes papillate. Microconidia not produced. Chlamydoconidia sometimes formed, in conidia, or intercalary in the hyphae, singly or in chains, 9–14 μm diam, smooth walled.

Distinctive features. Short, stout macroconidia are the prime feature distinguishing *Fusarium culmorum* from most other species. *F. culmorum* may be confused with *F. crookwellense* L.W. Burgess et al., but macroconidia of the latter species have a distinctly foot-shaped basal cell, whereas those of *F. culmorum* are shorter and stouter, and the basal cell is not distinctly foot-shaped.

Taxonomy. No teleomorph is known for this species.

Physiology. *Fusarium culmorum* has been reported to be psychrotrophic, growing down to 0°C, with an optimum at 21°C and a maximum of only 31°C (Arsvoll, 1975); however, Magan and Lacey (1984c) reported growth at 35°C. The minimum a_w for growth is 0.87 at 20–25°C and pH 6.5: at pH 4.0, growth did not occur below 0.90 a_w (Magan and Lacey, 1984a). A strain of *F. culmorum* produced deoxynivalenol optimally at 25°C, but only between 0.995 and 0.97–0.96 a_w (Hope and Magan, 2003; Hope et al., 2005). At 15°C, deoxynivalenol was produced in lower concentrations later in the growth cycle, but over a slightly greater a_w range (0.995 to 0.95–0.94 a_w). The dynamics of nivalenol production for this strain was similar (Hope and Magan, 2003). Zearalenone production by *F. culmorum* was reported to be optimal above 25°C (Bottalico et al., 1982). *F. culmorum* is very tolerant of low O_2 tensions (Magan and Lacey, 1984b). Radiation resistance of *F. culmorum* was relatively high: up to 0.8 kGy were needed for a tenfold reduction in spore numbers on grain, and up to 1.39 kGy on media (O'Neill et al., 1991).

Mycotoxins. *Fusarium culmorum* produces a variety of mycotoxins: indeed the list in our files is of more than 40 compounds. However, the most important toxins confirmed to be produced by this species (Nelson et al., 1983; Marasas et al., 1984; Desjardins, 2006; Leslie and Summerell, 2006) are deoxynivalenol, nivalenol and their derivatives (Abramson et al., 2001; Hestbjerg et al., 2002; Chandler et al., 2003; Jennings et al., 2004) and zearalenone (Bakan et al., 2001; Hestbjerg et al., 2002; Llorens et al., 2004a; Brinkmeyer et al., 2005). Moniliformin production was reported by Scott et al. (1987) but was not detected in 42 isolates of *F. culmorum* from Canada by Abramson et al. (2001). Reports of production of type A trichothecenes (T-2 toxin, HT-2 toxin) have not been substantiated (Leslie and Summerell, 2006).

The existence of two chemotypes of *Fusarium culmorum*, those that produce deoxynivalenol and those that produce nivalenol (Miller et al., 1991), has been confirmed by molecular studies. Within the trichothecene gene cluster, isolates possessing the *Tri7* and *Tri13* genes produce nivalenol and related compounds, whereas sequences in the *Tri3*, *Tri5* and *Tri6* genes are associated with deoxynivalenol production (Chandler et al., 2003; Jennings et al., 2004; Quarta et al., 2005, 2006). Of 55 European isolates examined by Quarta et al. (2005), 11 were the nivalenol chemotype, and the remainder were deoxynivalenol producers. Jennings et al. (2004) examined 153 isolates from England and Wales, and found that the DON chemotype was dominant over the NIV chemotype (59% vs 41%, respectively). Lauren et al. (1992) examined 45 isolates of *F. culmorum* from New Zealand soil and pasture and found none produced deoxynivalenol or its monoacetyl isomers. Two chemotypes were identified, one producing diacetylnivalenol with culmorin as the major metabolite accounted for 95% of isolates, while the other chemotype produced diacetoxyscirpenol.

Ecology. This species has a worldwide distribution in soil and as a pathogen of cereals and other hosts, with a higher incidence in temperate climates (Domsch et al., 1980; Nelson et al., 1983; Leslie and Summerell, 2006). It is an important component of the cohort of *Fusarium* species that cause head blight of wheat and associated cereal crops in Europe, Canada, China and other areas with cool weather during the growing season (Desjardins, 2006). In wheat it causes extensive internal damage to the grain, and reductions in flour yield and baking quality (Meyer et al., 1986). *F. culmorum* was reported as the dominant *Fusarium* species on barley in Europe (see Pitt and Hocking, 1997). It also occurs in triticale (Perkowski et al., 1988). *F. culmorum* has been identified as a component of Fusarium ear rot of maize in Europe (Logrieco

et al., 2002). *F. culmorum* was one cause of crown rot in bananas (Wade et al., 1993), and is a minor cause of spoilage of apples and pears (Snowdon, 1990).

References. Domsch et al. (1980); Nelson et al. (1983); Desjardins (2006); Leslie and Summerell (2006).

Fusarium equiseti (Corda) Sacc. Fig. 5.26

Teleomorph: *Gibberella intricans* Wollenw.

Colonies on CYA filling the whole Petri dish, often to the lid, of dense to floccose white mycelium; reverse pale or pale salmon. Colonies on MEA covering the whole Petri dish, of open, floccose white to pale brown mycelium; reverse pale, or sometimes showing areas of pale greyish red. Colonies on G25N 12–20 mm diam. At 5°C, colonies of 1–4 mm diam produced. At 37°C, usually no growth, although in isolates from the tropics, colonies up to 35 diam Produced.

On PDA, colonies of dense to floccose mycelium, white to pale salmon, becoming brown with age, with a central mass of orange to brown sporodochia, sometimes surrounded by poorly defined sporodochial rings; reverse pale salmon, often with a brown central area and brown flecks. On DCPA, colony appearance usually dominated by salmon, orange or brownish

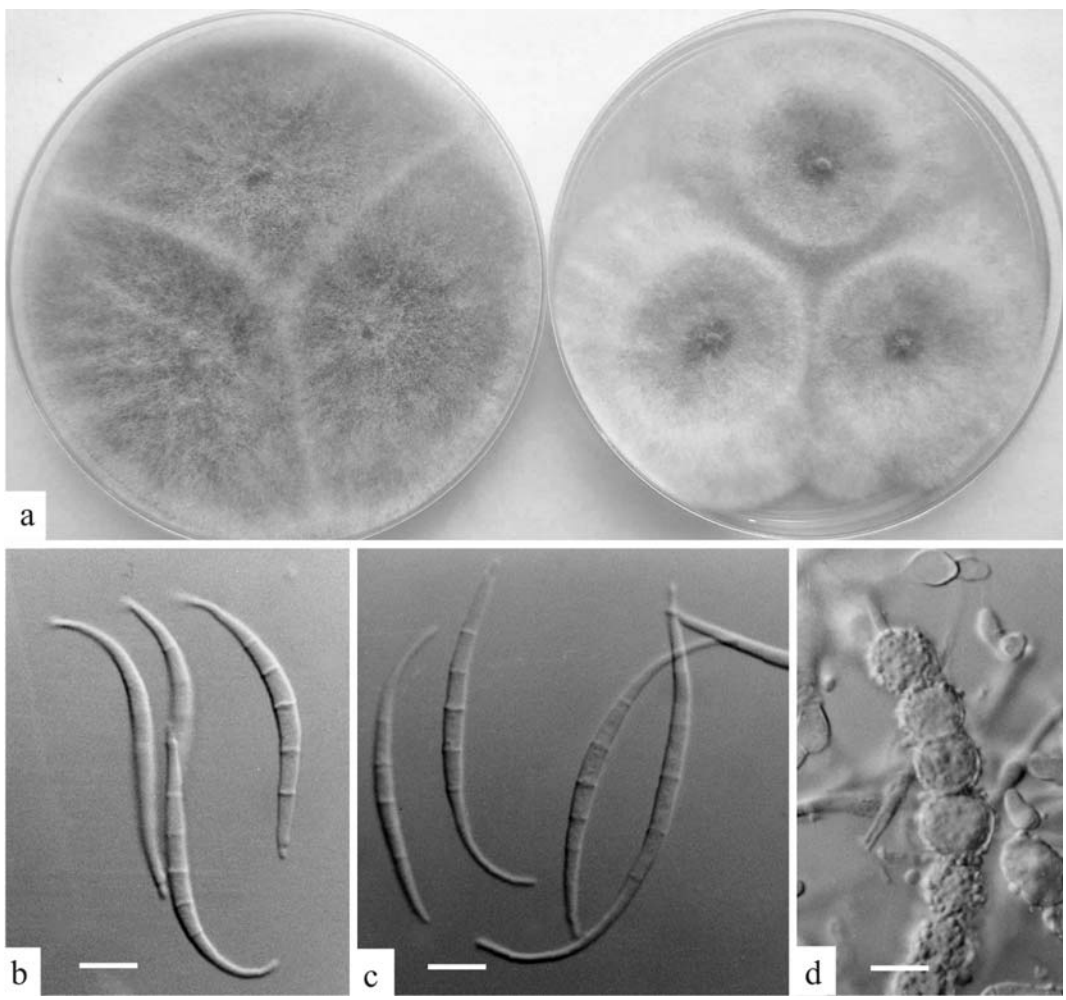


Fig. 5.26 *Fusarium equiseti* **(a)** colonies on PDA and DCPA, 7 d, 25°C; **(b, c)** macroconidia, bar = 10 µm; **(d)** chlamydoconidia, bar = 10 µm

sporodochia centrally and in poor to well defined concentric rings; mycelium low and sparse, coloured white or pale salmon; reverse pale.

Macroconidia distinctly curved, often with a "hunchbacked" appearance, with five to seven septa, ranging from relatively short in some isolates to very long in others, with a wide range of sizes often present in a single isolate, basal cell distinctly foot-shaped, often with an elongated heel, apical cell elongated and curved, in long spores becoming filamentous; chlamydoconidia usually produced abundantly, in chains or clumps; microconidia absent.

Distinctive features. *Fusarium equiseti* produces distinctly curved macroconidia which are often elongate, especially in the basal and apical cells. Colonies on PDA are floccose, usually with at least a central mass of orange sporodochia, and with reverse pale salmon, often flecked with brown. Chlamydoconidia are usually abundant; microconidia are not produced.

Taxonomy. The teleomorph of *Fusarium equiseti* is *Gibberella intricans* Wollenw. However, there are few records of its occurrence in nature (Booth, 1971).

Physiology. *Fusarium equiseti* grows strongly at 30°C (Burgess et al., 1994) but, from observations in our laboratory, most isolates grow poorly or not at all at 37°C. The minimum a_w for growth has been reported to be 0.92 a_w (Chen, 1966). Growth occurred at pH 3.3 but not 2.4, and was still rapid at pH 10.4 (Wheeler et al., 1991). Tripathi et al. (1999) reported growth between pH 2.0 and 11.00. Growth in N_2 with <1% O_2 was 90% of that in air (Hocking, 1990).

Mycotoxins. *Fusarium equiseti* has been reliably reported to produce a number of mycotoxins. Trichothecene production in *F. equiseti* is variable, with both type A trichothecenes (diacetoxyscirpenol and related compounds, T-2 toxin, HT-2 toxin and neosolaniol) and type B trichothecenes (deoxynivalenol, 15-acetyldeoxynivalenol, fusarenone-X and nivalenol) reliably reported (see Pitt and Hocking, 1997; Langseth et al., 1999; Hestbjerg et al., 2002; Kosiak et al., 2005; Desjardins, 2006). Zearalenone, moniliformin, beauvericin, fusarochromanone and related compounds have also been reported (see Pitt and Hocking, 1997; Logrieco et al., 1998a; Desjardins, 2006), but production of butenolide is questionable (Desjardins, 2006).

Ecology. A cosmopolitan soil fungus, *Fusarium equiseti*, has a distribution extending from Alaska to the tropics (Domsch et al., 1980). It has been isolated from a variety of plants, particularly cereals, where it may cause stem and root rots (Leslie and Summerell, 2006). *F. equiseti* has been identified as a minor component of maize ear rot in Europe, and head blight of wheat and associated grains in Europe and North American (Desjardins, 2006). It has been reported from various cereal grains including wheat, maize, barley, rye and rice (see Pitt and Hocking, 1997; Pitt et al., 1998a) and also from peanuts, walnuts, soybeans, cowpeas, sorghum, oilseeds and herbs (see Pitt and Hocking, 1997). It has been isolated from tomatoes (Okoli and Erinle, 1989) and capsicums (Adisa, 1983; Hashmi and Ghaffar, 1991), and has been reported as contributing to soft rot of bell peppers in India (Shukla and Sharma, 2000) and crown rot of bananas (Wallbridge, 1981; Jiménez et al., 1993). It has also been implicated as a cause of rots of cucurbit fruits in contact with soil (Burgess et al., 1994), dry rots of potato tubers (El-Hassan et al., 2004) and soft rot of pumpkins (Elmer, 1996).

F. equiseti has caused spoilage of UHT processed fruit juices, due to its ability to grow in very low O_2 tensions (Hocking, 1990).

References. Domsch et al. (1980) as *Gibberella intricans*; Nelson et al. (1983); Desjardins (2006); Leslie and Summerell (2006).

Fusarium graminearum Schwabe Fig. 5.27

Teleomorph: *Gibberella zeae* (Schwabe) Petch

Colonies on CYA filling the whole Petri dish, often to the lid, of dense, floccose mycelium coloured greyish rose, greyish yellow or paler; reverse usually orange red to greyish ruby, though sometimes pale brownish pink. Colonies on MEA filling the whole Petri dish, often reaching the lid at the margins at least, of dense to openly floccose mycelium, in shades of greyish rose and greyish yellow to golden brown; reverse orange brown to yellowish brown, sometimes paler at the margins. Colonies on G25N 20–30 mm diam, occasionally more. At 5°C, colonies of 5–12 mm diam produced. No growth at 37°C.

On PDA, colonies filling the whole Petri dish, of dense, floccose mycelium coloured olive brown,

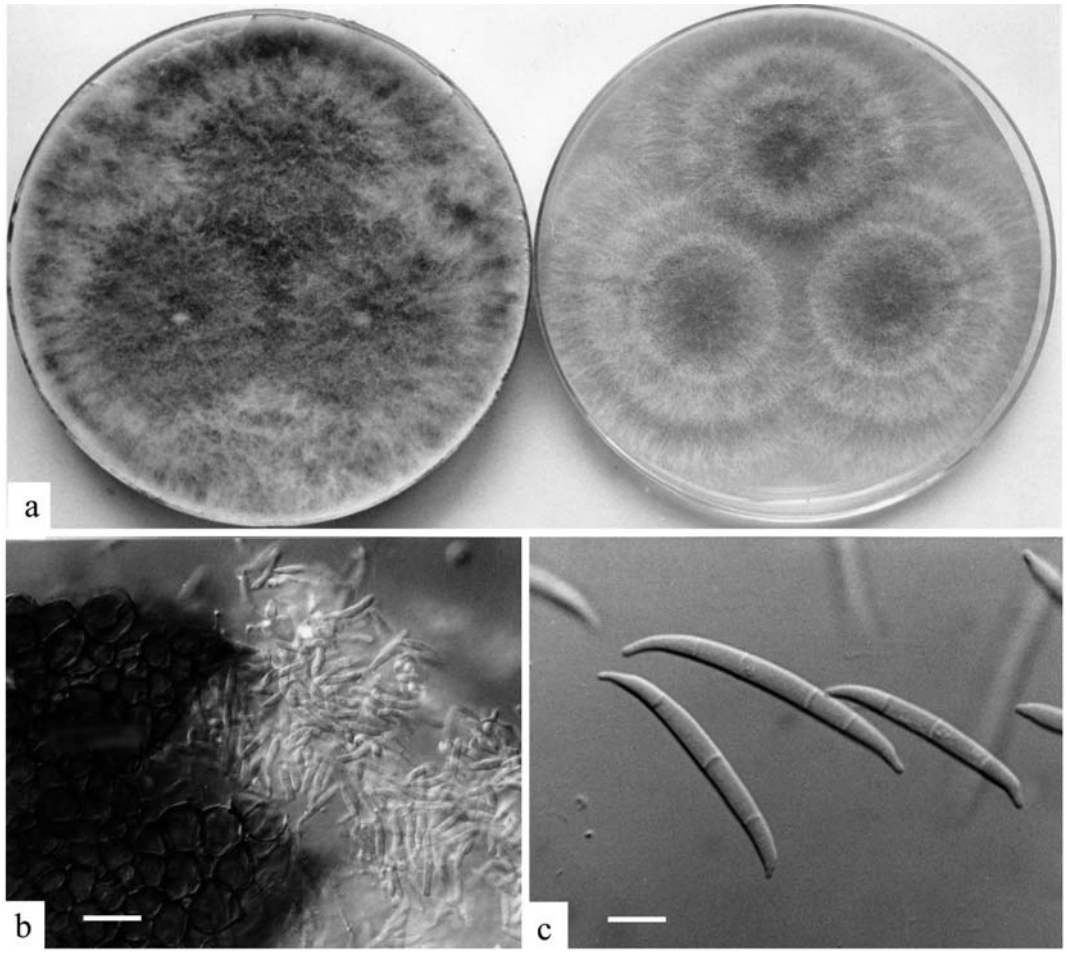


Fig. 5.27 *Fusarium graminearum* **(a)** colonies on PDA and DCPA, 7 d, 25°C; **(b)** *Gibberella zeae* perithecium and ascospores, bar = 25 μm; **(c)** macroconidia, bar = 10 μm

yellowish brown, reddish brown or pale salmon, or in combinations of those colours; sometimes with a central mass of red brown to orange sporodochia; reverse ruby to dark ruby centrally, sometimes violet brown. On DCPA, colony appearance dominated by salmon to orange sporodochia in concentric rings, overlaid by sparse, floccose, pale salmon mycelium.

Macroconidia usually with five septa, sometimes less, thick walled, straight to moderately curved, with the ventral surface almost straight and a smoothly arched dorsal surface; basal cell distinctly foot-shaped, apical cell tapered; chlamydoconidia formed tardily in some isolates; microconidia absent.

Distinctive features. On PDA, colonies of *Fusarium graminearum* are usually highly coloured, with dense to floccose greyish rose to golden brown mycelium and dark ruby reverse. Macroconidia are relatively straight and thick-walled, with a foot-shaped basal cell. Microconidia are not produced.

Taxonomy. The teleomorph of *Fusarium graminearum* is *Gibberella zeae* (Schwabe) Petch. Francis and Burgess (1977) described two populations of *F. graminearum*, which they termed Group 1 and Group 2. Group 2 isolates, the real *F. graminearum*, are responsible for head scab of wheat and also cause destructive ear rot of maize (Burgess et al., 1981; Desjardins, 2006; Leslie and Summerell, 2006). Group 1 isolates, which cause root and crown rot of wheat, were given species status and described as *Fusarium pseudograminearum* O'Donnell and T. Aoki (teleomorph *Gibberella coronicola*

T. Aoki and O'Donnell) by Aoki and O'Donnell (1999a, b). *F. graminearum* is homothallic and forms perithecia readily in nature and in culture on Carnation Leaf Agar (Leslie and Summerell, 2006), whereas *F. pseudograminearum* is heterothallic and does not. O'Donnell et al. (2004) proposed that the "*F. graminearum* clade" be split into nine different species, based on geographical and molecular data; however, the morphological distinctions between these species are small. This proposal has not been taken up by all *Fusarium* researchers (Leslie and Summerell, 2006). The complete genome of *F. graminearum* has been sequenced and is available (Broad Institute, 2003).

Physiology. The optimal temperature for growth of *Fusarium graminearum* is between 24 and 26°C on both liquid and solid media at pH 6.7–7.2 (Booth, 1971), and 25°C on irradiated wheat grains (Ramirez et al., 2006a). The minimum a_w for growth is close to 0.90 at 15–25°C (Cuero et al., 1987; Ramirez et al., 2006a). The minimum pH for growth is temperature-dependent, near 3.0 at 25 and 37°C, and 2.4 at 30°C. The maximum is near 9.5 at 37°C, but greater than 10.2 at the lower temperatures (Wheeler et al., 1991).

Mycotoxins. *Fusarium graminearum* produces numerous mycotoxins: almost 50 toxic compounds have been reported. The most important economically are type B trichothecenes – deoxynivalenol (DON) and its derivatives 3-acetyldeoxynivalenol (3-AcDON) and 15-acetyldeoxynivalenol (15-AcDON), nivalenol (NIV) and its derivatives and zearalenone (see Marasas et al., 1984; Pitt and Hocking, 1997; Desjardins, 2006; Leslie and Summerell, 2006). See Desjardins (2006) for a comprehensive review of trichothecene mycotoxins. Other toxins reported include aurofusarin, culmorins, fusarin C and steroids (see Pitt and Hocking, 1997; Leslie and Summerell, 2006). Type A trichothecenes, including T-2 toxin, HT-2 toxin, diacetoxyscirpenol and neosolaniol, have been reported from some isolates. However, production of these toxins by *F. graminearum* was queried by Nelson et al. (1983) and remains in doubt.

Based on production of the different trichothecenes, *Fusarium graminearum* has been divided into two chemotypes, chemotype I for deoxynivalenol producers and chemotype II for nivalenol producers. Strains producing 3-acetyldeoxynivalenol are classified as chemotype IA and those producing 15-acetyldeoxynivalenol as chemotype IB (Ichinoe et al., 1983; Logrieco et al., 1988; Miller et al., 1991). North American strains are predominantly chemotype IB (Abbas et al., 1989b; Mirocha et al., 1989b; Miller et al., 1991; Abramson et al., 1993; Desjardins, 2006). This chemotype also predominates in the Ukraine (Leonov et al., 1990) and New Zealand (Lauren et al., 1992). Chemotype IA predominates in China (Miller et al., 1991; Zhang et al., 2007b), Argentina (Ramirez et al., 2006b) and Italy (Logrieco et al., 1988) while in Japan (Sugiura et al., 1990) and Poland (Visconti et al., 1990) both chemotypes IA and IB are commonly isolated. Chemotype II occurs only rarely in North America (Miller et al., 1991) but is more common elsewhere, including New Zealand (Lauren et al., 1992), Australia (Blaney and Dodman, 1988, 2002; Tan et al., 2004), Japan (Sugiura et al., 1990), Korea (Lee et al., 1986; Kim et al., 1993), Italy (Logrieco et al., 1988) and South Africa (Sydenham et al., 1991).

Ramirez et al. (2006a) reported that deoxynivalenol production occurred most rapidly at 25°C, but the maximum amount was produced at 30°C. The a_w range for deoxynivalenol production was 0.95–0.995 a_w. Llorens et al. (2004b) reported that 28°C was optimal for deoxynivalenol production, but nivalenol and 3-acetyldeoxynivalenol were produced optimally at 20 and 15°C, respectively. Zearalenone production was optimal at 20°C (Llorens et al., 2004a).

Other toxins produced by *Fusarium graminearum* include butenolide, culmorin, sambucinol, calonectrin and related compounds, fusarins and a number of other minor metabolites (Desjardins, 2006).

Ecology. *Fusarium graminearum* is primarily a pathogen of gramineous plants, particularly wheat, in which it causes crown rot and head scab. Reports have come from Australia, Canada, the United States, Japan, Korea, South Africa and many European countries (see Pitt and Hocking, 1997; Desjardins, 2006; Leslie and Summerell, 2006). *F. graminearum* also causes cob rot and stalk rot of maize in many countries leading to DON and zearalenone formation in maize (see Pitt and Hocking, 1997; Leslie and Summerell, 2006). Occurrence of *F. graminearum* in barley has been less frequent, but it is believed to be one cause of gushing in beer (Niessen et al., 1992). Other sources include sugar beets (Bosch and Mirocha, 1992), soybeans (Clear et al., 1989; Jacobsen et al., 1995),

sorghum (Onyike and Nelson, 1992) and triticale (Perkowski et al., 1988). *F. graminearum* has been reported to cause dry rot in potatoes (Ali et al., 2005) and postharvest rots in pumpkins (Elmer, 1996). Crown rot of bananas may also be due to *F. graminearum* (Wallbridge, 1981; Jiménez et al., 1993).

Quorn, a mycoprotein produced for human consumption in the United Kingdom, is produced from a strain of *Fusarium* which was originally identified as *F. graminearum*. This strain, which does not produce any trichothecene toxins, has since been assigned to a different species, *F. venenatum* Nirenburg (O'Donnell et al., 1998). For reviews of this topic see Edwards (1993); Trinci (1994) and Wiebe (2004).

References. Domsch et al. (1980) (as *Gibberella zeae*); Nelson et al. (1983); Desjardins (2006); Leslie and Summerell (2006).

Fusarium longipes Wollenw. & Reinking Fig. 5.28

Colonies on CYA and MEA covering the whole Petri dish, plane, deep and floccose or sometimes

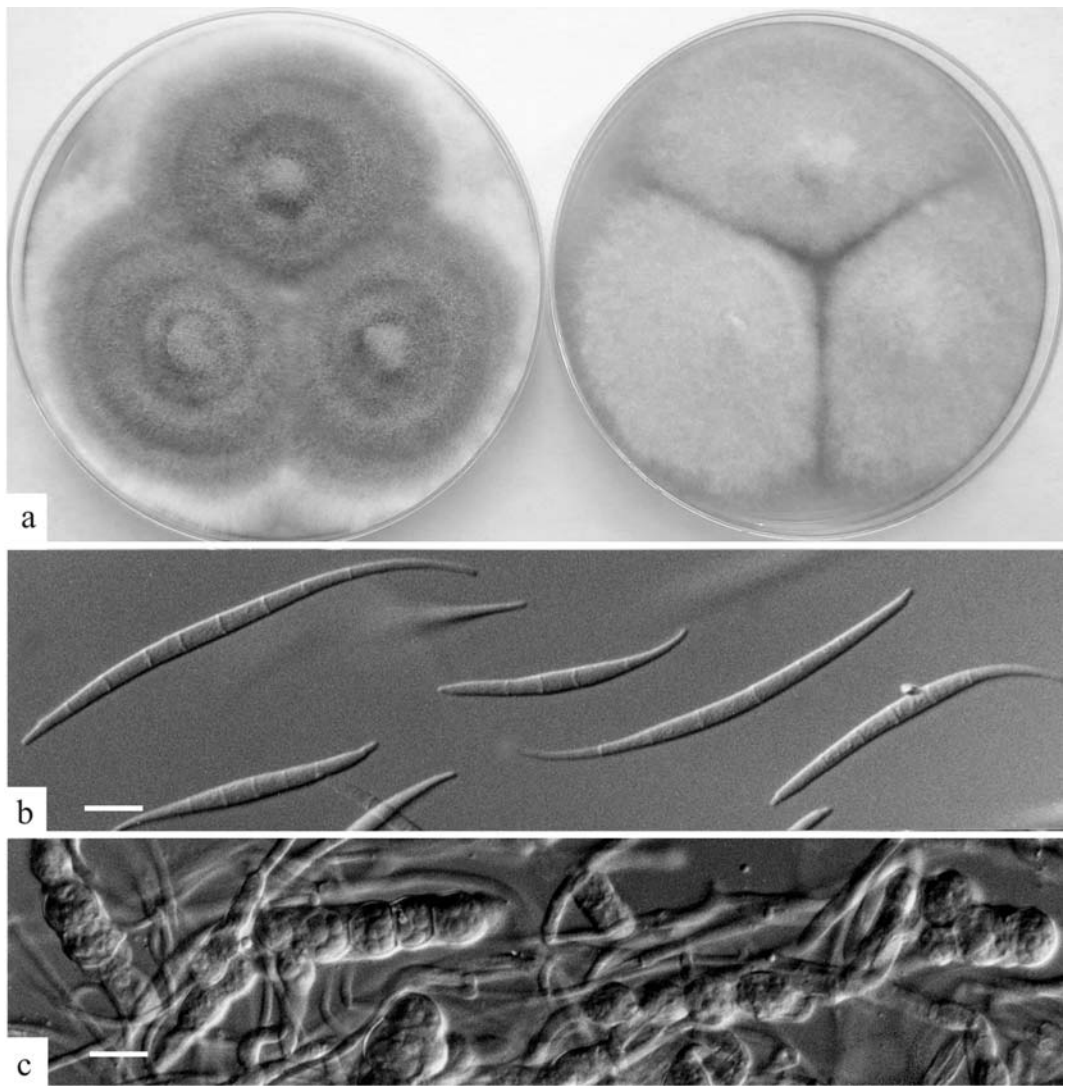


Fig. 5.28 *Fusarium longipes* **(a)** colonies on PDA and DCPA, 7 d, 25°C; **(b)** macroconidia, bar = 10 µm; **(c)** chlamydoconidia, bar = 10 µm

funiculose, mycelium pale orange to pale red or dull reddish, reverse on CYA brownish orange to light brown, on MEA deep red to brownish red. On G25N, colonies 15–22 mm diam, of floccose, pale orange mycelium, reverse in similar colours. No germination at 5°C. At 37°C, colonies of variable size, up to 65 mm diam, of white to very pale pink mycelium, reverse dull yellow to pinkish brown.

On PDA, colonies covering the whole Petri dish, similar to those on CYA, reverse dull red to dark red. On DCPA, colonies 55–65 mm diam, sparse, mycelium pale pink, showing abundant orange sporodochia containing macroconidia, reverse dull yellow to brown.

Macroconidia on DCPA strongly curved, 50–80 × 4–6 μm, with four to six septa; apical cells usually with a long papilla or a long slender whip-like end; basal cells with a deep notch and long "foot". Microconidia not produced. Chlamydoconidia produced by some isolates, in chains or small clusters, spherical to ellipsoidal or irregular in shape, 7–12 μm wide, with thin smooth walls, becoming rough in age.

Distinctive features. Pale orange to pale red mycelium on CYA and MEA, fast growth at 37°C and very long, slender, strongly curved macroconidia are the main distinguishing features of *Fusarium longipes*.

Taxonomy. No teleomorph is known for this species.

Physiology. No studies on the physiology of this species are known to us.

Mycotoxins. This appears to be a nontoxigenic species (Wing et al., 1993; Nelson et al., 1994), although Logrieco et al. (1998a) reported beauvericin production by one isolate.

Ecology. *Fusarium longipes* principally comes from tropical soils (Burgess et al., 1994; Sangalang et al., 1995). It has been reported from peanuts from West Java (Dharmaputra and Retnowati, 1996), and we isolated it from 13% of Indonesian peanut samples, at up to 16% of kernels in infected samples and 1% of all kernels examined (Pitt et al., 1998a). It was also found at low levels in Indonesian maize and cowpeas, Thai maize, mung beans, cassava and sesame seed, and paddy rice from the Philippines (Pitt et al., 1993, 1998a). *F. longipes* has also been reported from cereals in Iran (Zare and Ershad, 1997).

References. Nelson et al. (1983); Leslie and Summerell (2006).

Fusarium oxysporum Schltdl. Fig. 5.29

Colonies on CYA 50–70 mm diam, sometimes covering the whole Petri dish, moderately deep, of floccose white to greyish mycelium; reverse pale to pale greenish grey. Colonies on MEA 65–70 mm diam, often covering the whole Petri dish, of floccose white to pale greyish magenta mycelium; reverse greyish magenta to dark purple, often paler at the margins. Colonies on G25N 12–16 mm diam, occasionally larger. At 5°C, germination to formation of microcolonies. At 37°C, no growth, or colonies up to 5 mm diam formed.

On PDA, colonies of white, pale salmon or pale mauve mycelium, sometimes dense and floccose, sometimes low, often with salmon sporodochia in a central mass, and sometimes in one or two poorly defined concentric rings also; reverse pale salmon, often mauvish centrally, sometimes dark magenta. On DCPA, colonies low, sometimes with concentric rings of sparse, floccose aerial mycelium, powdery with microconidia on the agar surface, or sometimes uniformly low with little aerial mycelium and then with appearance dominated by surface macroconidia.

Macroconidia only slightly curved, usually with three septa, occasionally more, thin walled, with notched or foot shaped basal cells and short, sometimes hooked, apical cells; microconidia abundant, fusiform to kidney-shaped, produced in false heads from short, stout monophialides. Chlamydoconidia produced singly or in pairs.

Distinctive features. *Fusarium oxysporum* produces abundant fusiform to kidney-shaped microconidia in false heads from short, stout, flask-shaped phialides in the aerial mycelium. The colony reverse on PDA is usually mauve, violet or greyish magenta.

Taxonomy. Many plant pathogenic races exist in *Fusarium oxysporum*; these are called *formae speciales* (abbreviated f. sp.). Recent DNA sequence analyses have indicated that some of these are polyphyletic (Summerell et al., 2003). The most important of these are discussed by Booth (1971) and Leslie and Summerell (2006). No teleomorph is known.

Physiology. Domsch et al. (1980) reported an optimum growth temperature between 25 and 30°C for *Fusarium oxysporum*, with a minimum above 5°C, and a maximum at or below 37°C. The

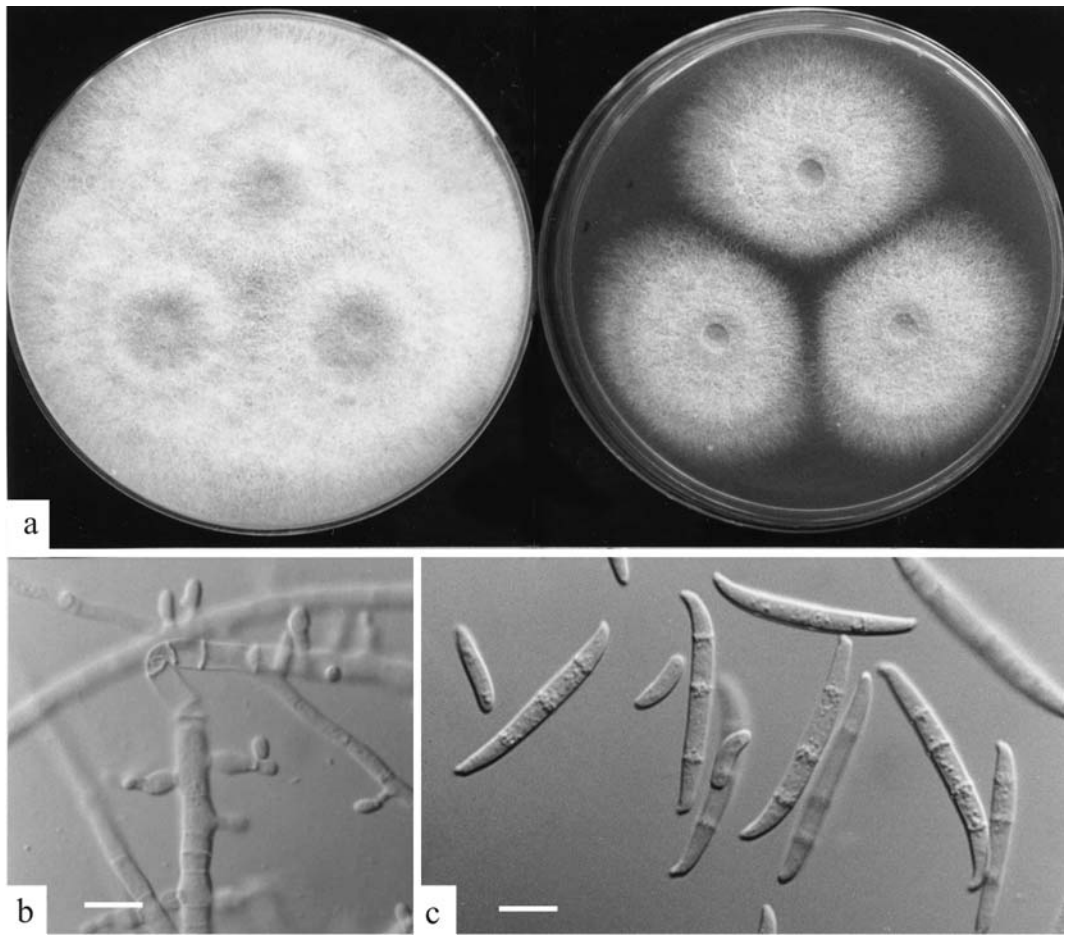


Fig. 5.29 *Fusarium oxysporum* **(a)** colonies on PDA and DCPA, 7 d, 25°C; **(b)** phialides, bar = 10 μm; (c) macroconidia and microconidia, bar = 10 μm

optimum pH for growth is 7.7 with the wide range of pH 2.2–9.0 being tolerated (Domsch et al., 1980). The minimum a_w for growth is 0.89 at 20°C, after a germination time of 2 months (Schneider, 1954). Growth in N_2 with <1% O_2 was 97% of that in air (Hocking, 1990). Some growth occurred in 99% CO_2, with only trace amounts of O_2 and N_2 present (Hocking, 1990). Atmospheres of 20–40% CO_2 with 5 or 1% O_2 reduced colony growth rate of *F. oxysporum* by 40–50%, but ergosterol content was reduced by 80–90% under these conditions (Taniwaki et al., 2001a). *F. oxysporum* chlamydoconidia do not exhibit exception heat resistance, despite its ability to cause spoilage in beverages (see below). In experiments in our laboratory, survival was recorded from mixed suspensions of microconidia and chlamydoconidia after heating at 63°C for up to 48 s, but not after exposure to 65°C for 6 s in 20° Brix sucrose solution at pH 4.2. *F. oxysporum* was more resistant to dry heat, with sporadic survival detected after exposure to 140°C for 60 s (our unpublished data).

Mycotoxins. *Fusarium oxysporum* has been widely reported to produce moniliformin (Marasas et al., 1979; Abbas et al., 1989b, 1991; Mirocha et al., 1989a; Chelkowski et al., 1990) and zearalenone (Per, 1995; see also Pitt and Hocking, 1997), although production of the latter compound appears to be in doubt as *F. oxysporum* does not possess the *tri5* gene required for trichothecene biosynthesis (Tan and Niessen, 2003). *F. oxysporum* isolates reported to produce wortmannin (Abbas and Mirocha, 1988; Abbas et al., 1989a, 1992; Gunther et al., 1989) have been re-examined and classified as *F. torulosum* (Thrane, 2001). Other mycotoxins reported

to be produced by *F. oxysporum* are beauvericin (Logrieco et al., 1998a; Moretti et al., 2002), type B fumonisins (Abbas et al., 1995; Proctor et al., 2004) and type C fumonisins (Seo et al., 1996).

Ecology. *Fusarium oxysporum* is a serious wilt pathogen of many crop plants, including sweet potatoes, cabbage and other crucifers, cucumbers and melons, oil and date palms, tomatoes, peas, soybeans and cowpeas, clovers, cotton and a variety of others (Booth, 1971; Nelson et al., 1981) and is geographically widespread (Sangalang et al., 1995; Leslie and Summerell, 2006). Considering these facts, its common occurrence in foods is not unexpected. It occurs in cereals, including maize in the United States, Australia, Turkey, South Africa and the Philippines; rice in India, Egypt and Indonesia; barley in Egypt and sorghum from Africa (see Pitt and Hocking, 1997). It is widespread on nuts: peanuts, pecans, hazelnuts and walnuts (see Pitt and Hocking, 1997).

This species is one cause of crown rot of bananas (Wallbridge, 1981; Jiménez et al., 1993; Boruah et al., 2004), and also occurs as a minor pathogen on citrus, pome fruits, tomatoes, melons (Snowdon, 1990) and pineapples (Biswal et al., 2007). Other sources include coriander seeds (Hashmi and Ghaffar, 1991), cacao beans (Ribeiro et al., 1986), navy beans (Tseng et al., 1996), adzuki and mung beans (Tseng and Tu, 1997), garlic (Rath and Mohanty, 1986), capsicums (Hashmi and Thrane, 1990), truffles (Bokhary et al., 1990), asparagus (Weber et al., 2006), cheese (Hocking and Faedo, 1992; Lund et al., 1995) and potatoes (Vrany et al., 1989; Kim and Lee, 1994; Venter and Steyn, 1998). *F. oxysporum* has been implicated as a cause of gill blackening disease in Kumma prawns (*Penaeus japonicus*) (Souheil et al., 1999).

Due to its ability to grow in low O_2 tensions, *Fusarium oxysporum* has caused spoilage of UHT-processed fruit juices (Hocking, 1990). This problem became widespread from 2001 onwards, and our laboratory investigated several spoilage incidents occurring in Australia and New Zealand in fruit, dairy- and soy-based aseptically filled beverages. We had anecdotal evidence of similar problems in Europe, Asia and North America. *F. oxysporum* appeared to be colonising the aseptic area of the packaging equipment, and once established, was very difficult to eliminate.

References. Booth (1971); Domsch et al. (1980); Nelson et al. (1981, 1983); Leslie and Summerell (2006).

Fusarium poae (Peck) Wollenw. Fig. 5.30

Colonies on CYA filling the whole Petri dish, deep, of moderately dense white to pale rose mycelium; reverse unevenly coloured, pale to rose red or deep red. Colonies on MEA filling the whole Petri dish, deep, of sparse, pale rose mycelium; reverse brownish orange or paler. Colonies on G25N 8–12 mm diam. At 5°C, germination to colonies up to 2 mm diam formed. No growth at 37°C.

On PDA, colonies moderately deep, of floccose, pale salmon to pale rose mycelium, darker centrally, reverse varying from pale salmon at the margins to greyish ruby centrally, or entirely dark ruby to dark magenta. On DCPA, colonies moderately deep, of floccose pale salmon mycelium in poorly defined concentric rings; sporodochia rarely present; reverse pale or, in highly pigmented isolates, with annular brownish red rings.

Macroconidia usually sparsely produced, varying in shape, mostly with three septa, occasionally more, slightly curved, with a foot-shaped basal cell. Microconidia abundant, spherical, often with a distinct papilla, occasionally also lemon-shaped, aseptate or with one septum, produced in the aerial mycelium from short flask-shaped phialides on compact branched stipes, often appearing like bunches of grapes when examined *in situ* under the low power microscope.

Distinctive features. The abundant production of spherical microconidia borne from flask-shaped phialides on compact, branched stipes distinguishes *Fusarium poae* from other species. It also has a distinctive odour, described as "fruity" or "sweet" (Leslie and Summerell, 2006).

Taxonomy. A group of cultures morphologically similar to *Fusarium poae*, referred to as "powdery" *F. poae*, have recently been assigned to a separate species *Fusarium langsethiae* Torp and Nirenburg (Torp and Nirenburg, 2004). This species has been isolated from oats, wheat and barley in cooler areas of Europe and Scandinavia. *F. langsethiae,* which does not have the characteristic sweet odour of *F. poae,* is also distinguished by its metabolic profile (Thrane et al., 2004) and by

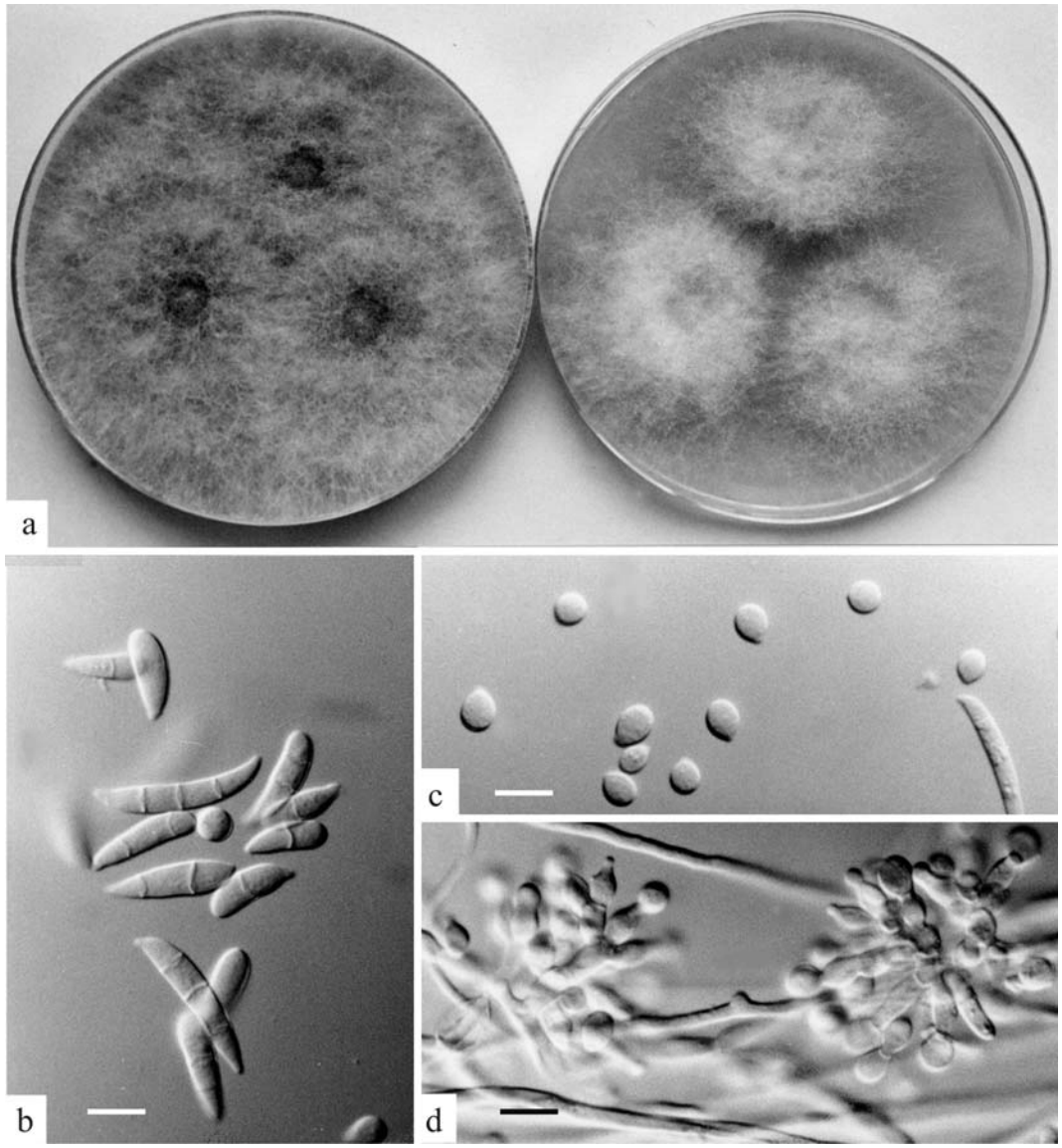


Fig. 5.30 *Fusarium poae* **(a)** colonies on PDA and DCPA, 7 d, 25°C; **(b)** macroconidia, bar = 10 μm; **(c)** microconidia, bar = 10 μm; **(d)** phialides, bar = 10 μm

molecular techniques (Schmidt et al., 2004). It appears to be more closely related to *F. sporotrichioides* than *F. poae* (Niessen et al., 2004).

Physiology. The optimum temperature for growth of *Fusarium poae* is near 25°C, with a minimum near 2.5°C and a maximum of 35°C (Kvashnina, 1976; Torp and Nirenburg, 2004). The minimum a_w for growth is near 0.90 between 17 and 25°C (Magan and Lacey, 1984c).

Mycotoxins. Reports of toxin production by *Fusarium poae* have been very variable. Reports that it was perhaps responsible for Alimentary Toxic Aleukia due to the production of T-2 toxin have largely been discounted, as *F. sporotrichioides*, the major producer of that toxin, was also commonly present during that disease syndrome, and in the haemorrhagic diseases that occurred in cattle, pigs and poultry in the United States in the 1960s (Marasas et al., 1984). However, it has been concluded more recently that certain strains of *F. poae* can indeed produce T-2 toxin (De Nijs et al., 1996; Langseth et al., 1999; Thrane et al., 2004).

Most strains of *Fusarium poae* produce nivalenol, diacetoxyscirpenol, monoacetoxyscirpenols, fusarenone-X and scirpentriol (Thrane et al., 2004), with some strains also producing beauvericin and enniatins (Thrane et al., 2004; Chelkowski et al., 2007). Logrieco et al. (1998a) also reported beauvericin production by *F. poae*. T-2 toxin is more often produced by *F. sporotrichioides* and *F. langsethiae* (Thrane et al., 2004; Desjardins, 2006).

Ecology. *Fusarium poae* mainly occurs in temperate regions, where it is found on woody seedlings or herbaceous and gramineous hosts (Booth, 1971; Leslie and Summerell, 2006). From foodstuffs, it has been reported most commonly from grains, especially wheat, from France, Canada, Japan, Ethiopia, Hungary, Poland, Germany and Norway (see Pitt and Hocking, 1997; Birzele et al., 2002; Bottalico and Perrone, 2002; Ioos et al., 2004; Kosiak et al., 2003, 2004). It has been implicated as a cause of panicle blight of oats in Poland (Mielniczuk, 2001), head blight of oats in Canada (Tekauz et al., 2004) and as a pathogen of barley in Argentina (Barreto et al., 2004). It has been associated with maize in cooler climates, where it may be involved in ear rot (see Pitt and Hocking, 1997; Logrieco et al., 2002; Chelkowski, et al., 2007). *F. poae* has also been recorded from soybeans in the United States (Abbas and Bosch, 1990), heart rot of sugar cane in South Africa, rice in Australia and decayed stored citrus fruit in Georgia, CIS (Booth, 1971).

References. Booth (1971); Domsch et al. (1980); Nelson et al. (1983); Desjardins (2006); Leslie and Summerell (2006).

Fusarium proliferatum (Matsush.) Nirenberg Fig. 5.31

Teleomorph: *Gibberella intermedia* (Kuhlman) Samuels

Colonies on CYA covering the whole Petri dish, plane, floccose, of pale orange mycelium, reverse light orange to pastel red. Colonies on MEA similar to those on CYA but much less dense, mycelium in similar colours, reverse orange, greyish red or violet brown. On G25N, germination or colonies up to 5 mm diam. At 5°C, no germination. At 37°C, colonies 5–20 mm diam, of pale orange mycelium, reverse usually orange.

On PDA, colonies covering the whole Petri dish, similar to on MEA, reverse light orange to greyish red. On DCPA, colonies 45–55 mm diam, of pale orange mycelium, plane, floccose, similar in texture to on CYA, orange sporodochia usually evident on the agar surface, reverse orange brown.

Macroconidia on DCPA slightly curved, with three to four septa, 40–50 × 3.0–4.0 μm, rounded or papillate at both ends, or sometimes with a notch at the basal end, with thin, smooth walls. Microconidia borne in chains and false heads from monophialides or polyphialides, ellipsoidal or bean-shaped, commonly 8–12 × 2.5–4.0 μm and nonseptate, occasionally longer and with one or more septa, with thin smooth walls. Chlamydoconidia not produced.

Distinctive features. Similar to *Fusarium verticillioides* in most respects, *F. proliferatum* is distinguished by the formation of microconidia from polyphialides. Microconidial chains are usually shorter in *F. proliferaturm* than *F. verticillioides*. Freshly isolated cultures form abundant macroconidia in sporodochia, but this feature is often lost after repeated transfer (Leslie and Summerell, 2006).

Taxonomy. Morphologically, *Fusarium proliferatum* is indistinguishable from *F. fujikuroi* Nirenburg. These two species can be differentiated using mating tests or by DNA sequencing. *F. fujikuroi*, which is associated mainly with rice, does not produce fumonisins, whereas most isolates of *F. proliferatum* do.

Physiology. The optimum pH and temperature for growth of *Fusarium proliferatum* are 5.5 and 25°C, respectively, with growth occurring at 5 and 37°C, but not 40°C on maize meal (Marín et al., 1996). The minimum a_w for germination reported by Marín et al. (1996) was 0.88, with no germination occurring at 0.85 a_w after 40 days. However, Samapundo et al. (2005a) reported a minimum a_w of 0.87 for growth of *F. proliferatum* on maize, with optimum growth occurring at 30°C at 0.97 a_w, the highest a_w tested. *F. proliferatum* was able to grow on irradiated maize kernels at 0.98 and 0.93 a_w in atmospheres containing up to 60% CO_2 with 20% O_2, although the lag time was longer and the growth rate slower than the controls (Samapundo et al., 2007a). Reduction of headspace oxygen from 20 to 2% (without elevated CO_2) had no effect on growth of *F. proliferatum,* but

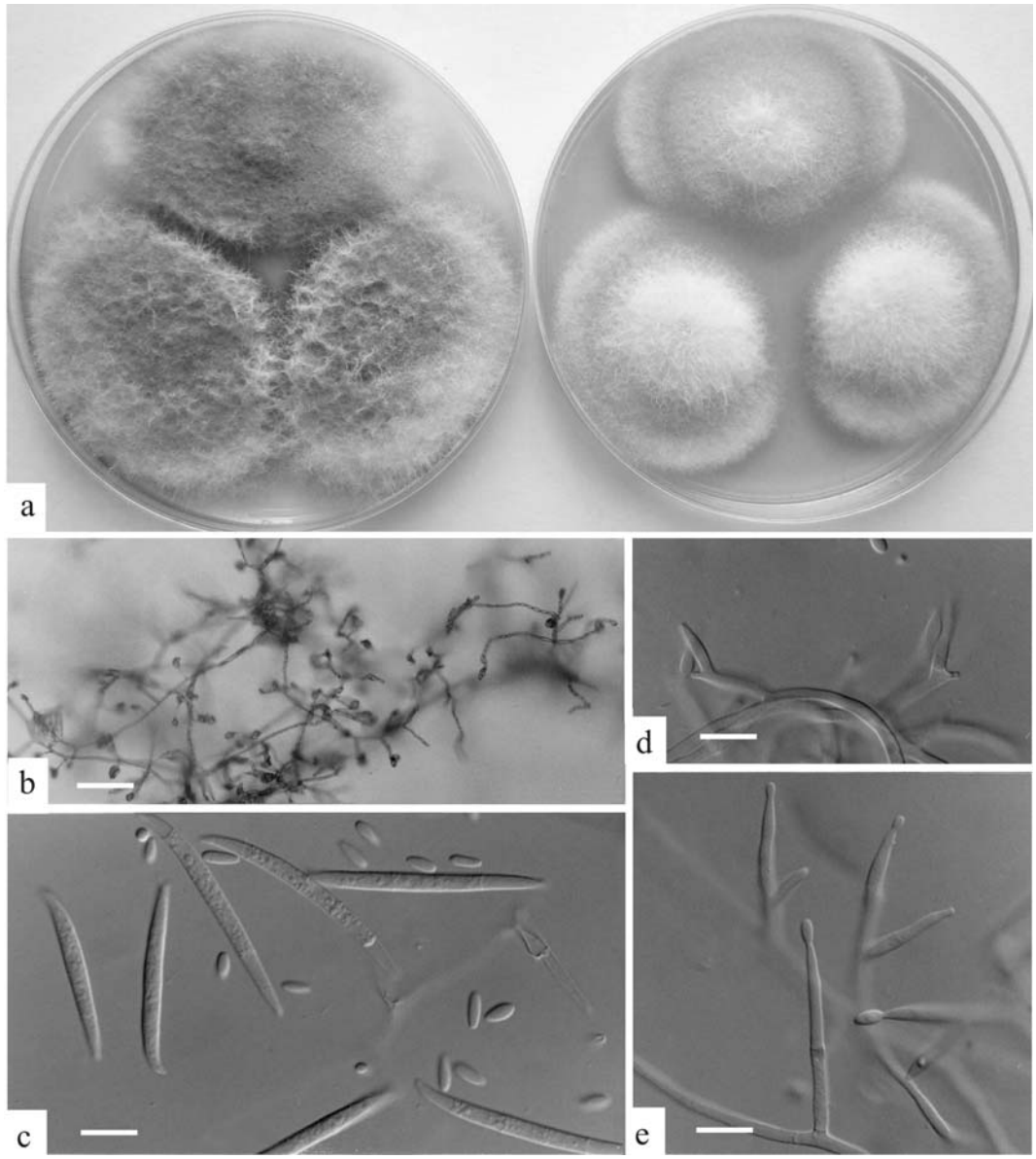


Fig. 5.31 *Fusarium proliferatum* **(a)** colonies on PDA and DCPA, 7 d, 25°C; **(b)** phialides bearing microconidia in chains and false heads in situ, bar = 50 μm; **(c)** macroconidia and microconidia, bar = 10 μm; **(d)** polyphialides, bar = 10 μm; **(e)** monophialides, bar = 10 μm

vacuum packaging and the incorporation of oxygen scavenging sachets prevented growth (Samapundo et al., 2007b). Application of 1% ammonium bicarbonate to maize at a_w values between 0.99 and 0.92 prevents growth and fumonisin production by *F. proliferatum* and was proposed as a possible treatment for stored maize (Samapundo et al., 2007c). The antioxidants propylparaben and butylated hydroxyanisole (BHA) can also inhibit growth and fumonisin production by *F. proliferatum* under some conditions (Etcheverry et al., 2002).

Fumonisin B_1 production was inhibited at headspace CO_2 concentrations of 40, 30 and 10% at 0.98, 0.95 and 0.93, respectively (Samapundo et al., 2007a). When headspace oxygen was controlled, optimum fumonisin production occurred

with 20% O_2 at 0.98 a_w, but at 0.95 and 0.93 a_w, more fumonisin was produced with 10% headspace O_2 (Samapundo et al., 2007b). Marín et al. (1995) reported fumonisin production was higher at 25 than at 30°C, and highest at the highest a_w tested (0.97), with low levels still produced at 0.92 a_w. Samapundo et al. (2005b) found that there was little difference in fumonisin production at 25 and 30°C, but lower amounts were produced at 15°C, irrespective of a_w value (between 0.97 and 0.92). Conversely, Melcion et al. (1998) observed maximal fumonisin production at 15°C on maize at very high a_w.

Mycotoxins. *Fusarium proliferatum* is a major producer of fumonisins B_1, B_2 and B_3 (Ross et al., 1990; see also Pitt and Hocking, 1997; Desjardins, 2006), and indeed strains of *F. proliferatum* are among the highest fumonisin producers (Leslie et al., 2004). Other mycotoxins produced include moniliformin, beauvericin, enniatins and fusarin (Marasas et al., 1986; Desjardins et al., 2000a; see also Desjardins, 2006).

Ecology. Reporting of *Fusarium proliferatum* in foods and feeds has increased sharply with the discovery of fumonisins and that this species is an important source of these toxins. It has been shown to colonise maize plants throughout the world, and is increasingly important in maize ear rot in Europe (see Bacon and Nelson, 1994; Desjardins, 2006; Leslie and Summerell, 2006). It was reported from sorghum in Nigeria by Onyike and Nelson (1992) and in pine nuts in Spain (Marín et al., 2007b). We have encountered this species in maize, mung beans, paddy rice and occasionally sorghum in Southeast Asia (Pitt et al., 1998a). *F. proliferatum* has also been reported as a cause of black point in wheat (Conner et al., 1996; Desjardins et al., 2007). It is a pathogen of bananas (Jiménez et al., 1993), onions (Toit et al., 2003; Stankovic et al., 2007), garlic (Dugan et al., 2003, 2007; Stankovic et al., 2007) and asparagus (Elmer, 2000; Gossman et al., 2005; Corpas-Hervias et al., 2006; Weber et al., 2006; Liu et al., 2007). Fumonisins have been reported in infected garlic (Seefelder et al., 2002) and asparagus (Logrieco et al., 1998b; Seefelder et al., 2002; Weber et al., 2006; Liu et al., 2007).

References. Nelson et al. (1983); Desjardins (2006); Leslie and Summerell (2006).

Fusarium semitectum Berk. & Ravenel Fig. 5.32

Fusarium incarnatum (Desm.) Sacc.
Fusarium pallidoroseum (Cooke) Sacc.

Colonies on CYA filling the whole Petri dish, sometimes to the lid at the edges, of dense, floccose, white, pale salmon or pale brown mycelium, sometimes powdery from macroconidia produced in the aerial mycelium; pale salmon sporodochia occasionally produced at the inoculation points; reverse pale salmon, yellowish, greyish yellow or with brown patches. Colonies on MEA filling the whole Petri dish, of floccose or funiculose white to pale greyish yellow mycelium; reverse pale, sometimes greyish orange centrally or in patches. Colonies on G25N 15–20 mm diam. At 5°C, usually only microcolonies produced. Usually no growth at 37°C, but occasionally colonies of 2 mm diam formed.

On PDA, colonies of dense, floccose mycelium, coloured pale salmon, brown or yellow, sometimes with a powdery appearance from macroconidia in the aerial mycelium; reverse pale salmon, often brownish centrally. On DCPA, colonies in similar colours to those on PDA, but less dense, aerial mycelium often powdery with macroconidia, and occasionally a central area of salmon to orange sporodochia; reverse pale.

Macroconidia of two types produced: the first in the aerial mycelium, often from polyphialides, cigar- or spindle-shaped, straight or slightly curved, with four to five septa, and with poorly differentiated basal and apical cells; the second in sporodochia, more curved, with a foot-shaped basal cell and curved apical cell. Chlamydoconidia usually produced; microconidia not produced.

Distinctive features. The production of spindle- or cigar-shaped macroconidia from polyphialides in the aerial mycelium is the most distinctive feature of *Fusarium semitectum*. These macroconidia can be observed *in situ* with the low power microscope, and are often arranged in pairs in "V" shapes, resembling rabbit ears.

Taxonomy. Type material of *Fusarium semitectum*, examined by Booth and Sutton (1984), was found to be a *Colletotrichum* species, *C. musae*. However, type material of *F. pallidoroseum* (Cooke) Sacc., which they also examined, contained polyphialides and macroconidia typical of *F. semitectum*. Subsequently, Nirenberg (1990) pointed out that the species epithet *incarnatum* predates *pallidoroseum* by about 30

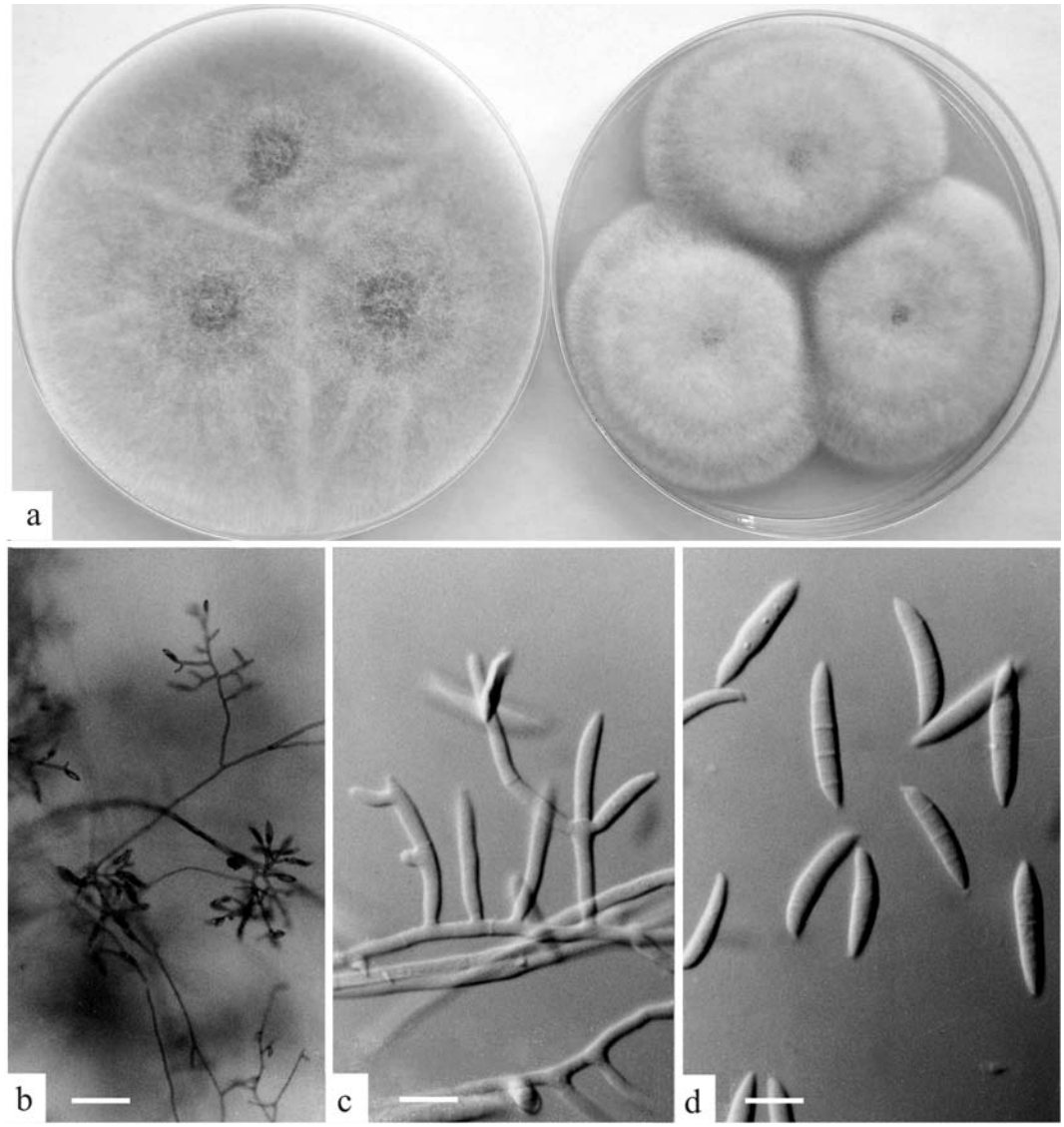


Fig. 5.32 *Fusarium semitectum* **(a)** colonies on PDA and DCPA, 7 d, 25°C; **(b)** phialides bearing macroconidia in aerial mycelium in situ, bar = 50 µm; **(c)** phialides, bar = 10 µm; **(d)** macroconidia, bar = 10 µm

years, so if the name were to be changed, it should be *F. incarnatum*. However, both Desjardins (2006) and Leslie and Summerell (2006) agree that *F. semitectum* is really a species complex, with possibly five intra-specific groups (Abd-Elsalam et al., 2003), and until such time as it is thoroughly examined both morphologically and phylogenetically, the name *F. semitectum* should continue to be used.

Physiology. The temperature range for growth of *Fusarium semitectum* is 3°C to ca. 37°C, with the optimum near 25°C (Kakker and Mehrotra, 1971; Marin et al., 1996; Shukla and Sharma, 2000). Cherian (2007) reported maximum rotting of bananas by *F. semitectum* occurred at 30°C.

Mycotoxins. Confusion still exists over the mycotoxins produced by *Fusarium semitectum* (Desjardins, 2006). Zearalenone production has been reported (see Marasas et al., 1984; Pitt and Hocking, 1997, Desjardins, 2006, Leslie and Summerell, 2006). Production of T-2 toxin has also been reported, but is unsubstantiated (Marasas et al., 1984; Desjardins, 2006). Japanese isolates of *F.*

semitectum have been reported to produce diacetoxyscirpenol, nivalenol, fusarenone-X and neosolaniol (Suzuki et al., 1980, 1981). Diacetoxyscirpenol production has also been reported by Molto et al. (1997).

Ecology. *Fusarium semitectum* is widespread in tropical and subtropical countries, although reports from foods are relatively uncommon. It has been found at low levels in maize in Italy (Logrieco et al., 1995) and the USA (Katta et al., 1995), and we have found high levels of *F. semitectum* in Thai maize (45% of samples, up to 22% infected grains in infected samples and 4% of all kernels examined; Pitt et al., 1993). Incidence in Indonesian maize was similar, but levels in Philippine maize lower (Pitt et al., 1998a). *F. semitectum* is one of the dominant fungi of pearl millet (Wilson, 2002; Jurjevic et al., 2007) and is common in peanuts (Joffe, 1969; Gilman, 1969; Oyeniran, 1980; Pitt et al., 1993; Dharmaputra and Retnowati, 1996). It has also been reported from rice (Desjardins et al., 2000a) and sorghum (Onyike and Nelson, 1992; Usha et al., 1994; Shabbir and Rajasab, 2004). It causes storage rots of various fruits (Snowdon, 1990), especially crown rot of bananas (see Pitt and Hocking, 1997; Vesonder et al., 1995; Marin et al., 1996; Cherian, 2007) and soft rot of bell peppers (Shukla and Sharma, 2000; Sharma and Shukla, 2003). It has also been reported to cause postharvest spoilage of tomatoes (Muniz et al. 2003; Chaturbhuj and Rai, 2005), passionfruit (Muniz et al., 2003), mushrooms (Kang et al., 2002) and disease in walnuts (Belisario et al., 1999). This species has also been isolated from pigeon peas (Maximay et al., 1992), soybeans (Vaamonde et al., 1987; and our unpublished data), black gram (Goyal and Jain, 1998), black beans (Castillo et al., 2004), coriander (Hashmi and Thrane, 1990; Hashmi and Ghaffar, 1991) and sunflower seeds (Shahnaz and Ghaffar, 1991), though with few reports in each case. Earlier reviews indicate isolations from citrus fruits, tomatoes, melons, cucumbers and potatoes (Booth, 1971; Domsch et al., 1980).

In our experience, *Fusarium semitectum* is much more common in tropical commodities than the above citations would indicate. This species appears to be endemic in paddy rice from Thailand (in 94% of samples examined, up to 44% of grains infected in infected samples and a very high 20% of all grains examined; Pitt et al., 1994) and Indonesia (60% of samples, up to 60% of grains in infected samples and 7% of all grains; Pitt et al., 1998a). It was the most common fungus in Thai mung beans (55% of samples; up to 76% infection in infected samples, and 15% infection overall). It was also common in Thai black beans (6% of all beans examined), sorghum (4% of all grains) and soybeans (2% of all beans; Pitt et al., 1994). Generally similar figures were found for infection in rice, soybeans, black beans and mung beans in Indonesia and the Philippines (Pitt et al., 1998a). Indeed, *F. semitectum* was by far the most common fungal invader of beans that we encountered in Southeast Asia.

References. Domsch et al. (1980); Nelson et al. (1983); Desjardins (2006); Leslie and Summerell (2006).

Fusarium solani (Mart.) Sacc. Fig. 5.33

Teleomorph: *Haemanectria haematococca* (Berk. & Broome) Samuels & Nirenberg

Colonies on CYA 60–65 mm diam, low, of moderately dense white mycelium, often covered in very fine droplets of clear exudate; a central, cream spore mass sometimes present; reverse pale, sometimes with bluish or greenish areas. Colonies on MEA 50–60 mm diam, low to moderately deep, of sparse, often slightly funiculose, white to pale violet mycelium; reverse pale, sometimes bluish grey centrally. Colonies on G25N 3–8 mm diam. No growth at 5°C. At 37°C, either no growth, or colonies up to 10 mm diam formed.

On PDA, colonies low to moderately deep, of white to cream mycelium in concentric rings, often alternating with rings of cream or bluish grey sporodochia; in some isolates the teleomorph produced, of dark orange perithecia scattered over the central area of the colony; reverse pale or with areas of turquoise grey or pale violet brown. On DCPA, colonies low, of sparse, white mycelium in annular rings, with a central mass of cream sporodochia; reverse pale to pale yellow brown.

Macroconidia abundant, stout, thick walled, with three to four, or less commonly five septa, straight, parallel sided for most of the length, apical cells blunt and rounded, basal cells either rounded, notched or sometimes distinctly foot-shaped.

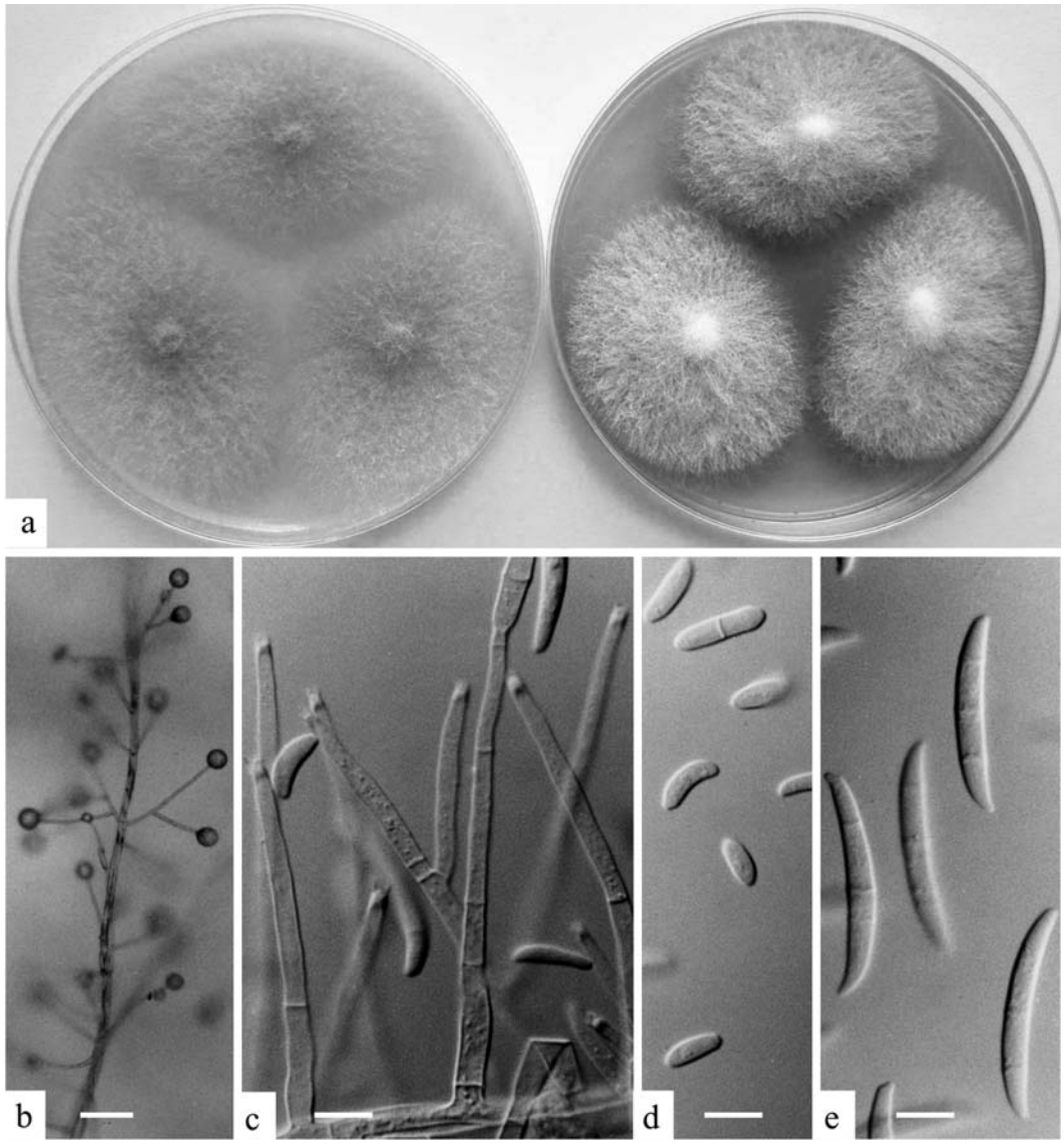


Fig. 5.33 *Fusarium solani* **(a)** colonies on PDA and DCPA, 7 d, 25°C; **(b)** phialides bearing macroconidia in false heads in situ, bar = 50 µm; **(c)** phialides, bar = 10 µm; **(d)** microconidia, bar = 10 µm; **(e)** macroconidia, bar = 10 µm

Microconidia usually abundant, ellipsoidal, fusiform or kidney-shaped, produced in false heads on very long, straight phialides. Chlamydoconidia produced singly or in pairs.

Distinctive features. *Fusarium solani* is the only *Fusarium* species which produces cream or bluish rather than salmon or orange sporodochia. The macroconidia are stout and straight or only slightly curved. Microconidia are produced in false heads (mucoid balls) on very long, slender phialides. This species is morphologically very similar to *F. oxysporum*. However, phialides in *F. solani* are much longer than those of *F. oxysporum*.

Taxonomy. The teleomorph of *Fusarium solani* is *Haemanectria haematococca* (Berk. and Broome) Samuels and Nirenberg, which produces orange brown perithecia. However, only about 1% of *F. solani* isolates produce the teleomorph in culture (Booth, 1983). Leslie and Summerell (2006) believe that there are a number of biological species within *F. solani* and that further work is required to clarify the taxonomy and phylogeny of this group.

Physiology. Domsch et al. (1980) noted that various authors have reported the optimum growth temperature for *Fusarium solani* to be between 27 and 31°C, with strong growth at 37°C. However, our observations indicate only weak growth at this temperature, and Chaturvedi et al. (2003) reported optimum growth at 25°C. Schneider (1954) reported growth of *F. solani* down to 0.90 a_w after a germination time of 8 weeks at 20°C.

Mycotoxins. Mouldy sweet potatoes (*Ipomoea batatas*) or extracts from them have been shown to be toxic to a variety of animals, including cattle (Wilson et al., 1970; Doupnik et al., 1971), chickens (Doupnik et al., 1971; Peckham et al., 1972) and mice (Boyd and Wilson, 1972). The toxic principles are believed to be furanoterpenoids, ipomeanols and ipomeanine (Nelson et al., 1983, 1994; Mawhinney et al., 2008). These compounds are not mycotoxins, but result from fungal metabolism of phytoalexins produced by the plant (see Leslie and Summerell, 2006). *Fusarium solani* has been reported to produce fusaric acid (Bacon et al., 1996) and the immunosuppressive compound cyclosporin (Sugiura et al., 1999).

Ecology. A cosmopolitan soil fungus, *Fusarium solani*, has frequently been isolated from subterranean crops such as potatoes, sweet potatoes, yams and peanuts (see Pitt and Hocking, 1997; Ismail, 2001; Peters et al., 2008). Hot water dipping (57.5°C, 20–30 min) can reduce the incidence of storage rots of potatoes caused by *F. solani* (Ranganna et al., 1998). *F. solani* may also invade a wide variety of other vegetable crops (Snowdon, 1991), especially cucurbits including muskmelons (Champaco et al., 1993) and squash (Assawah and Al-Zarari, 1984), and legumes such as beans, soybeans and peas (see Pitt and Hocking, 1997; Tseng and Tu, 1997). Other sources are diverse, including small grains, where it may be part of the cohort of *Fusarium* species causing head blight (Bottalico and Perrone, 2002), maize, sorghum, bananas, guavas, cassava, sugar beets, capsicums, garlic, and seeds of sunflower, sesame and coriander (see Pitt and Hocking, 1997). We have isolated *F. solani* from beer, shortening flakes, chilled water lines in a beverage production plant, the inside of a juice filling machine, and an air conditioning duct.

This species was widespread in Indonesian crops, especially beans, usually at about 1% of all seeds examined, in paddy rice, maize, peanuts and pepper (Pitt et al., 1998a).

Fusarium solani can attack keratin, and has been isolated from human fingernails and eyes (Domsch et al., 1980; de Hoog et al., 2000; Leslie and Summerell, 2006). It can be a serious pathogen of crustaceans, attacking and destroying the chitinous exoskeleton (Fisher et al., 1978; Gonzalez, 1995; Le et al., 2005) and has also been reported to cause disease in sea turtles (Cabañes et al., 1997; Castellá et al., 1999a; de Hoog et al., 2000).

References. Domsch et al. (1980); Nelson et al. (1983); Desjardins (2006); Leslie and Summerell (2006).

Fusarium sporotrichioides Sherb. Fig. 5.34

Colonies on CYA filling the whole Petri dish, deep, of floccose white to pale pink and brown mycelium; reverse pale salmon centrally, brownish at the margins. Colonies on MEA similar to those on CYA except more highly coloured; reverse violet brown centrally, paler at the margins. Colonies on G25N 12–15 mm diam. At 5°C, colonies 5–10 mm diam. No growth at 37°C.

On PDA, colonies of dense, floccose, salmon to pink mycelium, sometimes with a central mass of orange sporodochia; reverse greyish rose to burgundy, occasionally paler. On DCPA, colonies of floccose pale salmon mycelium in concentric rings; orange sporodochia sometimes present on the agar surface; reverse pale.

Macroconidia abundant, moderately curved, with three to five septa and with a curved, pointed apical cell and a notched or foot-shaped basal cell. Microconidia abundant, produced from polyphialides in the aerial mycelium, fusiform, broadly ellipsoidal or pyriform, the latter often produced only on PDA, often with a papilla at the base. Chlamydoconidia formed abundantly, singly or in chains or clumps, as cultures age.

Distinctive features. On PDA, *Fusarium sporotrichioides* has a similar appearance to *F. poae, F. chlamydosporum* and *F. tricinctum*. The production of both fusiform and pyriform microconidia from polyphialides distinguishes *F. sporotrichioides* from these closely related species. Pyriform microconidia are produced more commonly, and sometimes exclusively, on cultures on PDA. Colony reverses on PDA are always greyish rose or burgundy.

Physiology. Domsch et al. (1980) gave the optimum growth temperature of *Fusarium sporotrichioides* as 22.5–27.5°C, with a maximum of 35°C. Joffe (1962)

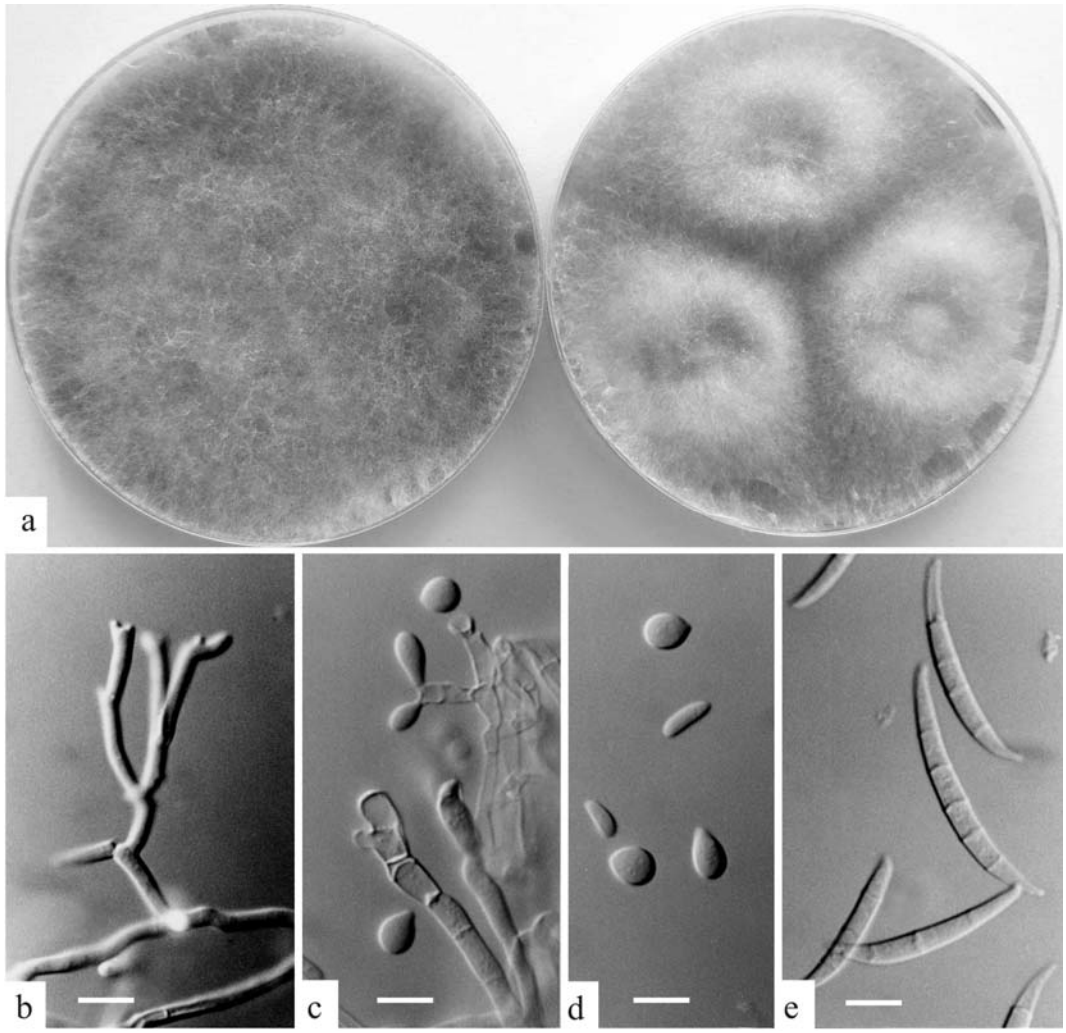


Fig. 5.34 *Fusarium sporotrichioides* **(a)** colonies on PDA and DCPA, 7 d, 25°C; **(b, c)** polyphialides, bar = 10 μm; **(d)** microconidia, bar = 10 μm; **(e)** macroconidia, bar = 10 μm

reported the growth of toxigenic isolates of this species down to –2°C. Schneider (1954) reported 0.88 a_w as the minimum for growth, after 8 weeks incubation at 20°C.

Mycotoxins. *Fusarium sporotrichioides* is regarded as the major cause of alimentary toxic aleukia (ATA) a devastating disease which occurred in the USSR during and after World War II, in times of extreme food shortage resulting in consumption of overwintered cereals (Joffe, 1978). Hundreds of thousands of people died from T-2 toxin produced by this species and *F. poae* (Marasas et al., 1984). *F. sporotrichioides* also produces a number of T-2 toxin derivatives, including HT-2, T-2 triol and T-2 tetraol, and biosynthetic intermediates such as neosolaniol, diacetoxyscirpenol and 15-monoacetoxyscirpenol (Thrane et al., 2004; Abramson et al., 2004; see also Pitt and Hocking, 1997; Desjardins, 2006; Leslie and Summerell, 2006). *F. sporotrichioides* has been used to elucidate the biochemistry and genetics of trichothecene biosynthesis (Desjardins et al., 1993), with the first trichothecene biosynthetic gene, trichodiene synthase (*TRI5*) cloned from *F. sporotrichioides* in 1989 (see Desjardins, 2006; Leslie and Summerell, 2006). Early reports of deoxynivalenol and nivalenol production have not been confirmed and may be due to misidentification of isolates (Desjardins, 2006). Other mycotoxins produced by *F. sporotrichioides* include aurofusarin, beauvericin and occasionally enniatins, but not nivalenol, fusarenone-X or fumonisins (Thrane et al., 2004; Desjardins, 2006).

Fusarium sporotrichioides has been implicated as the cause of mouldy corn toxicosis (haemorrhagic syndrome) in cattle, pigs and poultry in the USA and elsewhere, fescue foot in cattle feeding on winter pastures in the USA, Australia and New Zealand, akakabi-byo (scabby grain intoxication) and bean hulls poisoning in animals in Japan (Marasas et al., 1984; Wu et al., 1997).

Ramakrishna et al. (1996) reported that T-2 toxin formation by *F. sporotrichioides* on barley grain occurred optimally at 20°C and toxin production was stimulated by the presence of *Aspergillus flavus* or *Penicillium verrucosum*.

Ecology. While not so commonly isolated as some other species described here, *Fusarium sporotrichioides* is important because of its toxicity. It occurs more commonly in cool climates and, in foods, is almost entirely confined to grains. Historically, this species has been associated with rye in Europe (Joffe, 1978; Matossian, 1989). More recently, it has been reported most frequently from wheat, in Japan, Spain, Hungary, Poland, Canada, Ethiopia (see Pitt and Hocking, 1997) and Finland and Russia (Yli-Mattila et al., 2004). Other grain sources include maize (Logrieco et al., 1995, 2002; Adejumo et al., 2007; and see Pitt and Hocking, 1997), barley (Yli-Mattila et al., 2004; Bourdages et al., 2006), sorghum, wild rice (Nyvall et al., 1999) and cereal grains in general (see Pitt and Hocking, 1997; Langseth et al., 1998; Kosiak et al., 2003). This species has also been isolated from peanuts (Austwick and Ayerst, 1963), sugar beets (Bosch and Mirocha, 1992) and in our laboratory from Australian soybeans.

References. Domsch et al. (1980); Nelson et al. (1983); Desjardins (2006); Leslie and Summerell (2006).

Fusarium subglutinans (Wollenw. & Reinking) P.E. Nelson et al. Fig. 5.35

Fusarium moniliforme J. Sheld. var. *subglutinans* Wollenw. & Reinking

Fusarium sacchari (Butler) W. Gams var. *subglutinans* Wollenw. & Reinking

Teleomorph: *Gibberella subglutinans* (Edwards) P.E. Nelson et al.

Colonies on CYA covering the whole Petri dish, of dense white to very pale salmon mycelium, in degenerate cultures of low and sparse funiculose white mycelium; sometimes with a central orange spore mass; reverse pale, salmon or yellowish. Colonies on MEA less dense than on CYA, of white to pale salmon mycelium, sometimes powdery with microconidia; reverse pale yellow, salmon or violet grey. Colonies on G25N 5–12 mm diam, occasionally larger. At 5°C, germination to microcolony formation. No growth at 37°C.

On PDA, colonies low to moderately deep, of floccose to funiculose mycelium coloured white, pale salmon, pale pink or mauve, sometimes powdery with microconidia, reverse violet grey to deep violet centrally, paler at the margins, or uniformly pale. On DCPA, colonies low at the margins, floccose centrally, of pale salmon mycelium, sometimes overlying a thin layer of pale orange macroconidia on the agar surface; reverse pale.

Macroconidia slightly curved to almost straight, with three to five septa and thin, delicate walls, narrow, tapered apical cells and foot-shaped basal cells. Microconidia abundant, fusiform or broadly ellipsoidal, aseptate or with a single septum, produced in false heads from polyphialides and also from simple phialides. Chlamydoconidia not produced.

Distinctive features. The production of microconidia in false heads from polyphialides and the absence of chlamydoconidia distinguish *Fusarium subglutinans* from otherwise similar species such as *F. verticillioides* and *F. oxysporum*.

Taxonomy. *Fusarium subglutinans* was raised to species status by Nelson et al. (1983), previously having been regarded as a variety of *F. moniliforme* or *F. sacchari*. The teleomorph of *F. subglutinans* is *Gibberella subglutinans* (Edwards) P.E. Nelson et al. Recent phylogenetic studies indicate that there may be at least two cryptic species within *F. subglutinans* (Steenkamp et al., 2002; Desjardins et al., 2006).

Physiology. Martelleto et al. (1998) reported growth of *Fusarium subglutinans* between 10 and 30°C, with 25°C being optimal. Castellá et al. (1999b) found growth of *F. subglutinans* was optimal between 20 and 25°C on maize cultures, but on rice, growth was best at 15°C. Highest production of fusaproliferin was at 20°C on maize after 6 weeks (Castellá et al., 1999b).

Mycotoxins. Usually regarded as one of the less toxic of major *Fusarium* species, *F. subglutinans* has, however, been reported to produce moniliformin as its major toxin (see Pitt and Hocking, 1997; Kostecki et al., 1999; Sewram et al., 1999; Logrieco

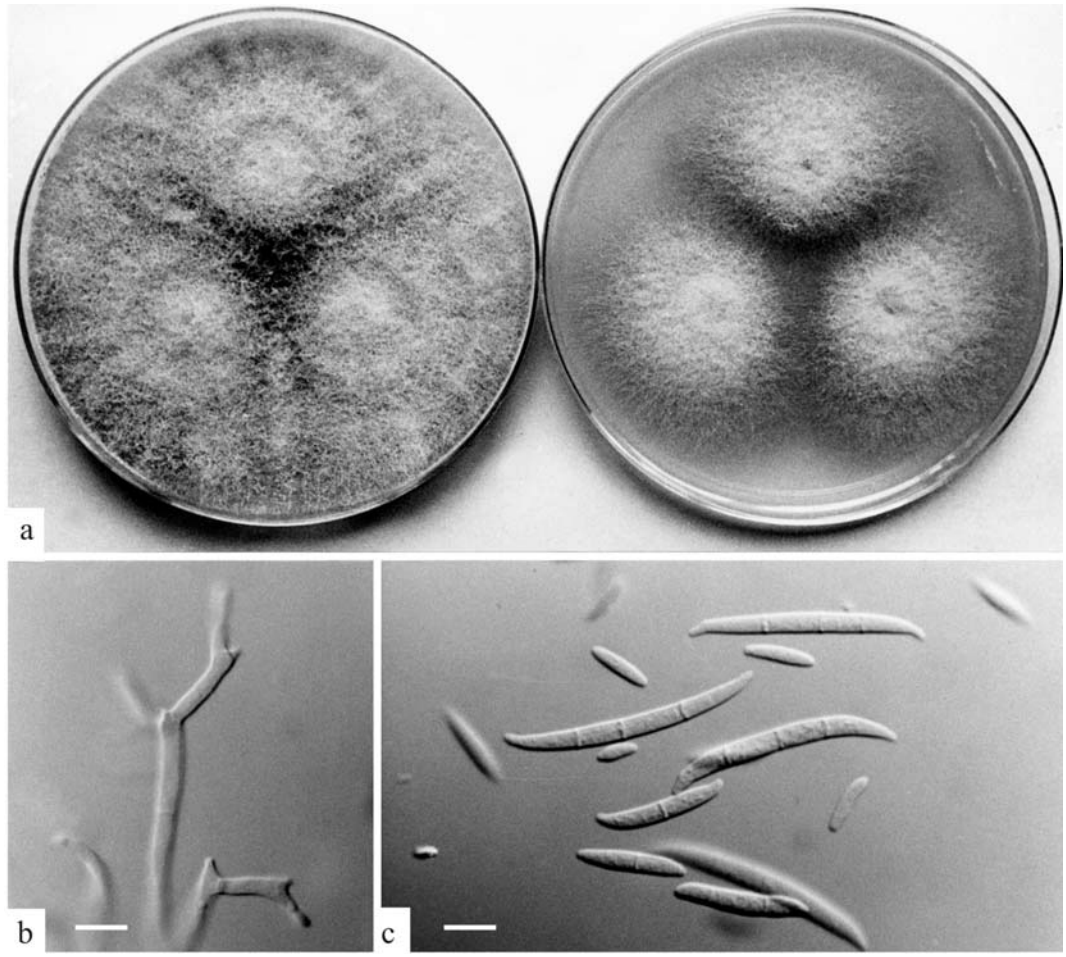


Fig. 5.35 *Fusarium subglutinans* **(a)** colonies on PDA and DCPA, 7 d, 25°C; **(b)** polyphialide**s**, bar = 10 μm; **(c)** macroconidia amd microconidia, bar = 10 μm

et al., 2002; Desjardins, 2006; Desjardins et al., 2006; Leslie and Summerell, 2006; Sørensen et al., 2007). Production of fumonisin B_1 has also been reported (Visconti and Doko, 1994), but levels are generally low (Reynoso et al., 2004). Other toxins reported are beauvericin and fusaproliferin (Moretti et al., 1995; Logrieco et al., 1998a; Castellá et al., 1999b; Kostecki et al., 1999; Shephard et al., 1999; Torres et al., 2001; Reynoso et al., 2004).

Ecology. Closely related to *Fusarium verticillioides, F. subglutinans* appears to have a similar host range, and a worldwide distribution. Its major food habitat is maize. It has been isolated from Europe (Italy, Logrieco et al., 1995; Austria, Lew et al., 1991; Poland, Logrieco et al., 1993b), the United States (Abbas et al., 1988, 1989b; Katta et al., 1995), Canada (Neish et al., 1983), the Caribbean (Julian et al., 1995), Peru (Logrieco et al., 1993a), Argentina (Torres et al., 2001; Reynoso et al., 2004, 2006), Mexico (Desjardins et al., 2000b; Morales-Rodriguez et al., 2007), South Africa (Marasas et al., 1978, 1979; Rheeder et al., 1995), Zimbabwe (Mubatanhema et al., 1999), Indonesia (Pitt et al., 1998a), Australia (Burgess et al., 1981; Blaney et al., 1986) and New Zealand (Hussein et al., 1991, 2002). *F. subglutinans* has also been isolated in Mexico from teosintes, the nearest wild relatives of maize (Desjardins et al., 2000b). *F. subglutinans* has been reported from wheat in Europe (Bottalico and Perrone, 2002; Ioos et al., 2004; Cosic et al., 2007), South Africa (Boshoff et al., 1998), Tanzania (van Dyk, 2004), Egypt (Gherbawy et al., 2006) and Iran (Moosawi-Jorf

et al., 2007), but it does not appear to play an active role in head blight.

Fusarium subglutinans is a pathogen of pineapples (Bolkan et al., 1979; Rohrbach and Taniguchi, 1984, Matos et al., 2000), bananas (Jiménez et al., 1993, 1997; Wade et al., 1993; Vesonder et al., 1995) and capsicums (bell peppers) (Hashmi and Thrane, 1990; Utkhede and Mather, 2004). Other sources have been sorghum (Onyike and Nelson, 1992), sugar beets (Bosch and Mirocha, 1992), traditional African vegetables (van der Walt et al., 2006), tomatoes and lemons (Muniz et al., 2003), and black pepper (Pitt et al., 1998a).

References. Booth (1971); Nelson et al. (1983); Desjardins (2006); Leslie and Summerell (2006).

Fusarium verticillioides (Sacc.) Nirenberg

Fig. 5.36

Fusarium moniliforme J. Sheld. (in part)

Teleomorph: *Gibberella moniliformis* Wineland

Colonies on CYA usually covering the whole Petri dish, low at the margins to moderately deep centrally, of floccose to funiculose white mycelium; reverse pale, or in shades of pale salmon or violet. Colonies on MEA usually covering the whole Petri dish, low to moderately deep centrally, of white or pale salmon funiculose mycelium, sometimes powdery with microconidia; reverse of some isolates pale, of others violet or greyish magenta. Colonies

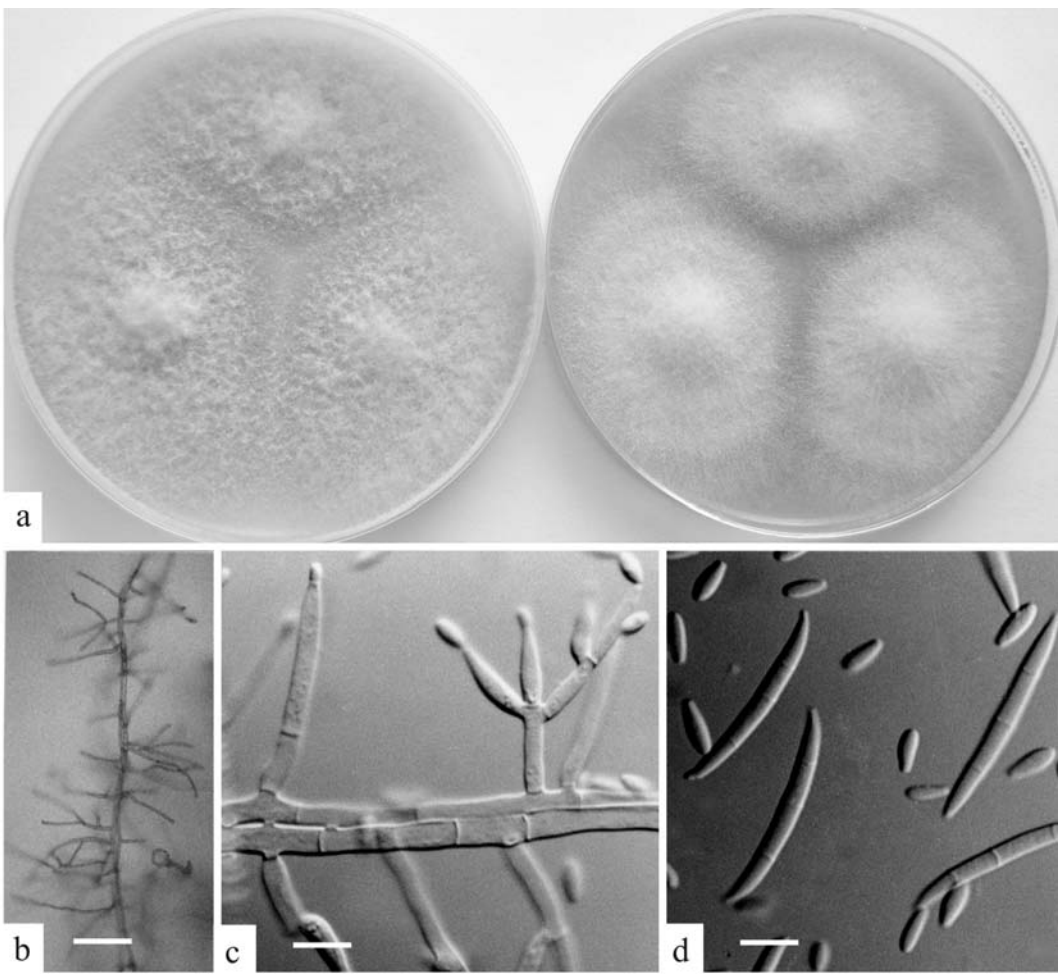


Fig. 5.36 *Fusarium verticillioides* **(a)** colonies on PDA and DCPA, 7 d, 25°C; **(b)** phialides bearing chains of microconidia, bar = 50 μm; **(c)** phialides, bar = 10 μm; **(d)** macro- and microconidia, bar = 10 μm

on G25N 5–12 mm diam. At 5°C, either germination or no growth. At 37°C, colonies of 4–10 mm diam produced.

On PDA, colonies of low, densely funiculose mycelium coloured white to pale salmon, usually powdery with chains of microconidia; reverse varying from isolate to isolate, pale salmon, greyish violet, brownish violet or deep violet; dark blue sclerotia produced by some isolates. On DCPA, colonies low, of thin, funiculose white to pale salmon mycelium in concentric rings, powdery with microconidia, and sometimes alternating with rings of macroconidia produced on the agar surface; reverse pale.

Macroconidia usually long and slender, almost straight, thin walled, with foot-shaped basal cells; microconidia fusiform to clavate, produced from long monophialides, forming chains readily seen *in situ* under the low power microscope (6× to 10×); chlamydoconidia not produced.

Distinctive features. The production of microconidia in long chains from relatively long phialides distinguishes *Fusarium verticillioides* from most other common *Fusarium* species. *F. proliferatum* is very similar to in appearance to *F. verticillioides* but is distinguished from it by the production of polyphialides bearing microconidia in short chains.

Taxonomy. In 1877, Saccardo described *Oospora verticillioides* from maize in Italy. In 1904, John Sheldon described *Fusarium moniliforme* from maize that was associated with animal toxicosis in the United States (see Desjardins, 2006), and for most of the twentieth century, the epithet *F. moniliforme* was used for this fungus. From an extensive study of the group of *Fusarium* species which included *F. moniliforme*, Nirenberg (1976) concluded that this species should be called *F. verticillioides*, but at the time, this proposal was not generally accepted. However, Seifert et al. (2003), representing the International Society for Plant Pathology and the International Committee on the Taxonomy of Fungi (ISPP/ICTF) Subcommittee on *Fusarium* Systematics, proposed that the name *F. verticillioides* be applied to the fumonisin producing fungus on maize, on the grounds that the name *F. moniliforme* represented an unacceptably broad species concept, and that *F. verticillioides* was undisputably the older name, which therefore had priority. This name is now generally accepted for isolates from maize and a few other sources (Leslie and Summerell, 2006). Morphologically similar species within the *Gibberella fujikeroi* complex, associated with different plant hosts and different mating types, have been described: *F. thapsinum* from sorghum, *F. sacchari* from sugar cane, *F. mangiferae* from mango and *F. fujikuroi* from rice, and these names have now been taken up by Leslie and Summerell (2006).

Physiology. The maximum temperature for growth of *Fusarium verticillioides* has been reported to be 32–37°C, the minimum as 2.5–5°C, and the optimum near 25°C (Nirenberg, 1976). The minimum a_w for growth was 0.87 at 25°C, after a 4-month germination time (Armolik and Dickson, 1956). Reduction of headspace oxygen from 20 to 2% had no effect on growth of *F. verticillioides*, but vacuum packaging with oxygen absorbing sachets completely inhibited growth (Samapundo et al., 2007b). Fumonisins B_1 and B_2 were produced down to at least 0.92 a_w (Marín et al., 1995) but were not detected at 0.89–0.91 a_w on irradiated maize grains (Marín et al., 1999). Maximum fumonisin production occurred with 15% oxygen at 0.976 a_w, but with 5% O_2, 0.93 was the optimum a_w (Samapundo et al., 2007b).

Mycotoxins. The major mycotoxin produced by *Fusarium verticillioides* is fumonisin B_1, the cause of leucoencephalomalacia in horses, pulmonary oedema in pigs and liver cancer in rats. It is a possible cause of human oesophageal cancer (Gelderblom et al., 1988; Sydenham et al., 1990; Shephard et al., 2007) and neural tube defects (Desjardins, 2006). For a comprehensive account of fumonisins, see Marasas (2001) and Desjardins (2006). Isolates of *F. verticillioides* from maize from many countries have been shown to produce fumonisins B_1 and B_2 (Bezuidenhout et al., 1988; Laurent et al., 1989; and see Pitt and Hocking, 1997; Desjardins, 2006). This species also commonly produces fusarins, fusaric acid and naphthoquinones, but not beauvarin, moniliformin or fusiproliferin (see Pitt and Hocking, 1997; Desjardins, 2006; Leslie and Summerell, 2006). Reports of zearalenone, diacetoxyscirpenol and deoxynivalenol production by *F. verticillioides* were discounted by Nelson et al. (1983). Recently identified isolates of *F. verticillioides* from banana rots (Mirete et al., 2004; Moretti et al., 2004) produce moniliformin but not

fumonisins (Patiño et al., 2006). These isolates need further characterisation.

Ecology. *Fusarium verticillioides* is widespread in the tropics and humid temperate areas of the world (Leslie and Summerell, 2006). This species is an endemic pathogen of maize, causing both stalk rot and cob rot which can be present at all stages of plant development. It has been isolated from maize kernels in every major location where it has been sought, including the United States, Canada, Mexico, Honduras, Ecuador (Pacin et al., 2002), Peru and Argentina; from Iran (Ghiasian et al., 2004; 2006), India, China, Nepal (Desjardins and Busman, 2006) and Taiwan; from Europe (Logrieco et al., 2002), and from Australia, South Africa and other countries on the African subcontinent (Adejumo et al., 2007; Bigirwa et al., 2007) (see also Pitt and Hocking, 1997; Desjardins, 2006; Leslie and Summerell, 2006). Incidence in individual maize kernels, where reported, has almost always been very high, e.g. 100% in Sardinia (Bottalico et al., 1995), 54% in mainland Italy (Logrieco et al., 1995), 97% in Thailand (Pitt et al., 1993), 92% in the Philippines and 73% in Indonesia (Pitt et al., 1998a).

Fusarium verticillioides (as *F. moniliforme* in earlier literature) has been reported as a pathogen of other Graminae, particularly sorghum (Leslie et al., 2005), rice (Desjardins et al., 2000a) and millet (Leslie et al., 2005) (see also Pitt and Hocking, 1997; Desjardins, 2006). It has been reported from several types of nuts, i.e. hazelnuts, pecans, peanuts and kola nuts (see Pitt and Hocking, 1997). *F. verticillioides* has also been reported from oilseeds: sunflower, amaranth, and soybeans; also spices, including coriander, fenugreek, cardamom and pepper (see Pitt and Hocking, 1997). It has also been found in mung beans (Pitt et al., 1994), biltong (van der Riet, 1976) and cheeses (Northolt et al., 1980).

Fusarium verticillioides (or *F. moniliforme*) has been reported to cause storage rot of oranges and other citrus (Snowdon, 1990), garlic (Gargi and Roy, 1988), yams (Ogundana, 1972), pineapples (Lim, 1983), bananas (Chorin and Rotem, 1961; Jiménez et al., 1993; Vesonder et al., 1995), asparagus (Snowdon, 1991) and dry rot of passion fruit (Lutchmeah, 1993). The molecular and chemical profiles of isolates of *F. verticillioides* that are pathogenic on bananas have been examined. Whilst banana isolates belong to mating population A (*G. moniliformis*), they are genetically distinct from *F. verticillioides* isolates from maize, produce moniliformin and are unable to produce fumonisins (Moretti et al., 2004; Mirete et al., 2004; Patiño et al., 2006).

In light of recent taxonomic changes, many of the above reports of *Fusarium moniliforme* occurrence may need to be verified.

References. Nirenberg (1976); Domsch et al. (1980); Nelson et al. (1983) (both under *F. moniliforme*); Desjardins (2006); Leslie and Summerell (2006).

5.18 Genus *Geotrichum* Link: Fr.

For a long time, the genus *Geotrichum* has been distinguished by its method of reproduction, which is exclusively the formation of arthroconidia from vegetative hyphae. Arthroconidia are cylindrical spores formed by septation of vegetative hyphae into short segments, which separate at maturity. Young colonies sometimes appear yeast-like on CYA, but soon spread rapidly. Recently, the definition of *Geotrichum* has been broadened to include some species which also produce blastospores (yeast-like cells), but these are not of interest here. The most recent taxonomy is that of de Hoog et al. (1986), in which 23 species are described, together with two teleomorphs, *Dipodascus* and *Galactomyces*. Only one species, *Geotrichum candidum*, is significant in foods (Fig. 5.37).

Geotrichum candidum Link: Fr. Fig. 5.37

Oidium lactis Fresen.
Oospora lactis (Fresen.) Sacc.

Teleomorph: *Galactomyces geotrichum* (E.E. Butler & L.J. Peterson) Redhead & Malloch

Colonies on CYA of variable size, 20–45 mm diam, very low and quite sparse, plane, of white mycelium, often leathery and difficult to dissect with a needle; reverse pale. Colonies on MEA 50–65 mm diam, similar to on CYA but of softer, yeast-like texture. On G25N, no growth to

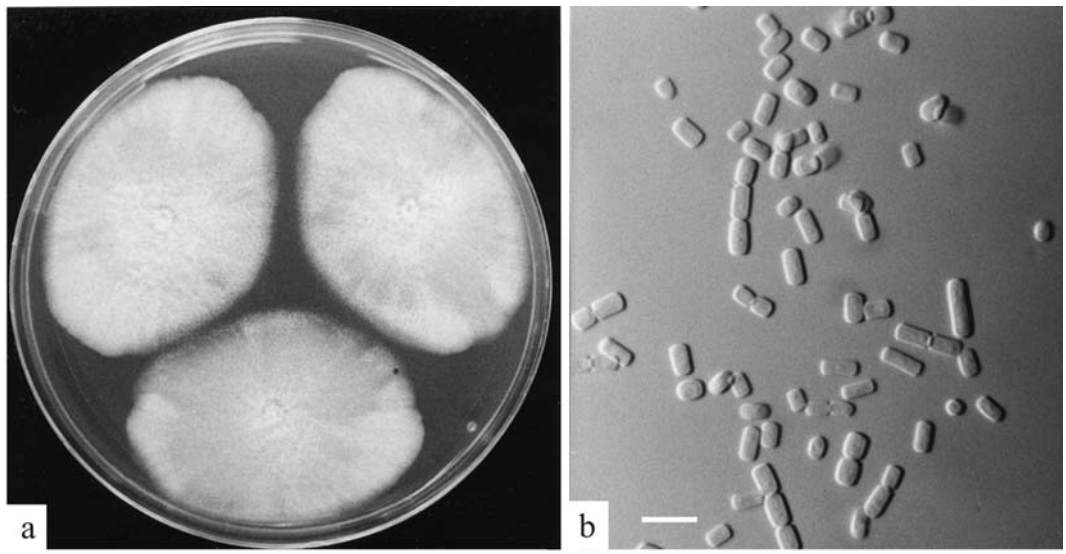


Fig. 5.37 *Geotrichum candidum* **(a)** colonies on MEA, 7 d, 25°C; **(b)** arthroconidia, bar = 10 μm

germination. At 5°C, no germination to colonies up to 4 mm diam, of dense white mycelium. At 37°C, usually no growth, occasionally sparse colonies up to 10 mm diam formed.

Conidiophores undifferentiated hyphae, at maturity fragmenting almost entirely to form arthroconidia; arthroconidia hyaline, cylindrical, sometimes developing rounded ends and thickened walls, commonly 5–8 × 2–5 μm, smooth walled.

Distinctive features. See genus description.

Taxonomy. Some isolates of *Geotrichum candidum* have been reported to form a teleomorph, *Galactomyces geotrichum*, which consists of single ascospores within solitary asci borne along fertile hyphae (de Hoog et al., 1986).

Physiology. *Geotrichum candidum* is restricted to habitats of high water availability, its minimum a_w for growth being 0.90 (Heintzeler, 1939). Growth occurred at 0.95 but not at 0.90 a_w (Plaza et al., 2003). Optimal growth temperatures are 25–30°C (Plaza et al., 2003), with maxima of 35–38°C (Domsch et al., 1980). The conidia have a very low heat resistance, with a D value of 30–40 min at 52°C (Beuchat, 1981b). Miller and Golding (1949) reported that *G. candidum* was able to grow at very low oxygen tensions, but not anaerobically.

Mycotoxins. This species is not known to produce any toxic compounds.

Ecology. *Geotrichum candidum* is a significant pathogen of citrus fruits during postharvest storage, causing sour rots (Butler et al., 1965; Hall and Scott, 1977; Snowdon, 1990). It occurs in fruit weakened by over maturity and long storage. Lemons and grapefruit are particularly susceptible, but a variety of other fruit can also be affected (Butler, 1960). Initial infection is mainly through injuries. The primary control measure is preventative, and lies in choosing sound, young fruit for long term storage (Hall and Scott, 1977). As *G. candidum* grows poorly below 10°C, cold storage can assist in control (Rippon, 1980, Plaza et al., 2003).

Geotrichum candidum causes spoilage of tomatoes (Okoli and Erinle, 1989; Snowdon, 1991), dried capsicums (Atanda et al., 1990) and sapodillas (Kusum and Geeta, 1990) in tropical countries. A wide variety of vegetables, including carrots, cucumbers, onions, peas and potatoes are also susceptible (Snowdon, 1991).

Geotrichum candidum has long been a problem in the canning and freezing industries. Known as "machinery mould" (Eisenberg and Cichowicz, 1977), it is a frequent contaminant of processing lines, and consequently of products such as frozen foods (Pitt and Hocking, 1997). Standard methods

have been established for estimating *G. candidum* in food processing machinery, by physical counting rather than microbiological techniques (Cichowicz and Eisenberg, 1974). Improvements in sanitation procedures have reduced this problem in recent years. Kure et al. (2004) concluded that air was the major source of *G. candidum* contamination in cheese factories in Norway.

This mould is a very common problem in raw milk in Europe especially when it is used in the manufacture of soft, fresh cheeses such as quarg or Roblochon (see Pitt and Hocking, 1997; Lopandic et al., 2006). Contamination of surface-ripened cheeses such as Brie and Camembert can also be a problem (Pitt and Hocking, 1997). However, recent surveys indicate that *G. candidum* may play an important role in the ripening of many soft and semi-hard cheeses as well as contributing desirable cheese flavours (Boutrou and Gueguen, 2005; Boutrou et al., 2006; Ghosh et al., 2006).

Geotrichum candidum has been isolated from a variety of other foods, including meats, raw and Parma hams, hard cheeses and traditional fermented foods, and Nigerian alcoholic beverages (see Pitt and Hocking, 1997). Low levels were found in maize, peanuts, sorghum, soybeans and black rice in Thailand (Pitt et al., 1993, 1994). It is of quite common occurrence in barley during malting (see Pitt and Hocking, 1997).

References. von Arx (1977); de Hoog et al. (1986).

5.19 Genus *Hyphopichia* Arx and van der Walt

Hyphopichia is a genus of yeast-like fungi, probably closely related to *Endomyces*, from which it differs by the production of hyphal fragments which produce yeast-like conidia by budding from spicules. There is a single species, *H. burtonii*.

Colonies on CYA 22–25 mm diam and on MEA 25–30 mm diam, of low and sparse to moderately dense white to pale grey mycelium, sometimes centrally umbonate; reverse pale. Colonies on G25N 10–12 mm diam, similar to those on CYA. No germination at 5°C. At 37°C, colonies 5–10 mm diam, similar to at 25°C; reverse pale.

Hyphopichia burtonii (Boidin et al.) Arx & van der Walt Fig. 5.38

Pichia burtonii Boidin et al.
Endomycopsis burtonii (Boidin et al.) Lodder & Kreger
Endomycopsis chodatii Wick.

Anamorph: *Candida chodatii* (Nechitsche) Berkhout
Cladosporium chodatii (Nechitsche) Sacc.
Trichosporon behrendii Lodder & Kreger
Trichosporon variabile (Lindner) Delitsch

Conidia borne from spicules (small projections) along the length of undifferentiated hyphae, yeast-like, ellipsoidal, pyriform or near cylindroidal, 2.5–5.0 × 1.5–2.5 μm, with thin, smooth walls; hyphal fragments also evident, 10–50 × 2.5–3.0 μm, still functioning as fertile hyphae, producing conidia (blastospores) from lateral spicules and terminal buds. Ascospores not observed in pure culture, two mating strains needed; ascospores hat-shaped, 1–4 per ascus.

Distinctive features. This species produces white, powdery, filamentous colonies which bear conidia from spicules on vegetative hyphae, and also by budding (yeast-like cells). It is distinguished from *E. fibuliger* by the fragmentation of hyphae into short lengths; these can also act as reproductive structures.

Taxonomy. This species and *Endomyces fibuliger* have rarely been placed in the same genus by taxonomists, but in culture the resemblance is unmistakable. Both grow at more or less the same rates and have similar habitats. One (*E. fibuliger*) is homothallic and the other heterothallic; however, the fragmenting of fertile hyphae in *Hyphopichia burtonii* is the only obvious difference in morphology.

The teleomorph of *Hyphopichia burtonii* has been seen only in the laboratory, after mixing of two mating strains. However, the teleomorph name is used here, both because it has become accepted in the recent literature and because there are several confusing anamorph names, at both genus and species level. Some recent taxonomies have included this species in the yeasts, as *Pichia burtonii* Boidin et al. (Kurtzman and Fell, 1998; Barnett et al., 2000).

Physiology. *Hyphopichia burtonii* grows more rapidly at 30°C than 20°C (Ramakrishna et al., 1993). The minimum is >5°C and growth occurs at 37°C. Growth occurs at least down to 0.90 a_w on barley grains (Ramakrishna et al., 1993).

Mycotoxins. This type of fungus is unlikely to produce toxic compounds.

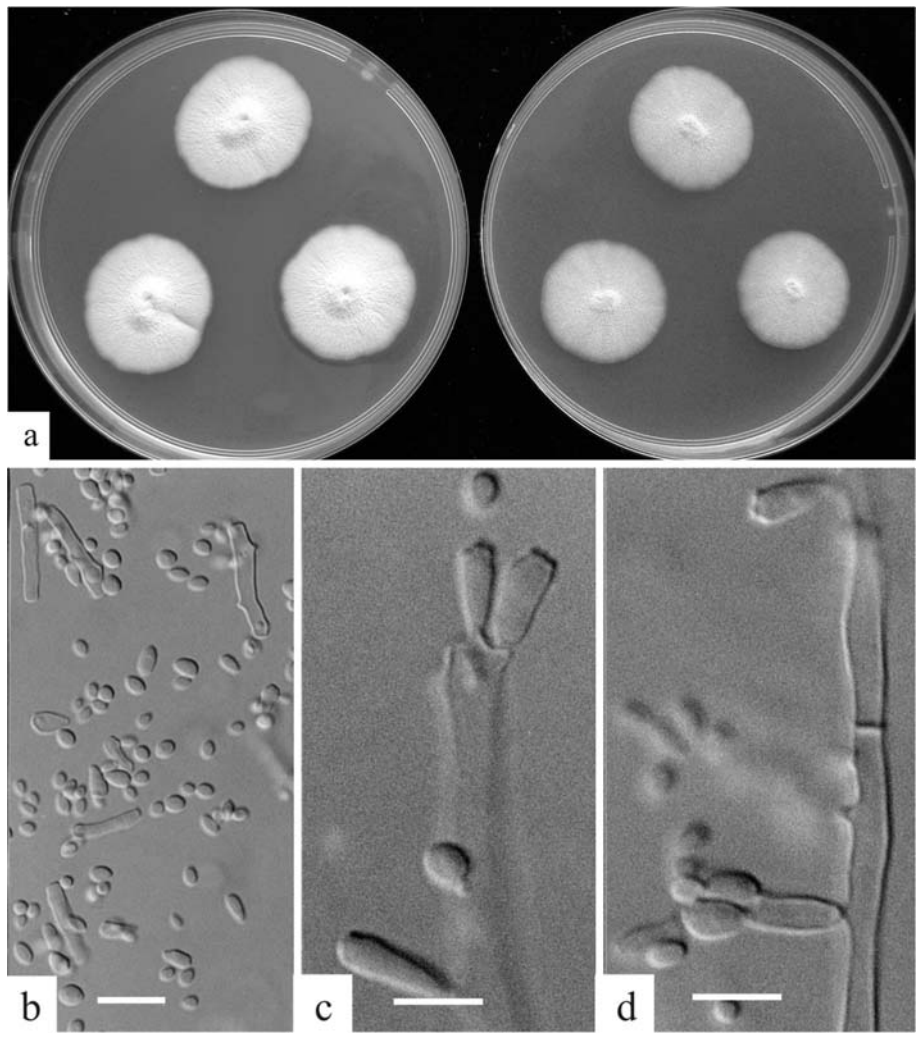


Fig. 5.38 *Hyphopichia burtonii* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b)** vegetative cells, bar = 10 µm; **(c,d)** vegetative cells, bar = 5 µm

Ecology. *Hyphopichia burtonii* is not uncommon in cereals and cereal products, especially packaged bread (Spicher, 1984a, 1985). In Europe, along with *Endomyces fibuliger*, it is known as "chalky mould" (Spicher, 1986b). It is also part of the flora of Parma hams during ripening (Simoncini et al., 2007).

Reference. Barnett et al. (1990).

5.20 Genus *Lasiodiplodia* Ellis and Everh.

Lasiodiplodia is the correct generic name for the fungus previously known as *Botryodiplodia theobromae* Pat. (Sutton, 1980). Both genus and species are characterised by distinctive large, striate conidia, as described below.

Lasiodiplodia theobromae (Pat.) Griffon & Maubl. Fig. 5.39

Botryodiplodia theobromae Pat.

Teleomorph: *Botryosphaeria rhodina* (Berk. & Curt.) Arx

Colonies on CYA and MEA usually filling the whole Petri dish with a loose to moderately dense weft of light to dark grey hyphae, adhering to the Petri dish lid; pycnidia borne beneath the agar surface (visible from the reverse), and sometimes

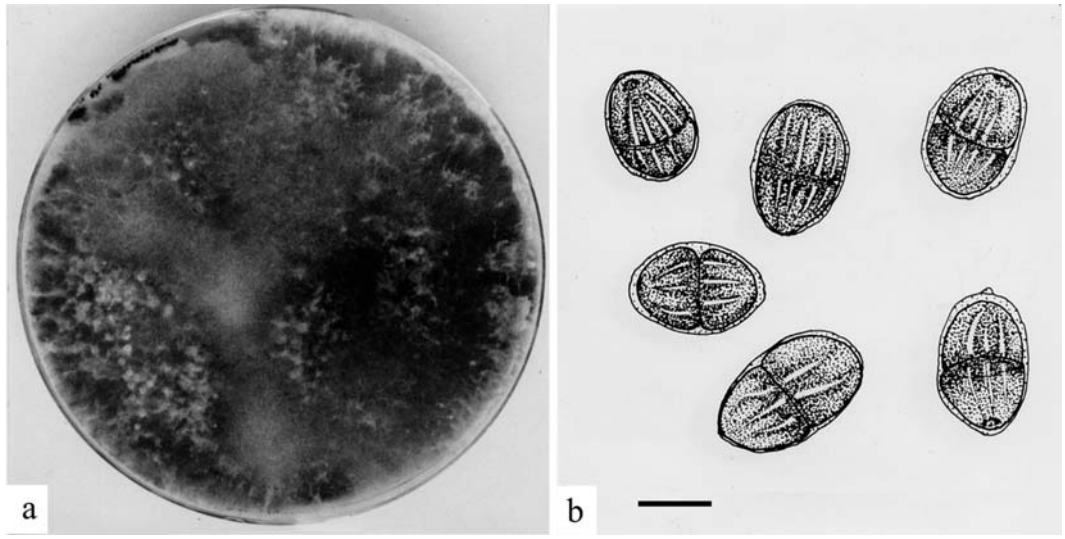


Fig. 5.39 *Lasiodiplodia theobromae* **(a)** colonies on CYA, 7 d, 25°C; **(b)** conidia, bar = 10 μm

at the base–lid interface or on the lid itself; reverse pale, black near pycnidia, uniformly grey-black elsewhere. On G25N, colonies 30–50 mm diam, occasionally more, low and spreading, with sparse white aerial mycelium; reverse pale or grey. No growth at 5°C. At 37°C, colonies greater than 50 mm diam, of low, dense mycelium, coloured grey or deep red; red brown soluble pigment and occasionally exudate produced; reverse deep red brown to almost black.

Reproductive bodies on CYA pycnidia, grey-black and roughly spherical, 200–400 μm diam, but on other media reportedly up to 5 mm diam (Punithalingam, 1976), or forming a stroma (Alasoadura, 1970), easily ruptured, of dark brown to black pseudoparenchymatous cells; conidia at 7 days ellipsoidal, 15–20 × 9–12 μm, with smooth, brownish walls and little ornamentation, at maturity (18–)20–30 × 10–15 μm, with a transverse septum and ornamented by longitudinal striations.

Distinctive features. *Lasiodiplodia theobromae* is distinguished in culture by fast growth on G25N and at 37°C: on G25N growth is sparse and usually colourless, but at 37°C it is dense, with mycelium grey and/or red. Microscopically, this species is distinguished by large ellipsoidal conidia which are borne in black pycnidia, and are ornamented with a transverse septum and longitudinal striations.

Taxonomy. A molecular study using a combined analysis of the ITS region and the translation elongation factor 1-α indicated that *Lasiodiplodia theobromae* as currently understood includes three species, distinguishable by differences in the size of their conidia (Alves et al., 2008).

This species is sometimes known by its teleomorph name, *Botryosphaeria rhodina*, but this state is not usually seen in culture.

Physiology. Alasoadura (1970) reported cardinal temperatures for *Lasiodiplodia theobromae* as minimum 15°C, optimum 28°C and maximum 40°C. This is difficult to reconcile with statements by Uduebo (1974) that "The vegetative growth at 37°C is scanty", or that this species does not grow at 35°C on potato dextrose agar (Adeniji, 1970b). Isolates of this species which we have studied grew rapidly at 37°C, and our observations are consistent with the statement that distribution of *L. theobromae* is mainly confined to the area between 40°N and 40°S (Punithalingam, 1976). Several references suggest that germination and growth of *L. theobromae* is confined to high water activities: vigorous growth observed on G25N in this study is at variance with this.

Mycotoxins. Mycotoxin production has not been reported.

Ecology. Punithalingam (1976) aptly described *Lasiodiplodia theobromae* as "an unspecialised virulent rot pathogen causing numerous diseases:

dieback, root rot, rot or decay of various fruits and storage rot of yams." Spoilage by *Lasiodiplodia* of avocados, bananas, grapes, guavas, mangoes, okra, papayas, passionfruit, traditional Nigerian foods and yams has been reported (see Pitt and Hocking, 1997). Control measures for fruits are well documented by Punithalingam (1976).

Lasiodiplodia theobromae has also been reported from pecans, peanuts, wheat and soybeans (see Pitt and Hocking, 1997). In our experience, it is of common occurrence in many tropical food commodities. We isolated it from 58% of Thai maize samples, at up to 46% of kernels in infected samples and in 7% of all kernels examined (Pitt et al., 1993). It was also common in Thai peanuts, mung beans and sorghum, with 30–33% of samples showing infection (Pitt et al., 1993, 1994). Indonesian crops were equally affected, particularly peanuts, where 47% of samples were positive, with up to 64% of kernels affected in those samples, and overall 9% of the more than 12,000 peanut kernels examined were infected with this species. More than 25% of maize, talo bean, velvet bean, black soybean and cowpea samples also showed infection (Pitt et al., 1998a).

References. Punithalingam (1976); Domsch et al. (1980), under *Botryosphaeria*.

5.21 Genus *Monascus* Tiegh.

Monascus is a genus of Ascomycetes characterised by the production of colourless to pale brown cleistothecia and aleurioconidia. Each cleistothecium is borne from a knot of hyphae on a well defined stalk, in 7 day old cultures resembling a clenched fist on a narrow forearm. Aleurioconidia occur singly or in short chains. Asci break down rapidly so that, when ascospores are mature, the impression under the microscope is of a sac filled with a mass of ellipsoidal, smooth walled, refractile spores.

Species of *Monascus* are best known for their role in the fermentative production of Oriental foods, of which red rice (ang-kak), rice wine and kaoliang brandy are the best known (Hesseltine, 1965; Lin, 1975). Three species were accepted in the most recent revision of the genus (Hawksworth and Pitt, 1983): two, *M. purpureus* Went and *M. pilosus* K. Saito ex D. Hawksw. & Pitt, are associated almost exclusively with fermented foods; the third, *M. ruber* Tiegh., is uncommon in fermented foods but of widespread occurrence elsewhere, and it sometimes causes food spoilage. Only *M. ruber* is treated here. For information on the other species, see Hawksworth and Pitt (1983). Further species have been described from Iraqi soils (Cannon et al., 1995) but these are not relevant to foods.

Monascus ruber Tiegh. Fig. 5.40

Colonies on CYA 20–32 mm diam, occasionally only 15 mm, plane, sparse, surface texture floccose to deeply floccose; mycelium white at first, then becoming pale brown as cleistothecia and aleurioconidia develop, in age sometimes becoming dark brown; brown soluble pigment sometimes produced; reverse sometimes uncoloured, usually brown to dark brown near sepia. Colonies on MEA 30–42 mm diam, plane and sparse, sometimes with little aerial growth but usually with deeply floccose mycelium, white at the margins, becoming brown to orange brown as cleistothecia and aleurioconidia develop; orange or brown soluble pigment sometimes produced; reverse pale at the margins, khaki or brownish orange at the centres. Colonies on G25N usually 16–20 mm diam, sometimes only 10 mm, plane, sparse and floccose; mycelium white; reverse uncoloured or brown. No growth at 5°C. At 37°C, colonies 12–30 mm diam, low and sparse, coloured as on CYA at 25°C, or with reverse deeper brown and sometimes with a reddish tinge.

Cleistothecia spherical, 30–60 μm diam, borne as a hyphal knot from a well-defined stalk, with cellular walls, becoming brown during maturation; ascospores ellipsoidal, hyaline, 5–7 × 4.0–4.5 μm, smooth walled. Aleurioconidia sometimes borne on pedicels from the sides of hyphae, but more commonly terminally, sometimes borne singly but more often in chains of up to 10 cells long, spherical to pyriform, often rounding at maturity, 10–14 μm diam or 10–18 × 8–14 μm, with thick, smooth, brown walls. Chlamydoconidia and arthroconidia produced by most isolates also.

Distinctive features. The cleistothecium produced by *Monascus* is distinctive, being borne as a fist-like hyphal knot on an arm-like stalk. *M. ruber* is distinguished from other *Monascus* species by relatively rapid growth, especially on MEA, and by brown pigmentation in the walls of cleistothecia and aleurioconidia.

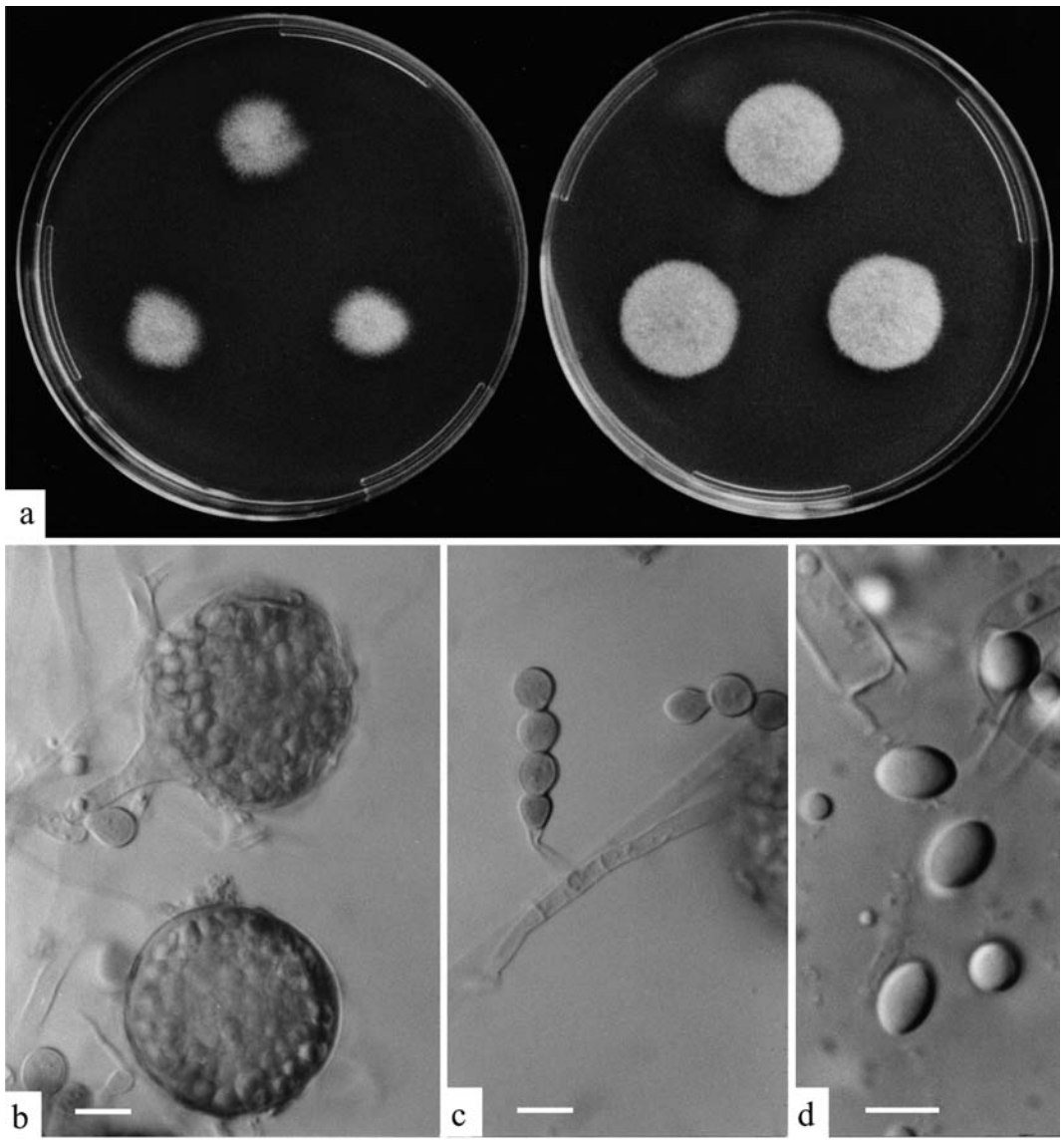


Fig. 5.40 *Monascus ruber* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b)** cleistothecia, bar = 10 µm; **(c)** aleurioconidia, bar = 10 µm; **(d)** ascospores, bar = 5 µm

Taxonomy. Sequencing of the ITS region and part of the β-tubulin gene indicate that *Monascus ruber* and *M. pilosus* should be considered as a single species (Park et al., 2004a).

Physiology. The cardinal temperatures reported for *Monascus ruber* are minimum 15–18°C; optimum 35°C and maximum near 45°C (Manandhar and Apinis, 1971; Panagou et al., 2003). *M. ruber* has been isolated from relatively concentrated substrates (such as high moisture prunes, ca. 0.85 a_w) and is probably weakly xerophilic. However, an isolate failed to grow in a solution of NaCl at 0.92 a_w (Panagou et al., 2003). Growth of *M. ruber* was little affected over the pH range 3.0–5.0 (Panagou et al., 2003) and was able to grow down to pH 2.2 at 30°C in high a_w (Panagou et al., 2005). The combined effect of temperature (25–35°C), water activity (0.999–0.92 a_w) and pH (2–6.8) was studied on gradient plates by Panagou et al. (2005), and also modelled against several equations (Panagou et al., 2003, 2005, 2007).

Ascospores of *Monascus ruber* are heat resistant. D_{80} in citrate buffer (pH 4) was 2.1 min. NaCl

provided some protection: D_{80} values were 0.88 min in 5.6% brine, but 1.04 min in 10.5% brine. *z* values over the temperature range 70–80°C varied from 7.4 to 7.9 C° under the conditions used (Panagou et al., 2002). They concluded that a heat process of 5 min at 80°C would assist the olive industry in producing a stable product.

Pigmentation has received a great deal of attention, having been used to distinguish a number of species utilised in Oriental fermentations. However, Carels and Shepherd (1977) showed that red pigments form during growth near neutral pH values, whereas cultures become orange if the pH of the fermenting food becomes strongly acid. Pigment stability is affected by pH, temperature, light, oxygen and water activity (Dufosse et al., 2005). The production of pigments by *Monascus* species has been reviewed by Miyake et al. (2008).

Mycotoxins. *Monascus ruber* (and *M. purpureus*) produce citrinin (Blanc et al., 1995a, b) both in submerged and solid state culture. This is of importance as red pigments produced by *Monascus* species are used as food colourants. Recent studies indicate that high pigment production can be achieved without citrinin synthesis by incorporating histidine in the growth media (Hajjaj et al., 2000; Xu et al., 2003). Wang et al. (2005) surveyed type cultures of 23 species of *Monascus* (most now regarded as synonyms) for their ability to synthesise citrinin and found that all, including *M. ruber*, produced citrinin.

Ecology. A widespread species, *Monascus ruber* has caused spoilage of high moisture Australian prunes (Hawksworth and Pitt, 1983). It was isolated quite frequently from Indonesian dried fish (Wheeler et al., 1986). Other sources include mayonnaise (Muys et al., 1966a), bread (Spicher and Isfort, 1988), table olives (Panagou et al., 2002), and minced meat, soft cheese, fruit sauce, spices, honey, cacao beans, soy beans, peas, palm kernels, maize and various animal feeds, and silage (data sheets, International Mycological Institute, Egham, Surrey; Hawksworth and Pitt, 1983).

References. Domsch et al. (1980); Hawksworth and Pitt (1983).

5.22 Genus *Moniliella* Stolk and Dakin

Moniliella is a genus of yeast-like fungi, characterised by the production of budding cells, thin walled arthroconidia and, in one species, by relatively large chlamydoconidia (up to 3× hyphal diameter). In some respects it is similar to *Hyphopichia*, but conidia are larger, and the hyphal fragments bearing conidia on spicules, characteristic of *Hyphopichia,* are absent. The latest taxonomy (de Hoog, 1979) accepts two species, *M. suaveolens* and *M. acetoabutans*. *M. suaveolens* is found in butter and margarine, and other substrates rich in oil, while *M. acetoabutans* is of interest because of its resistance to acetic acid and hence potential ability to spoil mayonnaise and other products preserved with acetic acid.

Key to *Moniliella* species included here

1	Spherical chlamydoconidia produced, 8–12 μm diam, with thick, brown walls; growth on malt acetic agar	*M. acetoabutans*
	Thick walled chlamydoconidia not produced; no growth on malt acetic agar	*M. suaveolens*

Moniliella acetoabutans Stolk & Dakin

Fig. 5.41

Colonies on CYA and MEA 22–30 mm diam, deeply floccose, especially on CYA, mycelium pure white; reverse yellow brown. Colonies on G25N 5–10 mm diam, deep but often mucoid, uncoloured. No growth at 5°C. Usually no growth at 37°C, occasionally colonies up to 5 mm diam.

Conidia of three types produced, budding cells from hyphal extremities, arthroconidia by differentiation of hyphal tips, and chlamydoconidia, in intercalary or terminal positions on hyphae, solitary or in short chains; budding conidia ellipsoidal, arthroconidia cylindroidal, both 5–9 μm long, chlamydoconidia spherical, 8–12 μm diam, with thick brown walls.

Distinctive features. *Moniliella acetoabutans* is morphologically distinguished by its three types of conidia: budding conidia like a hyaline *Cladosporium*; arthroconidia like *Geotrichum*, but not so extensive; and relatively large, brown walled chlamydoconidia. In culture, the definitive test for *M.*

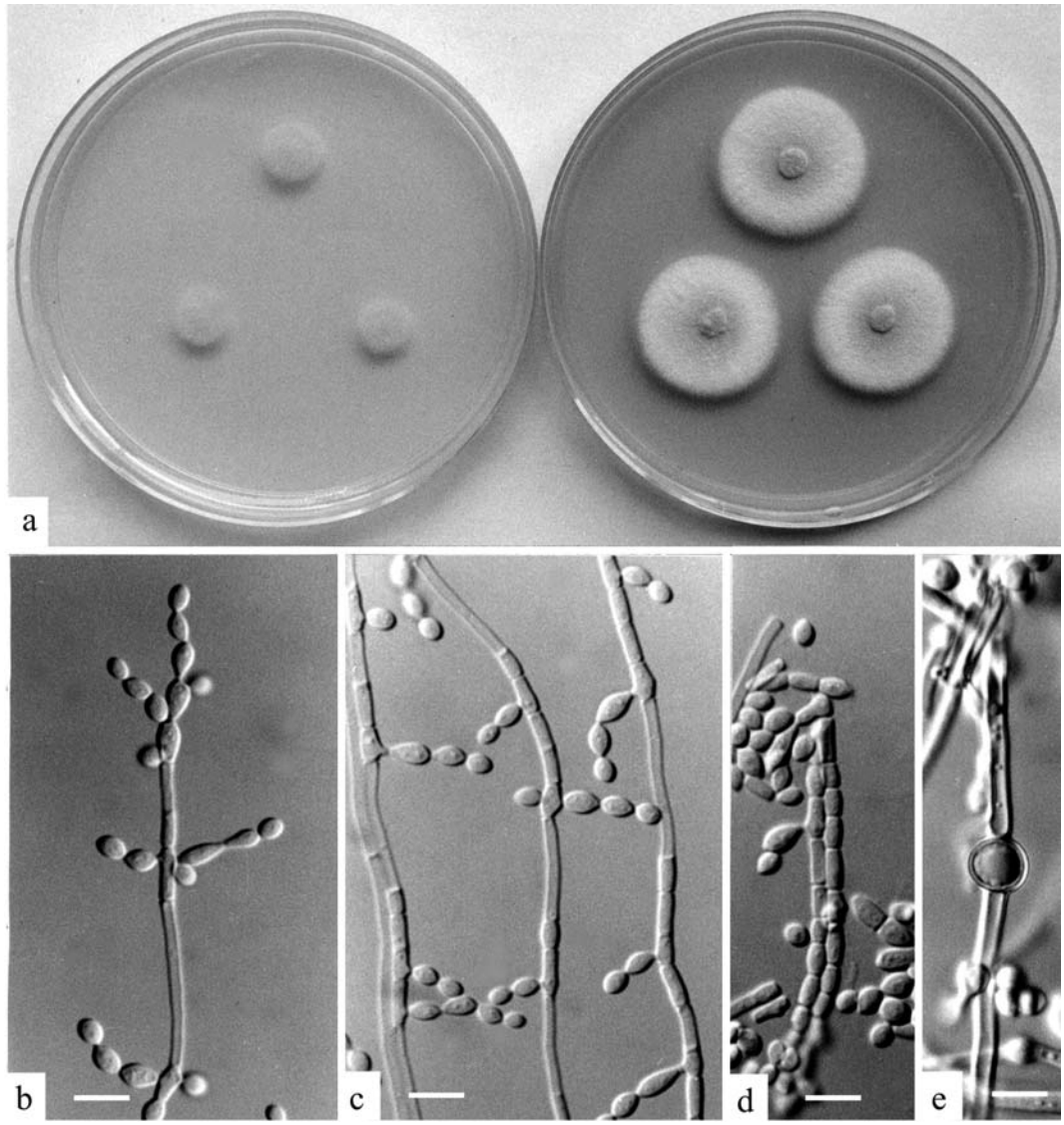


Fig. 5.41 *Moniliella acetoabutans* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b,c)** conidiophores producing budding cells (slide culture), bar = 10 µm; **(d)** arthroconidia, bar = 10 µm; **(e)** chlamydoconidia, bar = 10 µm

acetoabutans is its ability to produce quite rapidly growing white colonies on malt acetic agar – or even on MEA containing 2% acetic acid.

Physiology. This species appears to be unique in its tolerance of acetic acid and other weak acid preservatives. In our laboratory, *Moniliella acetoabutans* has been able to grow in MEA with 4% added acetic acid, to our knowledge a unique property. This species is capable of fermentative growth, like a true yeast, and also appears to be highly tolerant of acid pH.

Mycotoxins. Toxic compounds are not produced.

Ecology. Dakin (Stolk and Dakin, 1966) isolated *Moniliella acetoabutans* from spoiling sweet fruit sauce, and then a variety of other similar products. It also caused fermentative spoilage of a large production run of mayonnaise in Australia in 1971. The source was wooden vinegar tanks, where the fungus was surviving in 10% acetic acid, then growing on the wood as the tank level lowered and the acetic acid became diluted by evaporation. Data sheets from the International Mycological Institute, Egham, Surrey, record isolations from various acetic acid preserves.

Moniliella acetoabutans infections fortunately appear to be rare. They may be overcome either by holding vinegar or acetic acid in stainless steel tanks, or by pasteurising this ingredient before addition to product.

References. Stolk and Dakin (1966); Dakin and Stolk (1968); de Hoog (1979).

Moniliella suaveolens (Lindner) Arx Fig. 5.42

Cladosporium suaveolens (Lindner) Delitsch
Cladosporium butyri O.S. Jensen

Colonies on CYA and MEA highly variable in character, 15–40 mm diam, either white, floccose, sparse and persistently white, or low, dense, velutinous and olive, lightly to heavily sporing; reverse colourless or olive. Colonies on G25N 3–8 mm diam, of white mycelium. At 5°C, sometimes germination. At 37°C, no growth.

Conidiophores undifferentiated, bearing conidia in short, sometimes branched, acropetal chains (the youngest spore at the end), breaking up in liquid mounts; conidia subspheroidal or ellipsoidal to cylindroidal, or somewhat irregular, nonseptate, when ellipsoidal 9–13 × 7–10 μm, when cylindroidal commonly 15–20 × 5–7 μm, with smooth, slightly thickened walls.

Distinctive features. Unlike *Moniliella acetoabutans*, this species does not make chlamydoconidia. It is not preservative resistant, and is normally associated with oils and oil-based foods.

Taxonomy. The taxonomy of *Moniliella suaveolens* is complicated by the fact that the two varieties accepted by de Hoog (1979) culturally bear little resemblance to each other. One (*M. suaveolens* var. *suaveolens*) produces low, spreading colonies, which remain white; the other (*M. suaveolens* var. *nigra*) forms compact, dense colonies, which rapidly become olive. Their incorporation into a single species by de Hoog (1979) was due to similarities in conidial production and conidia.

Physiology. No physiological studies are known to us.

Mycotoxins. Mycotoxins are not produced.

Ecology. This species has caused spoilage of margarine in Europe (Muys et al., 1966a, b; Bours and Mossel, 1973) and Australia (Hocking and Pitt, unpublished). It has been reported as one of the causes of chalky bread in Europe (Spicher, 1985, 1986b; Spicher and Isfort, 1987). It has occasionally been isolated from cheese (Chabalier et al., 1997; Torkar and Vengust, 2008).

Reference. de Hoog (1979).

5.23 Genus *Nigrospora* Zimm.

Characterised by the production of relatively large, solitary, jet black, smooth-walled, oblate conidia, *Nigrospora* occurs in nature mainly as a plant and seed pathogen, but is found also in air, soil and

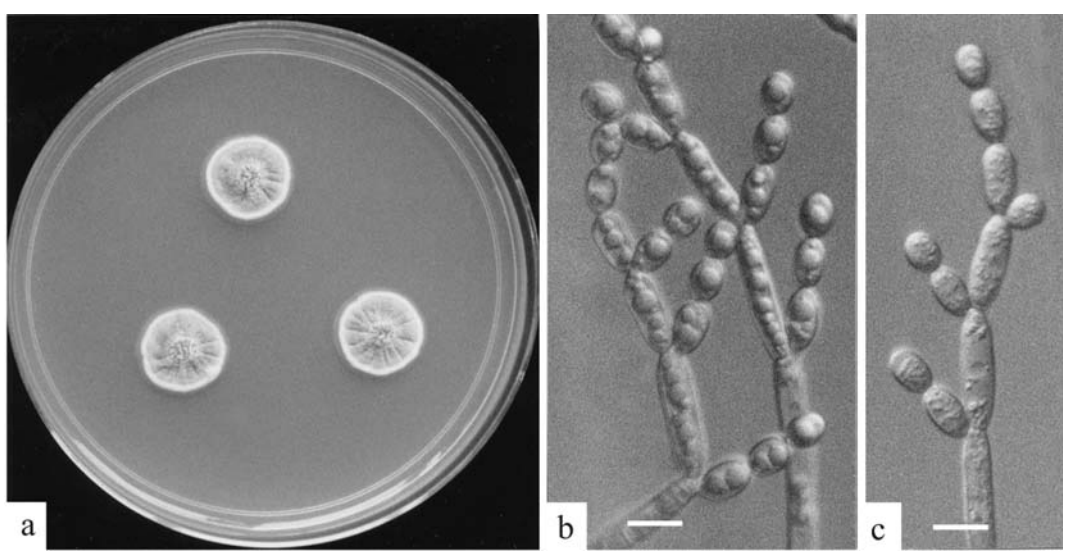


Fig. 5.42 *Moniliella suaveolens* **(a)** colonies on MEA, 7 d, 25°C; **(b,c)** vegetative cells (slide culture), bar = 10 μm

water. Two species are of significance in foods: *Nigrospora oryzae* and *Nigrospora sphaerica*.

Nigrospora oryzae (Berk. & Broome) Petch

Fig. 5.43

Teleomorph: *Khuskia oryzae* H.J. Huds.

Colonies on CYA and MEA covering the whole Petri dish, low to moderately deep, dense to floccose, mycelium flesh coloured or pale orange to pure grey; black conidia conspicuous at low magnifications; reverse pale, greyish orange or grey to deep bluish grey. Colonies on G25N usually 10–15 mm diam, white or mucoid. No growth at 5 or 37°C.

Conidiophores borne from aerial hyphae, short, dark walled, bearing conidia in isolation or in clusters from groups of irregular cells; conidia solitary, jet black, oblate, sometimes collapsing, mostly 12–15 µm long, with smooth, featureless walls, remaining attached or (on natural substrates) violently discharged.

Distinctive features. See genus description. *Nigrospora* is clearly distinguished from *Arthrinium*, which it superficially resembles, by its jet black conidia which lack surface features or markings.

Taxonomy. *Nigrospora oryzae* produces an ascomycete teleomorph, *Khuskia oryzae*, which is found only as a pathogen on certain plants.

Physiology. Optimum growth for *Nigrospora oryzae* has been recorded at 0.995 a_w and 25°C. *N. oryzae* sporulated at 0.98 and 0.995 a_w but not at lower a_w values (0.95, 0.90 or 0.85) at 25°C (Sempere and Santamarina, 2006). Inability to grow at low or high temperatures has been noted above.

Mycotoxins. Mycotoxin production has not been reported for *Nigrospora* species.

Ecology. As a plant pathogen on cereal crops, the widespread distribution of *Nigrospora oryzae* in cereal grains is to be expected. It has been reported from barley (Fakhrunnisa et al., 2006), wheat, other leguminous and cereal foods, pecans and various health foods (see Pitt and Hocking, 1997). It also causes a storage disease of apples in India (Khanna and Chandra, 1975).

We isolated *Nigrospora oryzae* frequently in surveys of Southeast Asian food commodities (Pitt et al., 1993, 1994, 1998a and unpublished). The highest level of occurrence was on paddy rice, where it was isolated from an average of 40% of samples, with up to 68% of infected kernels in infected samples and in 10% of more than 7,000 grains examined from Thailand, Indonesia and the Philippines. This species was isolated from high percentages of samples (25% or more) of maize, sorghum, soybeans, cashews and copra from Thailand, with overall infection rates of 2–5%. In Indonesia,

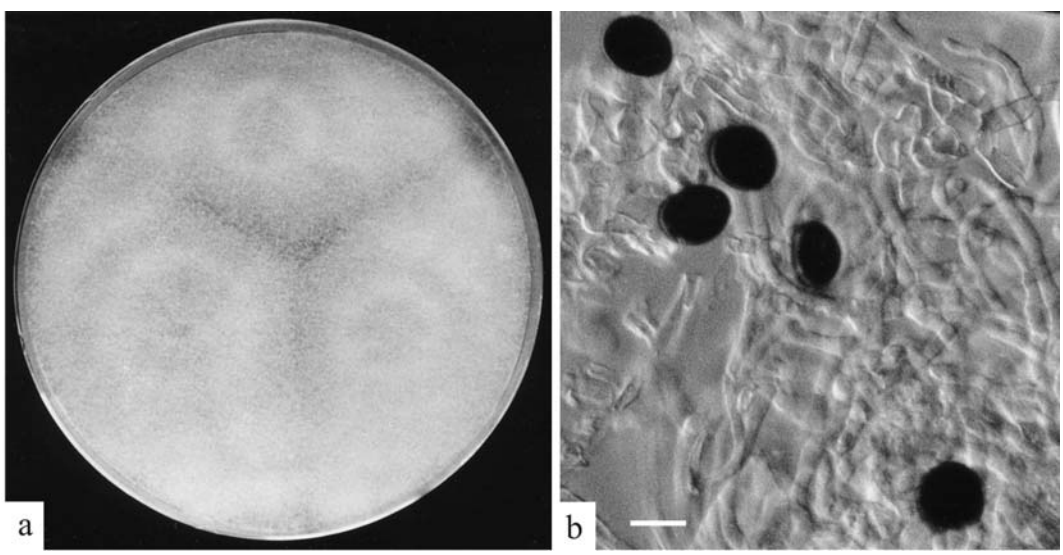


Fig. 5.43 *Nigrospora oryzae* **(a)** colonies on MEA, 7 d, 25°C; **(b)** conidia, bar = 10 µm

kemiri nuts showed infection at 5% overall, while maize, sorghum, soybeans, kidney beans, cowpeas and talo beans were all infected at the rate of 1% or more of all seeds examined (Pitt et al., 1998a). Maize, mung beans and black pepper from the Philippines showed similar levels of infection (our unpublished data).

Additional species. *Nigrospora spherica* (Sacc.) E.W. Mason is similar in culture to *N. oryzae*, but produces larger conidia, mostly 15–18 µm long. This species is associated with two diseases of bananas, crown rot (Wallbridge, 1981) and squirter (Hall and Scott, 1977; Snowdon, 1990), and *Nigrospora* rot of apples (Snowdon, 1990). It has also been recorded from wheat, peanuts, pecans, maize and biltong (see Pitt and Hocking, 1997).

Although less common in Southeast Asian food commodities than *Nigrospora oryzae*, we isolated *N. spherica* from kemiri nuts, maize, peanuts, milled rice, cowpeas and black soybeans in Indonesia (Pitt et al., 1998a), and from paddy rice, maize, soybeans and black pepper in the Philippines (our unpublished data).

References. Ellis (1971); Domsch et al. (1980).

5.24 Genus *Pestalotiopsis* Steyaert

Pestalotiopsis and the closely related genus *Truncatella* Steyaert (see below) are characterised by the formation of black acervuli containing relatively large fusiform conidia with three or four transverse septa and spiky appendages from one or both ends. Neither genus is encountered frequently in foods other than cereals or nuts, where they can be spoilage agents. In our experience, direct plating of cereals will frequently detect *Pestalotiopsis*, but dilution plating techniques are ineffective.

The name *Pestalotia* De Not. has frequently been incorrectly applied to foodborne fungi which should have been identified as *Pestalotiopsis* or, less frequently, *Truncatella*. In his comprehensive revision, Steyaert (1949) confined *Pestalotia* to a single species, not found in foods. Sutton (1980) accepted a single species in *Pestalotiopsis*, *P. guepinii*. However, many new species have been described since, often based on host specificity. Molecular studies on ITS and 5.8S DNA have shown that host specificity is not a sound basis for species delimitation in *Pestalotiopsis* (Jeewon et al., 2004).

Pestalotiopsis guepinii (Desm.) Steyaert

Fig. 5.44

Colonies on CYA and MEA growing rapidly, covering the whole Petri dish, plane and floccose; mycelium usually white, sometimes off-white to pale brown; reverse pale or in similar colours to the mycelium. Colonies on G25N 10–16 mm diam, of low, white mycelium. Sometimes germination or growth at 5°C. Usually no growth at 37°C.

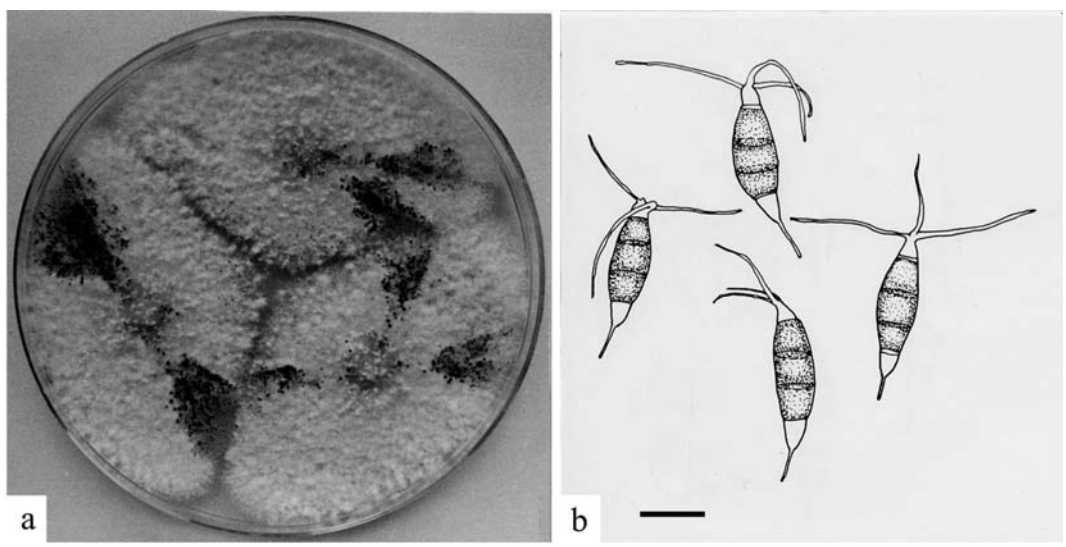


Fig. 5.44 *Pestalotiopsis guepinii* **(a)** colonies on CYA, 7 d, 25°C; **(b)** conidia, bar = 10 µm

Conidia produced in flat, black acervuli, borne just beneath the agar surface, opening irregularly at maturity, filled with a dense layer of conidia; conidia fusiform, five celled (four septate), 20–28 × 6–9 μm, the central 3 cells brown, 15–20 μm long, the apical and basal cells hyaline, the basal one with a single usually unbranched spike-like appendage and the apical one with two or more simple or branched spiky appendages.

Distinctive features. *Pestalotiopsis* shares with *Truncatella* the production in subsurface acervuli of relatively large fusiform conidia with appendages. In *Pestalotiopsis*, conidia have four septa, in *Truncatella*, three.

Physiology. Optimum sporulation of *Pestalotiopsis guepinii* was achieved on media containing 3% sucrose. In addition, reduced nitrogen concentration also favoured sporulation (Ebenezer et al., 2002).

Mycotoxins. Mycotoxin production has not been reported.

Ecology. There are few records under the name *Pestalotiopsis* in the food literature. Most relate to fruit spoilage: *Pestalotiopsis* sp. from crown rot of bananas and rots in litchis, canker in guavas, and tarry rots in pomegranates (Snowdon, 1990). Most reports of *Pestalotia* species, which probably refer to *Pestalotiopsis*, have come from pecans. Other *Pestalotia* records include wheat, rice, almonds and hazelnuts (see Pitt and Hocking, 1997).

We isolated *Pestalotiopsis guepinii* at low levels from Thai copra, maize, black beans and red beans (Pitt et al., 1993, 1994).

Additional genus. *Truncatella* Steyaert produces colonies similar to those of *Pestalotiopsis*, and produces similar conidiomata. Conidia are fusiform with three septa, and measure 15–20 × 6–8 μm; the two median cells are brown and 11–14 μm long; apical and basal cells are hyaline; and the basal cell is without appendages. Appendages from the apical cell are variable in number and branching.

No records of *Truncatella* in foods were found, but it is probable that some references to *Pestalotia* include *Truncatella* species.

Reference. Sutton (1980).

5.25 Genus *Phoma* Sacc.

Phoma is a large and variable genus, characterised by the production of small, single celled conidia in pycnidia. The pycnidia in *Phoma* are black, have one or more small ostioles (orifices), are produced beneath the agar surface, and exude the conidia in slime. Several *Phoma* species have been reported from foods, but until recently the taxonomy of the genus was uncertain, so identifications to species were often unreliable. A new monograph has clarified the taxonomy of the genus (Boerema et al., 2004). This book provides keys and descriptions of 223 species and subspecies in 10 sections, with a great deal of other information including methods for identifying species in natural habitats. A single species, *Phoma sorghina*, is described here as representative of the genus.

Phoma sorghina (Sacc.) Boerema Fig. 5.45

Colonies on CYA and MEA variable, usually 50–55 mm diam, of dense to floccose grey green or olive mycelium, with characteristic white to salmon pink tinges; reverse salmon or reddish. Colonies on G25N 8–10 mm diam, of sparse brown mycelium. No growth at 5 or 37°C.

Pycnidia produced abundantly on MEA, just beneath the agar surface, 300–400 μm diam, with one or more inconspicuous ostioles exuding conidia in a slimy matrix; conidia cylindroidal, 4–5 × 2–2.5 μm, hyaline, with thin, smooth walls.

Distinctive features. *Phoma* species produce black subsurface pycnidia which exude small cylindrical conidia in slime. These features are sufficient in the present context; however, see Sutton (1980) for a range of other similar genera.

Taxonomy. Taxonomic problems in *Phoma* have been addressed by Kovics et al. (2005) and a molecular taxonomy has recently been constructed (Aveskamp et al., 2006) for the 223 species and subspecies accepted by Boerema et al. (2004).

Physiology. Sporulation by *Phoma* species may be stimulated by growth on DCPA or PDA. Carnation Leaf Agar, developed for *Fusarium* species, induces sporulation in most *Phoma* isolates.

Mycotoxins. *Phoma sorghina* has been reported to produce tenuazonic acid (Shephard et al., 1991) and *P. lingam* the first naturally occurring epimonothiodiocoperazine, named sirodesmin H (Pedras et al., 1988). No studies have been reported on the significance, if any, of production of these toxins by these species.

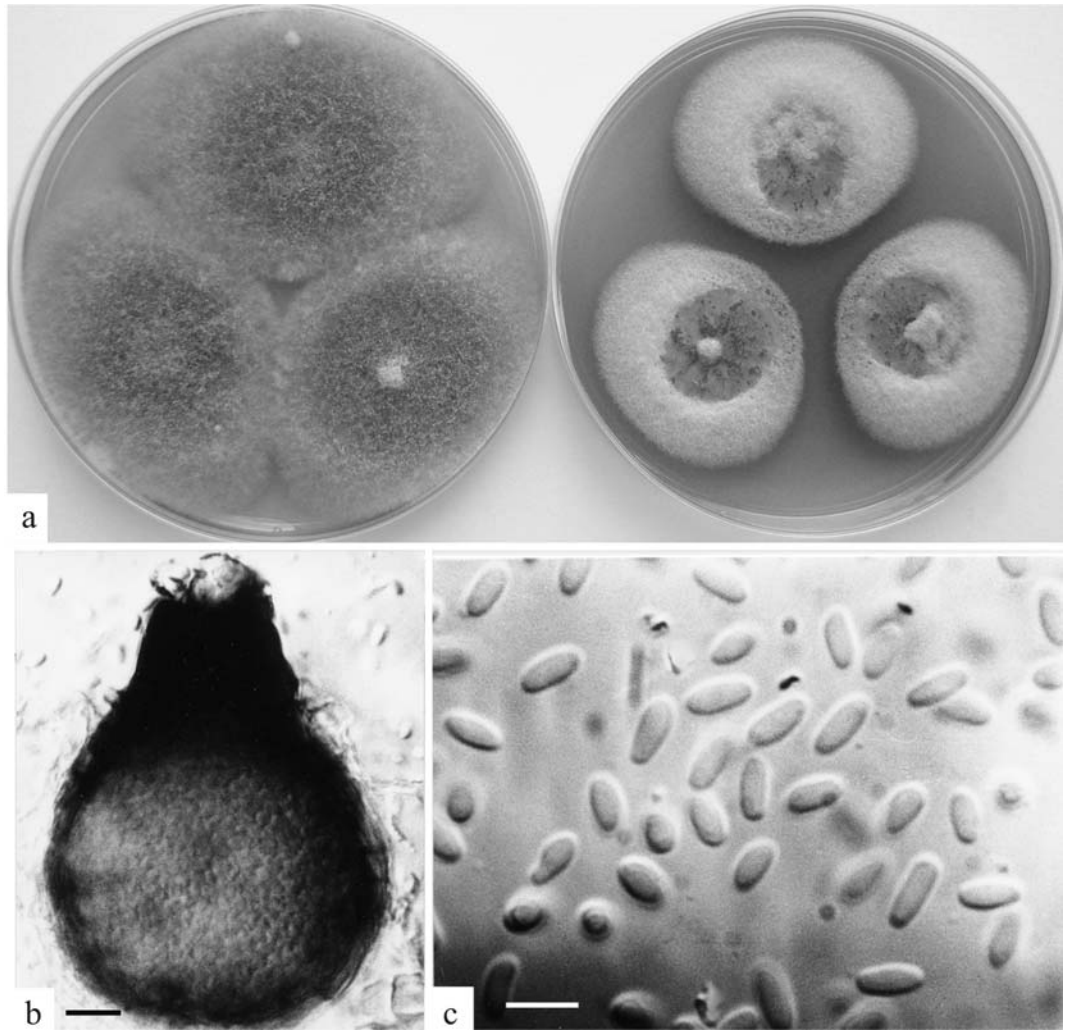


Fig. 5.45 *Phoma sorghina* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b)** pycnidium, bar = 10 µm; **(c)** conidia, bar = 5 µm

Ecology. *Phoma sorghina* is the principal *Phoma* species found in grain sorghum. Other Phomas, usually unidentified to species, have been reported from cereals, including barley, wheat, maize and rice (see Pitt and Hocking, 1997; Do Amaral et al., 2005; Perello and Moreno, 2005). Phomas, sometimes identified to species, have been reported from a wide variety of fruits and vegetables (Snowdon, 1990, 1991). Important are black rot of papayas due to *P. caricae-papayae* (Tarr) Punith., black rot of melons due to *Phoma cucurbitacearum* (Fr.) Sacc. (Snowdon, 1990), crown rot of bananas caused by *P. sorghina* and Phoma rot of sugar beets due to *P. betae* A.B. Frank. Other records have come from pecans, peanuts, apples, potatoes, fresh vegetables, raw milk, spoiled cheese, sunflower seed, coriander seed, infant cereal food, biltong and a variety of Japanese foods (see Pitt and Hocking, 1997).

Phoma species (unidentified) were common in paddy rice from Thailand (in 56% of samples, up to 44% of grains in infected samples and 8% of all grains examined) (Pitt et al., 1994). Similar levels occurred in sorghum from the Philippines and black soybeans from Indonesia (Pitt et al., 1998a and our unpublished data). Lower but significant levels (1–2% infection overall) were found in sorghum, mung beans and soybeans from Thailand, paddy rice and soybeans from the Philippines and paddy rice, mung beans, soybeans and talo beans from Indonesia (Pitt et al., 1994, 1998a and our unpublished data).

Although they occur in a variety of foods, *Phoma* species are most likely to cause spoilage in weather-damaged cereals.

References. Boerema et al. (2004).

5.26 Genus *Stemphylium* Wallr.

Like *Alternaria* and *Ulocladium, Stemphylium* produces conidia with both longitudinal and transverse septa. *Stemphylium* conidia are more or less ellipsoidal to short cylindroidal, like those of *Ulocladium*. Whereas those of *Ulocladium* are narrower at the base than the apex, and can be pointed at one end, to give a pyriform or apiculate shape, those of *Stemphylium* have rounded ends and are usually symmetrical longitudinally. For further details concerning the differences among these three genera, see the section dealing with *Alternaria* in this chapter, or Simmons (1967). In an extensive molecular study of *Alternaria, Stemphylium* and related genera (*Ulocladium, Embellisia* and *Nimbya*), Pryor and Gilbertson (2000) and Pryor and Bigelow (2003) provided strong molecular evidence that the genus *Stemphylium* is distinct from these other genera.

Species of *Stemphylium* occur commonly as weak parasites or saprophytes on a variety of plants and plant materials. The taxonomy of the genus has recently been revised: molecular methods showed good agreement with existing morphological taxonomy (Câmara et al., 2002). One species is treated here, *S. botryosum* (Fig. 5.46).

Stemphylium botryosum Wallr. Fig. 5.46

Teleomorph: *Pleospora tarda* E.G. Simmons

Colonies on CYA 50–65 mm diam, of low to somewhat floccose, rather sparse mycelium, olive to olive brown; reverse olive grey to dark grey. Colonies on MEA 50–70 mm diam, low to floccose, in the latter case sometimes with areas of white or pale yellow mycelium, elsewhere pale to dark grey; reverse light to dark grey, sometimes pale yellow or pinkish. Colonies on G25N 6–15 mm diam, pale to dark grey. At 5°C, usually germination. At 37°C, no growth.

Colonies on DCMA 35–45 mm diam, low to somewhat floccose, sparse, grey to dark grey in areas; reverse in similar colours.

Conidiophores undifferentiated, of variable length, with slightly swollen (up to 7 μm) tips bearing conidia successively, blown out from a pore as a hyaline cell, then septating both longitudinally and transversely, becoming thick-walled and detaching with maturity; the smaller conidia more or less spherical, with one to two septa, the larger ones at maturity usually short cylindrical, with rounded ends and straight or irregularly swollen sides, with two to four transverse and one to two longitudinal septa, sometimes near spherical or irregular in shape, overall ranging in size from 13 μm diam up to 30–32 × 16–20 μm, with brown walls, slightly roughened or with short spines, or occasionally with larger dark warts.

Distinctive features. As noted above, *Stemphylium* is distinguished by dark conidia with both transverse and longitudinal septa, with ends rounded, symmetrical from end to end. *S. botryosum* conidia characteristically are slightly constricted in the middle.

Taxonomy. The teleomorphs of this and other *Stemphylium* species are in the Ascomycete genus *Pleospora* (Simmons, 1969, 1985). They are not often seen in pure culture in the Petri dish. Metabolite profiles have been used to distinguish several *Stemphylium* species, including *S. botryosum* (Andersen et al., 1995).

Physiology. Potato dextrose agar supported abundant mycelial growth, whereas conidiogenesis was highest on Sabouraud maltose agar (Susuri and Doga-Gashi, 2003).

Mycotoxins. This species has been reported to produce a toxin called stemphol, but mammalian toxicity was not indicated (Solfrizzo et al., 1994).

Ecology. This and other *Stemphylium* species have been reported as pathogens on mangoes (Johnson et al., 1990), squash (Assawah and Zarari, 1984), sweet potatoes (Ravichandran and Sullia, 1983), asparagus and lettuce (Snowdon, 1991), tomatoes (Dodds et al., 1991; Muniz et al., 2003) and peppers (Muniz et al., 2003). *Stemphylium* species have also been isolated from malting barley (Sepitkova and Jesenska, 1986), lucerne seeds (Susuri and Doga-Gashi, 2003) and soybeans (our unpublished data).

References. Simmons (1967, 1969, 1985).

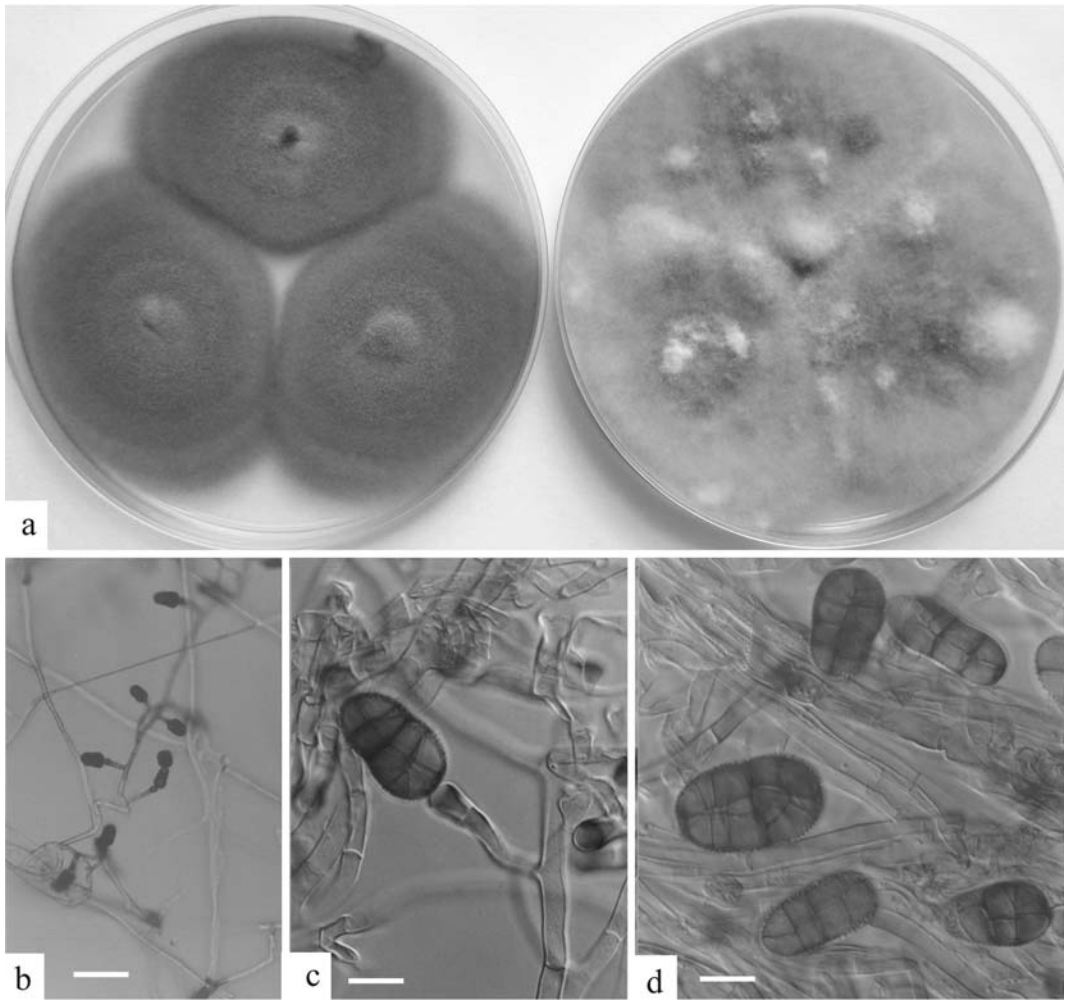


Fig. 5.46 *Stemphylium botryosum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b)** conidiophores and conidia in situ, bar = 50 µm; **(c,d)** conidia, bar = 10 µm

5.27 Genus *Trichoconiella* B.L. Jain

This genus was established to accommodate the fungus more commonly known as *Alternaria padwickii* (Ganguly) M.B. Ellis. Conidia of this species produce only transverse septa, excluding it from *Alternaria* (Jain, 1975).

Trichoconiella padwickii (Ganguly) B.L. Jain Fig. 5.47

Alternaria padwickii (Ganguly) M.B. Ellis

Colonies on CYA 30–45 mm diam, plane, dense to floccose, mycelium yellowish grey to light orange, sometimes with pale grey areas; yellow exudate sometimes produced; reverse pale or orange at the margins, centrally very dark brown to bluish black. Colonies on MEA 45–55 mm diam, plane, low to floccose, mycelium pale orange or grey; reverse pale or pinkish at the margins, overall bluish black. No growth on G25N or at 5°C. At 37°C, colonies up to 5 mm diam formed, or no growth.

Colonies on DCMA 35–55 mm diam, similar in appearance to on MEA, but mycelium mid to dark grey; reverse grey, dark grey or bluish black.

Conidia best observed on colonies grown on sterile wheat grains, borne singly from long hyphae, with five to six transverse septa, 40–55 × 9–11 µm between the tip and last septum, with the central

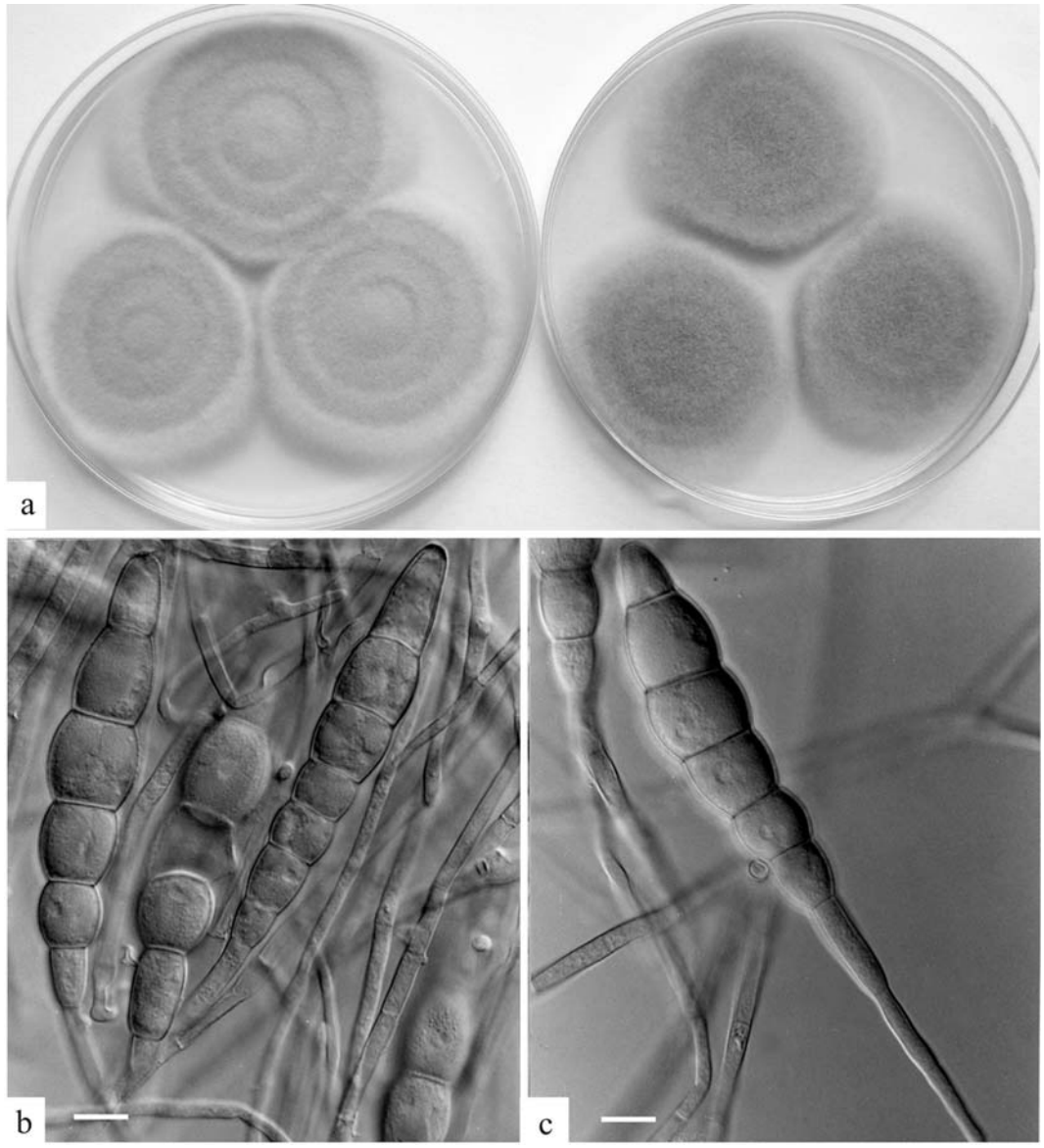


Fig. 5.47 *Trichoconiella padwickii* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b,c)** conidia, bar = 10 μm

cells larger, giving a narrowly ellipsoidal shape, and with smooth, brown walls; hyphae sometimes remaining attached to the conidium and then giving the impression of long appendages.

Distinctive features. This genus superficially resembles *Alternaria*, but produces conidia with transverse septa only. Long, whip-like hyphal appendages are also distinctive. *Trichoconiella padwickii* appears to be almost exclusively associated with rice.

Physiology. No reports of physiological studies are known to us.

Mycotoxins. Mycotoxins are not known to be produced.

Ecology. This species (by either generic name) has rarely if ever been reported from the food literature. However, we have found *Trichoconiella padwickii*, reported as *Alternaria padwickii*, to be endemic in rice from Southeast Asia. It was present in 9 of 18 samples of Thai paddy rice, with up

to 60% of grains in individual samples showing infection, and was found in 12% of all individual grains examined (Pitt et al., 1994). The incidence in paddy rice from Indonesia (71% of samples, 19% of all particles) and the Philippines (72% of samples, 23% of all particles) was even higher. Individual rice samples from Indonesia had up to 69% of particles infected by *T. padwickii*, while incidence was up to 90% of grains in samples from the Philippines (Pitt et al., 1998a and our unpublished data).

Ellis (1971) reported *Trichoconiella padwickii* (as *A. padwickii*) from rice grains from Egypt, India, Malaysia, Nigeria, Pakistan and Sabah, indicating that this species is very widespread. Occurrence on crops other than rice has rarely been reported.

References. Ellis (1971); Jain (1975); Domsch et al. (1980).

5.28 Genus *Trichoderma* Pers.

In this ubiquitous genus, reproduction is by small single-celled conidia produced from phialides which are arranged in irregular verticils, with the subterminal phialides borne more or less at right angles to the stipe. Colonies are low and spread rapidly. Mycelial growth is loose textured and characteristically develops irregularly, often appearing in tufts or isolated patches. Conidia, green in the common species, sometimes develop only after exposure to light.

Trichoderma species have usually been considered to be soil fungi, but the genus is now also known to include plant symbionts and fungal pathogens. Some are also of increasing importance as human pathogens. Interest in the genus has increased sharply in recent years, as some species have been proposed as biocontrol agents against plant pathogenic fungi.

Speciation in *Trichoderma* has proved to be exceptionally difficult. The most common species name in the literature, *Trichoderma viride* Pers., has often been incorrectly used (Rifai, 1969) and it is now considered to be a rare species (Jaklitsch et al., 2006). Conidia produced by *T. viride* have rough walls, whereas the majority of *Trichoderma* isolates produce conidia with smooth walls. Reports in the literature on *Trichoderma* species should be treated with caution, as green-spored *Trichoderma* isolates have often been called *T. viride* regardless of the texture of their conidial walls.

Because most *Trichoderma* isolates have similar morphology, taxonomy proved very difficult until the advent of molecular methods. The use of sequences from multiple gene loci has resulted in a large increase in species numbers, from 9 in 1970 to now over 80 (Samuels, 2006). Most species have been shown to have teleomorphs in *Hypocrea*, though few are found in laboratory culture (Samuels, 2006). The taxonomy of *Trichoderma* is still evolving: see Samuels (2006) for a clear overview of current understanding.

The most commonly isolated *Trichoderma* species is *Trichoderma harzianum* Rifai (Bisset, 1991a; Samuels, 2006). This is the species we have encountered most frequently from foods, and it is probable that most foodborne *Trichoderma* isolates belong in *T. harzianum*.

Care should be exercised in handling *Trichoderma* isolates in the laboratory. Conidia are small, are shed and dispersed readily, and contaminant colonies grow rapidly. Many Trichodermas produce powerful chitinases and cellulases and, in time, can overrun and destroy other cultures completely.

Trichoderma harzianum Rifai **Fig. 5.48**

Colonies on CYA and MEA generally covering the whole Petri dish, often irregular in outline or with isolated tufts evident, of white to yellow mycelium, with bright to dull yellow green conidia developing over the whole surface or in patches or tufts; reverse pale or yellowish. Colonies on G25N less than 5 mm diam, with growth weak. At 5°C, usually no growth, occasionally germination. No growth at 37°C.

Conidiophores consisting of highly branched structures, with a stipe bearing branches and the branches rebranching, all approximately at right angles, to form a pyramidal shape, with each branch bearing phialides irregularly; phialides ampulliform, commonly 5–7 × 3.0–3.5 μm, larger when borne apically, bearing conidia singly, not in chains; conidia often adhering in small clusters, spheroidal, subspheroidal, or sometimes broadly ellipsoidal,

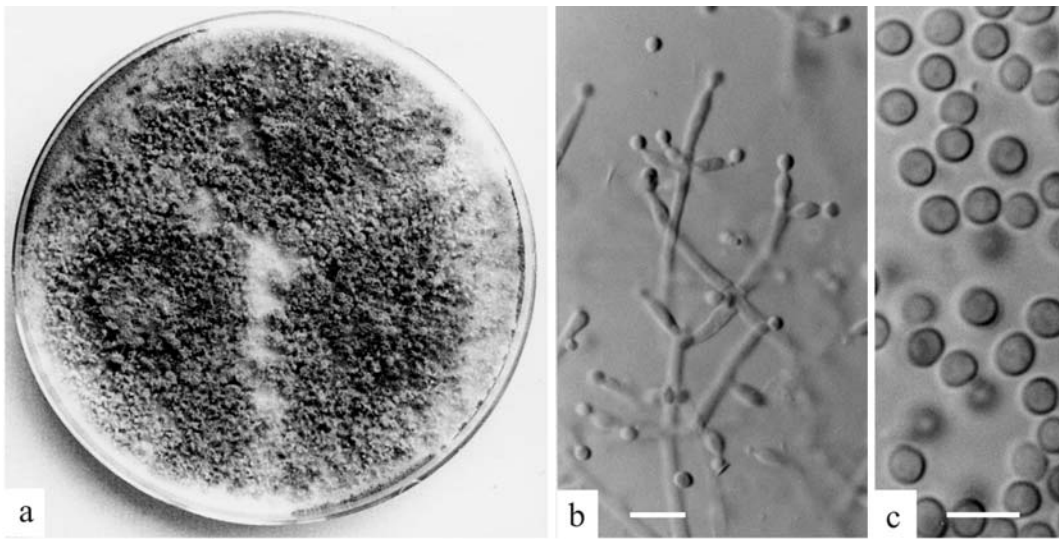


Fig. 5.48 *Trichoderma harzianum* **(a)** colonies on MEA, 7 d, 25°C; **(b)** conidiophores, bar = 10 µm; (**c)** conidia, bar = 5 µm

2.5–3.2(–4.0) µm diam or in length, smooth walled. Solitary aleurioconidia also formed by some isolates, spherical to broadly ellipsoidal, 6–8 µm diam.

Distinctive features. *Trichoderma harzianum* produces conidiophores which are compactly branched in a pyramidal shape, the base being highly branched, and the apex usually bearing a solitary phialide. Conidia are smooth-walled, nearly spherical and less than 3.5 µm long.

Taxonomy. The teleomorph of *Trichoderma harzianum* is *Hypocrea lixii*. This is not seen in laboratory culture (Samuels, 2006).

Physiology. Because of the confusion in the literature over the identity of *Trichoderma* isolates, it is possible that much of the information reported for *T. viride* actually is based on studies on *T. harzianum*. Domsch et al. (1980) reported the optimum growth temperature for *T. harzianum* as approximately 30°C, with a maximum near 36°C. Our observations indicate a minimum growth temperature at or slightly above 5°C. The minimum a_w for growth is 0.91 at 25°C (Griffin, 1963).

Mycotoxins. Mycotoxin production by *Trichoderma harzianum* has not been reported.

Ecology. *Trichoderma harzianum* is frequently isolated from cultivated and forest soils in all parts of the world (Domsch et al., 1980). It has been reported from rotting tubers of cassava (Ekundayo and Daniel, 1973) and as a cause of spoilage in apples (Penrose et al., 1984). It has been reported from salmon and peas (data sheets, International Mycological Institute, Egham, Surrey). We found *T. harzianum,* at levels up to 1% of all grains or nuts examined, in maize from Thailand and Indonesia and sorghum from Indonesia. It was encountered at low levels in Philippine maize, Thai peanuts and Indonesian cowpeas (Pitt et al., 1993, 1994, 1998a). *T. viride* was not encountered at all.

Trichoderma harzianum is no doubt widely distributed in foods, but it has often been reported as *T. viride*.

References. Bisset (1984, 1991a, b; Samuels, 2006).

5.29 Genus *Trichothecium* Link

Trichothecium is a distinctive genus with a single common species, *T. roseum,* characterised by sparse, pinkish colonies and conidia formed in a unique V-formation on long stipes. *T. roseum* is a

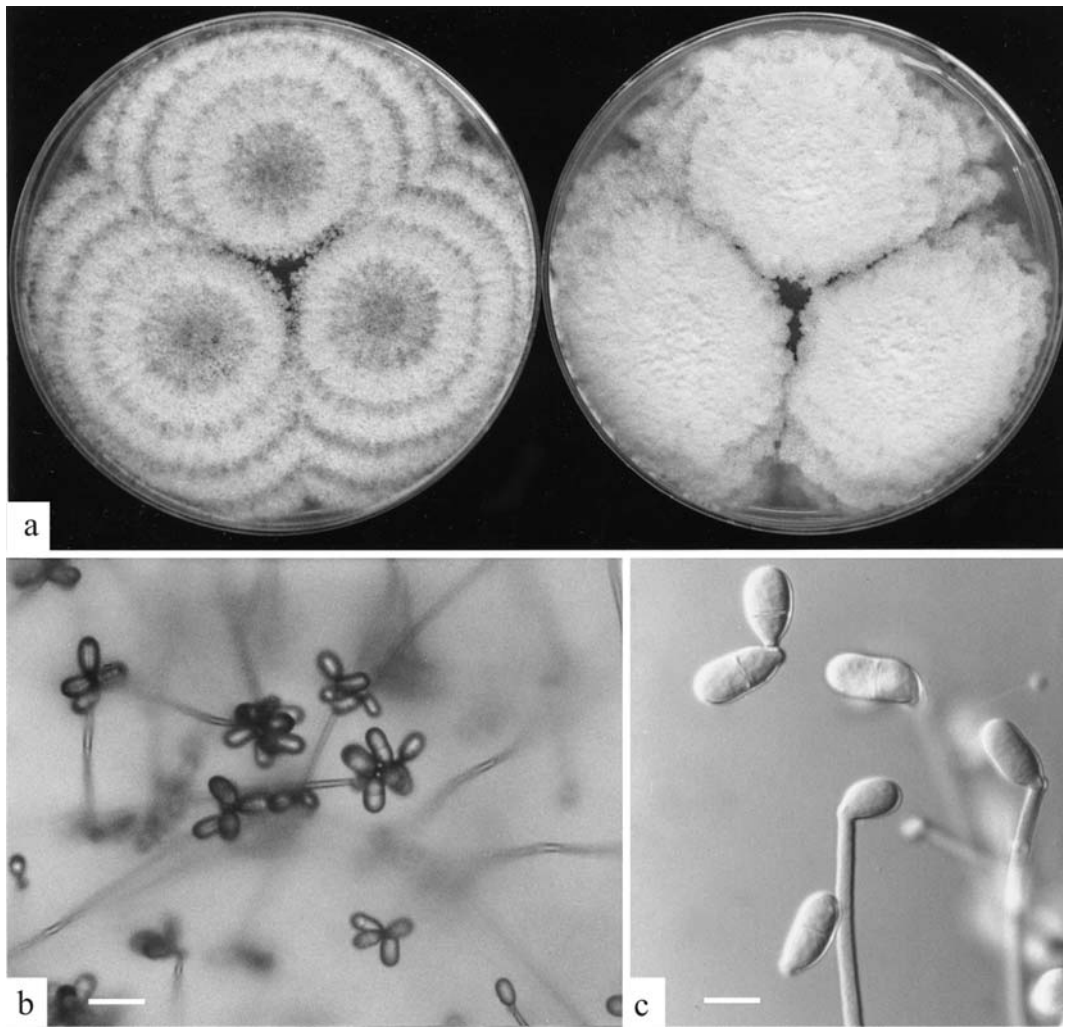


Fig. 5.49 *Trichothecium roseum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b)** conidiophores and conidia in situ, bar = 25 μm; **(c)** conidia, bar = 10 μm

common saprophyte in damp and decaying habitats and is sometimes a weak pathogen of both plants and animals.

***Trichothecium roseum* (Pers.) Link** **Fig. 5.49**

Colonies on CYA and MEA 50–60 mm diam, low and often sparse, characteristically coloured orange pink near salmon; reverse similarly coloured, or less intense, or brownish. Colonies on G25N barely macroscopic, 1–2 mm diam at most. At 5°C, no germination to germination. No growth at 37°C.

Conidiophores long, simple hyphae, bearing conidia at the tip successively, each formed as a blown-out cell below the previous one, offset from the hyphal axis and adhering loosely to form characteristic short, V-shaped chains; conidia approximately ellipsoidal to pyriform, with a single transverse septum, 16–20 × 8–12 μm, and with thin, smooth walls.

Distinctive features. See genus preamble.

Physiology. Domsch et al. (1980) reported growth temperatures for *Trichothecium roseum* as minimum 15°C, optimum 25°C and maximum 35°C. Our data on growth at low temperature is at

variance with this, indicating a minimum growth temperature of 5°C or near. Growth occurs down to 0.90 a_w (Snow, 1949).

Mycotoxins. *Trichothecium roseum* has been reported to produce trichothecene toxins, including trichothecin, trichothecolon and 12,13-epoxy-4-(1-oxobut-2-enyloxy)-trichothec-9-ene (Konishi et al., 2003). Some of these toxins have been found in grapes and wheat in measurable quantities, but as spoilage or heavy growth of *T. roseum* is rare, they are unlikely to be significant in human health. However, their presence at quite low levels in grape musts has been reported to inhibit wine yeasts (Flesch et al., 1986).

Ecology. As a ubiquitous and readily recognised saprophyte, *Trichothecium roseum* has been isolated from a variety of foods. Cereals are a common source, including barley, wheat, maize, sorghum and paddy rice (see Pitt and Hocking, 1997). Other important sources include apples (Valletrisco and Niola, 1983) and grapes (see Pitt and Hocking, 1997; Serra et al., 2005; Blancard et al., 2006). This species has caused spoilage of a wide variety of other fruits and vegetables from time to time, but is usually only a minor pathogen (Snowdon, 1990, 1991). It has also been isolated from meat products, cheese, beans, hazelnuts, pecans and pistachios (see Pitt and Hocking, 1997). We isolated *T. roseum* from sorghum, cashews, paddy and milled rice, peanuts, maize and soybeans in Southeast Asia, but always at a low incidence (Pitt et al., 1998a). In our experience, levels of *T. roseum* in foods other than fruits are usually low, and spoilage exceptional.

Reference. Domsch et al. (1980).

5.30 Genus *Ulocladium* Preuss

Ulocladium, in common with *Alternaria* and *Stemphylium*, produces conidia with both longitudinal and transverse septa. Those of *Ulocladium* are narrower at the base than the apex, and can be pointed at one end, to give a pyriform or apiculate shape, while those of *Stemphylium* have rounded ends and are usually symmetrical longitudinally. Conidia of *Alternaria* are definitely club-shaped (Simmons, 1967). Although this distinction has been maintained by Simmons (2007), molecular analyses have indicated that *Ulocladium* cannot be separated from *Alternaria* (Pryor and Gilbertson, 2000; de Hoog and Horré, 2002; Pryor and Bigelow, 2003).

Ulocladium botrytis is treated here as representative of the genus.

Ulocladium botrytis Preuss Fig. 5.50

Colonies on CYA and MEA 60 mm or more diam, plane, dense to somewhat floccose, dark olive to very dark grey, reverse blue black. On G25N, colonies 6–10 mm diam, of dense olive to dark grey mycelium, reverse blue black. At 5°C germination to formation of microcolonies. At 37°C, colonies 15–20 mm diam, olive grey, reverse grey brown or blue black.

On DCMA, colonies 30–40 mm diam, similar to those on CYA.

Conidia borne singly from nodes or pores on knobby or geniculate dark conidiophores, on CYA and DCMA variable in size and shape, often roughly spherical, 9–12 μm diam, occasionally single celled, usually with four or more septa, but also characteristically ellipsoidal, 15–25 × 9–10 μm, and with lateral and longitudinal or irregular septa, usually with only a small basal protrusion but occasionally stalked and resembling *Alternaria*, dark brown, with thick, often very rough walls.

Distinctive features. As noted above, the genus *Ulocladium* is distinguished from *Alternaria* and *Stemphylium* by conidial shape. Conidia of *Ulocladium* are obovoid, i.e. narrower at the base than at the apex. *U. botrytis* is the only species at all common in foods.

Physiology. No physiological studies on *Ulocladium* species are known to us.

Mycotoxins. No mycotoxins are known to be produced. None of 52 *Ulocladium* isolates produced altertoxins or tenuazonic acid, toxic metabolites produced by many *Alternaria* species (Andersen and Hollensted, 2008).

Ecology. *Ulocladium* species are infrequently found in foods. They have been isolated from hazelnuts and walnuts, pistachios, peanuts and barley

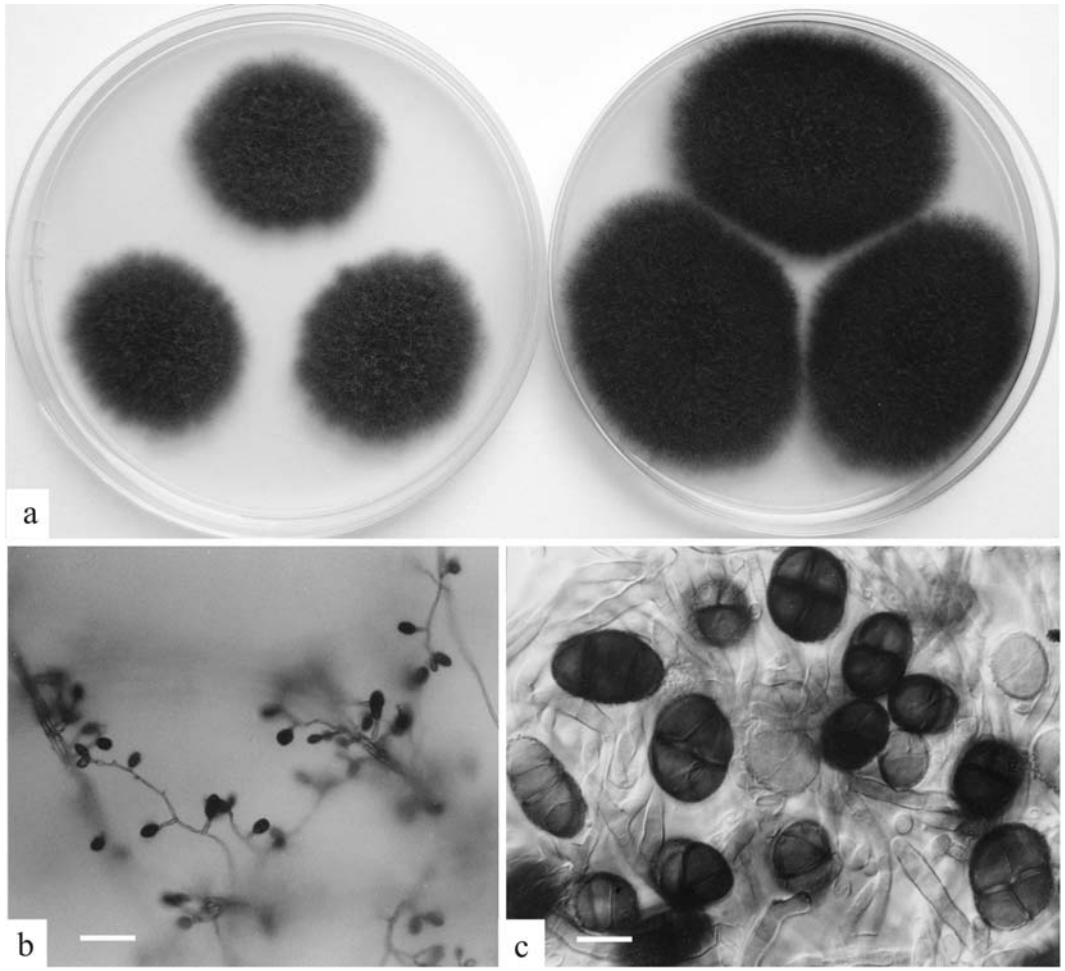


Fig. 5.50 *Ulocladium botrytis* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b)** conidiophores and conidia in situ, bar = 50 µm; **(c)** conidia, bar = 10 µm

(see Pitt and Hocking, 1997). *U. chartarum* can cause rots in melons (Snowdon, 1990). We isolated *Ulocladium* species, at low levels, from cashews, red beans, maize, peanuts and soybeans (Pitt et al., 1993, 1994, 1998a).

References. Simmons (1967).

Chapter 6
Zygomycetes

Zygomycetes (currently the phylum Zygomycota) are relatively primitive fungi characterised by the production of solitary spores, *zygospores*, as their teleomorph. Zygomycetes of significance here are characterised by hyphae with few if any cross walls (septa): the hyphae are essentially unobstructed tubes. Absence of septa facilitates rapid translocation of nutrients and organelles such as mitochondria and nuclei between sites of growth, nutrient adsorption and spore formation. In consequence, Zygomycetes are also characterised by rapidity of growth. Many species are able to fill a Petri dish with loosely packed mycelium and to produce mature spores within 2 days of inoculation.

Zygospores are large (usually greater than 30 μm diam), dark walled, distinctive bodies (Fig. 6.1) on which Zygomycetes rely for long-term survival. Formation of zygospores by the majority of species encountered in food spoilage requires mating by two strains, so zygospores are not commonly observed in the pure cultures used for identification. A few species such as *Rhizopus sexualis* do produce zygospores in pure culture, and this is a valuable taxonomic aid. Perhaps because they are physically difficult to separate from other more abundant spore types, little is known about the physiological properties of zygospores, such as resistance to heat and chemicals.

Zygomycetes embrace a wide variety of fungi, with diverse habitats. Almost all require high water activities for growth. In damp situations, their rapid growth habit provides a selective advantage over most, more advanced fungi with septate hyphae. Many are found on dung or as insect pathogens. For a guide to genera of Zygomycetes which can be grown in the laboratory, see O'Donnell (1979).

The genera of Zygomycetes commonly found in foods are confined to a single order, Mucorales.

Order Mucorales. The order Mucorales is monophyletic (having a single evolutionary origin) with all genera closely related genetically (White et al., 2006). However, classification within the order is less certain, as molecular studies have indicated poor correlation with traditional morphological taxonomy (O'Donnell et al., 2001). All of the families and many of the genera within the order cannot be sustained using molecular data.

Family Mucoraceae. At the same time, O'Donnell et al. (2001) consider that all of the genera included here fit within modern concepts of the family Mucoraceae, which is now being broadened to include many of the other previously recognised families (White et al., 2006). As the task of reordering the genera within this family remains incomplete, the generic taxonomy provided here is based on traditional systems. Comments on the taxonomic status of each genus is included under that name as appropriate.

Although some species within this family produce **zygospores** as a sexual state in culture, traditional classification has been based on asexual (anamorphic) reproduction. Asexual reproduction in the order Mucorales is primarily by **sporangiospores**, which are typically borne within sporangia. **Sporangia** (Fig. 6.2a) are closed sacs, borne on stipes (stalks). Stipes are often termed **sporangiophores**, although this name is more appropriately applied to the whole fruiting structure in line with conventional terminology in other asexual fungi.

J.I. Pitt, A.D. Hocking, *Fungi and Food Spoilage*, DOI 10.1007/978-0-387-92207-2_6,
© Springer Science+Business Media, LLC 2009

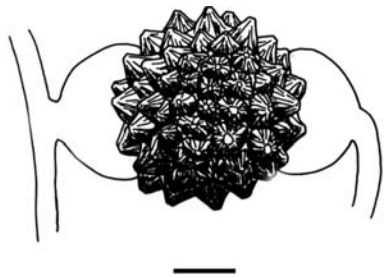


Fig. 6.1 Zygospore of *Rhizopus sexualis*, bar = 50 μm

Stipes may be borne singly or in clusters from fertile hyphae, may be branched or unbranched or may grow out from beneath a terminal sporangium to produce a succession of sporangia (sympodial branching, Fig. 6.2b).

Sporangia appear under the low-power microscope as small (less than 1 mm diam) brown to black spheres distributed on long stipes throughout the aerial mycelium. Sporangial walls disintegrate in age, releasing the sporangiospores.

In microscopic mounts from 7 day old cultures, intact sporangia are rare: the structures mostly seen at stipe apices are **columellae** (Fig. 6.2c), formed within the sporangia and remaining intact in age. The manner in which columellae collapse after sporangial disintegration provides useful taxonomic information.

In one genus, *Syncephalastrum*, sporangiospores are borne in cylindrical **merosporangia** in a radial array on the columella surface (Fig. 6.2d). Under low magnifications, these structures have some resemblance to the fruiting structures of *Aspergillus*. In another, *Thamnidium*, sporangiospores are borne both in normal sporangia and in small **sporangioles**, formed in clusters (Fig. 6.2e), often on the same stipe as a sporangium. In *Cunninghamella*, sporangioles are also borne in clusters, on spicules, but never produce sporangiospores. Here the sporangiole itself acts as the reproductive unit.

Some species in the Mucoraceae also produce **chlamydoconidia** as a second type of anamorph. These are cylindrical to spherical cells with relatively thick walls formed in hyphae and stipes, sometimes in great numbers (Fig. 6.2f). They probably function as a resting stage, more resistant to light, heat and desiccation than sporangiospores, and are probably analogous to similar structures formed by Hyphomycetes.

Identifying genera in Mucoraceae. One theme of this book is to provide a standardised system for identification of food spoilage fungi. Where possible, identification is achieved after 7 days incubation, by a single macroscopic and microscopic inspection of a standard set of Petri dishes. The

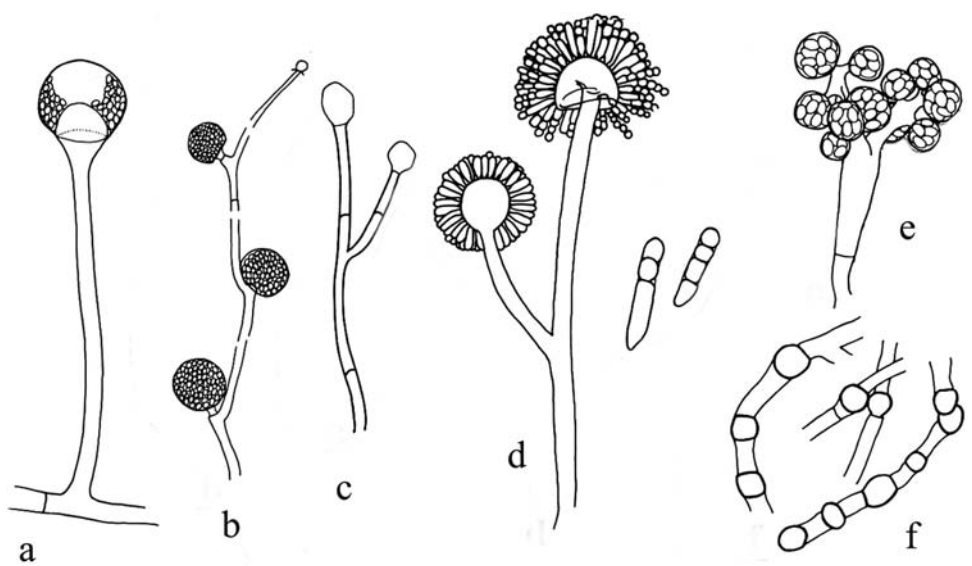


Fig. 6.2 Anamorphic reproductive structures in genera of Mucorales: **(a)** sporangium; **(b)** sympodial branching; **(c)** columellae; **(d)** merosporangium; **(e)** sporangioles; **(f)** chlamydoconidia

keys to Mucoraceae hereunder are accordingly based on this system.

To the experienced mycologist it will be obvious that because they grow so rapidly, isolates of Mucoraceae can be identified much earlier than 7 days. However, a second theme of this book is the facilitation of fungal identifications by the bacteriologist who does not instinctively recognise a *Mucor* from a *Monascus*, so the standard 7 day schedule has been maintained here. There is an unexpected bonus: the characteristic shapes of columellae collapsing in age simplifies recognition of some genera (Fig. 6.3).

Significant genera. Seven genera in the family Mucoraceae are treated here: *Absidia* Tiegh., *Cunninghamella* Matr., *Mucor* P. Micheli: Fr., *Rhizomucor* (Lucet and Costantin) Vuill., *Rhizopus* Ehrenb., *Syncephalastrum* Schröter and *Thamnidium* Link. These genera are differentiated primarily by the morphology of their sporangiophores and sporangia.

Rhizoids, mucous and contamination. Other useful features for distinguishing some of these genera are the production of rhizoids and the secretion of mucous material. Rhizoids are short root-like structures produced by *Rhizopus* at the base of each sporangiophore (Fig. 6.3c). *Mucor* species do not produce rhizoids, whereas *Absidia* and *Rhizomucor* species produce them irregularly.

As the name implies, *Mucor* produces sporangiospores in a layer of mucous which causes them to adhere to the colony when disturbed or picked with a needle. *Absidia* and *Rhizomucor* are similar, but *Rhizopus* produces dry spores.

If a *Mucor* culture fills the Petri dish the lid can be removed with minimal disturbance to the culture; however, the rhizoids of *Rhizopus* adhere to the lid and part of the culture will detach with it. Beware! Carry out this operation away from your inoculating area. Dry spores, which are aerially dispersed when plates are opened, and very rapid growth make *Rhizopus* species a serious source of laboratory contamination.

The seven genera of Mucoraceae considered here are keyed below and then treated in alphabetical order.

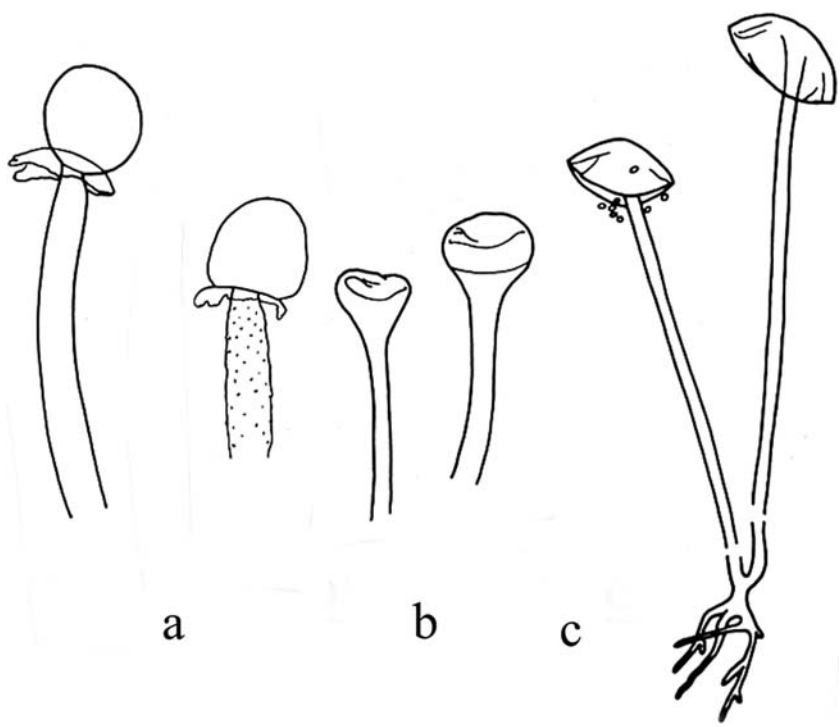


Fig. 6.3 Collapse of columellae in age: **(a)** little collapse (*Mucor, Rhizomucor*); **(b)** funnel shapes (*Absidia*); **(c)** umbrella shapes (*Rhizopus*)

Key to genera of the family Mucoraceae included here

1	All reproductive cells borne on spicules around vesicles	2
	Some or all reproductive cells sporangiospores, borne within sporangia (Fig. 6.2a, b, c)	3
2 (1)	Reproductive cells sporangiospores, borne in cylindrical sacs (merosporangia; Fig. 6.2d) (at low magnification reminiscent of *Aspergillus*)	*Syncephalastrum*
	Reproductive cells single-celled spherical sporangioles	*Cunninghamella*
3 (1)	As well as sporangia, clusters of smaller sacs (sporangioles, Fig. 6.2e) present	*Thamnidium*
	Only sporangia, or columellae derived from sporangia, present	4
4 (3)	Columellae retaining approximately spherical shape after sporangiospore discharge (Fig. 6.3a), sporangiospore walls smooth or spiny	5
	Columellae collapsing to form funnel or umbrella shapes, sporangiospore walls smooth or striate	6
5 (4)	Sporangiospores rarely exceeding 5 µm in long axis	*Rhizomucor*
	Sporangiospores commonly exceeding 5 µm in long axis	*Mucor*
6 (4)	Columellae collapsing inwardly from the apex to form a funnel shape (Fig. 6.3b), sporangiospore walls smooth	*Absidia*
	Columellae collapsing outwardly to form an umbrella shape (Fig. 6.3c), sporangiospore walls striate	*Rhizopus*

6.1 Genus *Absidia* Tiegh.

The genus *Absidia* produces a distinctive type of columella which widens gradually at the junction with the stipe, outside the circumference of the sporangium (Fig. 6.4b). In other Zygomycete genera considered here, the junction of stipe and columella is abrupt, and the columella is wholly within the sporangial wall. In age, columellae of *Absidia* frequently collapse inward from the apex to form funnel-shaped structures.

Absidia species form rhizoids, irregular root-like outgrowths at the bases of the stipes, but these are less conspicuous and less regular than in *Rhizopus*

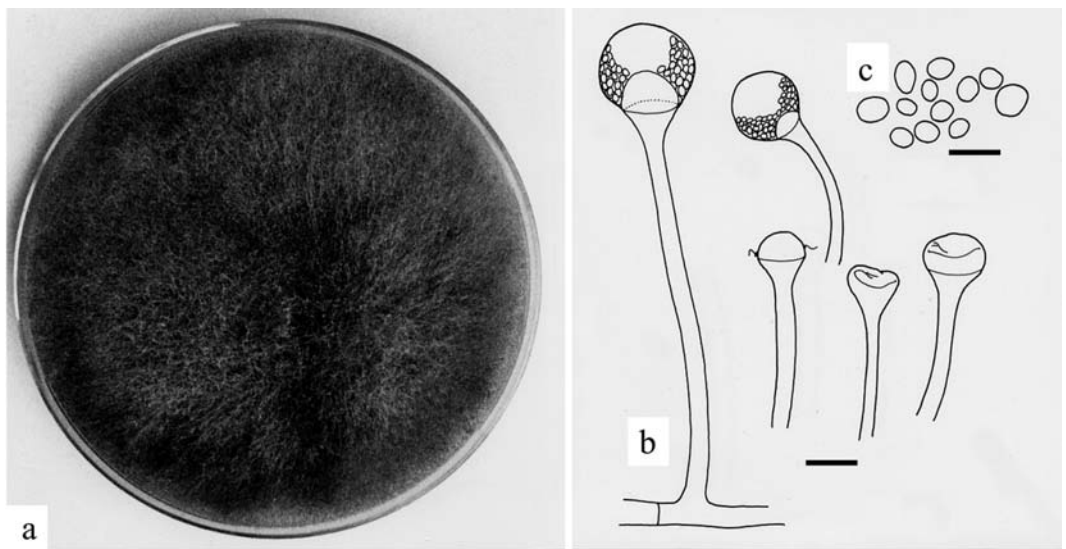


Fig. 6.4 *Absidia corymbifera* **(a)** colonies on CYA, 7 days, 25°C; **(b)** sporangia and columellae, bar = 25 µm; **(c)** sporangiospores, bar = 10 µm

species. Only one *Absidia* species, *Absidia corymbifera*, is at all common in foods. Several recent authors have concluded that *Absidia* is polyphyletic (evolutionarily diverse) based on molecular studies (e.g. Voigt et al., 1999; O'Donnell et al., 2001; Kwasna et al., 2006) and that *A. corymbifera* is not closely related to most other species in the genus and would be better classified in *Rhizomucor*. However, this name remains in common use.

Absidia corymbifera (Cohn) Sacc. & Trotter Fig. 6.4

Absidia ramosa J.J. Ellis & Hesselt.

On CYA, colonies covering the Petri dish, low and sparse, white to pale brown or grey; reverse colourless. On MEA, colonies filling the whole Petri dish with deep floccose mycelium, coloured mid-grey by sporangia; reverse pale. On G25N, colonies 10–30 mm diam, sparse and floccose, coloured as on MEA. No growth at 5°C. At 37°C, colonies covering the whole Petri dish, similar to those at 25°C.

Sporangiophores borne from aerial hyphae, stipes sometimes irregularly branched; sporangia hyaline, 15–50 μm diam, appearing pyriform due to external conical columellae; columellae pyriform 10–30 μm diam, sometimes with small projections on the apices or with collarettes above the base, in age often collapsing inward from the apex to form funnel-shaped structures; sporangiospores hyaline, broadly ellipsoidal to spheroidal, 3–6 μm long, smooth walled.

Distinctive features. See genus description.

Physiology. Evans (1971) recorded growth temperatures for *Absidia corymbifera* as minimum 14°C, maximum 50°C and optimum near 40°C. This species is able to germinate and grow down to 0.88 a_w (Hocking and Miscamble, 1995). Growth in nitrogen (<1% O_2) was similar to that in air (Hocking, 1990).

Mycotoxins. Mycotoxin production has not been reported.

Ecology. *Absidia corymbifera* is a weak human and animal pathogen, with a very wide host range and capable of infecting many body organs (de Hoog et al., 2000). From foods, most isolations have been from wheat, barley, malted barley and cereal products such as flour and bran. It has also been found in meat products and biltong, cassava, hazelnuts and sunflower seeds and, as *A. ramosa*, in pecans (see Pitt and Hocking, 1997). It has been recorded as a pathogen of peaches (Singh and Prashar, 1988). *A. corymbifera* is probably widespread in the tropics: Oyeniran (1980) recorded isolations from cocoa, palm kernels and maize. We have found it, always at low levels, in sorghum and mung beans from Thailand (Pitt et al., 1993, 1994); in peanuts, kemiri nuts, milled rice and coriander from Indonesia (Pitt et al., 1998a); and peanuts, paddy, milled rice, soybeans and black pepper from the Philippines (our unpublished data).

References. Ellis and Hesseltine (1966); Nottebrock et al. (1974); de Hoog et al. (2000).

6.2 Genus *Cunninghamella* Matr.

This genus is unusual in that sporangiophores give rise to sporangioles (small sporangia) which do not differentiate into sporangiospores but themselves act as the conidial stage. The sporangioles are borne on spicules (spikes) from vesicles; the vesicles are borne terminally or irregularly on the sporangiophores. A recent monograph by Zheng and Chen (2001) lists 15 species, and a molecular study of 12 of these showed good agreement with traditional speciation (Liu et al., 2001). Zheng and Chen (2001) report that a great deal of literature confusion exists over the names of species, in particular with the common species *Cunninghamella bertholletiae* and *C. elegans*. These two species are distinguishable by small morphological differences and by the fact that *C. bertholletiae* grows to 42°C or more, while the maximum growth temperature for *C. elegans* is usually 35°C. Although the name *C. elegans* is most commonly seen in the food literature, our experience indicates that *C. bertholletiae* is the most common species, along with *C. echinulata*. *C. bertholletiae* is described below: *C. echinulata* differs by the production of sporangioles with conspicuously spiny walls (Zheng and Chen, 2001).

Cunninghamella bertholletiae Stadel Fig. 6.5

Colonies on CYA and MEA covering or filling the whole Petri dish, sparse, mycelium off-white to beige, reverse colourless to pale yellow. On G25N, ranging from no growth to colonies 15 mm diam, of sparse, translucent mycelium. At 5°C, no

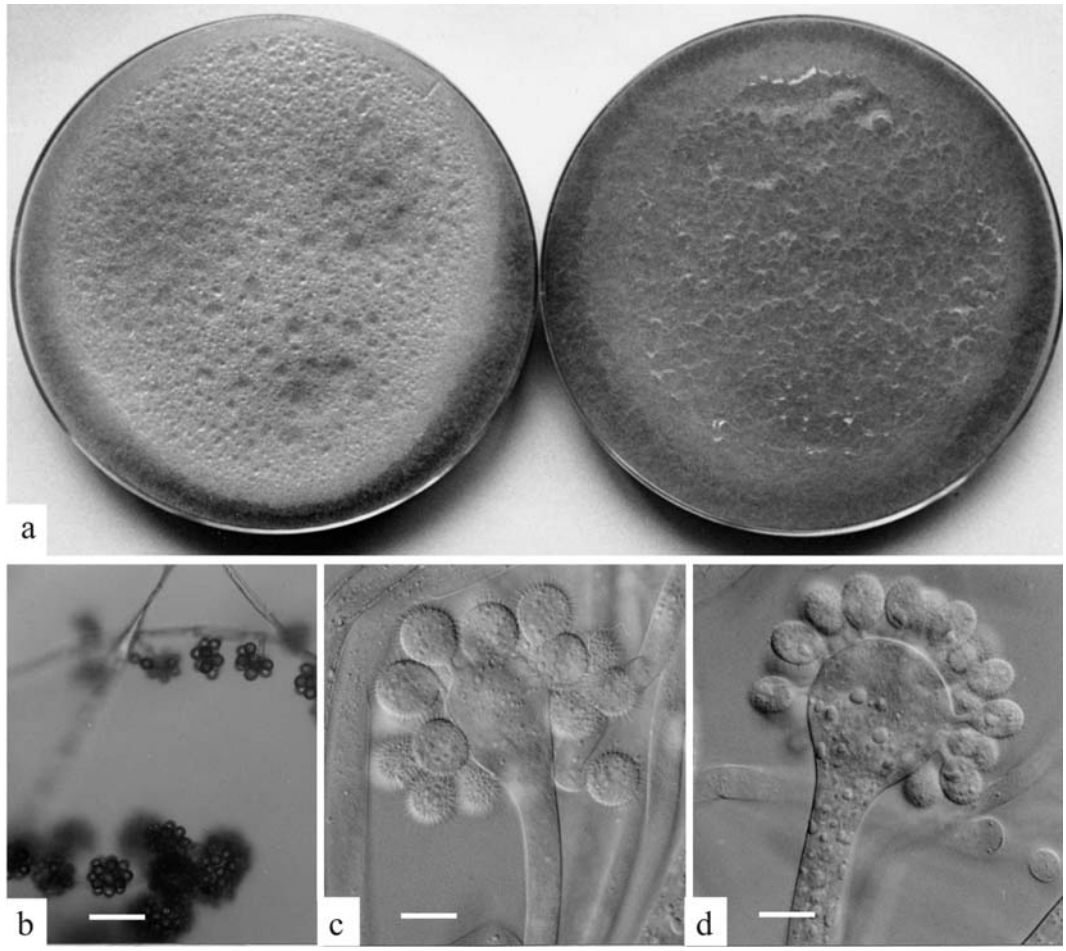


Fig. 6.5 *Cunninghamella bertholletiae* **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b)** developing sporangioles in situ, bar = 50 μm; **(c, d)** vesicles and mature sporangioles, bar = 10 μm

germination. At 37°C, colonies similar to those on CYA at 25°C, mycelium off-white to grey, reverse pale yellow to brown.

Sporangiophores long, bearing solitary vesicles terminally and in irregular verticils subterminally or along the length; sporangioles borne from spicules, breaking at maturity, spherical to ellipsoidal, 7–11 μm diam or 9–13 μm long, with smooth to finely roughened walls.

Zygospores not commonly seen in pure culture, *fide* Zheng and Chen (2001) irregularly ellipsoidal to spherical, brownish, 20–45 μm diam, with rough walls.

Distinctive features. See genus description.

Taxonomy. Most *Cunninghamella* species are heterothallic, producing zygospores only when two mating types from the same species are grown together.

Physiology. *Cunninghamella bertholletiae* grows up to 42–45°C. Growth in soil is confined to above –90 bars (ca. 0.93 a_w) (Kouyeas, 1964).

Mycotoxins. Mycotoxins are not produced.

Ecology. In common with other *Cunninghamella* species, *C. bertholletiae* mostly occurs in tropical and subtropical soils (Zheng and Chen, 2001). *C. echinulata* was a cause of rotting in kola nuts (Adebajo, 1994). We have seen *C. bertholletiae* at low levels in maize from the Philippines and peanuts from Indonesia (Pitt et al., 1998a and unpublished).

References. O'Donnell (1979); Domsch et al. (1980).

6.3 Genus *Mucor* P. Micheli: Fr.

As traditionally described, *Mucor* is a very common and widespread genus in nature, occurring in soils, decaying vegetation, dung and many other moist habitats where rapidly growing fungi have an advantage. Recent molecular studies (Voigt et al., 1999; O'Donnell et al., 2001; Kwasna et al., 2006) have indicated that *Mucor* includes at least two phylogenetically distinct groups. However, the species included here, insofar as they have been examined, appear to fall into the major *Mucor* clade, so the traditional description is used here. Unlike those of *Absidia*, sporangia of *Mucor* have columellae borne wholly within the sporangial wall; the columellae collapse irregularly, if at all, in age (Fig. 6.3a). Unlike *Rhizopus* species, Mucors do not produce rhizoids. In species of interest here, sporangiospores are longer than 5 μm and have walls which are smooth or spiny, but not striate. This is in contrast to *Rhizopus* species, which commonly produce sporangiospores with striate walls.

Some *Mucor* species are able to grow and weakly ferment under anaerobic conditions and occasionally cause spoilage of beverages in this manner. Growth is yeast-like in appearance (Fig. 6.6), although individual cells are much too large to be mistaken for a true yeast. Inoculation of such cells onto aerobic media produces normal growth. Yeast-like growth has also been reported to occur when *Mucor* and some related genera grow in the presence of high sodium chloride concentrations (Tresner and Hayes, 1971). At least 20 species of *Mucor* have been reported from foods. Five appear to be most significant: *Mucor circinelloides*, *M. hiemalis*, *M. piriformis*, *M. plumbeus* and *M. racemosus*. These species are keyed and described below.

General reference. Schipper (1978a).

Key to *Mucor* species included here

1	Strong growth at 37°C	*M. circinelloides*
	No growth at 37°C	2
2 (1)	Columellae frequently with small irregular projections on the apices; sporangiospores with minute spines	*M. plumbeus*
	Columellae without apical projections; sporangiospores smooth walled	3
3 (2)	Columellae 50–100 μm diam; growth on G25N weak or absent	*M. piriformis*
	Columellae usually less than 50 μm diam; colonies on G25N greater than 10 mm diam	4
4 (3)	Chlamydoconidia abundant, often dominating microscopic appearance	*M. racemosus*
	Chlamydoconidia present in low numbers or absent	*M. hiemalis*

Mucor circinelloides Tiegh. Fig. 6.7

On CYA, colonies 60 mm diam or more, often spreading across the whole Petri dish, but growth relatively low and sparse, appearing pale grey or yellowish; reverse uncoloured. On MEA, colonies filling the whole Petri dish, in colours similar to those on CYA. On G25N, colonies 15–25 mm diam, low and relatively dense, golden yellow in both obverse and reverse. At 5°C, colonies 4–10 mm diam, low and sparse. At 37°C, colonies 20–40 mm diam, sparse and floccose, with colours more brown than at 25°C.

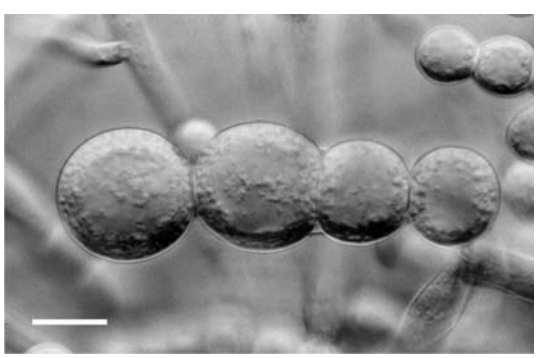


Fig. 6.6 *M. plumbeus* showing yeast-like growth, in liquid culture under anaerobic conditions, bar = 25 μm

Sporangiophores borne from aerial hyphae, stipes commonly branched, often sympodially, sporangia spherical, 25–50 μm diam, sometimes up to 80 μm; columellae roughly spherical, up to 50 μm diam; sporangiospores hyaline, ellipsoidal, mostly 4.5–7 μm long, smooth walled. Chlamydoconidia uncommon, spherical, cylindrical or rather irregular, up to 15 μm diam. Zygospores not formed in pure culture.

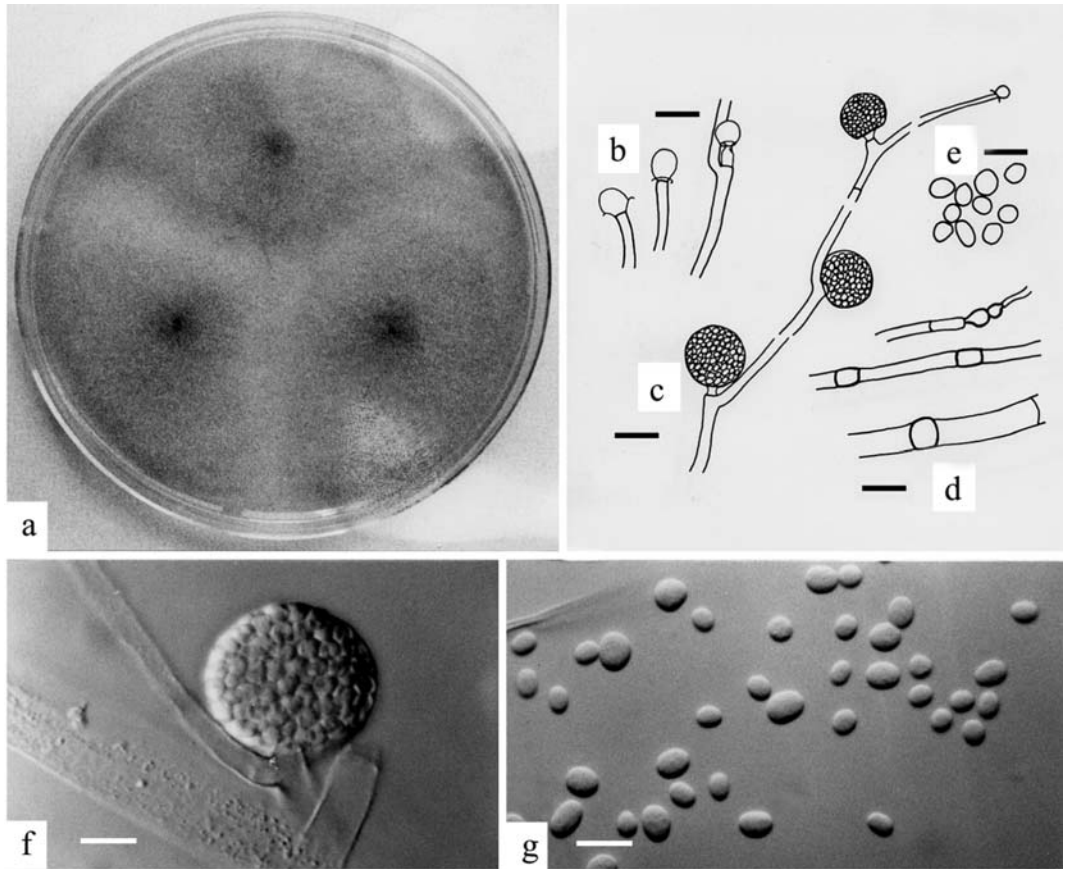


Fig. 6.7 *Mucor circinelloides* **(a)** colonies on CYA, 7 days, 25°C; **(b)** columellae; **(c)** sporangia; **(d)** chlamydoconidia; **(e)** sporangiospores; **(f)** sporangium; **(g)** sporangiospores, all bars = 10 μm

Distinctive features. *Mucor circinelloides* has numerous characters in common with *M. hiemalis*. Unlike *M. hiemalis*, *M. circinelloides* grows well at 37°C (20 mm or more in 7 days), grows weakly at 5°C (less than 10 mm vs. greater than 20 mm in 7 days) and commonly produces sympodially branched stipes.

Taxonomy. DNA sequence data from the ITS1 and 2 regions indicate that *Mucor circinelloides* is correctly placed in the genus *Mucor* (Kwasna et al., 2006).

Physiology. Tresner and Hayes (1971) reported that *Mucor circinelloides* grew in media containing 15% (w/v) NaCl (=0.90 a_w) but not 20% (=0.86 a_w). On a medium with glucose as controlling solute, this species germinated and grew down to 0.90 a_w (Hocking and Miscamble, 1995).

Mycotoxins. This species is not known to produce mycotoxins.

Ecology. This species has been reported frequently as an animal pathogen and occasionally as a human pathogen (de Hoog et al., 2000). It can cause spoilage in cheese and yams (see Pitt and Hocking, 1997) and can be pathogenic on mangoes (Johnson et al., 1990) and peaches (Restuccia et al., 2006). It has been isolated from meat, hazelnuts and walnuts and also from maize, mung beans, soybeans and barley (Pitt et al., 1993, 1994, 1998a; see Pitt and Hocking, 1997).

References. Schipper (1976); Domsch et al. (1980).

Mucor hiemalis Wehmer **Fig. 6.8**

On CYA, colonies spreading across, and sometimes filling, the whole Petri dish, growth relatively

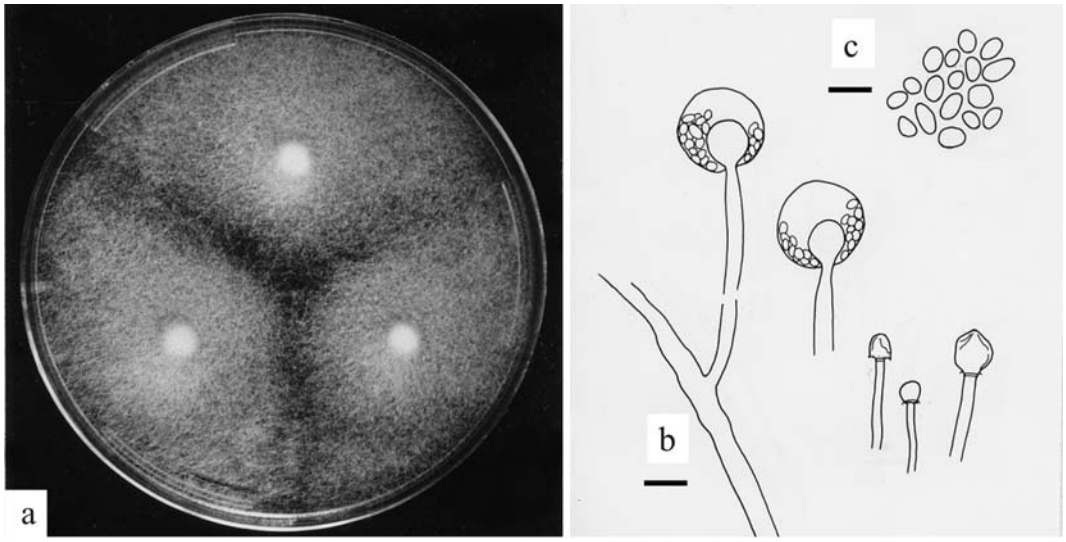


Fig. 6.8 *Mucor hiemalis* **(a)** colonies on CYA, 7 days, 25°C; **(b) s**porangia and columellae, bar = 25 μm; **(c)** sporangiospores, bar = 10 μm

sparse, greyish; reverse pale. On MEA, colonies filling the whole Petri dish, relatively dense, greyish to distinctly yellow; reverse yellow to golden yellow. On G25N, colonies 10–15 mm diam, moderately dense, bright yellow in both obverse and reverse. At 5°C, colonies 20–30 mm diam, low and sparse. No growth at 37°C.

Sporangiophores borne aerially, stipes generally unbranched, less commonly sympodially branched; sporangia up to 60 μm diam; columellae ellipsoidal, 15–30 μm diam; sporangiospores hyaline, narrowly to broadly ellipsoidal or reniform (kidney shaped), 5–11 μm long, smooth walled. Chlamydoconidia uncommon, spherical to cylindrical or irregular, up to 15 μm diam. Zygospores not formed in pure culture.

Distinctive features. *Mucor hiemalis* is similar to *M. circinelloides*, but grows more rapidly at 5°C and does not grow at 37°C. Moreover, it produces larger sporangiospores which are sometimes reniform and usually has unbranched stipes.

Taxonomy. DNA sequence data from the ITS1 and 2 regions indicate that *Mucor hiemalis* is correctly placed in the genus *Mucor* (Kwasna et al., 2006).

Physiology. Growth of some *Mucor hiemalis* isolates occurs at temperatures below 0°C (Joffe, 1962).

Mycotoxins. Mycotoxin production has not been reported.

Ecology. *Mucor hiemalis* has been reported as a rare cause of cutaneous mycosis (de Hoog et al., 2000). This species causes rots in guavas (Ito et al., 1979), carrots and cassava (Snowdon, 1991). It has been reported from spoilage of yoghurt due to inward collapse of containers (Foschino et al., 1993) and cheese (Hayaloglu and Kirbag, 2007). It has also been reported from fresh vegetables (Lugauskas et al., 2005), from chestnuts (Jermini et al., 2006), hazelnuts and soybeans (see Pitt and Hocking, 1997), from wheat based fast foods in Nigeria (Fapohunda and Ogundero, 1990), from preprepared airline food in Egypt (Saudi and Mansour, 1990) and from chocolate confectionery in Italy (Dragoni et al., 1989). We have isolated it at low levels from Thai and Indonesian maize and paddy rice (Pitt et al., 1993, 1998a).

Reference. Schipper (1973).

Mucor piriformis A. Fisch. Fig. 6.9

On CYA, colonies low and sparse, spreading across the Petri dish or discrete; mycelium colourless, overall colour buff from sporangia; reverse pale. On MEA, colonies 35–60 mm diam, coloured grey or brownish; reverse pale. On G25N, colonies less than 7 mm diam, or growth absent. At 5°C, colonies

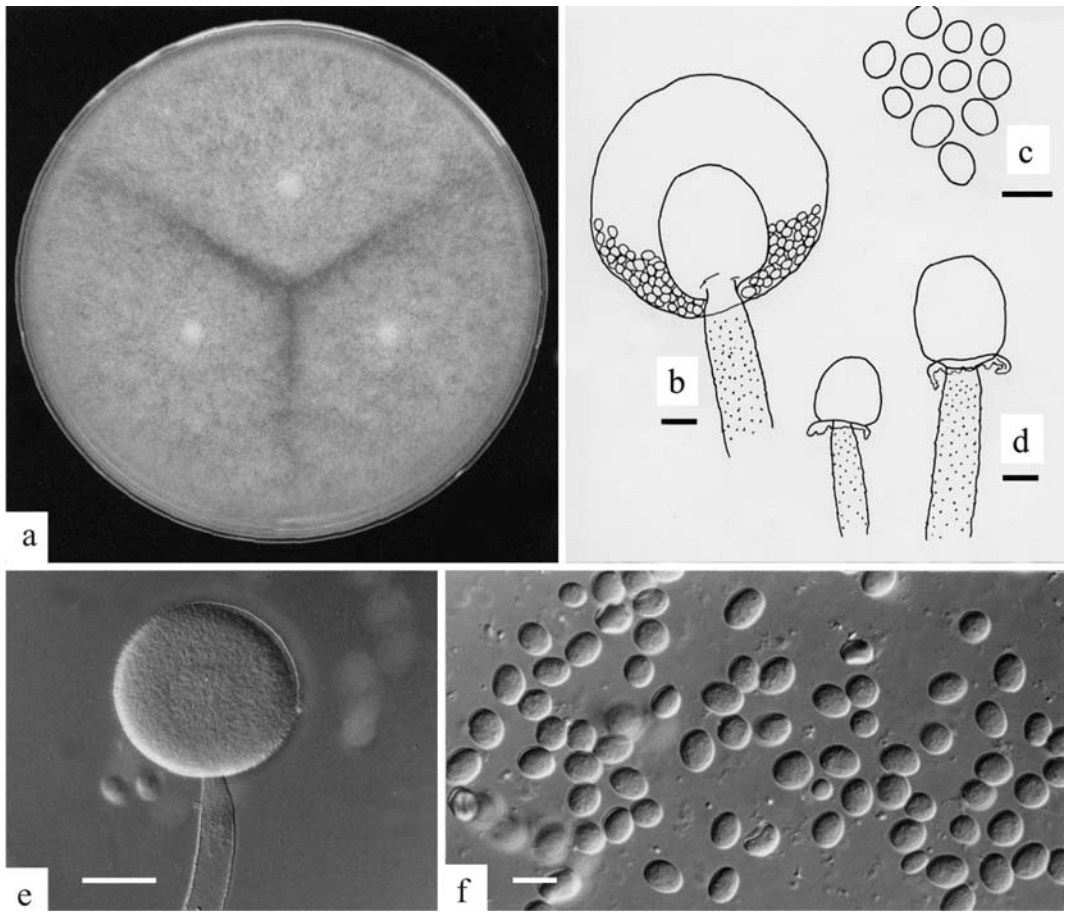


Fig. 6.9 *Mucor piriformis* **(a)** colonies on MEA, 7 days, 25°C; **(b) s**porangia, bar = 25 µm; **(c)** columellae, bar = 25 µm; **(d)** sporangiospores, bar = 10 µm; **(e)** developing sporangium, bar = 25 µm; **(f)** sporangiospores, bar = 10 µm

30–45 mm diam, low and sparse, or with some aerial hyphae. No growth at 37°C.

Sporangiophores on CYA borne from surface hyphae, stipes short, broad, often sympodially branched and with encrusted walls; sporangia up to 150 µm diam, with spinulose walls; columellae spheroidal to short cylindroidal, 25–80(–100) µm diam or in length, the larger ones collapsing irregularly; sporangiospores hyaline, spherical to broadly ellipsoidal, 6–12(–20) µm diam or in long axis, smooth walled. Chlamydoconidia uncommon; zygospores not formed in pure culture.

Distinctive features. *Mucor piriformis* grows rapidly on CYA at 5°C, but relatively poorly under the standard 25°C incubation conditions. This is especially noticeable on G25N, where growth is weak or absent. Mycelium on CYA at 25°C is often contorted and highly branched. Sporangia, columellae and many sporangiospores are larger than those of other common *Mucor* species.

Physiology. *Mucor piriformis* is a psychrophile. Cardinal temperatures are minimum, near 0°C, optimum 20–21°C, maximum 26°C (Michailides and Spotts, 1990). Mycelium and sporangiospores were inactivated by heating to 43–46 and 52–55°C, respectively (Michailides and Ogawa, 1989).

Mycotoxins. Mycotoxin production has not been reported.

Ecology. *Mucor piriformis* is a destructive pathogen of fresh strawberries (Snowdon, 1990; Pitt and Hocking, 1997) and causes rotting of cold stored pears, apples and tomatoes (see Pitt and Hocking, 1997), plums (Borve and Vangdal, 2007) and yams (Amusa and Baiyewa, 1999; Iwata, 2006). A variety of

treatments for control of pear spoilage have been proposed: dips in hot water (Michailides and Ogawa, 1989), a hot water pressure process (Spotts et al., 2006), salt and surfactant solutions (Spotts and Cervantes, 1989), prestorage drench in thiabendazole (Lennox et al., 2004) or treatment of wash water with chlorine dioxide or peracetic acid (Roberts and Reymond, 1994; Mari et al., 2003). This species has recently been reported as a dominant species in the mycoflora of cassava and yam chips (Gnonlonfin et al., 2008).

References. Schipper (1975); Michailides and Spotts (1990).

Mucor plumbeus Bonord. **Fig. 6.10**

Mucor spinosus Tiegh.

On CYA and MEA, colonies at least 50 mm diam, low to deep, often spreading across the Petri dish; mycelium colourless, overall colour pale to deep grey from sporangia; reverse colourless. Colonies on G25N 20–35 mm diam, low, moderately dense, white to pale yellow brown; reverse pale. At 5°C, colonies 8–15 mm diam, low and sparse. No growth at 37°C.

Sporangiophores borne from surface or aerial hyphae, stipes unbranched or branched sympodially,

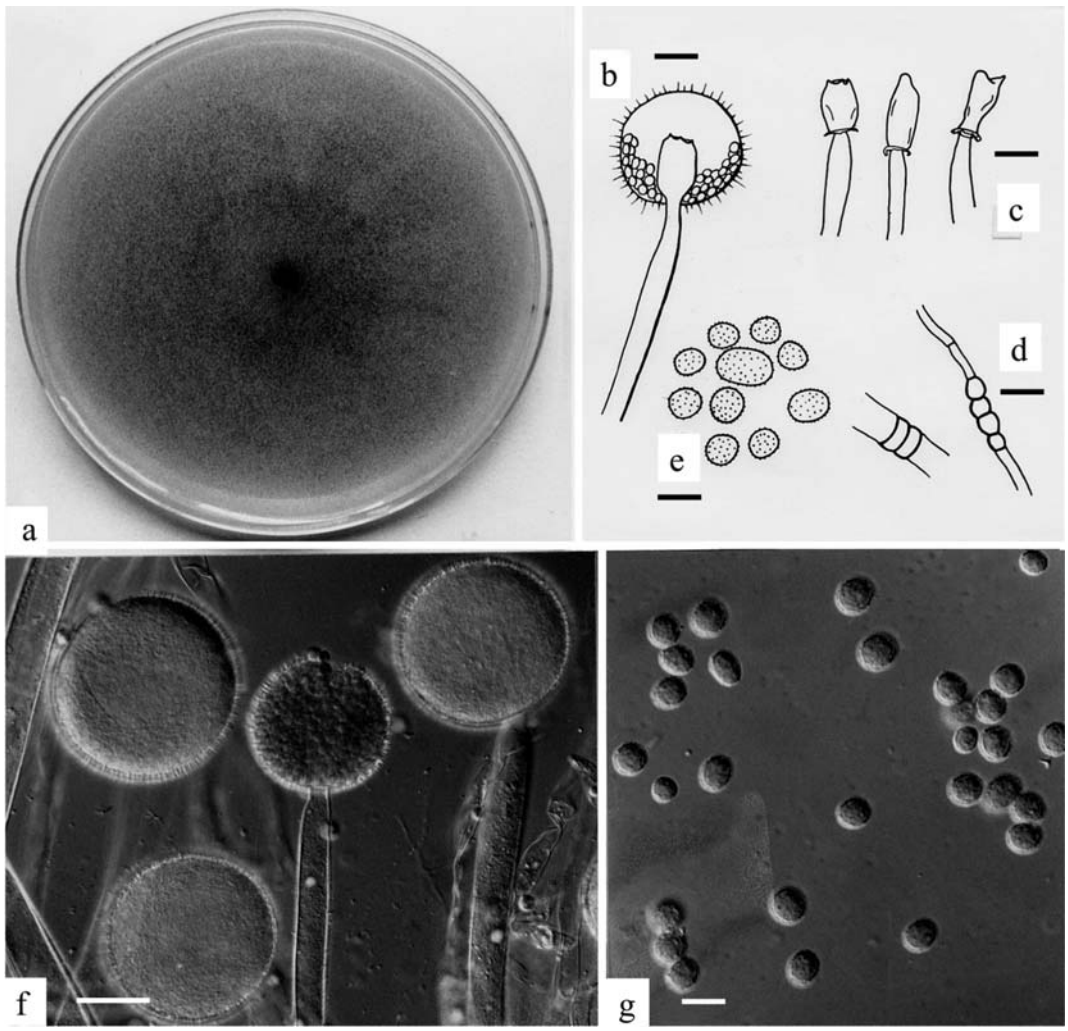


Fig. 6.10 *Mucor plumbeus* **(a)** colonies on CYA, 7 days, 25°C; **(b) s**porangia, bar = 25 µm; **(c)** columellae, bar = 25 µm; **(d)** chlamydoconidia, bar = 25 µm; **(e)** sporangiospores, bar = 10 µm; **(f)** sporangia, bar = 25 µm; **(g)** sporangiospores, bar = 10 µm

sporangia dark greyish brown, up to 80 μm diam, with spiny walls; columellae pyriform to ellipsoidal or short cylindroidal, up to 50 × 30 μm, often with irregular projections at the apices; sporangiospores brown, spheroidal, commonly 7–8(–12) μm diam, with walls rough or minutely spiny. Chlamydoconidia uncommon; zygospores not formed in pure culture.

Distinctive features. *Mucor plumbeus* is distinguished by its grey colour, columellae with irregular apical projections and brown sporangiospores with rough or spiny walls.

Taxonomy. DNA sequence data from the ITS1 and 2 regions indicate that *Mucor plumbeus* is correctly placed in the genus *Mucor* (Kwasna et al., 2006).

Physiology. Panasenko (1967) reported growth of *Mucor plumbeus* from 4 or 5 to 35°C, with an optimum of 20–25°C. The minimum a_w for growth was reported to be 0.93 by Snow (1949). Growth in N_2 (<1% O_2) was 80% of that in air; some growth occurred in an atmosphere of ≥97% CO_2, with only trace amounts of O_2 (Hocking, 1990). As measured by colony diameters, growth on cheddar cheese in an atmosphere of 20% CO_2 and 5% O_2 was 50% of that in air, and growth in 20% CO_2 and 1% O_2, 40% CO_2 and 5% O_2 and 40% CO_2 and 1% O_2 was 40, 50 and 30% of that in air, respectively (Taniwaki et al., 2001a).

Mycotoxins. Mycotoxins are not known to be produced.

Ecology. *Mucor plumbeus* has been reported to spoil cheese (Northolt et al., 1980; Devoyod, 1988) and has been observed to cause anaerobic spoilage of apple juice in our laboratory. Meat (Gros et al., 2003; Pitt and Hocking, 1997), nuts and cereals are other commodities from which this species, and its synonym *M. spinosus*, have been reported (see Pitt and Hocking, 1997). It was isolated at low levels from black rice in Thailand, soybeans in the Philippines and coriander in Indonesia (Pitt et al., 1994, 1998a).

Reference. Schipper (1976).

Mucor racemosus Fresen. Fig. 6.11

On CYA and MEA, colonies spreading across the Petri dish, low to moderately deep; mycelium colourless, overall colour light to mid brown from sporangia and chlamydoconidia; reverse light brown. On G25N, colonies 25–40 mm diam, low, moderately dense, similar in colour to those on CYA. At 5°C, colonies 12–25 mm diam, low and sparse. No growth at 37°C.

Sporangiophores borne from surface or aerial mycelium, stipes branched sympodially or irregularly, sporangia up to 80 μm diam, light brown, with encrusted walls; columellae ellipsoidal to pyriform, up to 40 μm long; sporangiospores hyaline to pale brown, broadly ellipsoidal to subspheroidal, commonly 5–8 μm diam, smooth walled. Chlamydoconidia and arthroconidia formed abundantly, 5–20 μm or more in diam or long axis. Zygospores not formed in pure culture.

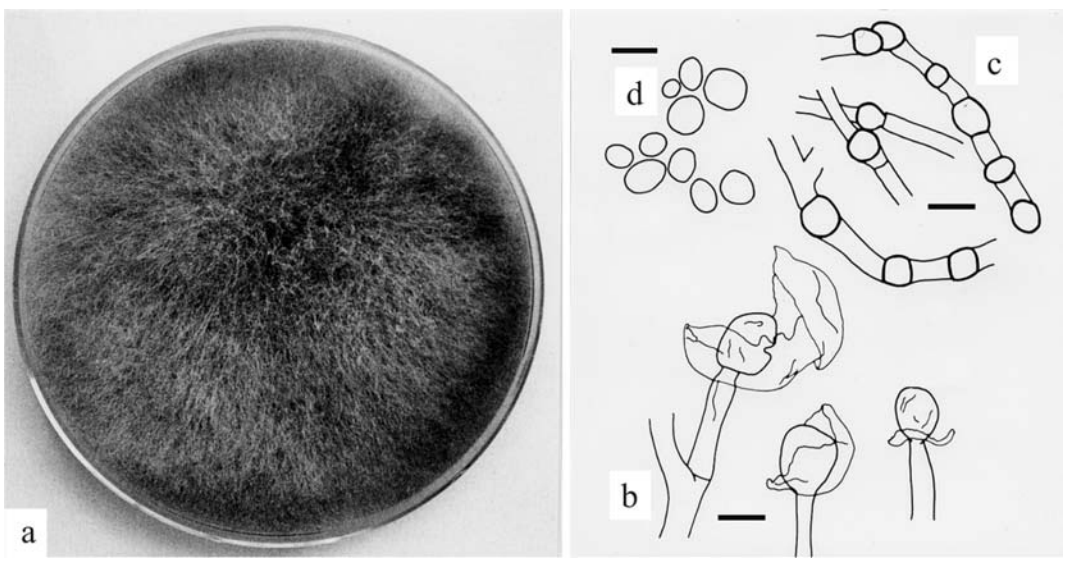


Fig. 6.11 *Mucor racemosus* **(a)** colonies on CYA, 7 days, 25°C; **(b)** columellae, bar = 25 μm; **(c)** chlamydoconidia, bar = 25 μm; **(d)** sporangiospores, bar = 10 μm

Distinctive features. *Mucor racemosus* is similar in many respects to *M. plumbeus*. *M. racemosus* differs by faster growth on G25N, brown not grey colony colouration, the much greater abundance of chlamydoconidia, smooth sporangiospores and the absence of irregular projections on columellae.

Taxonomy. DNA sequence data from the ITS1 and 2 regions indicate that *Mucor racemosus* is correctly placed in the genus *Mucor* (Kwasna et al., 2006).

Physiology. *Mucor racemosus* grows between –3 or –4°C and 30–35°C, with an optimum of 20–25°C (Panasenko, 1967). The minimum a_w for growth is 0.92 (Panasenko, 1967).

Mycotoxins. No mycotoxins are produced.

Ecology. *Mucor racemosus* is responsible for a spongy soft rot of cool stored sweet potatoes, potatoes and citrus (Chupp and Sherf, 1960). It has been reported, along with *M. hiemalis*, from spoilage of yoghurt due to inward collapse of containers (Foschino et al., 1993) and as a cause of spoilage of cheese (Devoyod, 1988; and in our laboratory) and cheesecake (Piskorska-Pliszczynska and Borkowska-Opacka, 1984). It has also been found contaminating cheese (Kivanç, 1992; Lund et al., 1995; Cantoni et al., 2003), salami (Cantoni et al., 2007), raisins (Youssef et al., 2000), frozen and processed meats, salted horse meat, fermenting cacao beans, maize, barley, soybeans and paddy rice (see Pitt and Hocking, 1997).

Reference. Schipper (1976).

6.4 Genus *Rhizomucor* (Lucet and Costantin) Vuill.

Schipper (1978b) revived the genus *Rhizomucor* for three species previously accepted in *Mucor*, but distinguished by the production of stolons and by their thermophilic nature. Molecular studies (Voigt et al., 1999) support this concept. *Rhizomucor* species are significant in foods mainly in tropical regions. The most common species in foods is *Rhizomucor pusillus*; *R. miehei* is also mentioned.

Rhizomucor pusillus (Lindt) Schipper Fig. 6.12

Mucor pusillus Lindt

On CYA, colonies sometimes 25–35 mm diam, of colourless to white floccose mycelium surmounted by brown sporangia, or sometimes covering the whole Petri dish and then similar to that on MEA. On MEA, colonies covering the whole Petri dish, low and relatively sparse, pale to mid-grey; reverse pale, yellowish or greenish grey. On G25N, growth sparse, not exceeding 5 mm diam, or absent. No growth at 5°C. At 37°C, colonies similar to those at 25°C on MEA, but more dense, brown to dark grey.

Sporangiophores borne from surface hyphae, stipes sometimes appearing unbranched, but usually extensively and irregularly branched; poorly formed rhizoids sometimes apparent but not adjacent to the

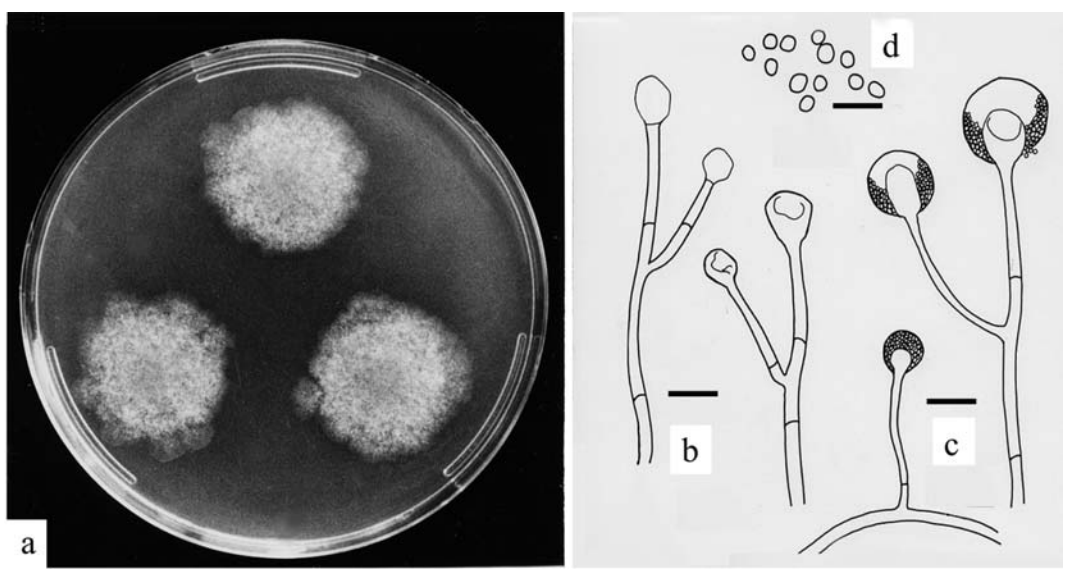


Fig. 6.12 *Rhizomucor pusillus* **(a)** colonies on CYA, 7 days, 25°C; **(b)** columellae, bar = 25 μm; **(c)** sporangia, bar = 25 μm; **(d)** sporangiospores, bar = 10 μm

stipe bases, a distinction from *Rhizopus*; sporangia spherical, brown or grey, 40–60(–80) μm diam; columellae spherical, ellipsoidal or pyriform, 20–45 μm diam, sometimes collapsing irregularly (similar to *Mucor* species); sporangiospores hyaline, spherical to broadly ellipsoidal, 3–4 μm diam, smooth walled. Zygospores produced by occasional isolates, black, broadly ellipsoidal, 60–70 μm diam.

Distinctive features. *Rhizomucor* species are most readily distinguished by rapid growth at 37°C and by their sporangiospores, which are small (less than 5 μm diam) and smooth walled.

Physiology. *Rhizomucor pusillus* is a thermophile. Growth has been reported at temperatures as high as 60°C (Crisan, 1973). Optimum growth conditions are 37–42°C, with a lower limit of 20°C (Panasenko, 1967; Evans, 1971).

Mycotoxins. Mycotoxin production is unknown.

Ecology. *Rhizomucor pusillus* is a frequent agent of bovine mycotic abortion and a rare cause of human zygomycosis (de Hoog et al., 2000). This species appears to be of widespread though uncommon occurrence in foods. Reported sources include cereals (Lugauskas et al., 2006), kola nuts (Adebajo and Popoola, 2003), various spices (Mandeel, 2005), olives (Roussos et al., 2006), meat products, pecans, hazelnuts and walnuts, sunflower seeds and various tropical products (see Pitt and Hocking, 1997). It is used in cheese manufacture in Japan (Koizumi, 2001). We isolated *R. pusillus* from mung beans in Thailand and kemiri nuts in Indonesia (Pitt et al., 1994, 1998a).

Additional species. *Rhizomucor miehei* (Cooney and R. Emers.) Schipper (synonym *M. miehei* Cooney and R. Emers.) has occasionally been isolated from foods (Kuthubutheen, 1979; Ogundero, 1981). It is similar in most characters to *R. pusillus*, including thermophily. *R. miehei* produces sporangia up to 50(–60) μm diam with spiny walls, with columellae rarely larger than 30 μm diam.

Reference. Schipper (1978b).

6.5 Genus *Rhizopus* Ehrenb.

The genus *Rhizopus* needs little introduction to food microbiologists, because *R. stolonifer* is among the most obvious moulds encountered on Petri dishes inoculated with food materials. Coarse, rampant growth and rapidly maturing spores make this species a source of endless contamination to the unwary laboratory worker. Other *Rhizopus* species are less common in foods and cause less nuisance.

Rhizopus species, especially *R. oligosporus*, have been used for millennia to modify basic foods in the Orient. The subject of fermented foods is outside the scope of this book: for further information see, for example, Hesseltine (1965), Gray (1970), Beuchat (1987) or Rombouts and Nout (1995).

Rhizopus is distinguished from other genera in the order *Mucorales* by the formation of rhizoids (short root-like appendages), which are conspicuous at the base of the sporangiophores; by columellae which often collapse into umbrella shapes in age; and by dry sporangiospores with striate walls. The most recent complete taxonomic treatment is by Zheng et al. (2007a) who accepted 10 species. Molecular phylogenetic analyses have recently been carried out by Abe et al. (2006) and Liou et al. (2007), with good agreement with the morphological conclusions by Zheng et al. (2007a). Five species are considered here and keyed below: *R. microsporus*, *R. oligosporus, R. oryzae*, *R. sexualis* and *R. stolonifer*. Because of its ubiquitous nature, *R. stolonifer* is described first.

Key to *Rhizopus* species included here

1	Abundant zygospores present on CYA and MEA	*R. sexualis*
	Zygospores not produced	2
2 (1)	Rapid growth at 37°C; spores commonly less than 8 μm diam	3
	Growth at 37°C weak or absent; spores commonly 8–20 μm diam	*R. stolonifer*
3 (2)	Columellae up to 100 μm diam; spores 5–8 μm long	*R. oryzae*
	Columellae not more than 80 μm diam; spores less than 5 μm long	4
4 (3)	Columellae up to 75 μm diam; spores less than 4 μm long, with spinose walls; source food fermentations and fermented foods	*R. oligosporus*
	Columellae less than 40 μm diam; spores 3–5 μm long, with finely striate walls; source other types of foods	*R. microsporus*

Rhizopus stolonifer (Ehrenb.: Fr.) Lindner — Fig. 6.13

Rhizopus nigricans Ehrenb.

On CYA, colonies covering the whole Petri dish, sometimes low and sparse, with black sporangia only at the margins, sometimes filling the whole Petri dish and then similar to those on MEA; reverse pale. On MEA, colonies filling the whole Petri dish with floccose white mycelium bearing conspicuous sporangia, at first white, then with maturation rapidly becoming black, either distributed uniformly or concentrated at dish peripheries; reverse uncoloured. On G25N, colonies similar to that on MEA but less dense. At 5°C, spores barely germinating. At 37°C, usually no growth, sometimes colonies up to 15 mm diam, very thin and sparse.

Sporangiophores borne in groups of three to five from clusters of rhizoids, stipes unbranched, robust and up to 3 mm long, with brown walls; sporangia 100–350 μm diam, usually spherical; columellae roughly spherical, up to 200 μm diam, in age often collapsing downwards and outwards to produce umbrella shapes; sporangiospores commonly 8–20 μm in long axis, pale brownish, with striate walls.

Distinctive features. *Rhizopus stolonifer* is distinguished by its habit, with coarse hyphae and rampant growth at 25°C, and by sporangia which are white when first formed but which change to black with maturity. Sporangiospores are large, with striate walls. In contrast to that at 25°C, growth at 5 and 37°C is weak or absent.

Taxonomy. Twenty nine isolates identified as *Rhizopus stolonifer* or possibly related species were phylogenetically analysed by RAPD analysis (Vágvölgyi et al., 2004) and ca. 30 different isolates by Liou et al. (2007) using DNA sequences from the D1/D2 of rDNA. These analyses divided *R. stolonifer* and some varieties into two or four clades. In each case, the isolates commonly seen in foods remained in *R. stolonifer*.

Physiology. *Rhizopus stolonifer* has been reported to grow from 4.5 or 5°C up to 30°C (Schipper, 1984) or 35–37°C (Pierson, 1966, and our observations), with an optimum near 25°C. However, Zheng et al. (2007a), who tested 36 isolates of *R. stolonifer*, reported maximum growth temperatures of only 30–32°C. This species germinated down to 0.84 a_w at 25°C, but growth was very slow, and was absent at 30°C (Hocking and Miscamble, 1995). It produced the fastest mycelial growth we have recorded: at 25°C and an a_w in excess of 0.99, the radial growth rate reached 2 mm/h (i.e. nearly 5 cm/day). Like some other species in the Mucorales, *R. stolonifer* can grow under anaerobic conditions (Stotzky and Goos, 1965); however, an atmosphere of 80% O_2 + 20% CO_2 halved its growth rate at 25°C after 40 h (Hoogerwerf et al., 2002).

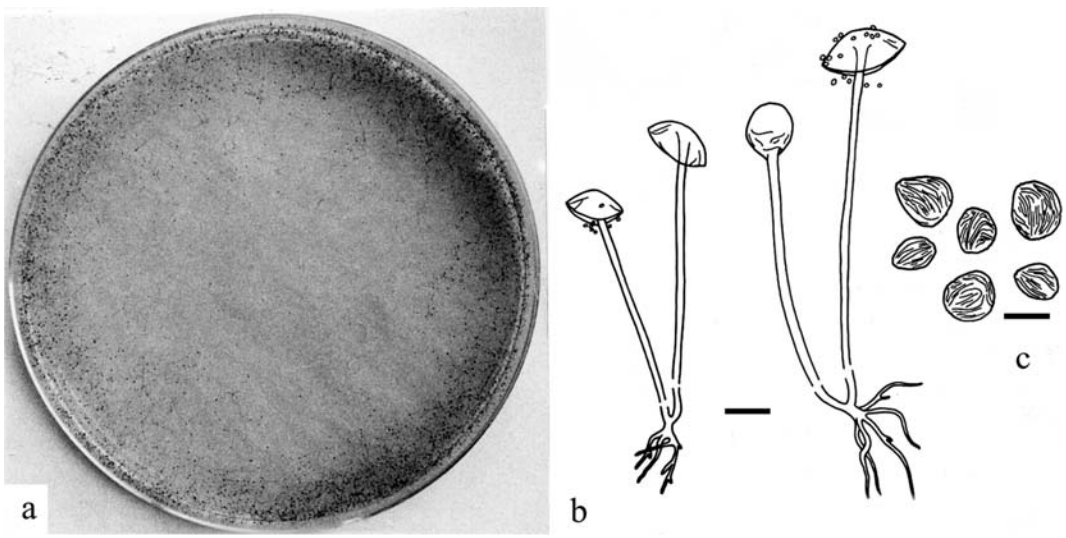


Fig. 6.13 *Rhizopus stolonifer* **(a)** colonies on CYA, 7 days, 25°C; **(b)** sporangiophores with rhizoids and collapsed columellae, bar = 25 μm; **(c)** sporangiospores, bar = 10 μm

Mycotoxins. *Rhizopus stolonifer* (a single isolate tested) was moderately toxic to ducklings (Rabie et al., 1985). The possible toxins involved have not been elucidated.

Ecology. *Rhizopus stolonifer* is by far the most commonly occurring species of the order Mucorales in foods. It causes destructive rots of a wide range of fruits (Snowdon, 1990, 1991). The most important are fresh berries, especially strawberries (Beneke et al., 1954; Dennis et al., 1979; Harris and Dennis, 1980), and stone fruits of all kinds (Snowdon, 1990; Singh and Shukla, 2005). Tomatoes, eggplant and capsicums are also seriously affected in some countries (Kassim, 1987; Snowdon, 1991). These diseases occur postharvest and are commonly known as "transit rot". Whole cases of fruit can decay in just a few days. Spoilage is usually initiated through damage and then spreads by contact. Control usually relies on preharvest or postharvest sprays or dips of fungicides. Thiabendazole (Benlate) or 2,6-dichloro-4-nitroaniline (Dichloran or Botran) provides reasonable control (Snowdon, 1990, 1991 and see Pitt and Hocking, 1997). Other treatments suggested include hot water dips and irradiation (Nicoue et al., 2004), ultraviolet light (Pan et al., 2004) and chlorine dipping (Sabaa-Srur et al., 1993). Control of postharvest rot has been satisfactorily achieved with applications of yeasts such as *Metschnikowia fructicola* (Karabulut et al., 2004) and *Cryptococcus laurentii* (Zhang et al., 2007a). Transgenic tomatoes with reduced polygalacturonase activity appear to have a higher resistance to transit rot (Sanders et al., 1992).

Pectic enzymes of *Rhizopus stolonifer* survive the canning process normally applied to fruit. If even a small proportion of infected fruit is processed, these enzymes can cause softening and spoilage of canned apricots (Harper et al., 1972).

Many types of vegetables are also susceptible to spoilage by *Rhizopus stolonifer* (Snowdon, 1991). Beans and peas, carrots, sweet potatoes, yams and cassava (see Pitt and Hocking, 1997) are all highly susceptible.

Isolation of this species has been reported from many other food sources, including wheat and barley (Lugauskas et al., 2006), soybeans (Tariq et al., 2005), peanuts (Gachomo et al., 2004), hazelnuts (Senser, 1979), pecans and bran (see Pitt and Hocking, 1997), spices (Mandeel, 2005), cheese (Hayaloglu et al., 2007) and meat products (see Pitt and Hocking, 1997). *R. stolonifer* is a cosmopolitan fungus, particularly in tropical and subtropical regions, in almost all kinds of fresh, moist or partially dried foods.

References. Schipper (1984); Zheng et al. (2007a).

Rhizopus microsporus Tiegh. Fig. 6.14

On CYA and MEA, colonies covering or filling the whole Petri dish, of fine, pale grey mycelium, becoming dark grey as sporangia mature; reverse pale to dull yellow. On G25N, germination to microcolony formation. No growth at 5°C. At 37°C, colonies filling the whole Petri dish, similar to those on CYA or MEA at 25°C.

Sporangiophores borne singly or in groups of two to three from clusters of poorly formed rhizoids, stipes unbranched, relatively short, commonly 200–500 μm long, with brown walls; sporangia black, 100–200 μm diam, usually spherical; columellae 20–35 μm diam, spherical, some remaining so in age, others collapsing downwards, to form umbrella shapes; sporangiospores ellipsoidal, 3–5 μm long, with thin, striate walls.

Distinctive features. *Rhizopus microsporus* differs from *R. stolonifer* by shorter stipes and smaller columellae, by the absence of white immature sporangia and by strong growth at 37°C. It is distinguished from *R. oryzae* by production of smaller columellae and smaller spores. *R. oligosporus* is very similar to *R. microsporus*, but produces larger columellae and smooth to spinose spores.

Taxonomy. Sporangia up to 80 μm and spores up to 6.5(–7.5) μm long were described by Schipper and Stalpers (1984). This species was divided into six varieties by Zheng et al. (2007a), one of which, *Rhizopus oligosporus*, is considered here to be a separate species. Other varieties are uncommon or unknown from foods.

Physiology. *Rhizopus microsporus* grows up to 46–48°C (Schipper and Stalpers, 1984; Zheng et al., 2007a). The lower limit for growth is 0.90 a_w (Hocking and Miscamble, 1995).

Mycotoxins. Wilson et al. (1984) were the first to report the production of rhizonin A, a nonspecific hepatotoxin, by *Rhizopus microsporus*. However, a recent study demonstrated that the toxin was not

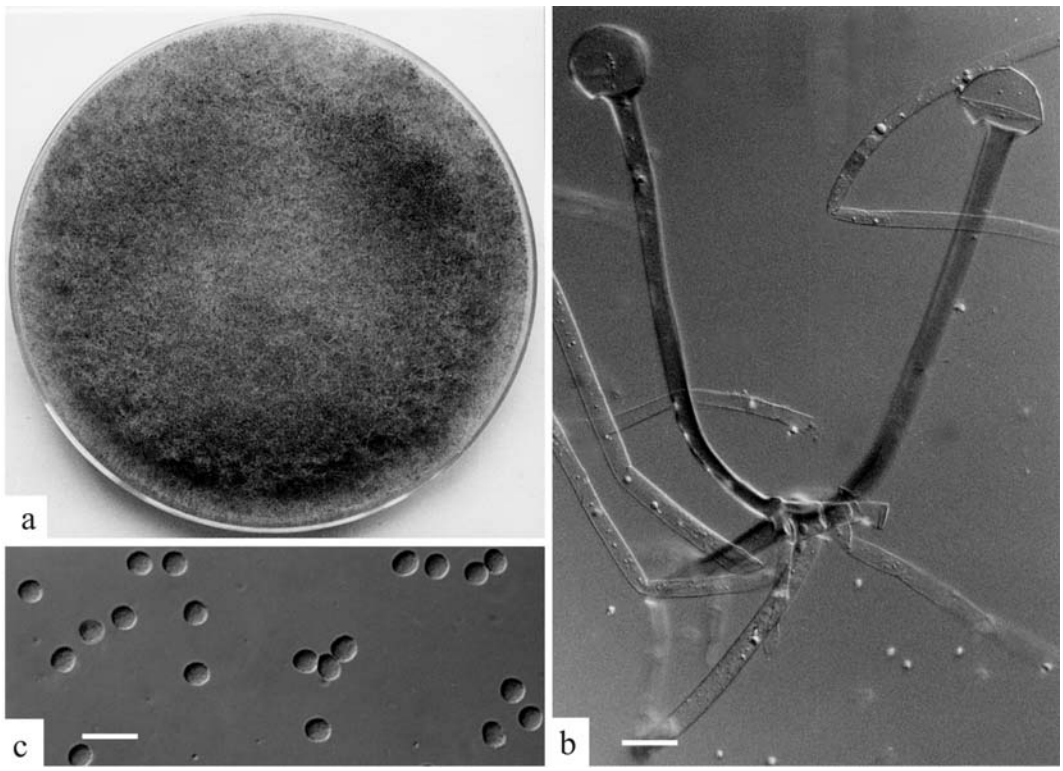


Fig. 6.14 *Rhizopus microsporus* **(a)** colonies on CYA, 7 days, 25°C; **(b)** sporangiophores with rhizoids, bar = 25 μm; **(c)** sporangiospores, bar = 10 μm

actually synthesised by the fungus, but by a bacterial symbiont belonging to the genus *Burkholderia* (Partida-Martinez et al., 2007). This finding is of interest as species of *Rhizopus* are frequently used in the food industry for the preparation of fermented foods such as tempe and sufu.

Ecology. *Rhizopus microsporus* causes human zygomycoses, particularly cutaneous and gastrointestinal infections (de Hoog et al., 2000). This species appears to be uncommon in foods from temperature zones; however, we isolated it quite frequently from tropical commodities. It was found in 18% of maize samples from the Philippines and from 3% of all kernels examined. It was also present, at lower numbers, in peanuts, paddy rice, soybeans and black pepper from the Philippines, and maize, peanuts, paddy and milled rice, soybeans, mung beans and coriander from Indonesia (Pitt, 1998a and our unpublished data).

Reference. Schipper and Stalpers (1984); Zheng et al, (2007a).

Rhizopus oligosporus Saito Fig. 6.15

Colonies on CYA 50–60 mm diam, not usually covering the whole Petri dish, low, plane and sparse, with ill-defined margins, pale brown, sporangia sparsely produced, brown, sometimes enclosed in clear to brown droplets; reverse uncoloured to pale brown. Colonies on MEA covering the whole Petri dish, sometimes reaching, and adhering to, the lid, coloured dark grey to black, sporangia abundant, black; reverse uncoloured. On G25N, sometimes germination. At 5°C, no germination. At 37°C, colonies covering the whole Petri dish in a low, very sparse, often cobwebby growth; reverse uncoloured.

Sporangiophores best observed on MEA, stipes 150–400 μm long, usually with well developed short rhizoids at or near the base, terminating in dark sporangia, 80–120 μm diam, at 7 days usually broken with spores dispersed; columellae persistent, often split, but remaining spheroidal to pyriform,

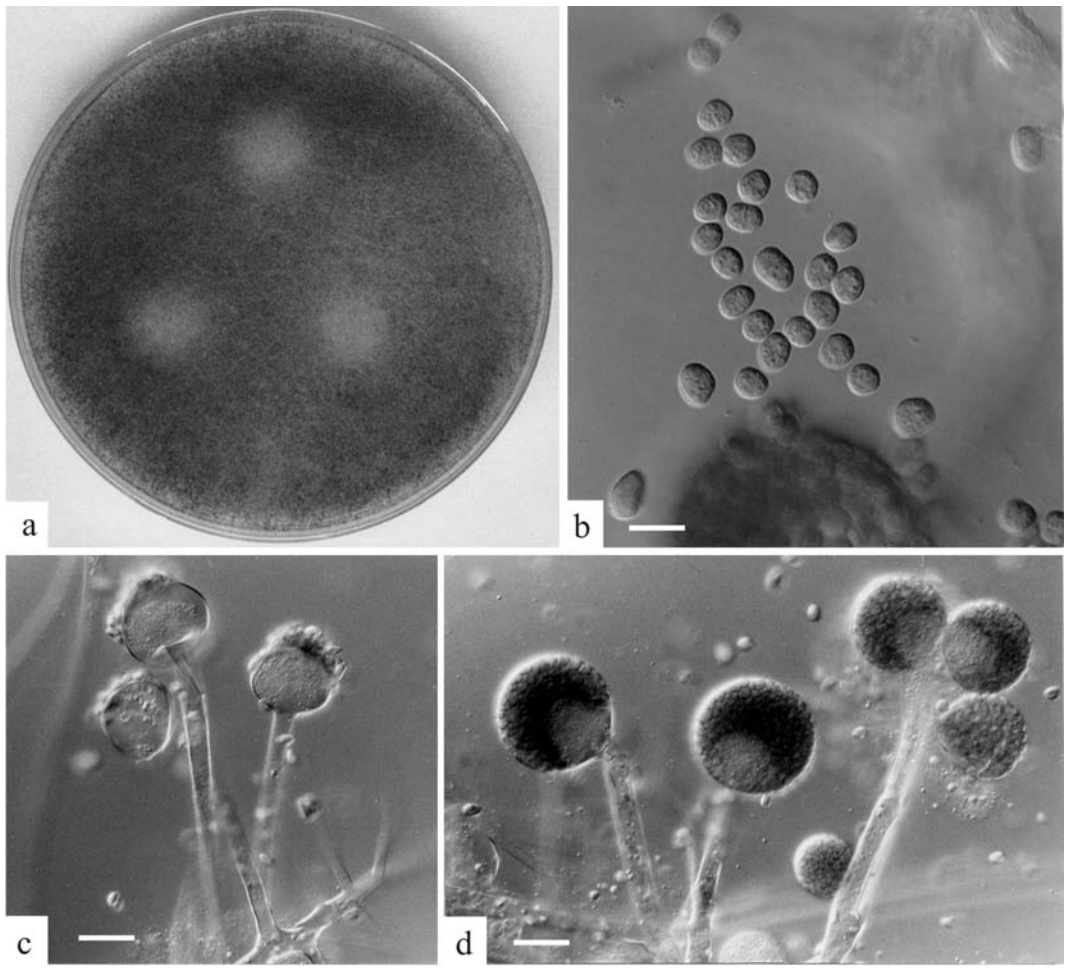


Fig. 6.15 *Rhizopus oligosporus* **(a)** colonies on MEA, 7 days, 25°C; **(b)** sporangiospores, bar = 10 μm; **(c)** developing sporangia, bar = 25 μm; **(d)** sporangia, bar = 25 μm

25–75 μm diam or long; sporangiospores spherical to subspheroidal, 3.0–3.5 μm diam, with thin, finely spinose walls.

Distinctive features. In common with *Rhizopus microsporus, R. oligosporus* differs from *R. stolonifer* by shorter stipes and smaller columellae, by the absence of white immature sporangia and by strong growth at 37°C. It is distinguished from *R. oryzae* by production of smaller columellae and smaller spores. *R. oligosporus* produces larger columellae than isolates of *R. microsporus* and forms smooth to spinose spores.

Taxonomy. Schipper and Stalpers (1984) reduced *Rhizopus oligosporus* to the status of a variety of *R. microsporus*, and this was maintained by Zheng et al. (2007a). However, this taxon deserves species status as a domesticated species used in, and probably confined to, food fermentations. Its derivation from *R. microsporus* seems likely, as the two taxa share many properties. Using a detailed light and scanning electron microscopy study, Jennessen et al. (2008) have illustrated criteria for separating *R. oligosporus* from related species. Sporangiospores of *R. oligosporus* are often larger and less regularly shaped than those of other species. This is likely to be the result of domestication (Jennessen et al., 2008).

Physiology. Maximum growth temperature of 13 isolates was 45–49°C (Zheng et al., 2007a).

Mycotoxins. No mycotoxins are known to be produced. Weak toxicity to ducklings was reported by Rabie et al. (1985). However, only a single isolate was tested.

Ecology. *Rhizopus oligosporus* is used in food fermentations, the most notable product being tempeh,

which is produced in Southeast Asian countries, especially in Indonesia. Cooked, dehulled soybeans are soaked for 2–3 days, then inoculated from a previous batch or, more commonly now, with a starter culture, usually *R. oligosporus*. Fermentation takes place under ambient (tropical) conditions, preferably with air temperatures of 25–28°C for 36–48 h (Hesseltine, 1965, 1991; Ko and Hesseltine, 1979; Beuchat, 1987; Nout and Rombouts, 1990). Feng et al. (2007) reported the production of volatiles by *R. oligosporus* during the fermentation of soybean and barley tempeh. *R. oligosporus* has also been used to increase the functional properties of foods and reduce the levels of allergenic proteins in buckwheat (Handoyo et al., 2006). As noted above, *R. oligosporus* appears to be a domesticated fungus. It has rarely, if ever, been reliably reported from sources other than fermented foods.

References. Hesseltine (1965); Schipper and Stalpers (1984); Zheng et al. (2007a).

Rhizopus oryzae Went & Prins. Geerl. Fig. 6.16

Rhizopus arrhizus A. Fisch.

On CYA and MEA, colonies filling the whole Petri dish with fine greyish mycelium and small blackish grey sporangia; reverse pale. On G25N, colonies 30–60 mm diam, or occasionally filling the whole Petri dish, relatively low and sparse. No growth at 5°C. Colonies at 37°C covering and sometimes filling the Petri dish, similar to that on CYA or more sparse.

Sporangiophores borne in clusters of one to three from rhizoids, with stipes up to 1500 μm long, usually unbranched; sporangia spherical, up to 150 μm diam, white at first then becoming greyish black at maturity; columellae usually spherical, up to 100 μm diam, pale brown, in age often collapsing downwards to form umbrella shapes; sporangiospores brown, of variable shape, ellipsoidal to broadly fusiform or irregularly angular, commonly 5–8 μm long, with striate walls.

Distinctive features. This species is distinguished from *Rhizopus stolonifer* by its smaller sporangia and spores and by much faster growth at 37°C. It differs from *R. microsporus* and *R. oligosporus* by longer stipes, larger collumellae and larger spores.

Taxonomy. Schipper and Stalpers (1984) concluded that *Rhizopus oryzae* was the correct name for this species, considering *R. arrhizus* to be a doubtful name, placing this latter species in synonymy with *R. oryzae*. Their treatment is followed here, as *R. oryzae* is by far the more common name in the literature. In the latest taxonomy, Zheng et al.

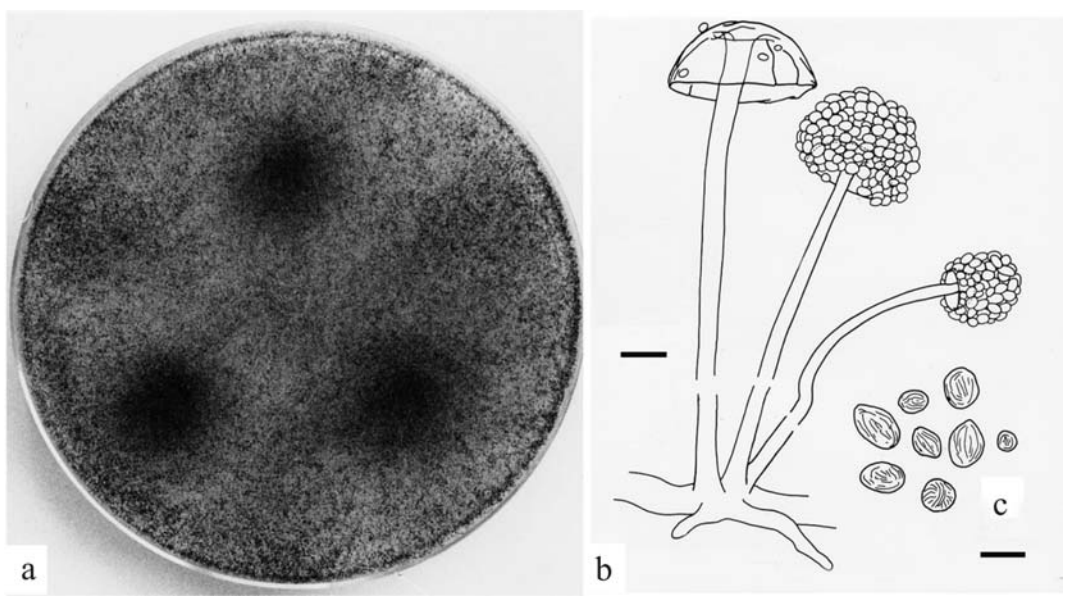


Fig. 6.16 *Rhizopus oryzae* **(a)** colonies on CYA, 7 days, 25°C; **(b)** sporangia and columellae, bar = 25 μm; **(c)** sporangiospores, bar = 10 μm

(2007a) have taken up *R. arrhizus* based on neotypification by Ellis (1985), so that usage of this name may increase in the future. However, it is not clear if Ellis' neotypification is valid.

Physiology. *Rhizopus oryzae* has been reported to grow from 7 to 42°C or 45°C, with the optimum near 37°C (Panasenko, 1967; Gleason, 1971; Schipper, 1984; Zheng et al., 2007a). Its minimum a_w for growth is 0.88 (Hocking and Miscamble, 1995). Growth under optimal conditions is exceptionally fast (up to 1.6 mm/h), although slower than that of *R. stolonifer* (Hocking and Miscamble, 1995). *R. oryzae* produces lipase D, an enzyme which hydrolyses triglycerides of fatty acids and acts as a catalyst for the esterification of fats and oils to improve their physical properties. The toxicity of lipase D produced by *R. oryzae* was examined in rats for 13 weeks and no ill effects were detected (Flood and Kondo, 2003).

Mycotoxins. Maize meal on which isolates of *Rhizopus oryzae* had been grown was toxic to ducklings and rats, causing growth depression (Rabie et al., 1985). The toxin responsible has not been elucidated.

Ecology. *Rhizopus oryzae* is an important agent of human zygomycoses, causing mainly rhinocerebral infections (de Hoog et al., 2000). It has been isolated quite frequently from foods, often as *R. arrhizus*. It appears to be associated with many of the rots in fruits and vegetables usually attributed to *R. stolonifer*

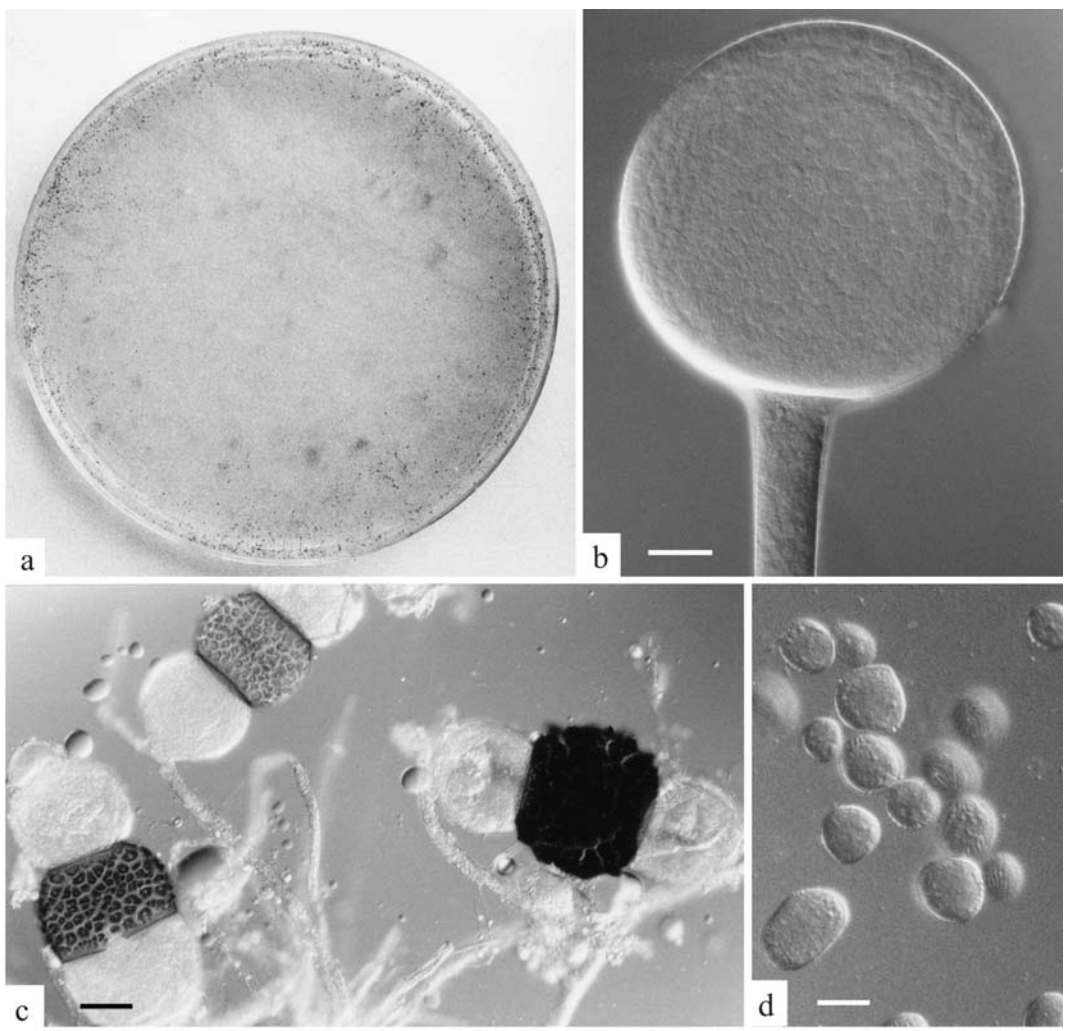


Fig. 6.17 *Rhizopus sexualis* **(a)** colonies on CYA, 7 days, 25°C; **(b)** sporangium, bar = 25 µm; **(c)** zygospores, bar = 50 µm; **(d)** sporangiospores, bar = 10 µm

(Snowdon, 1990, 1991). From our observations, it is more common in tropical commodities than is *R. stolonifer*. It was especially common in peanuts, being isolated from 21% of all peanut kernels examined from Indonesia, 15% of those from Thailand and 14% of those from the Philippines. It was growing in 25% of all copra pieces from Thailand and 9% of kemiri nuts from Indonesia. It was present in lower numbers in maize, various kinds of beans, sorghum and cowpeas (Pitt et al., 1993, 1998a). Other reports include maize (Askun, 2006), peanuts, pecans, hazelnuts, pistachios, wheat, barley, potatoes, sapodillas and various other tropical foods (see Pitt and Hocking, 1997).

References. Schipper (1984); de Hoog et al. (2000); Zheng et al. (2007a).

Rhizopus sexualis (G. Sm.) Callen Fig. 6.17

On CYA, colonies covering the whole Petri dish, very low and sparse, of white to greyish mycelium; sporangia in limited numbers, pale; black zygospores conspicuous; reverse uncoloured. On MEA, colonies similar to that on CYA, though more dense. On G25N, colonies at least 40 mm diam, sometimes covering the whole Petri dish, often as vigorous as on CYA or MEA, and of similar appearance, but zygospores usually absent. No growth at 5 or 37°C.

Sporangiophores borne from rhizoids, one to three per cluster, stipes up to 1500 μm long, usually unbranched; sporangia spherical, 50–150 μm diam, white, becoming grey; columellae spherical to ellipsoidal, up to 100 μm diam or long, in age collapsing to umbrella shapes; sporangiospores subspheroidal, ellipsoidal or angular, 5–12(–25) μm long, with thick, grey, striate walls. Zygospores commonly occurring, 80–180 μm diam, black, with adjacent cells (suspensors) approximately spherical, but at maturity appearing hemispherical.

Distinctive features. *Rhizopus sexualis* is distinguished from other foodborne species of *Rhizopus* and *Mucor* by the profuse production of conspicuous black zygospores.

Physiology. Schipper (1984) reported that this species only grows below 30°C; however, Zheng et al. (2007a) reported 26–27°C as the maximum growth temperature. Relatively fast growth on G25N perhaps indicates a relatively low minimum a_w for growth.

Mycotoxins. Mycotoxin production has not been reported.

Ecology. *Rhizopus sexualis* causes a soft rot of strawberries (Harris and Dennis, 1980) and occasionally of other fruit.

References. Schipper (1984); Zheng et al. (2007a).

6.6 Genus *Syncephalastrum* J. Schröt.

Syncephalastrum produces sporangiospores in cylindrical sacs (merosporangia) attached around spherical vesicles, providing a superficial resemblance to *Aspergillus niger* at low magnifications. In other respects, this genus resembles *Mucor*. There is one species, *S. racemosum*.

Syncephalastrum racemosum J. Schröt. Fig. 6.18

On CYA and MEA, colonies covering the whole Petri dish, sparse to grey; reverse pale or yellowish brown. On G25N, colonies 20–30 mm diam, dense to floccose, grey; reverse pale. No growth at 5°C. Colonies at 37°C filling the whole Petri dish, dark grey; reverse yellow brown.

Sporangiophores borne from aerial hyphae, stipes long and branched or produced as short side branches from fertile hyphae; sporangial heads 30–80 μm diam, with sporangiospores formed in a single line within cylindrical sacs (merosporangia) borne on spicules around the vesicle; vesicles spherical or nearly so, 10–50 μm diam, brown, with walls smooth except at merosporangium attachment points, usually collapsing irregularly; sporangiospores adhering in chains of up to 10, becoming brown, irregular in size and shape, spherical to cylindrical, 3.0–5.0(–10) μm diam or long, smooth walled.

Distinctive features. See genus description.

Physiology. Good growth has been reported between 17 and 40°C (Domsch et al., 1980): no doubt growth limits are rather wider than these figures. This is one of the Mucorales most tolerant to low a_w: the minimum for growth is 0.84 a_w (Hocking and Miscamble, 1995).

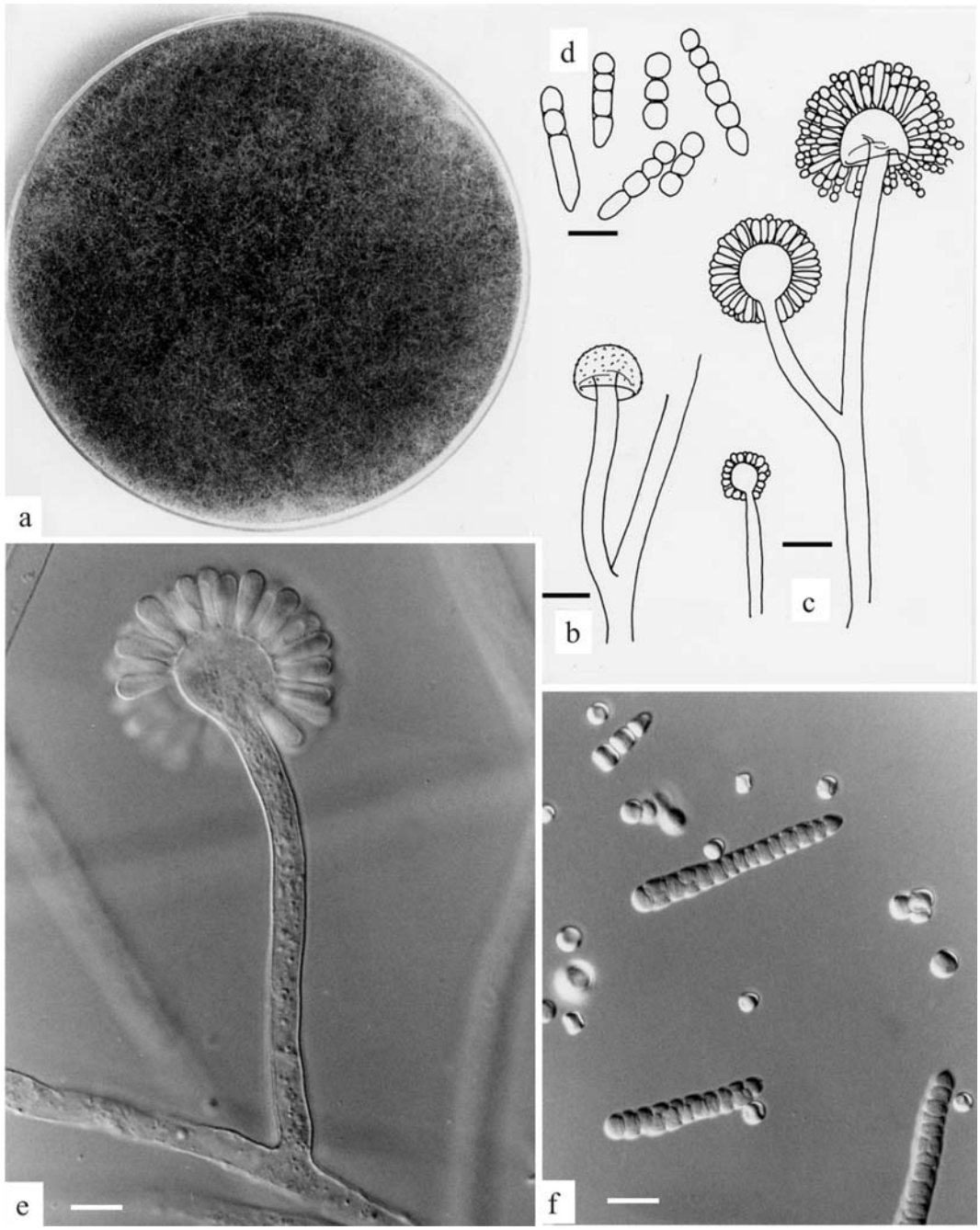


Fig. 6.18 *Syncephalastrum racemosum* **(a)** colonies on CYA, 7 days, 25°C; **(b)** columellae, bar = 25 μm; **(c)** stages in merosporangium formation, bar = 25 μm; **(d)** sporangiospores, bar = 10 μm; **(e)** developing merosporangium, bar = 10 μm; **(f)** sporangiospores, bar = 10 μm

Mycotoxins. It was reported by Makun et al. (2007b) that two of three isolates of *Syncephalastrum* were mildly toxic to mice (one of three injected with extracts died). No other details are known.

Ecology. Reported isolations from foods have not been numerous, but this species is of widespread occurrence nevertheless. Principal reported sources have been from nuts (see Pitt and Hocking, 1997),

young coconuts (Waje et al., 2005), cereals, spices (Elshafie et al., 2002), fermented foods, processed meats and capsicums (see Pitt and Hocking, 1997). We isolated this species from 10% of all coriander seeds examined from Indonesia and 4% of all kemiri nuts. It was present in 2% of all sorghum grains from Thailand; 1% of Thai mung beans; Philippine maize, soybeans and black pepper; and Indonesian peanuts. It was present at lower levels in soybeans, black and red beans, cassava and copra from Thailand and mung beans, maize and milled rice from Indonesia (Pitt et al., 1994, 1998a). Recent reports include cashews (Adebajo and Diyaolu, 2003), millet (Makun et al., 2007) and chickpeas (Dawar et al., 2007b).

References. Benjamin (1959); O'Donnell (1979).

6.7 Genus *Thamnidium* Link

As well as large columellate sporangia, *Thamnidium* produces sporangioles, small sporangia without columellae, borne on highly branched structures. Sporangioles in *Thamnidium* contain sporangiospores, similar to those borne in sporangia. There is a single species, *T. elegans*.

Thamnidium elegans Link Fig. 6.19

On CYA, colonies usually covering the whole Petri dish, sparse but quite deep due to the production of long sporangiophores, coloured grey to pale brown; reverse pale. On MEA, colonies 30–50 mm diam, similar to those on CYA but with longer, larger sporangiophores. On G25N, colonies variable, from germination only to colonies 10 mm diam, low and dense. At 5°C, colonies 15–35 mm diam, of low mycelium. No growth at 37°C.

Sporangiophores very large, borne from surface hyphae, stipes bearing sporangia or sporangioles or both; sporangia brown, 150–250 μm diam; columellae roughly spherical, 40–80 μm diam, or sometimes larger, collapsing or tearing irregularly; sporangioles 12–25 μm diam, rough walled; sporangiospores narrowly to broadly ellipsoidal, 6–15 μm long, with thin, smooth walls.

Distinctive features. See genus description.

Physiology. *Thamnidium. elegans* is psychrophilic, growing down to at least 1°C (Brooks and Hansford, 1923). The maximum temperature for growth is about 27°C (Gleason, 1971).

Mycotoxins. Mycotoxin production has not been reported.

Ecology. *Thamnidium elegans* has traditionally been associated with cool stored meat, on which it occurs as long "whiskers". Improved temperature control of meat storage has virtually eliminated this problem in developed countries. However, where meat is traditionally hung for curing, festoons of *T. elegans* can still sometimes be seen. Whether this constitutes "spoilage" depends on definitions. Spoilage of Taleggio

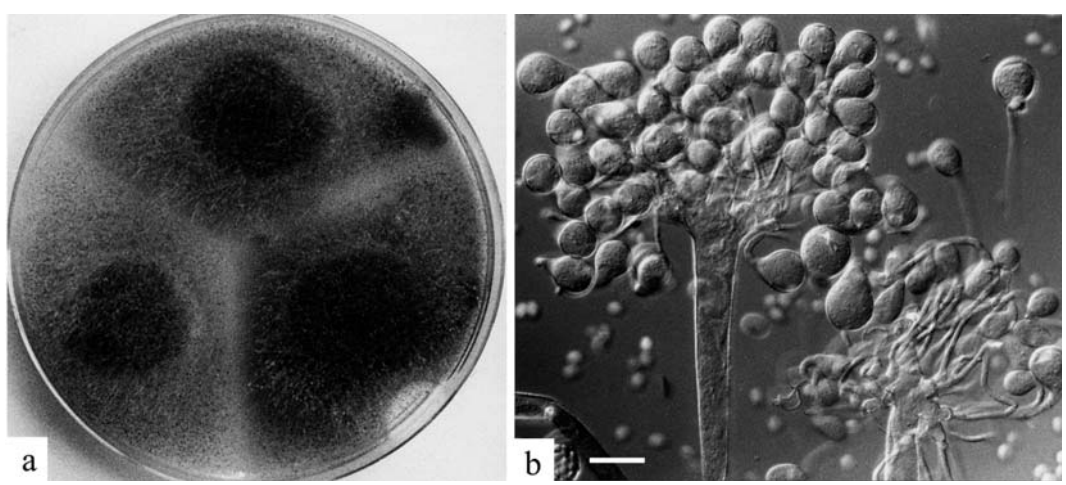


Fig. 6.19 *Thamnidium elegans* **(a)** colonies on CYA, 7 days, 25°C; **(b)** sporangiophore, developing sporangioles and sporangiospores, bar = 25 μm

cheese (a traditional, washed rind, soft, red pigmented cow's milk cheese from Italy) has been reported (Dragoni et al., 1997). We isolated *T. elegans* from coriander from Indonesia, where it was found in 1% of seeds examined (Pitt et al., 1998a).

References. Hesseltine and Anderson (1956); O'Donnell (1979).

Chapter 7
Penicillium and Related Genera

Grouped in this chapter are genera which produce conidia in a structure termed a **penicillus** (Latin, little brush). A penicillus consists essentially of a well-defined cluster of phialides or similar cells bearing small, single-celled, dry conidia in chains. The phialides are either attached to a stipe directly or through one or more stages of branching. Branches are of generally similar diameter to stipes (Fig. 7.1). This definition covers four genera here: *Penicillium*, *Geosmithia*, *Paecilomyces* and *Scopulariopsis*. Genera with greatly enlarged stipe apices, e.g. *Aspergillus,* are excluded, as are genera in which phialides are not borne in clusters or which produce wet, adherent conidia, e.g. *Fusarium, Trichoderma*.

The degree of relatedness of these four genera treated here is not high, but they are conveniently considered together. A variety of characteristics enable them to be distinguished. They are keyed out here on the basis of differences in phialide shape and conidial shape and colour.

Key to genera producing penicilli

1	Mature conidia truncate or flattened at the base; colonies white, buff or brown	*Scopulariopsis*
	Conidia not truncate at the base, symmetrical from end to end; colony colour various	2
2 (1)	Mature conidia spherical to ellipsoidal, in shades of blue, green and/or grey	*Penicillium*
	Mature conidia ellipsoidal to fusiform or cylindroidal, not blue, green or grey	3
3 (2)	Phialides cylindrical, rough walled, necks truncate, mature conidia cylindroidal	*Geosmithia*
	Phialides gradually tapering, smooth walled, often with long necks angled away from the phialide axis, conidia ellipsoidal to fusiform	*Paecilomyces*

Teleomorphs. The genera in this chapter are all associated with Ascomycete teleomorphs. Treated here are *Byssochlamys*, *Eupenicillium* and *Talaromyces*. *Microascus*, the teleomorph of *Scopulariopsis*, does not occur in foods.

Byssochlamys is the teleomorph of certain species of *Paecilomyces*, while *Eupenicillium* is associated with *Penicillium* species. *Talaromyces* teleomorphs are produced by some species of *Geosmithia*, *Paecilomyces* and *Penicillium*, but those in *Paecilomyces* are not found associated with foods. It should be emphasised that production of teleomorphs by any of these three anamorph genera is the exception rather than the rule.

Species producing teleomorphs are discussed here under the teleomorph name, as the teleomorph is usually the dominant form or is more significant in foods because of properties such as heat resistance.

Key to teleomorph genera producing anamorphs with penicilli

1	Asci produced within a discrete macroscopic structure, a cleistothecium or gymnothecium	2
	Asci produced openly, solitarily or in groups on hyphae	*Byssochlamys*
2 (1)	Asci produced within a cleistothecium, a body with a solid cellular wall	*Eupenicillium*
	Asci produced within a gymnothecium, with walls of fine, closely woven hyphae	*Talaromyces*

J.I. Pitt, A.D. Hocking, *Fungi and Food Spoilage*, DOI 10.1007/978-0-387-92207-2_7,
© Springer Science+Business Media, LLC 2009

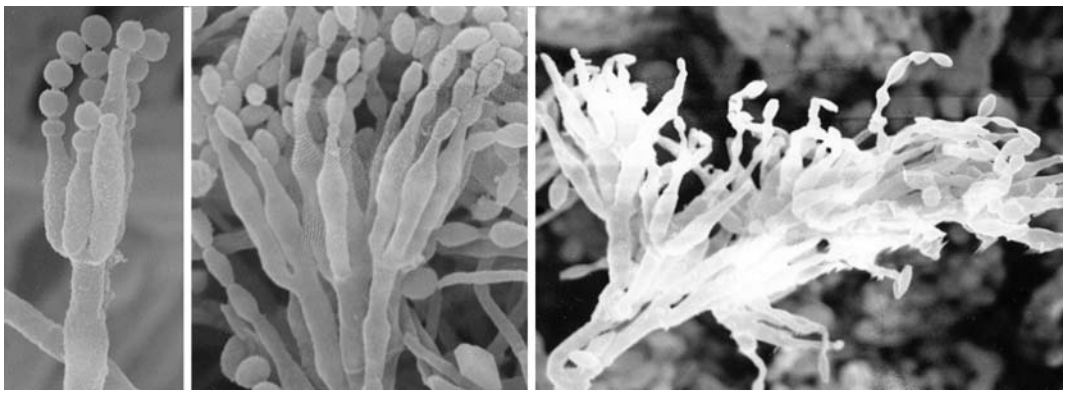


Fig. 7.1 Scanning electron micrographs of various types of penicilli, showing the relationship between the diameter of the stipe and the other elements of the penicillus

In contrast with the very large genus *Penicillium*, the other genera considered in this chapter include few species of significance in foods. The smaller genera are considered below in alphabetical order, followed by *Penicillium*.

7.1 Genus *Byssochlamys* Westling

Byssochlamys has the distinction of being almost uniquely associated with food spoilage, and in particular with the spoilage of heat processed acid foods. *Byssochlamys* species have been reported only rarely from other sources.

Byssochlamys is an Ascomycete genus characterised by the absence of cleistothecia, gymnothecia or other bodies enveloping asci during development. Asci in *Byssochlamys* are borne in open clusters, in association with, but not surrounded by, unstructured wefts of fine, white hyphae.

Techniques for isolating *Byssochlamys* rely on a heat treatment to inactivate other fungal spores which are less heat resistant. These techniques have been described in Chapter 4.

Three species of *Byssochlamys* are significant in food spoilage: *B. fulva, B. nivea* and *B. spectabilis*. *B. nivea* is readily distinguished from the others by its white colonies. It also forms ascospores readily in the laboratory, when incubated for 10 days at 25 or 30°C (but not 37°C). *B. fulva* and *B. spectabilis* form brown colonies of quite similar appearance. *B. fulva* usually forms ascospores in the laboratory when grown at 30°C (but not at 25 or 37°C). *B. spectabilis* does not usually make ascospores in laboratory culture, as it is heterothallic, i.e. usually two isolates must be mated to form the teleomorph. For this reason, *B. spectabilis* is discussed under its anamorph *Paecilomyces variotii*.

How do we know we have a *Byssochlamys* if it does not produce ascospores? Presumptive evidence of a *Byssochlamys* can be made from plates at 25 or 37°C if the isolate has come from heat processed foods or raw materials. If a brown *Paecilomyces* is isolated after a sample has been heat treated at 75°C, it is probably *B. spectabilis,* which is included under *Paecilomyces variotii* later in this chapter. This aspect is discussed further under "heat resistant fungi" in Chapter 12.

Key to *Byssochlamys* species included here

1	Colonies on CYA and MEA persistently white to cream	*B. nivea*
	Colonies on CYA and MEA predominantly buff to brown	2
2.	White asci and ascospores produced on MEA at 30°C after 7–10 days; conidia mostly cylindroidal	*B. fulva*
	Colonies remaining buff to brown, no obvious teleomorph; conidia mostly ellipsoidal	*Byssochlamys spectabilis* See *Paecilomyces variotii*

Byssochlamys fulva Olliver & G. Sm. Fig. 7.2

Anamorph: *Paecilomyces fulvus* Stolk & Samson

Colonies on CYA and MEA at least 60 mm diam, often covering the whole Petri dish, relatively sparse, low or somewhat floccose; conidial production heavy, uniformly coloured olive brown or paler; reverse in similar colours or pale. Colonies on G25N 5–10 mm diam, texture variable, low and sparse to deep and floccose, coloured white or as on CYA. No growth at 5°C. At 30 and 37°C, colonies usually covering the whole Petri dish, low and sparse, coloured as on CYA or brighter; reverse in similar colours.

Teleomorph single asci borne from, but not enveloped by, wefts of contorted white hyphae, best developed at 30°C on MEA, maturing in 7–12 days, occasionally formed at 25°C in fresh isolates but maturing slowly if at all; asci spherical to subspheroidal, 9–12 μm diam; ascospores ellipsoidal, 5–7 μm long, smooth walled. Anamorph reproductive structures penicilli, best observed at 25°C, borne from surface hyphae or long, trailing, aerial hyphae; stipes 10–30 μm long; phialides of nonuniform appearance, flask shaped or narrowing gradually to the apices, 12–20 μm long; conidia mostly cylindroidal or doliiform (barrel shaped), usually narrow and 7–10 μm long, but sometimes longer, wider or ellipsoidal from individual phialides, smooth walled.

Distinctive features. In culture at 30°C, *Byssochlamys fulva* is distinguished by rapidly growing brown colonies with areas of fine white hyphae, in which asci are produced in open clusters. At 25°C, colonies may not produce the white hyphae, and then they closely resemble *Paecilomyces variotii* (teleomorph *Byssochlamys spectabilis*). The simplest microscopic distinguishing feature is that *P. variotii* produces ellipsoidal not cylindroidal conidia. Moreover, *P. variotii* does not readily produce a teleomorph in culture.

Physiology. The major physiological characteristic which makes *Byssochlamys fulva* significant in food mycology is the heat resistance of its ascospores. First noted by Olliver and Rendle (1934), who described this species, and very carefully documented by Hull (1939), this property has been extensively studied in more recent work. These studies were comprehensively reviewed by Beuchat and Rice (1979).

Many variables can affect the heat resistance of *Byssochlamys fulva*, which can vary markedly from isolate to isolate (Bayne and Michener, 1979; Hatcher et al., 1979). Factors such as pH, water activity (see Dijksterhuis and Samson, 2006) and the presence of preservatives also have an effect. Ascospores are more susceptible to heat if the pH is low, and/or if preservatives such as sodium benzoate or potassium sorbate are present (El-Geddawy, 2005). On the other hand, high levels of sugar have a protective effect (El-Geddawy, 2005). For *B. fulva*, a *D* value of between 1 and 12 min at 90C° (Bayne and Mitchener, 1979) and a *z* value of 6–7°C (King et al., 1969) are practical working values. Ascospores of *B. fulva* isolated from canned tomato paste were more heat resistant in tomato juice than in phosphate buffer (Kotzekidou, 1997a). Treatment at 80°C for 1 hour had no effect on the viability of ascospores, however, in the presence of CO_2, a considerable reduction (80%) in ascospore numbers was observed (Ballestra and Cuq, 1998).

Byssochlamys fulva ascospores are also very resistant to high pressure, with resistance increasing with ascospore age (Chapman et al., 2007). After treatment at 600 MPa for 10 min, a 4–5 log reduction was achieved for 3 week old ascospores, whereas the same treatment applied to 9 week old ascospores resulted in a 1-log reduction and 15 week old ascospores were activated after pressure treatment (Chapman et al., 2007).

The second physiological characteristic which makes *Byssochlamys fulva* an outstanding spoilage fungus is its ability to grow at very low oxygen tensions. This ability, shared with *B. nivea*, but apparently not with other common heat resistant moulds, provides *Byssochlamys* species with a selective advantage in products such as canned, bottled or cartoned fruits and fruit juices. Our observations suggest that in the presence of very low levels of oxygen, these species grow anaerobically and produce CO_2. A small amount of oxygen contained in the headspace of a jar or bottle, or the slow leakage of oxygen through a package such as a Tetra-Brik can provide sufficient oxygen for these fungi to grow. The production of gas may cause visible swelling and spoilage of the product.

Byssochlamys fulva can grow extensively in atmospheres containing 20, 40 or 60% CO_2 and less than 0.5% O_2. In 20% CO_2, colonies reached 40–80 mm diameter in 15–30 days, depending on the medium. In 60% CO_2 and less than 0.5% O_2,

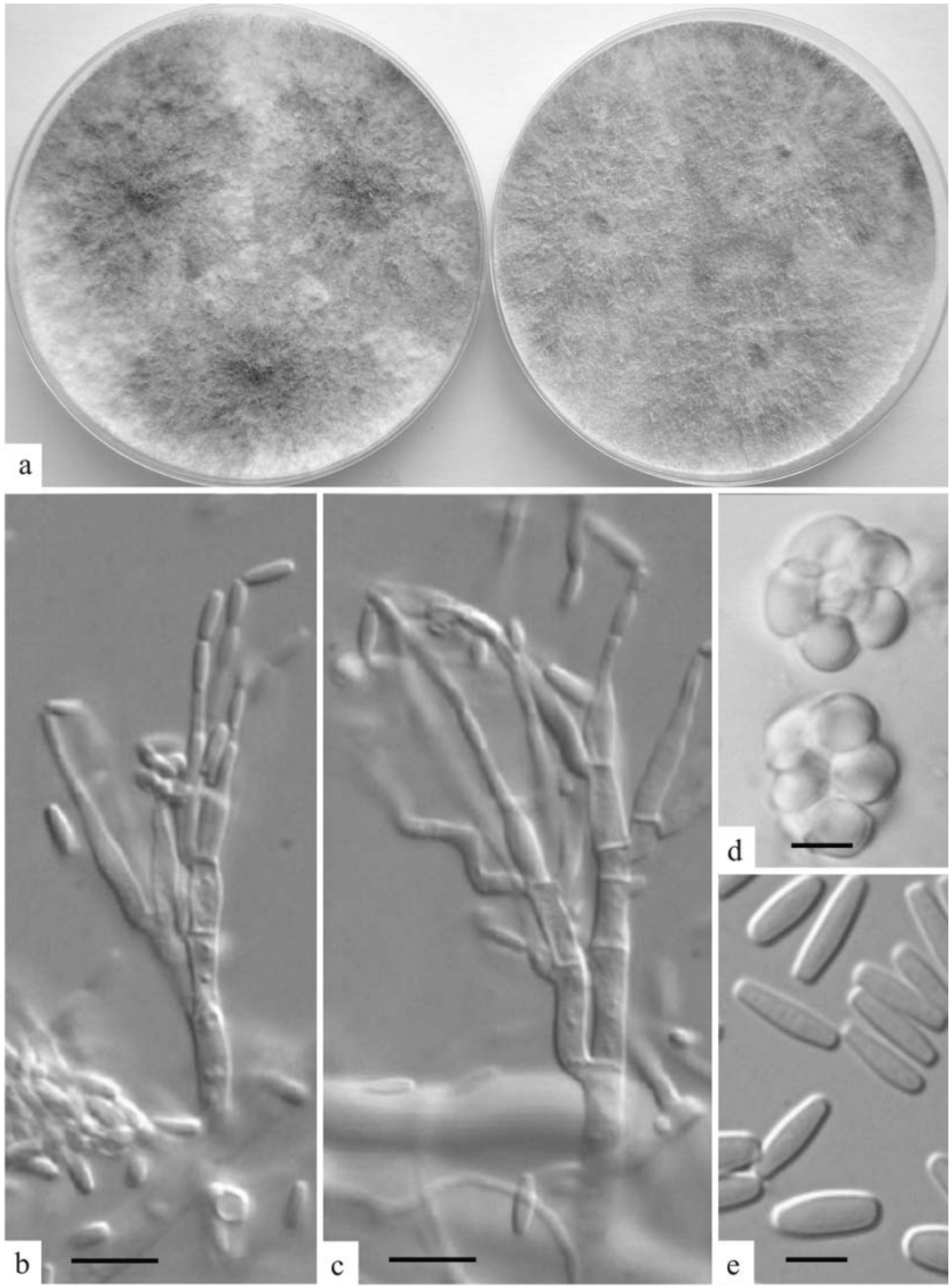


Fig. 7.2 *Byssochlamys fulva* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** penicilli, bars = 10 µm; **(d)** asci and ascospores, bar = 5 µm; **(e)** conidia, bar = 5 µm

colonies of 20–40 mm diameter were formed in 30 days; colonies in 80% CO_2 and 20% O_2 were similar (Taniwaki, 1995; Taniwaki et al., 2001a).

The small amount of growth formed while residual oxygen is consumed in canned or bottled fruit may be sufficient to allow the production of pectinolytic enzymes. The first sign of spoilage by *Byssochlamys* species is usually a slight softening of the fruit. This progresses until total disintegration takes place, due to the production of powerful pectinases by the fungus (Hull, 1939; Beuchat and Rice, 1979). Off odours, and a slightly sour taste may develop and, as noted above, gas production may occur. It is rare for canned and bottled fruit to be spoiled by fungi other than *Byssochlamys* species.

Mycotoxins. Rice et al. (1977) reported patulin production by some isolates of *Byssochlamys fulva*, however, there is no published evidence that patulin has been produced by growth of *B. fulva* in commercially processed fruit juices. Very low levels of patulin were produced under atmospheres containing less than 0.5% O_2 and 20–60% CO_2 (Taniwaki, 1995). A recent survey revealed that none of the 11 isolates of *B. fulva* examined produced patulin in vitro (Puel et al., 2007), a result also demonstrated by Houbraken et al. (2006). *B. fulva* synthesises byssochlamic acid (Houbraken et al., 2006).

Ecology. Spoilage caused by *Byssochlamys fulva* was first recognised in canned strawberries in England in the 1930s. Olliver and Rendle (1934) investigated the incidence of *B. fulva* in fruits, in other canning ingredients and in packing materials. Although strawberries and plums seemed to be particularly affected, *B. fulva* was also found on other fruits such as berries, currants and apples (Olliver and Rendle, 1934). Hull (1939) found *B. fulva* on leaves, fruits and straw from strawberry fields, and also on mummified fruit. Olliver and Rendle (1934) and Hull (1939) concluded that soil acts as the primary reservoir for *Byssochlamys* ascospores, and that fruit which came in contact with soil directly or from rain splash were susceptible to contamination.

Byssochlamys fulva has been reported from several other areas of the world, notably the United States, where it has occurred in grape products (King et al., 1969; Splittstoesser et al., 1971), and Australia, as the cause of spoilage of canned strawberries (Richardson, 1965), fruit juices and fruit-based baby foods. The main Australian source of *B. fulva* has been shown to be passionfruit juice (Hocking and Pitt, 1984). Effective measures to overcome this problem include washing of fruit before juice extraction, rejection of difficult to clean wrinkled fruit and screening of juices for heat resistant ascospores (Cartwright and Hocking, 1984; Kotzekidou, 1997a). For details of screening methods see Chapter 4.

The high heat resistance of *Byssochlamys fulva* and *B. nivea* ascospores has led to the study of other approaches for deactivating them. Microwave heating (used to pasteurise packaged ravioli) had little effect on *B. nivea* (Dragoni et al., 1990). Intermittent use of dimethyldicarbonate was suggested (van der Riet and Pinches, 1991) after it was found that a single high dose (1000 mg/l) had no effect on ascospores of *B. fulva* (van der Riet et al., 1989). Approximately 1.2 kGy of ionising radiation produced one decimal reduction in viable ascospores of two resistant strains of *B. fulva*; it was estimated that more than 7 kGy would be needed for an effective pasteurising process. Apple juice containing high numbers of spores and treated with 5 kGy spoiled after 3 months (van der Riet and van der Walt, 1985).

References. Samson (1974); Beuchat and Rice (1979); Hocking and Pitt (1984); Houbraken et al. (2006).

Byssochlamys nivea Westling **Fig. 7.3**

Anamorph: *Paecilomyces niveus* Stolk & Samson

Colonies on CYA 40–50 mm diam, low and quite sparse, white to slightly grey; reverse pale to mid brown. Colonies on MEA covering the whole Petri dish, low and sparse, white to creamish, with small knots of dense hyphae; reverse pale to brownish. On G25N, usually only microscopic growth. No growth at 5°C. At 30°C on CYA, colonies covering the whole Petri dish, similar to on MEA at 25°C, but often more dense, enveloping distinct knots of dense hyphae. At 37°C, colonies 50–70 mm diam, low to floccose, moderately dense, white to cream, reverse pale to brown.

Teleomorph similar to that of *Byssochlamys fulva* except for slightly smaller asci (8–11 μm diam) and ascospores (4–6 μm diam), maturing in 10–14 days at 25°C and in 7–10 days at 30°C, but rarely found at 37°C. Anamorphs of two kinds produced, aleurioconidia and penicilli; aleurioconidia borne singly, common at 30 and 37°C, spherical to pyriform, 7–10 μm

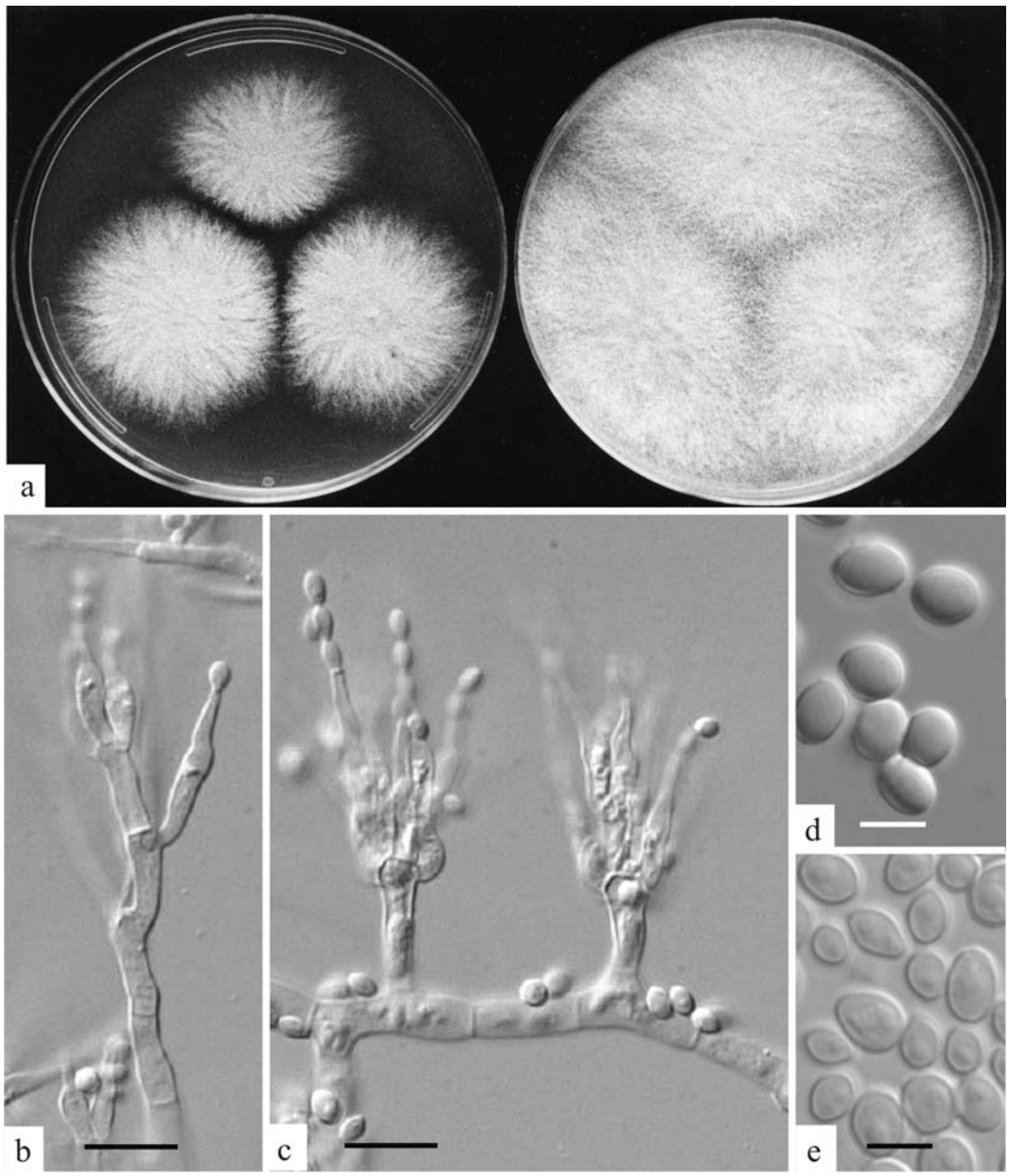


Fig. 7.3 *Byssochlamys nivea* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** penicilli, bars = 10 μm; **(d)** ascospores, bar = 5 μm; **(e)** conidia, bar = 5 μm

diam; penicilli sparsely produced, with short stipes bearing irregular metulae and phialides or phialides alone, and with phialides sometimes borne solitarily from hyphae as well; phialides 12–20 μm long, cylindrical then gradually tapering; conidia ellipsoidal to pyriform, 3–6 μm long, smooth walled.

Distinctive features. *Byssochlamys nivea* is readily distinguished from *B. fulva* by its persistently white colonies. It differs from other fungi by forming three characteristic types of reproductive structures: aleurioconidia, penicilli and solitary asci.

Physiology. The minimum a_w for growth of *Byssochlamys nivea* was 0.92 at 21°C, 0.89 at 30°C and 0.87 a_w at 37°C (Roland and Beuchat, 1984). So far as has been established, the physiology of *B. nivea* is similar to that of *B. fulva*. Like *B. fulva*, it

causes spoilage of heat processed acid foods under conditions of low oxygen tension. *B. nivea* ascospores are reported to be marginally less heat resistant than those of *B. fulva* (Put and Kruiswijk, 1964; Beuchat and Rice, 1979). A *D* value at 88°C of 0.75–0.8 min, with *z* values ranging from 4.0–6.1C°, was determined by Casella et al. (1990). See also Engel and Teuber (1991) and Quintavalla and Spotti (1993). *B. nivea* ascospores exhibited pressure resistance similar to that reported for *B. fulva* (Butz et al., 1996; Chapman et al., 2007).

Remarks about the physiology of *Byssochlamys fulva* probably apply equally well to *B. nivea*. Growth of *B. nivea* in atmospheres containing 0.5% O_2 and 20, 40 or 60% CO_2 was somewhat slower than observed for *B. fulva*, while growth on 20% O_2 and 80% CO_2 was rather faster (Taniwaki, 1995). Taniwaki et al. (2001a) showed that *B. nivea* was capable of continued growth at 40% CO_2 with <0.5% O_2.

Mycotoxins. *Byssochlamys nivea* may produce patulin (Rice et al., 1977), byssochlamic acid and mycophenolic acid (Houbraken et al., 2006), but none of these have been reported to be significant in foods. Several studies have investigated the effect of physical parameters on patulin production, including the effect of headspace in glass jars of heat processed grape juice (Rice, 1980), controlled atmospheres (Orth, 1976; Taniwaki, 1995), temperature, a_w and preservatives (Roland and Beuchat, 1984; Roland et al., 1984). Minimum a_w values for patulin production were 0.92 at 21°C and 0.87 at both 30 and 37°C (Roland and Beuchat, 1984). Ayesha and Viswanath (2006) reported the production of patulin in sugar cane juice inoculated with *B. nivea*. Patulin synthesis was greater at 20°C than 35°C, and more patulin was produced in juice than in commercial broth.

Ecology. *Byssochlamys nivea* appears to be a less common problem in foods than *B. fulva*. It has mostly been reported from European sources: from sweet cider in Switzerland, from fruit juices in Denmark, from bottled strawberries in the Netherlands and packaged ravioli in Italy (see Pitt and Hocking, 1997). Spoilage of the latter product was prevented by packing in an atmosphere of 60% CO_2, 39.4% N_2 and 0.05% O_2 (Dragoni and Cantoni, 1988). *B. nivea* has also been isolated from passionfruit juice and from cream cheese in Australia (A.D. Hocking, unpublished).

References. Put and Kruiswijk (1964); Samson (1974); Beuchat and Rice (1979); Hocking and Pitt (1984); Houbraken et al. (2006).

7.2 Genus *Eupenicillium* F. Ludw.

The name *Eupenicillium* was applied to the very hard (sclerotioid) cleistothecial state produced by certain *Penicillium* species as early as 1892. However, for many years it remained common practice to name both teleomorph and anamorph species producing a *Penicillium* anamorph by their *Penicillium* name. As well as conflicting with provisions of the International Code of Botanical Nomenclature, this practice ignored the effects of the teleomorph on cultural appearance, longevity, heat and chemical resistance, etc. These important characteristics are readily overlooked if the *Penicillium* name is used.

Benjamin (1955) revived the name *Carpenteles* Langeron 1922 for *Penicillium* species producing cleistothecia. This name slowly gained acceptance until Stolk and Scott (1967) showed that the obscure *Eupenicillium* F. Ludw. 1892 was the correct name for this Ascomycete genus. Scott and Stolk transferred all *Carpenteles* species to *Eupenicillium* and described 16 new species (Scott and Stolk, 1967; Stolk and Scott, 1967). Despite vigorous opposition from some taxonomists (Raper, 1957; Fennell, 1973), the use of teleomorph names for *Penicillium* (and *Aspergillus*) species where Ascomycete states are present is now firmly established (Pitt, 1979b; Samson and Pitt, 1985; Pitt and Samson, 1993; Pitt and Hocking, 1997; Pitt et al., 2000).

Scott (1968) published a monograph of *Eupenicillium*. Pitt (1974b, 1979b, 2000), while providing new keys and descriptions, adhered closely to Scott's species concepts. Stolk and Samson (1983) published a revised taxonomy with greatly modified species concepts. Acceptance was limited, as Scott's concepts appear sound. The close relationships between some *Eupenicillium* species, some *Penicillium* species producing sclerotia (cleistothecia which never mature) and indeed some strictly anamorphic species were recognised first by Pitt (1974b). Chemotaxonomy (Frisvad et al., 1990a) and molecular studies (LoBuglio et al., 1993) have added weight to this concept, which probably has phylogenetic significance (Pitt, 1995).

Eupenicillium is characterised by the production of macroscopic (100–500 μm diam), smooth walled, often brightly coloured cleistothecia, in association with a *Penicillium* anamorph. In many species cleistothecia become rock hard as they develop, and may remain so for many weeks or months, finally maturing from the centre to yield numerous eight spored asci. Because of this delayed

maturation, taxonomy is very difficult if based on the morphology of mature ascospores, as the keys of Scott (1968) and Stolk and Samson (1983) were. For this reason, Pitt (1974b, 1979b) introduced synoptic keys to *Eupenicillium*, which permit identification of many *Eupenicillium* species from cultural and microscopic characters.

Most *Eupenicillium* species are soil fungi, and of little interest to the food microbiologist. However, they have occurred from time to time as survivors of heat processing. Williams et al. (1941) recorded that a new species, *Penicillium lapidosum* (stone-like, an apt name), was causing spoilage of canned blueberries. It possessed highly heat resistant sclerotia (immature cleistothecia). This fungus was later shown to produce a *Eupenicillium* state. Two points are worth noting: first, it was the immature cleistothecium itself which was acting as the heat resistant body; and second, most *Eupenicillium* species produce heat resistant ascospores. Fortunately, they find their way into heat processed foods only rarely.

Eupenicillium species have been isolated as heat-resistant contaminants of fruit juices on several occasions (Anon., 1967; van der Spuy et al., 1975; Hocking and Pitt, 1984, and unpublished). No particular species appears to be responsible, and growth of the fungus in the product has been rare. As a cause of food spoilage, *Eupenicillium* ascospores can be safely ignored unless an unusual set of circumstances leads to excessive contamination of some raw material or product.

Of the 37 *Eupenicillium* species recognised by Pitt (1979b), five are treated here: *E. brefeldianum*, *E. cinnamopurpureum*, *E. hirayamae*, *E. javanicum* and *E. ochrosalmoneum*. *E. cinnamopurpureum* and *E. javanicum* are relatively common. Because *Penicillium* species that produce sclerotia cannot be distinguished from *Eupenicillium* species after 7 days incubation, *P. raistrickii*, *P. sclerotiorum*, *P. simplicissimum* and *P. thomii* are included in this key.

Key to *Eupenicillium* and sclerotigenic *Penicillium* species included here

1	Colonies on CYA less than 20 mm diam, coloured deep brown	*E. cinnamopurpureum*
	Colonies on CYA more than 20 mm diam, not coloured deep brown	2
2 (1)	Colonies with bright yellow or orange mycelium and/or reverse colours	3
	Colonies with mycelium and reverse uncoloured, or in colours other than bright yellow or orange	5
3 (2)	Penicilli strictly monoverticillate	4
	Penicilli biverticillate or sometimes irregular	*E. ochrosalmoneum*
4 (3)	Growth on CYA at 37°C similar to at 25°C	*E. hirayamae*
	No growth at 37°C	*P. sclerotiorum*
5 (2)	Penicilli monoverticillate	6
	Penicilli biverticillate	7
6 (5)	Growth at 37°C	*E. javanicum* *E. brefeldianum*
	No growth at 37°C	*P. thomii*
7 (5)	Usually growth at 37°C, conidial walls rough	*P. simplicissimum*
	No growth at 37°C, conidia with smooth walls	*P. raistrickii*

Eupenicillium cinnamopurpureum D.B. Scott & Stolk **Fig. 7.4**

Penicillium pusillum G. Sm.
Penicillium cinnamopurpureum S. Abe ex Udagawa

Anamorph: *Penicillium phoeniceum* J.F.H. Beyma

Colonies on CYA 15–20 mm diam, of closely textured, sulcate or wrinkled white to brown mycelium, usually enveloping numerous brown to pinkish cleistothecia; conidial production sparse, coloured greyish green (25C-D3, 26D2); clear exudate and purple soluble pigment typically produced; reverse pink, purple or cinnamon, rarely pale or dull orange. Colonies on MEA 13–15 mm diam, plane, usually with a central area of cinnamon to brown cleistothecia surrounded by white mycelium and few to numerous penicilli; colours

as on CYA except reverse sometimes dull yellow or orange, usually also with some purple areas. Colonies on G25N 8–12 mm diam, of dense white mycelium, conidial production light to moderate, grey green; reverse purple, pink or cinnamon. At 5°C, usually no germination. At 37°C, colonies 5–8 mm diam, of white mycelium or, rarely, no growth.

Cleistothecia 150–250 µm diam, pinkish cinnamon to brown, becoming hard, maturing very slowly; ascospores ellipsoidal, pale yellow, 3.0–3.5 µm long, with spinulose walls and with two low longitudinal flanges. Conidiophores borne from surface mycelium, stipes 20–150 µm long, strictly monoverticillate, smooth walled, terminating in enlarged (4–5 µm) apices; phialides ampulliform to acerose, 8–12 µm long, gradually tapering; conidia subspheroidal to ellipsoidal, 2.0–3.5 µm long, with smooth or finely roughened walls.

Distinctive features. *Eupenicillium cinnamopurpureum*, together with its anamorph *Penicillium phoeniceum*, is a distinctive and readily recognised species. It grows slowly on CYA and MEA, stipes are strictly monoverticillate, are apically enlarged and bear long, gradually tapering phialides. Most isolates produce brown cleistothecia, which mature very slowly.

Taxonomy. Some isolates fail to produce cleistothecia at all, but are still readily recognisable as

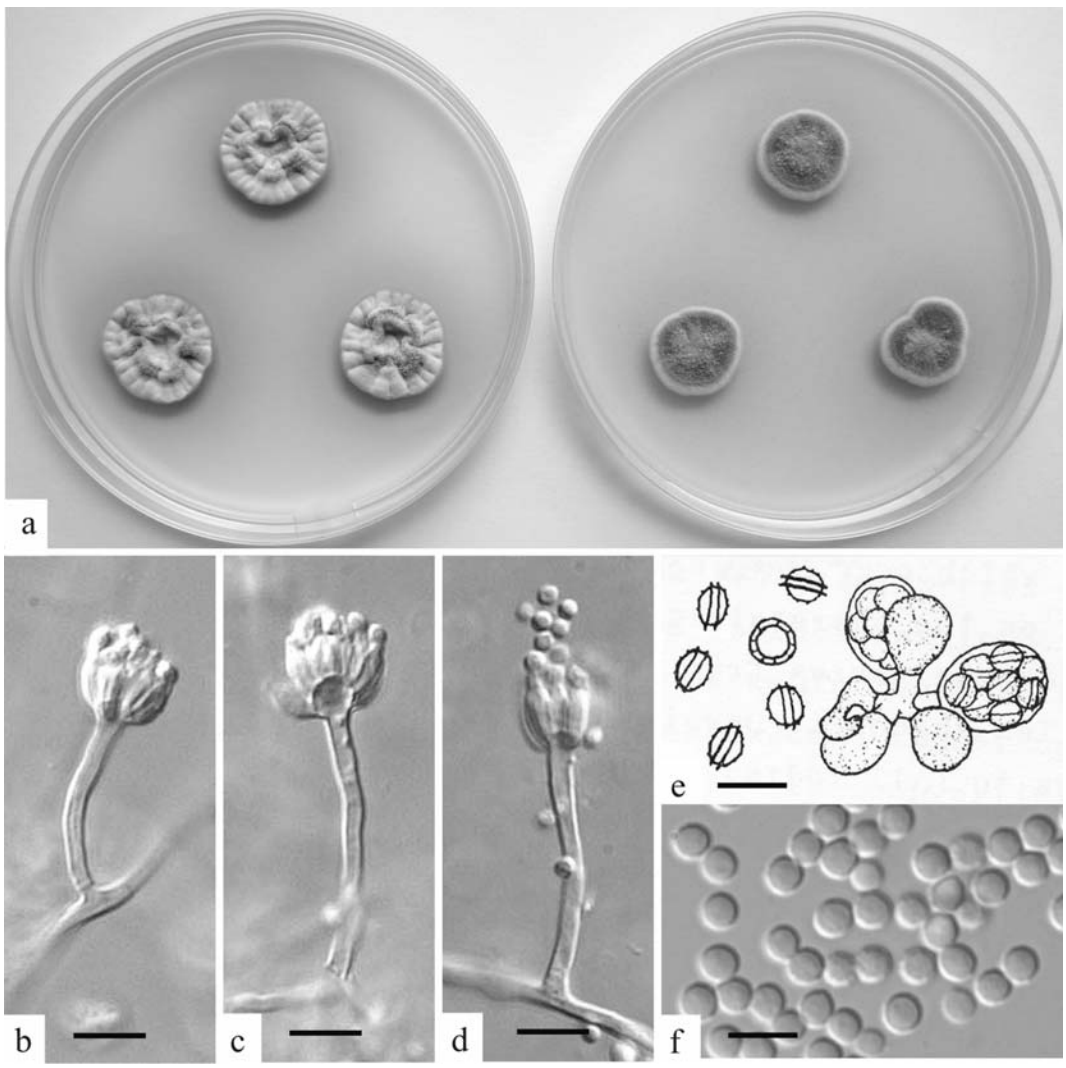


Fig. 7.4 *Eupenicillium cinnamopurpureum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 µm; **(e)** asci and ascospores, bar = 5 µm; **(f)** conidia, bar = 5 µm

belonging here. *Penicillium phoeniceum* is the appropriate name in such cases.

Physiology. This species is among the most xerophilic of the Penicillia, growing down to 0.78 a_w in glycerol media (Hocking and Pitt, 1979), or 0.82 a_w in salt (25% w/v; Udagawa and Tsuruta, 1973). From growth data in Pitt (1979b: 67), this species will grow at temperatures between 4–6°C and 35–38°C, with some isolate to isolate variation.

Mycotoxins. Mycotoxin production has not been reported.

Ecology. A very widely distributed species, *Eupenicillium cinnamopurpureum* has been isolated from flour (Graves and Hesseltine, 1966; as *Penicillium* sp.), rice (Udagawa, 1959; as *P. cinnamopurpureum*), dried beans and rice, especially in long term storage (Udagawa and Tsuruta, 1973; Tsuruta and Saito, 1980, as *P. pusillum*), dried peas (Smith, 1939, as *P. pusillum*) and stored maize kernels (Wicklow et al., 1998). We isolated this species quite frequently from Southeast Asia: from soybeans, red beans and copra in Thailand, from maize, peanuts, soybeans and black pepper in the Philippines, and from maize, peanuts, kemiri nuts, mung beans, soybeans and red kidney beans in Indonesia. The strict anamorph *P. phoeniceum* was found in black rice from Thailand and milled rice from Indonesia (Pitt et al., 1994, 1998a).

Eupenicillium cinnamopurpureum and *Penicillium phoeniceum* were amongst the most commonly isolated Penicillia in Australian wheat and various milling fractions (flour, semolina, bran and wheat germ) during a survey of the microbiology of flour milling (Hocking, unpublished).

References. Udagawa (1959); Udagawa and Tsuruta (1973); Pitt (1979b, 2000).

Eupenicillium hirayamae D.B. Scott & Stolk Fig. 7.5

Anamorph: *Penicillium hirayamae* Udagawa

Colonies on CYA usually 22–28 mm diam, but up to 45 mm diam if cleistothecia absent, radially sulcate, of dense, brilliant yellow or orange mycelium usually enmeshing cleistothecia and overlaid by funicles (ropes) of fertile hyphae; conidial production moderate, dull green (28D3); exudate clear to pale yellow; reverse usually apricot to deep orange (5B6-A8). Colonies on MEA usually 15–22 mm diam, but up to 35 mm if cleistothecia absent, plane, otherwise similar to those on CYA. Colonies on G25N 8–14 mm diam, similar to those on CYA. No germination at 5°C. At 37°C, colonies 20–30 mm diam, mycelium centrally brown, otherwise similar to those produced at 25°C.

Cleistothecia buff to yellow, appearing orange or brown from adherent hyphae, 250–300 μm diam or up to 400 μm long, hard, maturing after 4–6 weeks or more; ascospores small and ellipsoidal, yellow, 2.2–3.0 μm long, with rough walls and two small longitudinal flanges. Conidiophores borne from ropes of aerial hyphae, with stipes 10–50 μm long, smooth walled, strictly monoverticillate; phialides ampulliform, 6–8(–10) μm long; conidia subspheroidal, minute, 1.8–2.8 μm long, smooth walled.

Distinctive features. Relatively slowly growing, brilliant yellow and orange coloured colonies make *Eupenicillium hirayamae* a readily recognised species. Confirmation is provided by the formation of very similar colonies at 25 and 37°C and by the production of short monoverticillate conidiophores.

Taxonomy. Most isolates produce cleistothecia and in due course ascospores. A few isolates have been encountered which do not – interestingly colony growth is significantly faster. Such isolates are correctly identified as *Penicillium hirayamae*.

Physiology. We are not aware of any physiological studies on this species.

Mycotoxins. Mycotoxin production has not been reported.

Ecology. *Eupenicillium hirayamae* has mostly been isolated from cereals, from Thailand, United States South Africa and India.

References. Udagawa (1959); Pitt (1979b, 2000).

Eupenicillium javanicum (J.F.H. Beyma) Stolk & D.B. Scott Fig. 7.6

Penicillium javanicum J.F.H. Beyma (name invalid because it included the teleomorph).

Anamorph: *Penicillium indonesiae* Pitt

Colonies on CYA 30–45 mm diam, radially sulcate, consisting of dense, velutinous, pale yellow mycelium; cleistothecia abundant, enveloped by the mycelium; conidia sparse; exudate copious, brown; reverse olive green, often also with deep reddish

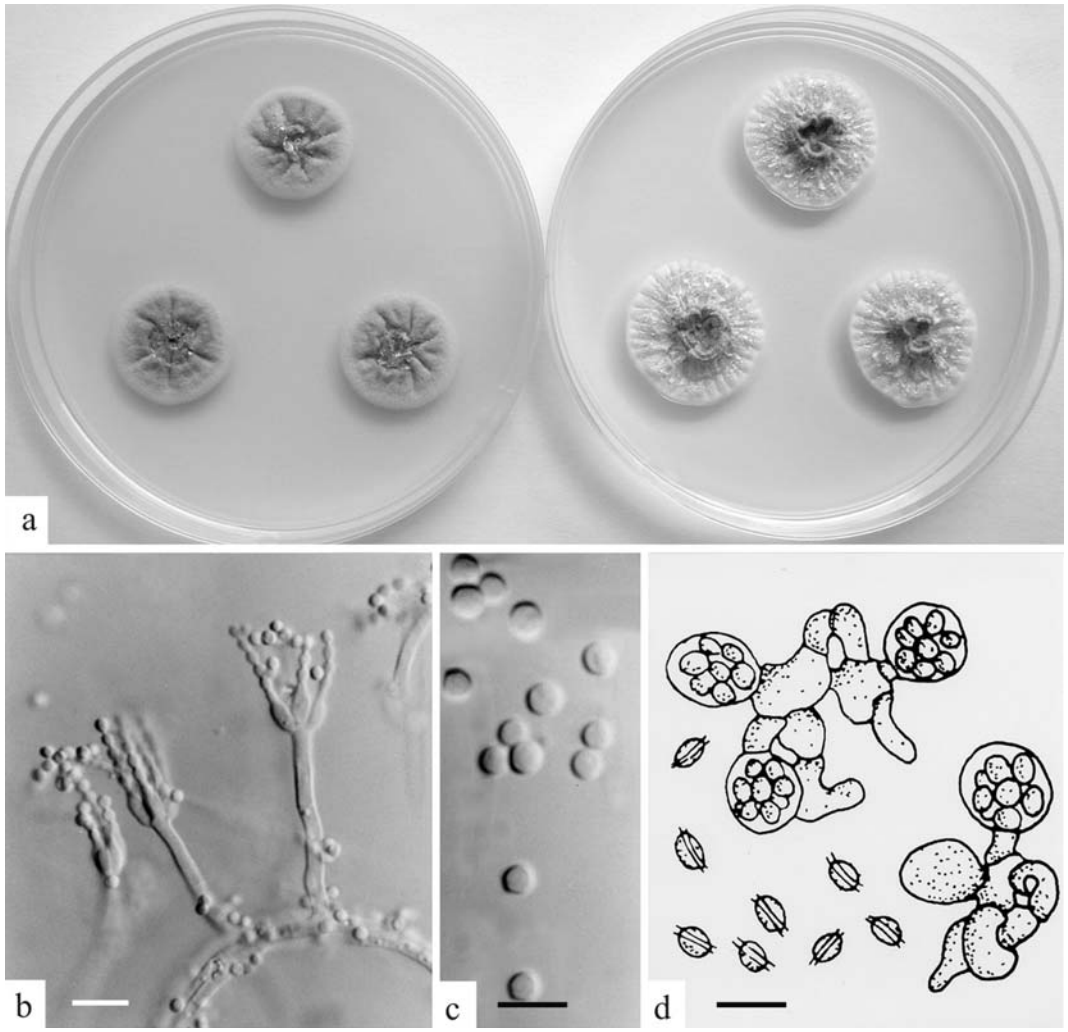


Fig. 7.5 *Eupenicillium hirayamae* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b)** penicilli, bar = 10 μm; **(c)** conidia, bar = 5 μm; **(d)** asci and ascospores, bar = 5 μm

brown areas. Colonies on MEA 30–50 mm diam, similar to those on CYA but mycelium brighter yellow and reverse usually deep olive brown. Colonies on G25N 9–14 mm diam, of floccose, pale yellow mycelium; reverse olive brown. No germination at 5°C. At 37°C, colonies 25–50 mm diam, radially sulcate or irregularly wrinkled, mycelium usually white but sometimes deep brown; cleistothecia sometimes present but penicilli absent; clear to brown or reddish exudate and reddish brown soluble pigment usually produced; reverse pale, yellow, reddish brown or deep brown.

Cleistothecia dull yellow to brown, 80–200 μm diam, maturing in 2–3 weeks; ascospores ellipsoidal, 2.5–3.0 μm long, with slightly roughened walls and a faint longitudinal furrow. Conidiophores borne from aerial hyphae, stipes 50–100 μm long, smooth walled, nonvesiculate, bearing monoverticillate penicilli; phialides ampulliform, 8–11 μm long; conidia subspheroidal, ellipsoidal or pyriform, 2.5–3.0 μm long.

Distinctive features. *Eupenicillium javanicum* grows rapidly at 37°C, produces sparse strictly monoverticillate penicilli and produces bright colours in exudate and reverse on CYA at 25°C. Mature ascospores are produced relatively rapidly, usually within 14 days at 25°C.

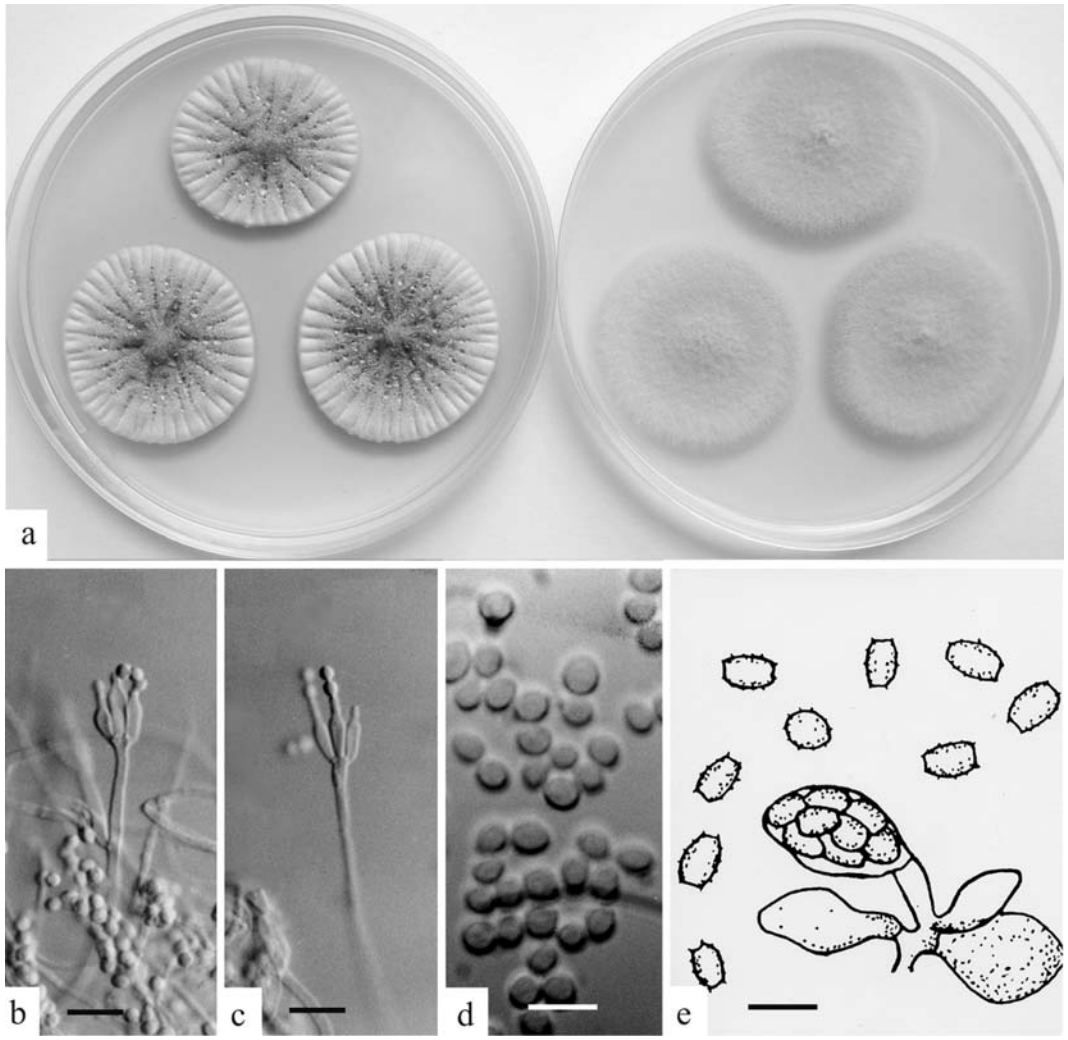


Fig. 7.6 *Eupenicillium javanicum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** penicilli, bars = 10 μm; **(d)** conidia, bar = 5 μm; **(e)** asci and ascospores, bar = 5 μm

Taxonomy. The anamorph name, necessary for nomenclatorial reasons at the time it was published (Pitt, 1979b), is hardly ever used.

Physiology. Tanaka et al. (2000) reported that *Eupenicillium javanicum* exhibited greater cellulase activity on soy bran medium than on crystalline cellulose medium.

Mycotoxins. Some isolates of *Eupenicillium javanicum* have been reported to produce xanthomegnin and a little palitantin (Frisvad et al., 1990a). This is unlikely to be of practical importance. A report of patulin production (Okeke et al., 1993) requires confirmation, especially as this compound was not found by Frisvad et al. (1990a). Two new compounds, namely eujavanoic acids A and B, were characterised from *E. javanicum*. The decalin derivatives were found to be active against *Aspergillus fumigatus* (Okamoto et al., 2004).

Ecology. This is one of the most common *Eupenicillium* species, abundant in soils, but occurring in foods rather rarely. It has been isolated from wheat and flour (Basu and Mehrotra, 1976), fermented and cured meats (Leistner and Ayres, 1968) and peanuts (Pitt et al., 1998a).

Additional species. *Eupenicillium brefeldianum* (B.O. Dodge) Stolk and D.B. Scott is closely related to *E. javanicum*; the two species produce colonies of similar sizes on the standard media. *E. brefeldianum* produces longer, sometimes metulate stipes, larger

cleistothecia (150–250 μm long) and ascospores (3–4 μm long) and pale orange mycelium. It lacks green reverse colours.

This species has been reported to cause spoilage of apple juice in South Africa by surviving pasteurising treatments (Anon., 1967; van der Spuy et al., 1975). It has also been isolated from peanuts (Joffe, 1969).

References. Stolk and Scott (1967); Pitt (1979b, 2000).

Eupenicillium ochrosalmoneum D.B. Scott & Stolk Fig. 7.7

Anamorph: *Penicillium ochrosalmoneum* Udagawa

Colonies on CYA 18–28 mm diam, plane or sulcate, with a moderately deep, dense layer of mycelium enveloping abundant cleistothecia, overlaid by sparse to abundant penicilli; mycelium white to yellow (2-4A7); conidia greyish green to dull green (27C-D3); clear to pale yellow exudate and pale to bright yellow soluble pigment sometimes produced; reverse yellow to cadmium orange (3-5A8). Colonies on MEA 15–20 mm diam, plane, low and sparse to moderately deep and dense, velutinous; mycelium white at the margins, elsewhere bright yellow, as on CYA; cleistothecia abundant, on the agar surface; conidial production sparse to moderate, coloured as on CYA; yellow soluble pigment

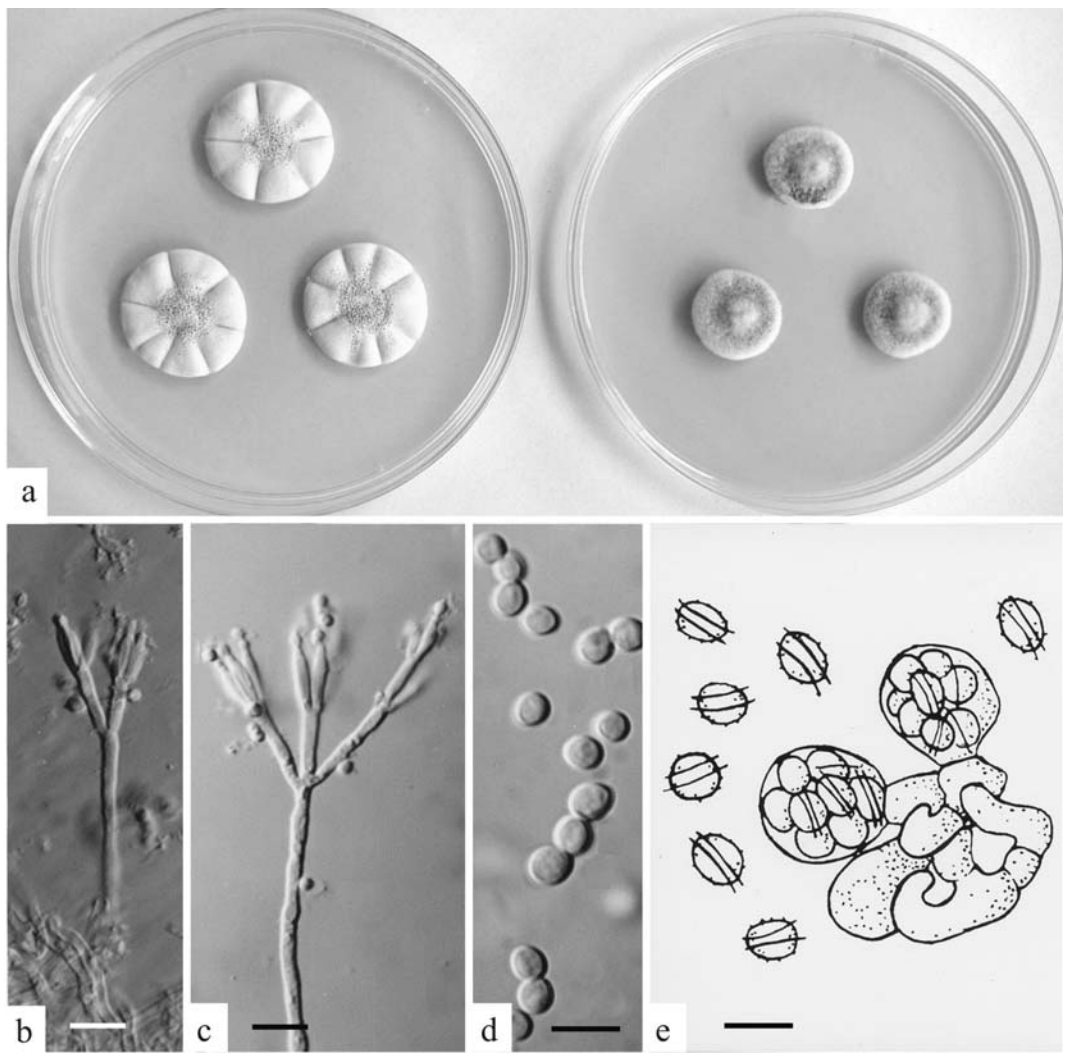


Fig. 7.7 *Eupenicillium ochrosalmoneum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** penicilli, bars = 10 μm; **(d)** conidia, bar = 5 μm; **(e)** asci and ascospores, bar = 5 μm

usually produced; reverse bright yellow to orange. Colonies on G25N 1–7 mm diam, of deep, dense white mycelium; reverse pale or yellow. No germination at 5°C. At 37°C, colonies 10–30 mm diam, radially sulcate or wrinkled, closely resembling growth on CYA at 25°C except for increased cleistothecial production by some isolates.

Cleistothecia pale to bright yellow or orange, 250–500 μm diam, sclerotioid, maturing in 4–6 weeks or more; ascospores ellipsoidal, 3.5–5.0 μm long, with walls smooth to roughened or spinulose, ornamented with two longitudinal flanges. Conidiophores borne from surface or aerial hyphae, stipes variable, (20–)40–200 μm long, smooth walled, bearing very irregular penicilli, basically biverticillate but with subterminal and intercalary metulae often present, the latter being indistinguishable from short monoverticillate conidiophores; phialides ampulliform-acerose 6–8(–10) μm long; conidia subspheroidal to apiculate, commonly 2.5–3.5 μm long, smooth walled, borne in disordered chains.

Distinctive features. *Eupenicillium ochrosalmoneum* forms slowly growing, bright yellow colonies on both CYA and MEA at 25°C. Growth on CYA at 37°C is similar in size and colouration to that at 25°C. These characteristics are shared with *E. hirayamae*: this latter species, however, produces strictly monoverticillate penicilli.

Physiology. *Eupenicillium ochrosalmoneum* is capable of growth down to 0.88 a_w (Hocking and Pitt, 1979).

Mycotoxins. This species is the second major producer of citreoviridin, a mycotoxin described in more detail under *Penicillium citreonigrum*. Citreoviridin produced by *E. ochrosalmoneum* has been found naturally occurring in US maize, at levels up to 2.8 mg/kg (Wicklow et al., 1988).

Ecology. This species has been reported from maize in North America (Wicklow and Cole, 1984; Wicklow et al., 1988) and pig feed in Slovakia (Labuda and Tančinová, 2003). We have seen both *Eupenicillium ochrosalmoneum* and its anamorph in Australian wheat and wheat fractions during a survey of the microbiological status of Australian flour mills (Hocking, unpublished). Its importance lies in the potential to produce citreoviridin under natural conditions.

References. Scott and Stolk (1967); Pitt (1979b, 2000).

7.3 Genus *Geosmithia* Pitt

Geosmithia was split from *Penicillium* by Pitt (1979a), because phialide shape and wall texture, and conidial shape and colour, are distinctive. Conidia in *Geosmithia* are strictly cylindrical and borne from usually rough walled, cylindrical phialides which have cylindrical necks. Except in one species, *G. namyslowskii* K.M. Zalessky, known only from a single isolate from soil, conidia totally lack the blue or green pigmentation so characteristic of *Penicillium*.

Recent molecular studies (Ogawa et al., 1997) and later papers have shown that Pitt's concept is polyphyletic and that some *Geosmithia* species are closely related to *Penicillium*. The species treated here, *G. putterillii*, remains in *Geosmithia* with the type species, *G. lavendula*.

The main habitat of *Geosmithia* species, including *G. putterillii*, is in association with bark beetles (Kolařík et al., 2004). However, *G. putterillii* is quite common on cereal products and may cause spoilage occasionally (Fig. 7.8).

Geosmithia putterillii (Thom) Pitt Fig. 7.8

Penicillium putterillii Thom

Colonies on CYA 25–35 mm diam, velutinous, floccose or somewhat funiculose, mycelium white or buff, conidial production moderate to heavy, off white to buff or very pale yellow; reverse dull yellow or olive brown. Colonies on MEA 20–30 mm diam, usually markedly funiculose, otherwise as on CYA. Colonies on G25N 15–18 mm diam, similar to those on CYA. At 5°C, no germination to formation of microcolonies. At 37°C, usually no growth, sometimes colonies to 8 mm diam.

Conidiophores borne from surface or aerial hyphae, stipes 20–100 μm long, with rough walls; penicilli often complex, with 2, 3 or more branch points; phialides rough walled, cylindrical, 8–10 μm long; conidia cylindrical, 4.0–5.0 × 2.0–2.5 μm, smooth walled.

Distinctive features. *Geosmithia putterillii* forms quite slowly growing off white to buff colonies, with irregular penicilli and distinctive cylindrical conidia.

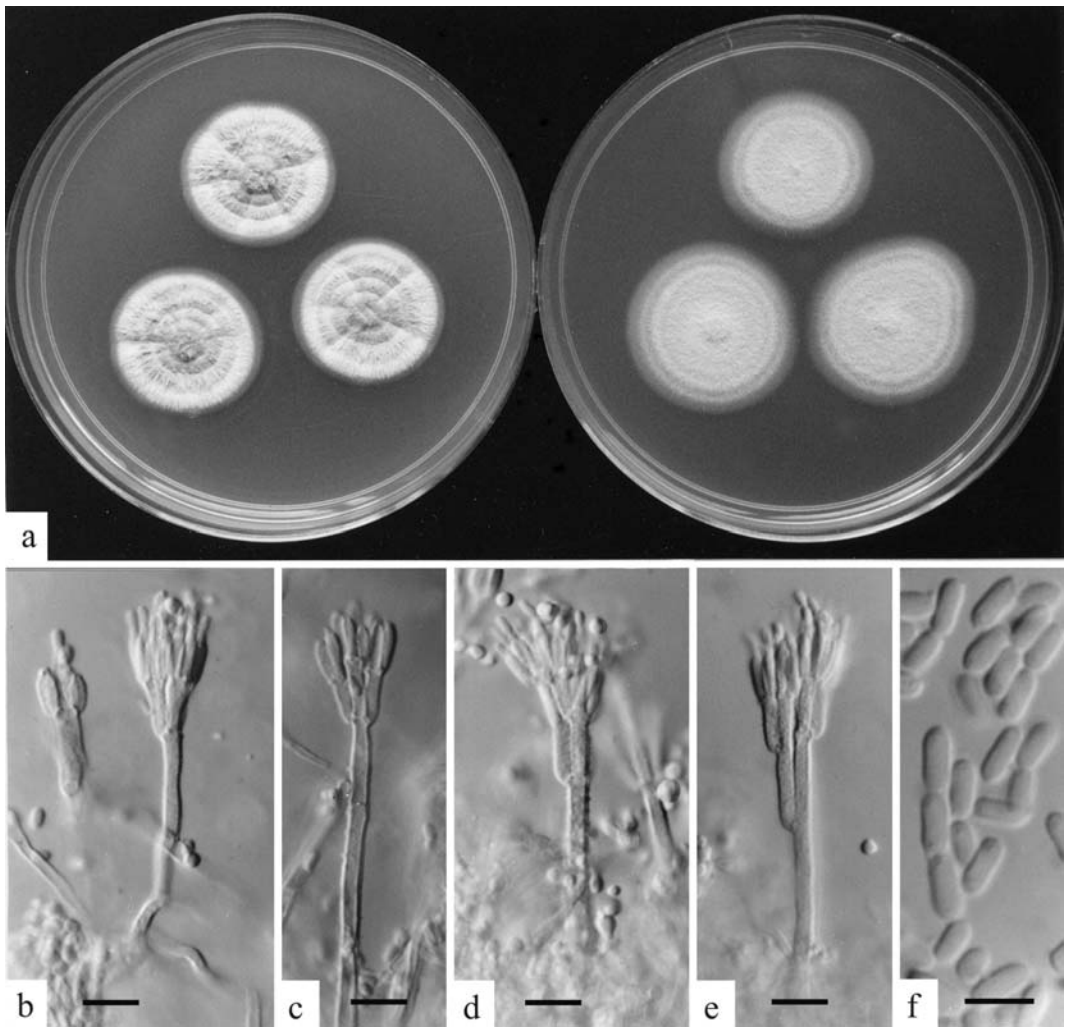


Fig. 7.8 *Geosmithia putterillii* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d, e)** penicilli, bars = 10 μm; **(f)** conidia, bar = 5 μm

Taxonomy. Kolařík et al. (2004) published a comprehensive molecular and morphological study of *Geosmithia putterillii* and related taxa.

Physiology. Information on the physiology of *Geosmithia putterillii* is lacking. However, judged by its isolation from a variety of dry substrates, this species is xerophilic.

Mycotoxins. Mycotoxin production has not been reported.

Ecology. Most foodborne isolations of this species have come from cereals and cereal products, katsuobushi and dried peas (see Pitt and Hocking, 1997), although Kolařík et al. (2004) report that it is normally associated with bark beetles. In our laboratory, it has twice been recorded as the cause of spoilage of Lebanese bread, on which it produces a profuse white growth. We have also isolated it from Indonesian soybeans (Pitt et al., 1998a). It has been reported (as *Penicillium pallidum*) as one cause of rots in melons (Snowdon, 1991).

Reference. Pitt (1979a); Kolařík et al. (2004).

7.4 Genus *Paecilomyces* Bainier

Paecilomyces was split from *Penicillium* by Bainier (1907a) on the basis of differences in phialide shape and conidial colour. *Paecilomyces* phialides have necks which are characteristically (although not exclusively) long and bent away from the phialide

axis. Unlike those produced by *Penicillium* species, conidia are never green or blue and are usually elongate, fusiform or ellipsoidal to cylindroidal.

Paecilomyces species are of importance as soil fungi and insect pathogens. Only three species are commonly isolated from foods: *P. variotii* and *P. lilacinus* are treated below. The teleomorph of *P. variotii* is *Byssochlamys spectabilis* (Houbraken et al., 2006). *P. variotii* is heterothallic, requiring culturing of (+) and (–) strains together to produce the teleomorph, so the *Byssochlamys* state is rarely seen in culture. The discovery of the teleomorph explains why *P. variotii* is often seen in heat-treated samples without evidence of a heat-resistant state (Houbraken et al., 2008). *P. fulvus* is described under its teleomorph, *Byssochlamys fulva*.

Key to *Paecilomyces* species included here

1	Colonies on CYA and MEA brown	2
	Colonies on CYA and MEA lilac or mauve	*P. lilacinus*
2 (1)	Conidia typically cylindrical; on CYA at 30°C, superficial white hyphae at colony centres	*P. fulvus* (see *Byssochlamys fulva*)
	Conidia ellipsoidal to fusiform; on CYA at 30°C, superficial white hyphae absent	*P. variotii*

Paecilomyces variotii Bainier **Fig. 7.9**

Teleomorph: *Byssochlamys spectabilis* (Udagawa & Suzuki) Samson et al.

Talaromyces spectabilis Udagawa & Suzuki

Colonies on CYA of variable size, 30–70 mm diam, plane, of low to floccose appearance, usually coloured uniformly brown or olive brown from conidia; reverse pale. Colonies on MEA 70 mm diam or more, otherwise very similar to those on CYA. Colonies on G25N 8–16 mm diam, similar to on CYA or of white mycelium. No germination at 5°C. At 37°C, colonies growing very rapidly, 60 mm or more diam, similar to those at 25°C, or with sporulation reduced; reverse pale.

Penicilli borne from aerial hyphae on short stipes, of irregular pattern, a cluster of phialides alone or with metulae and phialides or occasionally rami; phialides 12–20 μm long, tapering gradually, with collula often bent away from the axis; conidia mostly subspheroidal to ellipsoidal, sometimes cylindroidal or pyriform, usually 3.0–5.0 μm long, smooth walled.

Distinctive features. With its rapidly growing colonies, coloured uniformly brown to olive brown at both 25 and 37°C, and irregular penicilli with long phialides, *Paecilomyces variotii* is a readily recognised fungus. Only *P. fulvus* is similar: *P. variotii* is distinguishable by its broadly ellipsoidal rather than cylindrical conidia. When grown on MEA at 30°C for 10 days, *P. fulvus* usually produces its *Byssochlamys* state, whereas isolates of *P. variotii* do not usually produce asci. However, if *P. variotii* is isolated from a heat treated food or beverage, it is likely to be the anamorph of *Byssochlamys spectabilis* (Houbraken et al., 2008).

Taxonomy. Houbraken et al. (2006) reported that *Byssochlamys spectabilis* is the teleomorph of *Paecilomyces variotii*. The teleomorphic state is rarely seen in laboratory culture because *B. spectabilis* is heterothallic: production of ascospores requires co-culturing of (+) and (–) strains (Houbraken, et al., 2008). For a description of the teleomorphic state, see Udagawa and Suzuki (1994).

Physiology. *Paecilomyces variotii* is a xerophilic fungus, with growth recorded down to 0.80 a_w (Wheeler and Hocking, 1988). It grows between about 5 and 45–48°C, with an optimum at 35–40°C (Samson, 1974). Our unpublished experiments indicate an ability to grow under low oxygen tensions.

Mycotoxins. Houbraken et al. (2006) reported viriditoxin production by *Byssochlamys spectabilis*.

Ecology. This species is a ubiquitous contaminant of foods and raw materials. It has a strong association with edible oils, raw materials containing oil, bacon, peanuts and peanut cake (see Pitt and Hocking, 1997), margarine (Demirci and Arici, 2006) and cocoa beans (Wolcik-Stopczynska, 2006). Amongst many other reports, it has been recorded from cereals (Loiveke et al., 2004), bread, meat products, biltong, health foods and airline foods (see Pitt and Hocking, 1997). In our laboratory, we have frequently isolated *Paecilomyces variotii* from heat processed fruit

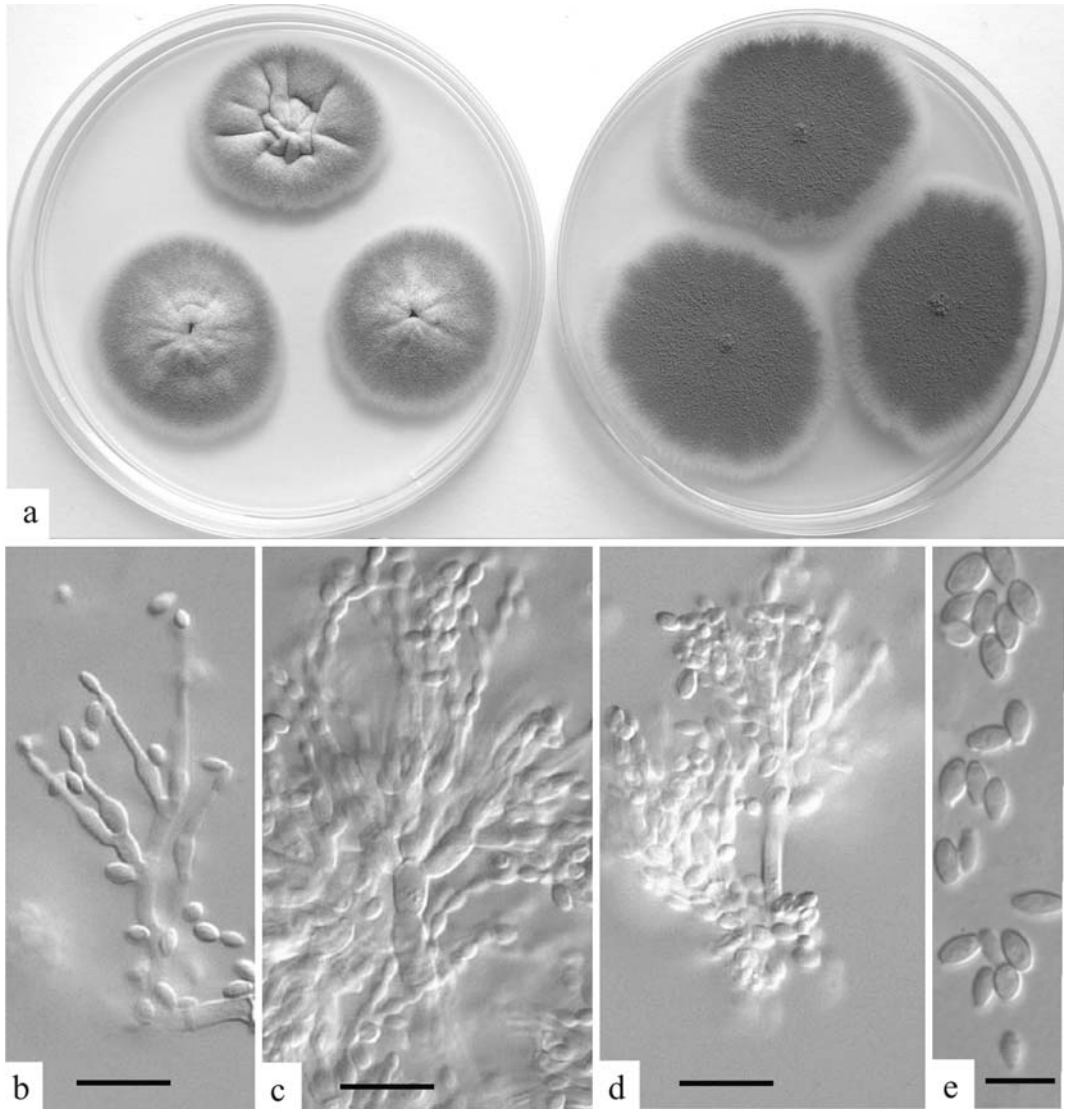


Fig. 7.9 *Paecilomyces variotii* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 µm; **(e)** conidia, bar = 5 µm

products and beverages, including sports drinks. It was found in 7% of all black peppercorns we examined from Indonesia (Pitt et al., 1998a), and at low levels in black beans, cassava, cashews and copra from Thailand (Pitt et al., 1994).

Paecilomyces variotii is notable for sorbate resistance and causes spoilage of margarine and processed cheeses due to production of 1,3-pentadiene from the degradation of sorbate (Sensidoni et al., 1994). It is also one cause of spoilage in dried fruits and other products. This is due to the "mouldy leather" odour of chloroanisole produced from chlorophenols (Tindale et al., 1989; Whitfield et al., 1991).

References. Samson (1974); Udagawa and Suzuki (1994); Houbraken et al. (2006, 2008).

Paecilomyces lilacinus (Thom) Samson Fig. 7.10

Penicillium lilacinum Thom

Colonies on CYA 25–35 mm diam, plane, dense to floccose; mycelium in marginal areas white, elsewhere pale pink or pinkish grey, sometimes also areas of bright pale yellow; conidial production sparse, pinkish brown; reverse pale, yellow or brown. Colonies on MEA 25–32 mm diam, low

and sparse, mycelium uniformly pinkish grey; reverse pale or centrally brown. Colonies on G25N 3–6 mm diam, low and dense. No germination at 5°C. At 37°C, response ranging from no growth to colonies up to 5 mm diam.

Conidiophores borne from aerial or surface hyphae, 200–600 μm long, with finely roughened walls, bearing irregular verticils of metulae both terminally and subterminally; phialides 7–10 × 2.5–3.0 μm, tapering to long, narrow collula; conidia ellipsoidal to fusiform, 2.5–3.0 × 2.0–2.2 μm, with smooth to finely roughened walls.

Distinctive features. This is the only pink species of *Paecilomyces* at all common in foods.

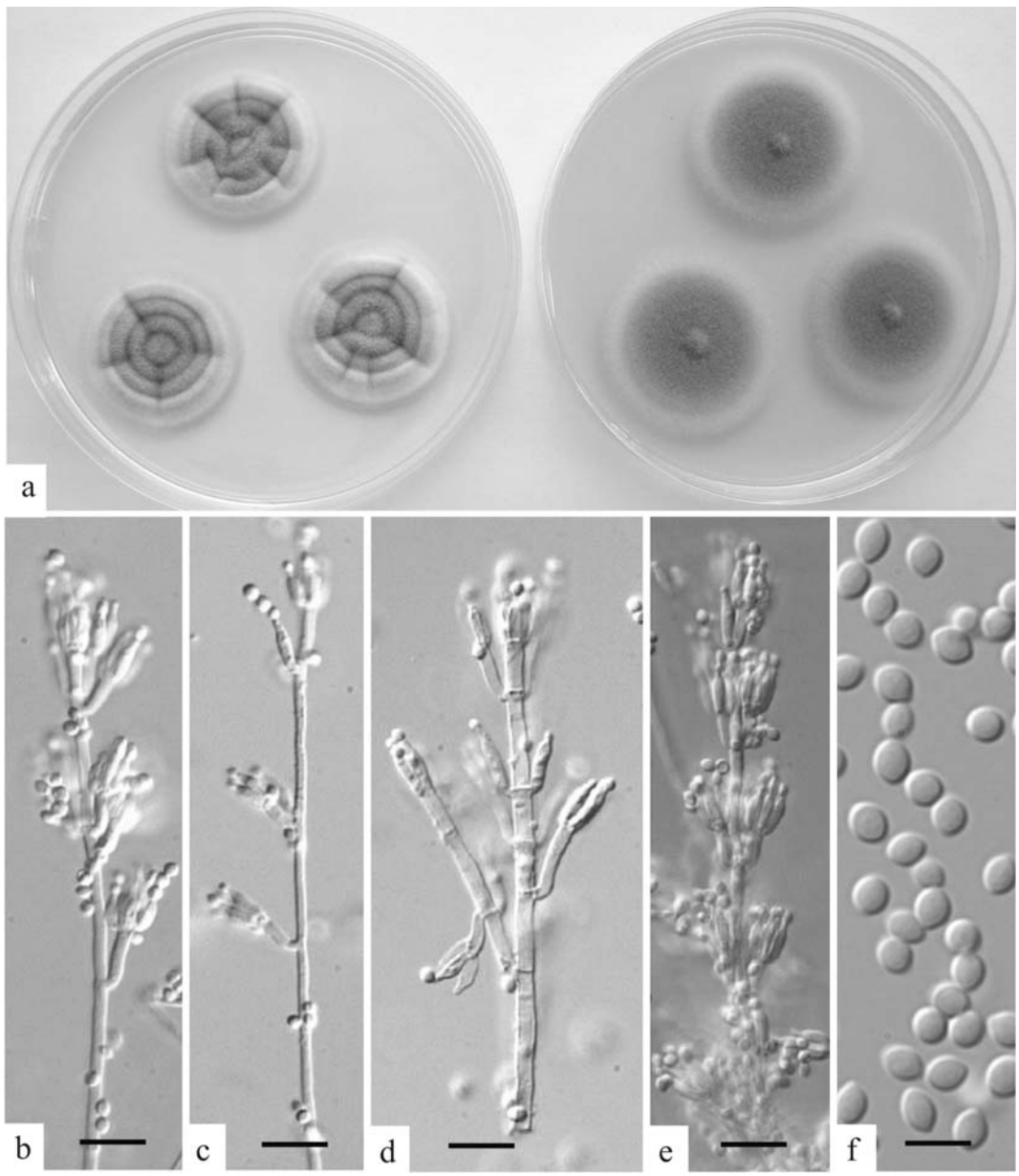


Fig. 7.10 *Paecilomyces lilacinus* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d, e)** penicilli, bars = 10 μm; **(f)** conidia, bar = 5 μm

Physiology. *Paecilomyces lilacinus* grows from 8 to 38°C and between pH 2 and 10 in submerged culture (Duncan, 1973). It is capable of growth down to at least 0.90 a_w in NaCl (Tresner and Hayes, 1971) or carbohydrate media (Panasenko, 1967).

Mycotoxins. Paecilotoxin is the only reported mycotoxin (Mikami et al., 1989). Its significance is unknown. Khan et al. (2003) recently tested one strain of *Paecilomyces lilacinus* for the production of paecilotoxin and were unable to show toxin production in that strain, suggesting that toxin synthesis may vary among isolates (Khan et al., 2003; Park et al., 2004b).

Ecology. Although a contaminant rather than a food spoilage fungus, *Paecilomyces lilacinus* has been isolated from a variety of foods: cereals, including wheat, flour and barley, peanuts and pecans, fresh figs, beans, frozen meat, salami (see Pitt and Hocking, 1997) and in our laboratory from bottled water and pandanus nuts from Papua New Guinea.

Reference. Samson (1974).

7.5 Genus *Scopulariopsis* Bainier

Like *Paecilomyces*, *Scopulariopsis* was segregated from *Penicillium* by Bainier (1907b); it is generally agreed that the differences from *Penicillium* are more fundamental. In *Scopulariopsis*, conidia are not extruded from phialides, but are cut off from annelides. The difference from a phialide is clearly evident: as each successive conidium is cut off from the tip of an annelide, a small amount of wall material remains, so that the annelide elongates, and in age shows a succession of faint rings or scars. A consequence is that conidia borne from annelides possess distinctive broad bases. In *Scopulariopsis*, this characteristic is readily seen under the microscope at high magnification.

Colonies of *Scopulariopsis* species range in colour from white to brown, but are never green or blue. Most species are broad ranging saprophytes, of common occurrence in decaying vegetation and soil. In comparison with their ubiquity in these habitats, they must be classed as relatively uncommon in foods. Only one species, *S. brevicaulis*, warrants description here.

Scopulariopsis brevicaulis (Sacc.) Bainier
Fig. 7.11

Colonies on CYA usually 40–50 mm diam, low, dense and velutinous, often irregularly wrinkled, coloured orange grey to brownish orange (5B2-C4); reverse bright yellow to orange brown. Colonies on MEA usually 40–50 mm diam, sometimes much smaller, 15–30 mm, low and sparse at the margins, sometimes centrally floccose, plane, coloured brownish orange (6C4-5); reverse yellow brown. Colonies on G25N 15–20 mm diam, low and dense, white or centrally yellow, reverse white to bright yellow. No growth at 5°C. At 37°C, colonies 7–20 mm diam, dense, often centrally raised, white or brown; reverse dull yellow.

Reproductive structures varying from single conidiogenous cells (annelides) to irregular penicilli, sometimes with well defined metulae and rami; conidia pyriform with a distinctly truncate base, clearly visible before release from the annelide, 5–8 μm diam, brown, with rough walls, sometimes adhering in short chains.

Distinctive features. Small single-celled conidia with truncate bases are characteristic of *Scopulariopsis*. Colonies of *S. brevicaulis* at 25°C are low, dense and brown. Growth at 37°C is slow. The brown, rough walled conidia are distinctive.

Physiology. The minimum a_w for growth of this species is 0.90 (Galloway, 1935). Better growth was observed in media supplemented with 0.1 to 1 M NaCl (Yoder et al., 2003).

Mycotoxins. Mycotoxins are not produced.

Ecology. Inagaki (1962) isolated *Scopulariopsis brevicaulis* from rice grains and rice flour, nonfat dried milk and butter. This species was reported to be a cause of spoilage in cheese (Northolt et al., 1980), along with other rarer species of *Scopulariopsis*. Other sources have been barley, wheat, salami, matured cheese, bacon and biltong (see Pitt and Hocking, 1997) and luncheon meats (Mohamed and Hussein, 2004). We have isolated this species from soybeans and black pepper from the Philippines, and peanuts and mung beans from Indonesia (Pitt et al., 1998a).

Reference. Morton and Smith (1963).

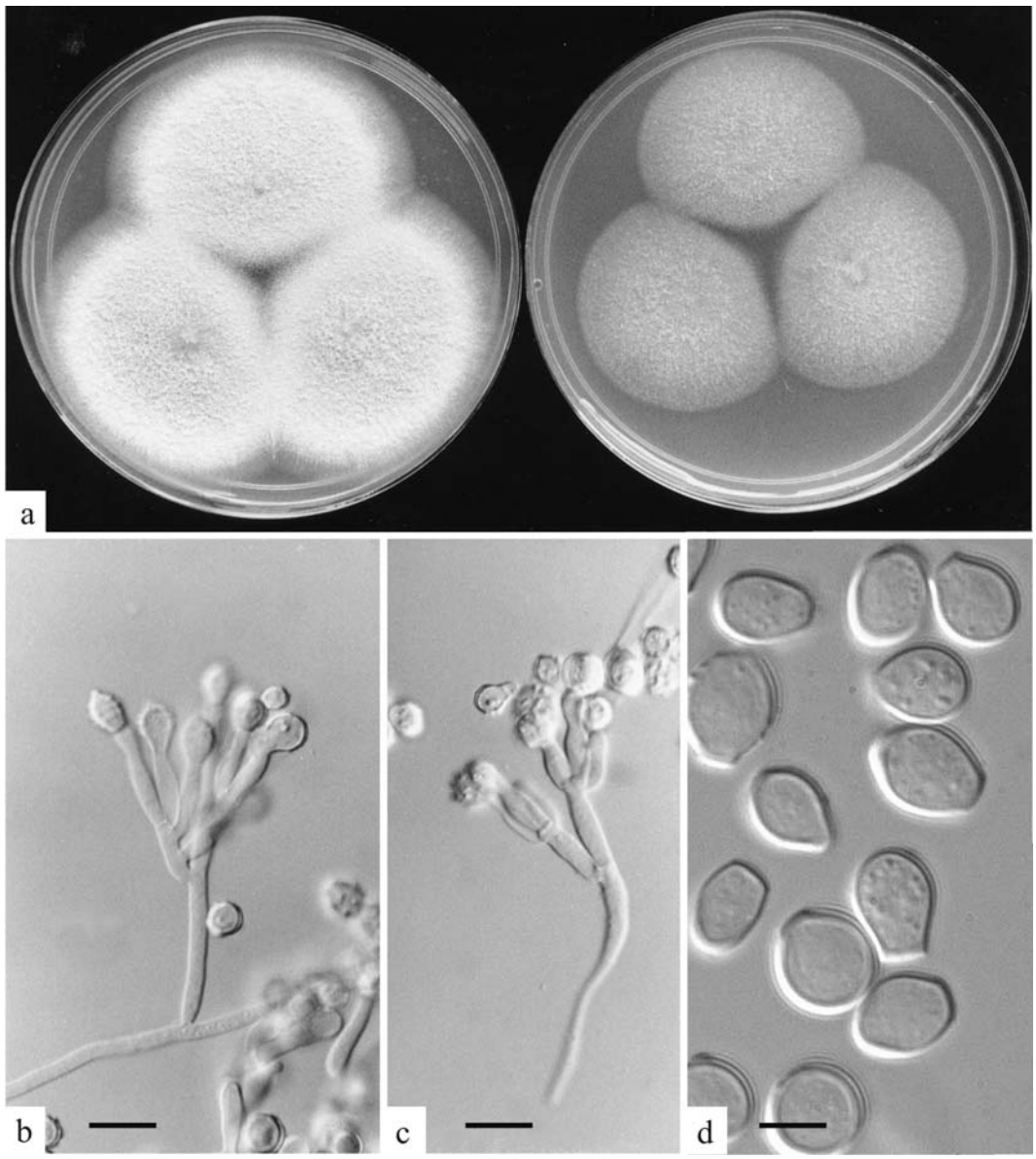


Fig. 7.11 *Scopulariopsis brevicaulis* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** penicilli, bars = 10 μm; **(d)** conidia, bar = 5 μm

7.6 Genus *Talaromyces* C.R. Benj.

The name *Talaromyces* is derived from the Greek word for a basket, which aptly describes the body in which asci are produced by this Ascomycete genus. Known as a gymnothecium, this ascocarp is composed of fine hyphae woven into a more or less closed structure of indeterminate size.

Talaromyces is characterised by the production of yellow or white gymnothecia in association with an anamorph characteristic of *Penicillium*, *Paecilomyces* or *Geosmithia*. The production of gymnothecia in association with other anamorphs characterises a variety of other genera, unknown in foods and outside the scope of this book.

As with *Eupenicillium*, until recently *Talaromyces* species with *Penicillium* anamorphs were commonly known by their *Penicillium* names. It is current practice for use of the anamorph names to be restricted to references to the anamorphs alone.

Talaromyces is a genus of about 25 species, mostly soil inhabiting. Their main interest to the food mycologist lies in their production of heat resistant ascospores and, consequently, their isolation from pasteurised fruit juices and fruit-based products (Hocking and Pitt, 1984). The most commonly isolated heat-resistant species is *T. macrosporus*, but we have also seen *T. trachyspermus* a number of times. The most common *Talaromyces* species in nature is *T. flavus*, which is occasionally isolated from commodities such as cereals. *T. wortmannii*, readily distinguished from *T. flavus* by its slower growth but similar in many other respects, is the only other species likely to be encountered in foods.

Key to *Talaromyces* species included here

1	Colonies exceeding 25 mm diam on MEA in 7 days	2
	Colonies not exceeding 25 mm diam on MEA in 7 days	3
2 (1)	Ascospores more than 5 μm long	*T. macrosporus*
	Ascospores less than 5 μm long	*T. flavus*
3 (1)	Colonies yellow	*T. wortmannii* (see *T. flavus*)
	Colonies white	*T. trachyspermus*

Talaromyces flavus (Klöcker) Stolk & Samson — Fig. 7.12

Penicillium vermiculatum P.A. Dang. (invalid name, teleomorph was included)
Talaromyces vermiculatus (P.A. Dang.) C.R. Benj.

Anamorph: *Penicillium dangeardii* Pitt

Colonies on CYA 18–30 mm diam, occasionally larger, plane, low and quite sparse to moderately deep and floccose; mycelium bright yellow, less commonly buff or reddish brown, in most isolates concealing developing gymnothecia; conidial production usually sparse and inconspicuous, but if more profuse, greenish grey; clear to reddish exudate present occasionally; reverse sometimes yellow, more usually orange, reddish or brown. Colonies on MEA 30–50 mm diam, generally similar to those on CYA but gymnothecia more abundant; reverse usually dull orange or brown, but sometimes deep brown or deep red. Colonies on G25N 2–7 mm diam, low, of sparse white mycelium; occasionally microcolonies or no growth. No germination at 5°C. At 37°C, colonies 20–45 mm diam, usually similar to those on CYA, but sometimes with white or brown mycelium or overlaid with grey conidia; gymnothecia absent; reverse yellow, orange or brown.

Gymnothecia of tightly interwoven mycelium, bright yellow, about 200–500 μm diam, closely packed, maturing within 2 weeks; ascospores yellow, ellipsoidal, 4.0–5.0 μm long, with spinose walls. Conidiophores borne from aerial hyphae, stipes 20–80 μm long, bearing terminal biverticillate or less commonly monoverticillate penicilli; phialides acerose, 10–16 μm long; conidia ellipsoidal to fusiform, 2.5–4.0 μm long, with smooth to spinulose walls.

Distinctive features. *Talaromyces flavus* and the closely related *T. macrosporus* grow relatively rapidly on MEA, produce bright yellow colonies at both 25 and 37°C, and usually produce abundant yellow gymnothecia, especially on MEA. *T. flavus* produces ascospores less than 5 μm long, smaller than those of *T. macrosporus*.

Physiology. Beuchat (1988) and Quintavalla and Spotti (1993) reported that the heat resistance of *Talaromyces flavus* ascospores (in both cases using strain FRR 1265) was substantially lower than that of isolates with larger ascospores now known to belong to *T. macrosporus*. No other studies have been published on the physiology of this species.

Mycotoxins. This species is not known to produce mycotoxins.

Ecology. This is by far the most common species of *Talaromyces* found universally in soils in warmer climates. Consequently it occurs in foods from time to time, most probably as a contaminant rather

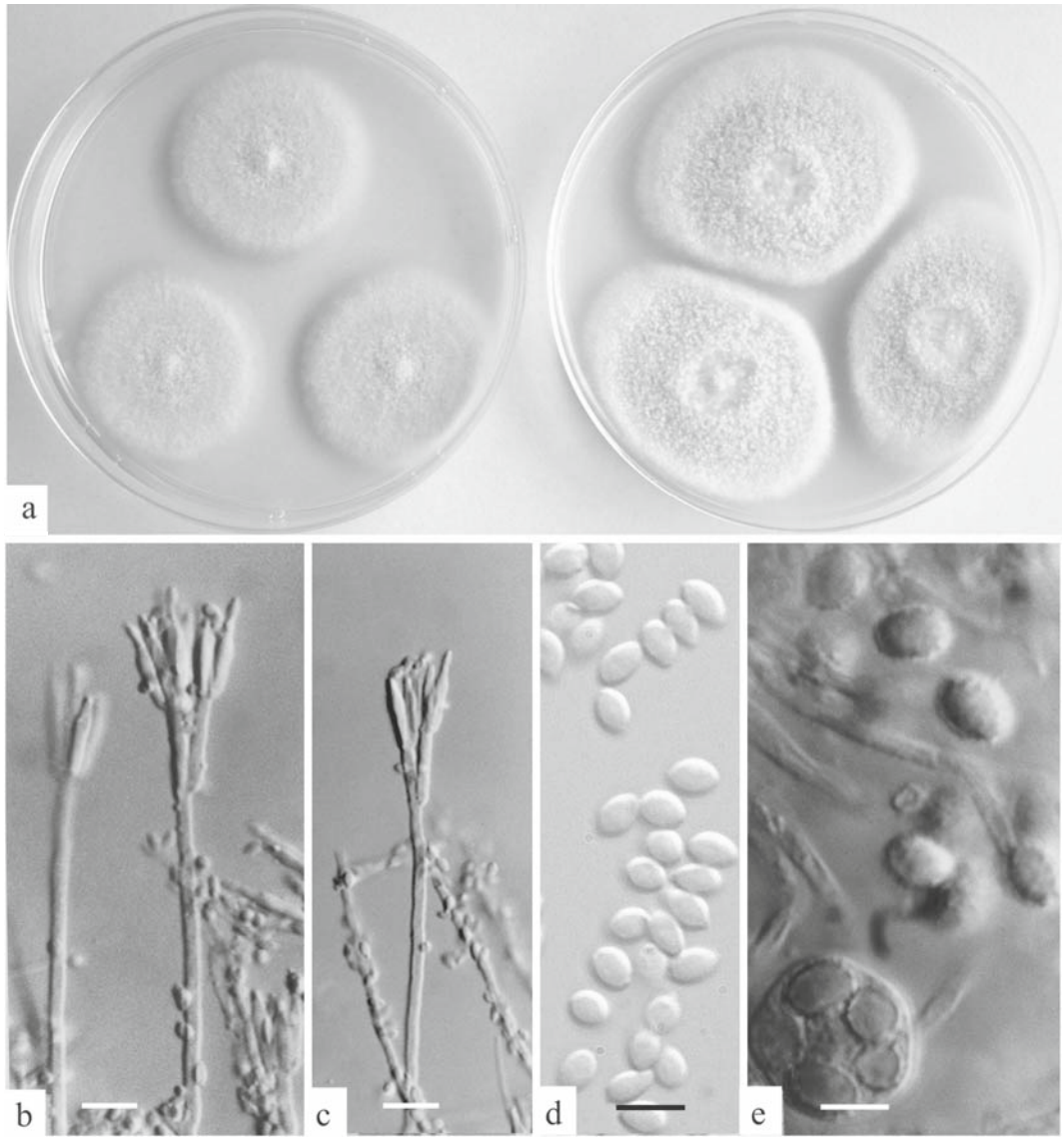


Fig. 7.12 *Talaromyces flavus* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** penicilli, bars = 10 μm; **(d)** conidia, bar = 5 μm; **(e)** ascus and ascospores, bar = 5 μm

than as a spoilage fungus. Reports of *T. flavus* as a heat-resistant fungus have always referred to *T. macrosporus* so far as can be established.

Additional species. *Talaromyces wortmannii* (Klöcker) Stolk and Samson (anamorph *Penicillium kloeckeri* Pitt; synonym *P. wortmannii* Klöcker) resembles *T. flavus* closely in colony colours, appearance, gymnothecia, ascospores and penicilli. However, the two species are readily distinguished by differences in growth rates: *T. wortmannii* produces colonies only 10–15 mm and 15–20 mm diam on CYA and MEA, respectively, at 25°C; at 37°C, colonies are less than 10 mm diam. Hocking and Pitt (1979) reported the minimum a_w for growth of *T. wortmannii* as 0.88 at 25°C.

Isolates of *Talaromyces wortmannii* produce the mycotoxin rugulosin (Frisvad et al., 1990b) (see *Penicillium rugulosum*), but this is unlikely to be of practical importance.

Like *Talaromyces flavus, T. wortmannii* is a soil fungus. It is widespread, but relatively uncommon. It sometimes occurs in foods, e.g. wheat, pecans and salami (see

Pitt and Hocking, 1997). There are no reports of spoilage, or of occurrence in pasteurised products.

References. Stolk and Samson (1972); Pitt (1979b).

Talaromyces macrosporus (Stolk & Samson) Frisvad et al. Fig. 7.13

Talaromyces flavus var. *macrosporus* Stolk & Samson

Anamorph: *Penicillium macrosporum* Frisvad et al.

Colonies on CYA of variable size, usually 20–40 mm diam, plane, floccose; mycelium pale to bright yellow (3A2-4); immature gymnothecia sometimes present, bright yellow (2-3A6); conidial production sparse and inconspicuous; exudate clear or more commonly red; soluble pigment usually present, orange red (8A6-8); reverse brown to dark brown, or, in the presence of soluble pigment, dark red approaching black. Colonies on MEA usually 45–55 mm diam, sometimes smaller, plane, usually floccose, mycelium pale yellow (3A2-4), usually enveloping abundant developing gymnothecia, bright yellow (2-3A6); exudate and soluble pigment absent; reverse often strongly coloured, orange, brown, red or olive. On G25N, microcolonies to colonies 7 mm diam formed. No germination at 5°C. At 37°C, colonies usually 40–50 mm diam, occasionally less, plane, floccose, mycelium white to pale yellow; gymnothecia not produced; red exudate and soluble pigment sometimes present; reverse pale, brown, or in the presence of soluble pigment, dark red.

Gymnothecia borne as a contiguous layer on the agar surface, or sometimes discrete, 1.0–1.5 mm diam, composed of fine, bright yellow hyphae, maturing within 2 weeks; ascospores ellipsoidal, commonly 5.0–6.5 μm long, with spinose walls. Conidiophores borne from aerial hyphae, sparsely produced, stipes 20–80 μm long, bearing terminal biverticillate or less commonly monoverticillate penicilli; phialides acerose, 10–16 μm long; conidia ellipsoidal to fusiform, 2.5–4.0 μm long, with smooth to spinulose walls.

Distinctive features. Like the closely related *Talaromyces flavus*, *T. macrosporus* grows relatively rapidly on MEA, produces bright yellow colonies at both 25 and 37°C, and usually forms abundant yellow gymnothecia. *T. macrosporus* produces ascospores that are more than 5 μm long.

Taxonomy. Established for strains of *Talaromyces flavus* with large ascospores (Stolk and Samson, 1972), *T. flavus* var. *macrosporus* was not recognised by Pitt (1979b). On the basis of differences in secondary metabolite production, Frisvad et al. (1990b) raised the variety to species status. Moreover, the quite frequent recovery of isolates with large ascospores from heat-processed foods (Hocking and Pitt, 1984) indicates a real physiological difference from isolates with small ascospores. *T. macrosporus* is recognised here for isolates with ascospores more than 5 μm long.

Physiology. From the food industry viewpoint, the main attribute of *Talaromyces macrosporus* is the very high heat resistance of its ascospores. Published decimal reduction times and *z* values vary: a D_{88} of 7–22 min, a D_{91} of 2.9–5.4 min (Beuchat, 1986), a D_{90} of 2–7 min and a *z* value of 10.3C° (King and Halbrook, 1987), a D_{90} of 2.2 min and a *z* value of 5.2C° (Scott and Bernard, 1987), a D_{80} of 190 min and a D_{90} of 6 min and a *z* value of 6.7C° (King and Whitehand, 1990). Dijksterhuis and Teunissen (2004) demonstrated a D_{85} of 30–100 min. These values are considerably higher than reported for *Byssochlamys* species (Beuchat, 1986). The influence of organic acids, including preservatives, on the heat resistance of *T. flavus* was reported by Beuchat (1988).

Ascospores of *Talaromyces macrosporus* contain trehalose, which is degraded to glucose following heat activation. Upon germination, glucose is released from the ascospores. The presence of trehalose in ascospores is thought to act as a stress protectant against dehydration and heat (Dijksterhuis et al., 2002). The effect of sugars such as trehalose in protecting vital enzymes from heat has been shown in other organisms such as *Neurospora crassa* (Dijksterhuis et al., 2002).

Dijksterhuis and Teunissen (2004) showed that high pressure treatments at 400–800 MPa activated ascospores to germinate. The authors explained that high pressure constituted a stress which induced germination, in a situation akin to heat (Dijksterhuis et al., 2002). Ascospore age also affects heat resistance, with mature ascospores being more resistant to heat. This phenomenon has also been shown in ascospore suspensions, where young ascospores stored in buffer developed increased heat resistance over time (Dijksterhuis and Teunissen, 2004).

Mycotoxins. This species has been reported to produce duclauxin, a secondary metabolite of

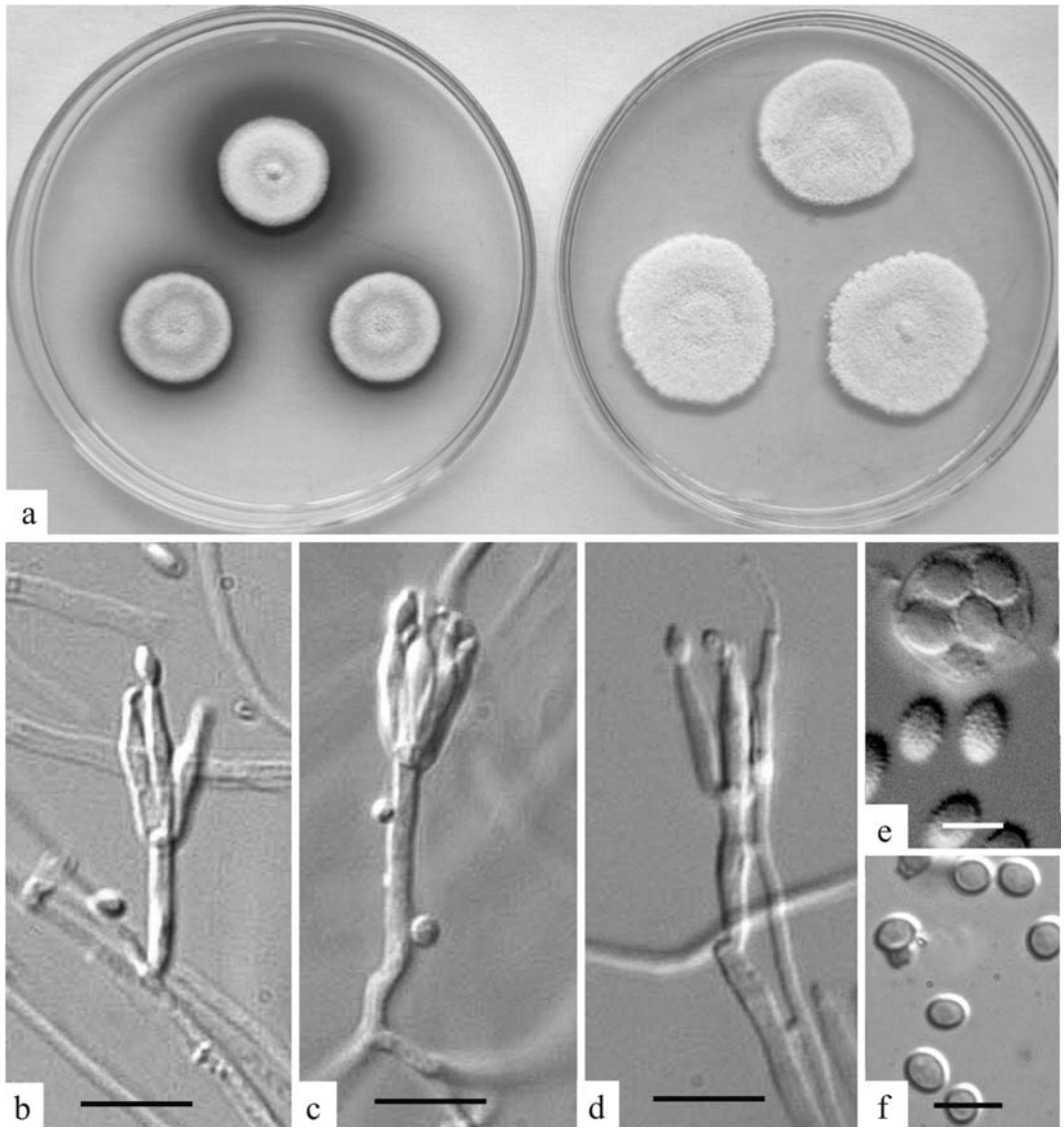


Fig. 7.13 *Talaromyces macrosporus* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 µm; **(e)** ascospores, bar = 5 µm; **(f)** conidia, bar = 5 µm

doubtful significance as a mycotoxin (Frisvad et al., 1990a).

Ecology. *Talaromyces macrosporus* (sometimes reported as *T. flavus*) has been reported fairly frequently from heat processed juices (see Pitt and Hocking, 1997), often as a cause of spoilage. *T. macrosporus* has rarely been reported away from heat processed juices. It has been found in low levels in pasteurised dairy products (Aydin et al., 2005).

References. Stolk and Samson (1972); Frisvad et al. (1990a).

Talaromyces trachyspermus (Shear) Stolk & Samson Fig. 7.14

Talaromyces spiculosporus (Lehman) C. R. Benj.

Anamorph: *Penicillium lehmannii* Pitt

Colonies on CYA 15–20 mm diam, occasionally smaller, plane, sparse at the margins, otherwise floccose; mycelium white; gymnothecia not produced; conidial production sparse; reverse pale to dull yellow brown. Colonies on MEA 20–25 mm diam, sparse at the margins but floccose and more

dense centrally; mycelium white to creamish; gymnothecia tardily produced, few if any conidial structures, reverse pale to deep orange brown. On G25N, no growth or microcolonies. No germination at 5°C. At 37°C, colonies 25–40 mm diam, sparse at the margins, but deeper and floccose centrally; mycelium white, usually enveloping developing gymnothecia; conidial formation sparse or absent; reverse pale to dull brown.

Gymnothecia of densely woven hyphae, cream to pale yellow when mature (2–3 weeks); 300–500 µm diam; ascospores uncoloured, ellipsoidal, 3.5–4.5 µm long, with spinose walls. Conidiophores borne from aerial hyphae, short stipes (5–20 µm long) bearing terminal penicilli, biverticillate or monoverticillate, sometimes with subterminal elements; phialides acerose, 8–15 µm long, conidia variable, ellipsoidal to fusiform, 2.5–4.5 µm long, with smooth walls.

Distinctive features. *Talaromyces trachyspermus* colonies are slow growing, pale, with straw coloured gymnothecia produced readily at 30–37°C after 7–14 days.

Physiology. Ascospores of *Talaromyces trachyspermus* are heat resistant. *T. trachyspermus* was isolated

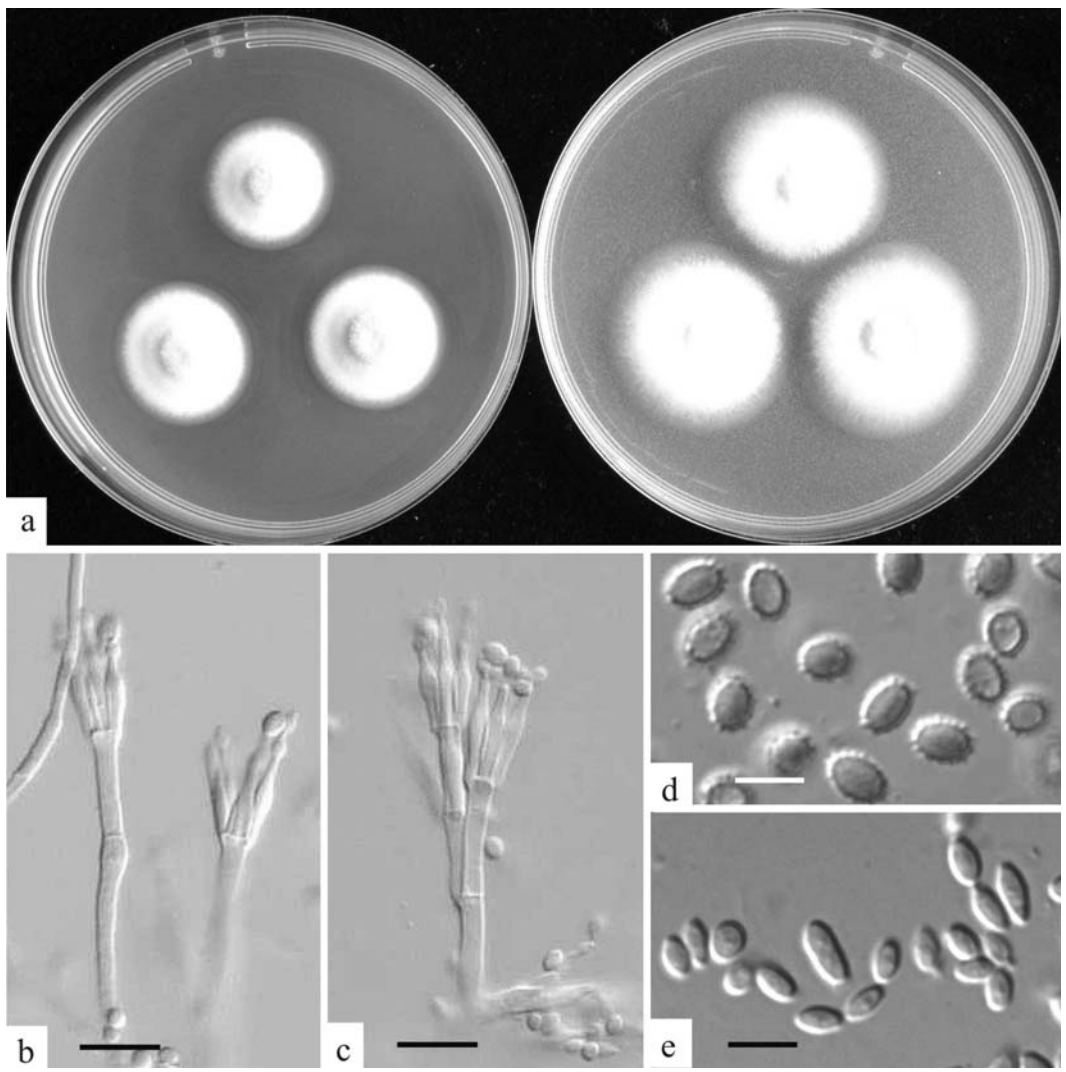


Fig. 7.14 *Talaromyces trachyspermus* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** penicilli, bars = 10 µm; **(e)** ascospores, bar = 5 µm; **(f)** conidia, bar = 5 µm

from pineapple concentrate heated at 80°C for 30 min (Enigl et al., 1993) and we have isolated it from a tea-based beverage that had received a heat treatment of 94°C for 30 sec. Jesenská et al. (1992) isolated *T. trachyspermus* from soil that had been heated at 70°C for 60 min, but not from samples that were heated at 80°C for 60 min. The D_{80} value for *T. trachyspermus* is probably in the order of 5–10 min with a *z* value of 2.6 ± 0.2C° (A.D. King, pers. comm.).

Mycotoxins. No mycotoxins have been reported from *Talaromyces trachyspermus*.

Ecology. The natural habitat for *Talaromyces trachyspermus* is soil. It has been reported from soil in Nepal (Minoura et al., 1975), India (Rajak et al., 1991) and the Slovak Republic (Jesenská et al., 1992). Isolates in the CBS catalogue have come from soil in Morocco, Nigeria and Germany and bird dung in Venezuela. Food sources include pineapple juice and concentrate (Enigl et al., 1993), canned strawberries, pasteurised drinking yoghurt and honey tea with ginsing (CBS catalogue). In our laboratory all isolates of *T. trachyspermus* have come from heat processed products: fruit juice jelly, pineapple slices, canned strawberries and an iced tea beverage.

References. Stolk and Samson (1972); Pitt (1979a).

7.7 Genus *Penicillium* Link

While it is arguable whether *Aspergillus* or *Penicillium* is of greater economic importance as a cause of food spoilage, it is certain that *Penicillium* is the more diverse genus, in terms of numbers of species and range of habitats.

Penicillium species are ubiquitous, opportunistic saprophytes. Nutritionally, they are supremely undemanding, being able to grow in almost any environment with a sprinkling of mineral salts, any but the most complex forms of organic carbon and a wide range of physico-chemical environments, i.e. a_w, temperature, pH and redox potential.

A majority of the described species are soil fungi, and their occurrence in foods is more or less accidental and rarely of consequence. However, quite a number of species are closely associated with human food supplies. Some species are more specialised: several are destructive pathogens on fruit (e.g. *Penicillium digitatum*, *P. expansum, P. italicum*); a few grow below 0.80 a_w (e.g. *P. brevicompactum*, *P. chrysogenum*, *P. implicatum*), at low oxygen tension (e.g. *P. roqueforti)* or are preservative resistant (*P. roqueforti*). Many are psychrotrophic and capable of causing food spoilage at refrigeration temperatures.

A problem with attempting to establish the incidence of *Penicillium* species in foods is that so many accounts have not identified Penicillia to species level; as a result many surveys of Penicillia in foods are of little value in the present context. Reports on the occurrence of *Penicillium* species in this book have therefore relied on a relative handful of papers which have provided detailed species lists. Given the taxonomic difficulties of the genus, it is unlikely that these reports are entirely accurate, but in our view they provide the best information available.

Taxonomy. *Penicillium* taxonomy has evolved rapidly since the last complete taxonomy (Pitt, 1979b) was published. Details of these developments may be found elsewhere (Pitt, 1989a; Pitt and Samson, 1990a, b, 1993); the important result is that consensus has been reached on the names and circumscriptions of most species. Publication of a list of "Names in Current Use (NCU)" means that names in *Penicillium*, *Aspergillus* and related genera now enjoy protection from earlier names under the International Code of Botanical Nomenclature (Pitt and Samson, 1993; Greuter et al., 1994: p. *x*). In consequence, the taxonomy of *Penicillium* has now become quite stable. Fortunately, all of these more recent changes have had little effect on the names of well known species. None of the *Penicillium* names used either in the previous edition of this book (Pitt and Hocking, 1997) or in the current taxonomy of common species (Pitt, 2000) have required change. However, the taxonomy of the subgenus *Penicillium* has recently been revised by Samson and Frisvad (2004). The number of species in this subgenus has been increased from about 25 to over 50.

Penicillium taxonomy is not easy for the inexperienced. The species commonly occurring in foods are mostly similar in colour and general colony appearance. Reproductive structures are small and often ephemeral. However, it is the authors' belief that identification of a high percentage of isolates to species level can be accomplished if isolates are grown under standardised conditions of medium and temperature and examined after a relatively short time (seven days), so that fruiting structures

and colony colours are at their best. Colony diameters are readily measured and provide very valuable information. The standard conditions used throughout this book were originally developed specifically for *Penicillium* taxonomy. The cultural conditions used and the general principles of colony examination, etc., have been outlined in Chapter 4.

As with the other genera described in this chapter, the reproductive structure characteristic of *Penicillium* is a conidiophore with a relatively delicate, distinct stipe terminating in a penicillus. The form of the penicillus determines the primary taxonomic division of the genus into subgenera.

To determine the subgenus to which a *Penicillium* isolate belongs, count the number of branch points between phialide (or conidial chain) and stipe, down the main axis of the penicillus. In the simplest subgenus, *Aspergilloides*, there is one branch point: conidia are borne from phialides (the primary conidiogenous cell), which in turn are borne directly from the stipe as a single whorl or verticil (Fig. 7.15a, b). Such penicilli are termed monoverticillate.

In its most complex form, the penicillus has characteristically three branch points (terverticillate), and in some species not infrequently four branch points (quaterverticillate), between phialide and stipe (Fig. 7.15c). Species with such penicilli are classified in subgenus *Penicillium*. As a useful check, nearly all the commonly encountered species in this subgenus characteristically grow to 18 mm in diameter or more on G25N medium in 7 days at 25°C.

Of intermediate complexity are biverticillate penicilli, i.e. ones having two branch points. In this category, there are two distinct subgenera, *Furcatum* and *Biverticillium* (Fig. 7.15d, e, f). Until familiarity breeds confidence, distinguish these two subgenera by observing the following characters: the relative lengths of phialides and their supporting cells (metulae); the number of metulae per stipe; colony diameters on G25N; and if all else fails, the shape of the phialides. The differences between these two subgenera are shown in Table 7.1.

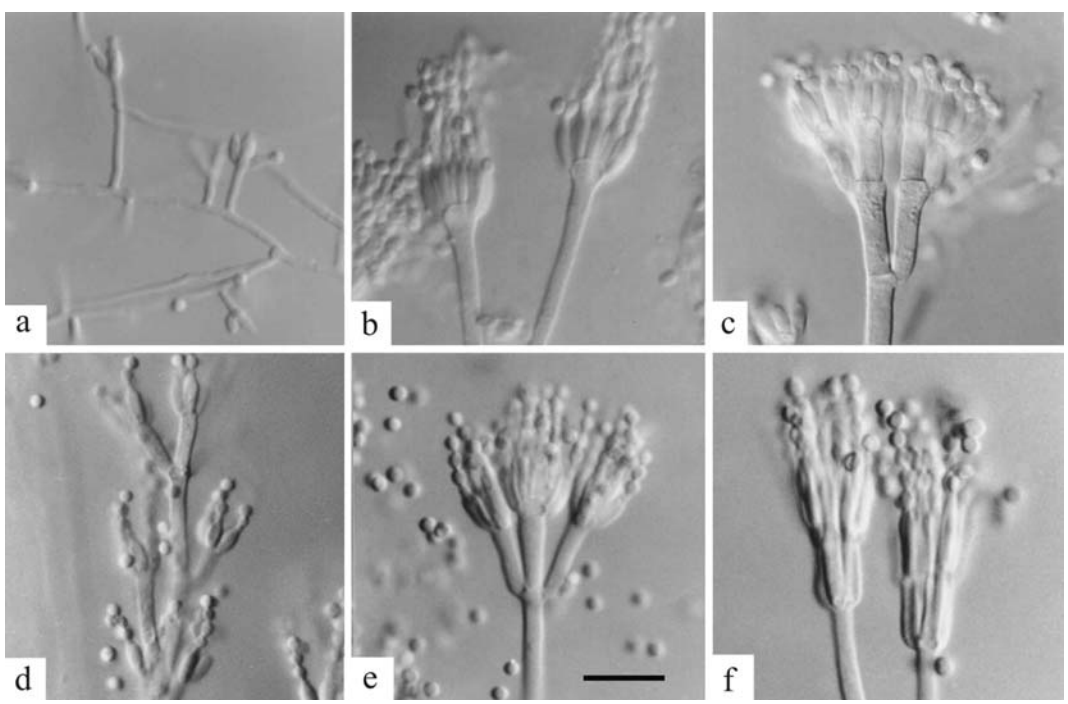


Fig. 7.15 Penicillus types in *Penicillium* species **(a, b)** monoverticillate; **(c)** terverticillate; **(d, e)** biverticillate, subgenus *Furcatum*; **(f)** biverticillate, subgenus *Biverticillium*; bar = 10 μm

Table 7.1 Characters distinguishing subgenus *Furcatum* from subgenus *Biverticillium*

	Subgenus *Furcatum*	Subgenus *Biverticillium*
Ratio of metula length to phialide length	Much greater than one	Approximately one (1–1.2)
Metulae per stipe	Not exceeding five	Usually exceeding five
Colony diam on G25N	9–18 mm	Less than 10 mm
Phialide shape	Flask shaped, gradually tapering to neck (ampulliform)	Parallel sided, abruptly tapering to neck(acerose)

Because of the size and complexity of the genus, no general key to *Penicillium* is given. Taxonomy is simplified by providing a key to subgenera here, and then keys to species in the preamble to each subgenus.

Key to subgenera of *Penicillium*

1	Penicilli monoverticillate or with only a minor proportion bearing metulae	Subgenus *Aspergilloides*
	Penicilli commonly biverticillate or more complex	2
2 (1)	Penicilli predominantly biverticillate or irregularly monoverticillate and biverticillate; colonies on G25N rarely exceeding 18 mm diam	3
	Penicilli predominantly terverticillate; colonies on G25N not usually less than 18 mm diam	Subgenus *Penicillium*
3 (2)	Penicilli biverticillate or, much less often, terverticillate; ratio of metula to phialide length near one; colonies on G25N less than 10 mm diam	Subgenus *Biverticillium*
	Penicilli biverticillate or irregularly monoverticillate and biverticillate; ratio of metula to phialide length much greater than one; colonies on G25N more than 9 mm diam (see also Table 7.1)	Subgenus *Furcatum*

7.7.1 Penicillium *subgenus* Aspergilloides *Dierckx*

In this subgenus conidiophores are strictly or predominantly monoverticillate, i.e. with phialides borne directly on the stipe, with only one branch point between stipe and conidial chain. In some species, metulae may be present on a proportion of stipes (less than 50%).

The distinction between species considered to produce monoverticillate or biverticillate penicilli is not always obvious. Some species produce penicilli on hyphal branches which can be interpreted either as short monoverticillate stipes or as metulae. The distinction made here is that if such a hypha, as well as producing intercalary branches, terminally produces a cluster of two or more branches at an acute angle, these are interpreted as metulae, the penicillus as biverticillate, and the species is placed in subgenus *Furcatum*. Where the hypha gives rise only to branches at right angles along its length and neither hypha nor branches have terminal clusters of metulae, the hypha is interpreted as a fertile hypha, each branch as a stipe, the penicilli as monoverticillate, and the species is classified in subgenus *Aspergilloides*.

One character used to differentiate species in subgenus *Aspergilloides*, but not elsewhere in the genus, is the presence or absence of terminal swellings (vesicles) on the stipes. A stipe is considered to be vesiculate when the terminal swelling is twice the stipe diameter or more. If in doubt, examine colonies grown on MEA, as vesicles are sometimes more obvious on this medium.

Key to *Penicillium* subgenus *Aspergilloides* species included here

1	Conspicuous orange, yellow, brown or purple mycelium, soluble pigment or reverse colours on CYA	2
	Colonies on CYA lacking bright colours	5
2 (1)	Colonies on CYA coloured brown and/or purple	*P. phoeniceum* (see *Eupen. cinnamopurpureum*)
	Colonies on CYA coloured orange or yellow	3

3 (2)	Stipes usually vesiculate	*P. sclerotiorum*
	Stipes not or rarely vesiculate	4
4 (3)	Colonies at 37°C more than 15 mm diam	*P. hirayamae* (see *Eupen. hirayamae*)
	Colonies at 37°C less than 15 mm diam or growth absent	*P. citreonigrum*
5 (1)	Colonies on CYA greater than 30 mm diam	6
	Colonies on CYA not greater than 30 mm diam	8
6 (5)	Stipes rough walled	*P. thomii*
	Stipes smooth walled	7
7 (6)	Growth at 37°C	*P. decumbens*
	No growth at 37°C	*P. glabrum*
		P. spinulosum
8 (5)	Stipes usually vesiculate	*P. implicatum*
	Stipes usually nonvesiculate	*P. restrictum*

Penicillium citreonigrum Dierckx **Fig. 7.16**

Penicillium citreoviride Biourge
Penicillium toxicarium L. Miyake (invalid name)

Colonies on CYA 20–28 mm diam, radially sulcate and often centrally wrinkled, dense and velutinous; mycelium white to bright yellow; conidia sparse to moderately abundant, greenish grey (27C-D2); exudate present only rarely, clear to pink; yellow soluble pigment typically produced; reverse usually brilliant yellow, occasionally yellow brown. Colonies on MEA 22–26 mm diam, plane to lightly sulcate, low, dense and velutinous; mycelium white, becoming yellow or buff centrally; conidial production moderate, in colours similar to those on CYA or slightly bluish; exudate produced rarely, clear to yellow; brown soluble pigment sometimes produced; reverse pale, brown or deep reddish brown. Colonies on G25N 11–14 mm diam, coloured similarly to those on CYA; reverse pale to brilliant yellow or brown. At 5°C, germination to formation of microcolonies. At 37°C, typically no growth, occasionally colonies up to 10 mm diam formed.

Conidiophores borne from floccose or less commonly funiculose aerial hyphae, stipes slim and delicate, 60–100 μm long, smooth walled, nonvesiculate, monoverticillate, occasionally with two metulae; phialides ampulliform, length varying with isolate, 5–12 μm long; conidia spherical or near, 1.8–2.8 μm diam, with walls smooth to very finely roughened, borne in short disordered chains.

Distinctive features. *Penicillium citreonigrum* produces compact yellow colonies which at most grow weakly at 5 and 37°C; stipes are slender, not apically enlarged; conidia are tiny and smooth walled.

Taxonomy. Raper and Thom (1949) used the name *Penicillium citreoviride* for this species. Pitt (1979b) took up the earlier valid name *P. citreonigrum*, and this has been accepted.

Physiology. The physiology of *Penicillium citreonigrum* has been little studied. Cardinal temperatures are below 5°C, 20–24°C and 37–38°C (ICMSF, 1996b). Ji et al. (2007) reported an optimum growth temperature of 27–30°C for *P. citreonigrum* (as *P. citreoviride*). They recorded growth at 0.80 a_w at 20, 25 and 30°C, but no growth occurred at 15°C at a_w values from 0.80 to 0.90. However, the authors allowed only 24 h for equilibration of the rice cakes (1.5 cm thick) from an initial a_w of 0.92, and as the actual a_w did not appear to have been measured, we would consider their data on effect of a_w on growth to be unreliable. The minimum a_w for growth is not known, but this species is probably a xerophile.

Mycotoxins. *Penicillium citreonigrum* is the major source of citreoviridin (El-Banna et al., 1987b), the cause of the Oriental disease known in Japan as acute cardiac beriberi. Recognised for the past three centuries (Ueno and Ueno, 1972), this disease frequently occurred in young healthy adults, and death could occur within a few days. The work of Sakaki in the 1890s (Ueno and Ueno, 1972) implicated mouldy "yellow rice" as a probable cause and led to a ban on the sale of yellow rice in Japan in 1910. The disease in Japan is now only of historical interest. Uraguchi (1969) and Ueno and Ueno (1972) showed that acute cardiac

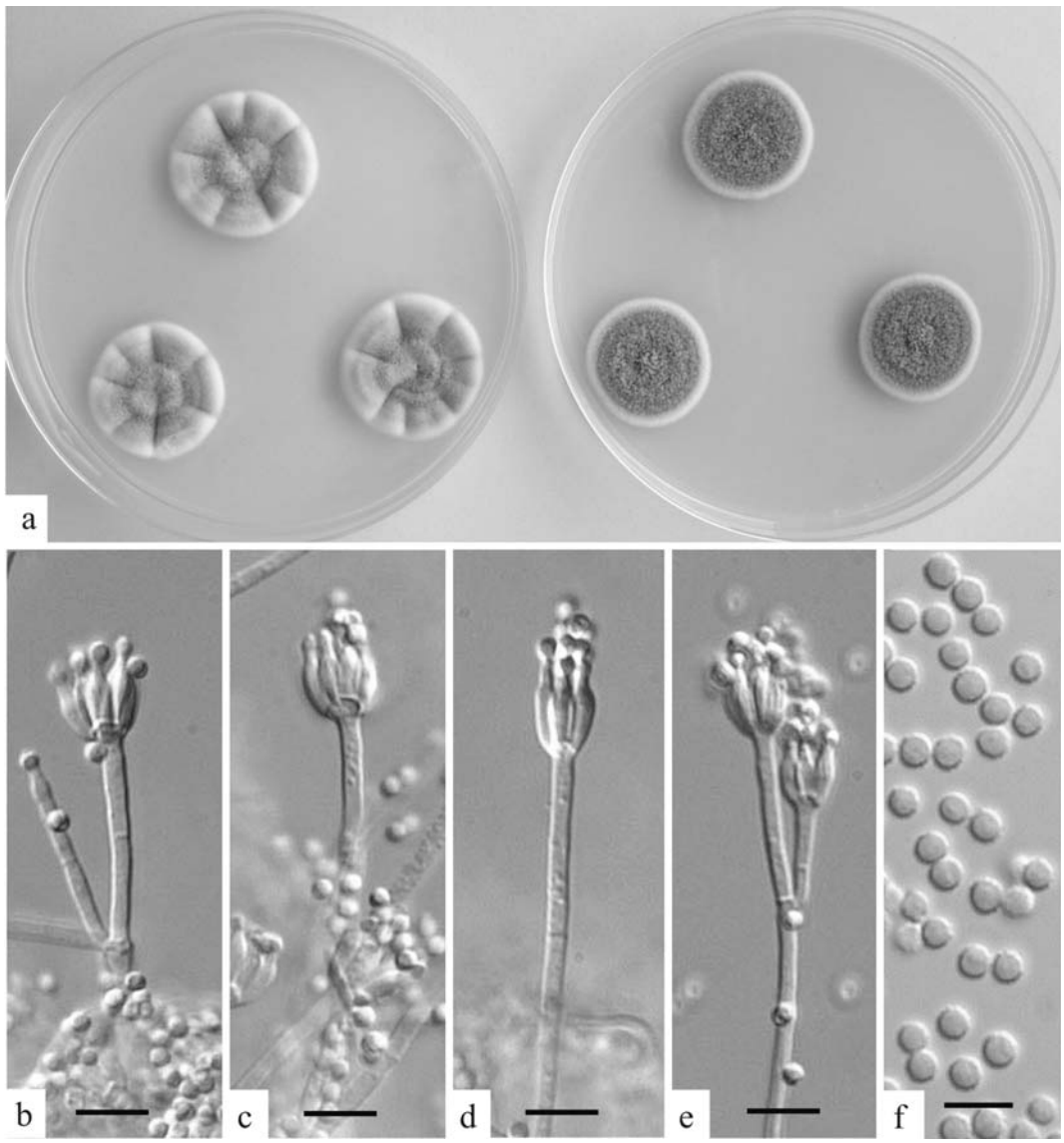


Fig. 7.16 *Penicillium citreonigrum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d, e)** penicilli, bars = 10 μm; **(f)** conidia, bar = 5 μm

beriberi was a mycotoxicosis due to the growth of *P. citreonigrum* in rice. Citreoviridin is produced from 10 to 37°C, with a maximum near 20°C (Ueno, 1972).

Also produced by *Eupenicillium ochrosalmoneum*, citreoviridin is an unusual molecule consisting of a lactone ring conjugated to a furan ring, with a molecular weight of 402 (Cole and Cox, 1981). It is a neurotoxin, acutely toxic to mice, with intraperitoneal and oral LD_{50}s of 7.5 and 20 mg/kg, respectively (Ueno and Ueno, 1972).

Ecology. Although not a commonly isolated species, *Penicillium citreonigrum* is very widely distributed. Its occurrence as a cause of spoilage of rice in Japan from time to time has been well documented. *P. citreonigrum* has been reported from other cereals by several authors, including Graves and Hesseltine (1966), Saito et al. (1971b) and Basu and Mehrotra (1976). It was isolated very occasionally in our laboratory during a survey of the microbiology of Australia wheat and flour milling (Hocking, unpublished). It has been isolated much less frequently from other foods: mung beans (Pitt et al., 1994), spices (Takatori et al., 1977) and jam (Udagawa et al., 1977). Magnoli et al. (2005)

isolated this species from pig feed, which consisted of 60% corn and also from rabbit feed in which corn was replaced by oats, alfalfa and barley.

References. Uraguchi (1971); Pitt (1979b, 2000).

Penicillium decumbens Thom **Fig. 7.17**

Colonies on CYA commonly 20–30 mm diam, occasionally 40 mm, low and dense, velutinous to lightly floccose; mycelium white to cream; conidial production light to moderate, greyish green to dull green (25C-D3); reverse pale, dull yellow brown or olive. Colonies on MEA 25–40 mm diam, usually plane, typically low and relatively sparse, less commonly floccose; moderate numbers of conidia produced, coloured as on CYA. Colonies on G25N 11–16 mm diam, usually rather sparse, in general terms similar to those on CYA. At 5°C, germination by a proportion of conidia up to formation of microcolonies. At 37°C, colonies 5–20 mm diam, velutinous to floccose, coloured white to grey green; reverse pale or brownish.

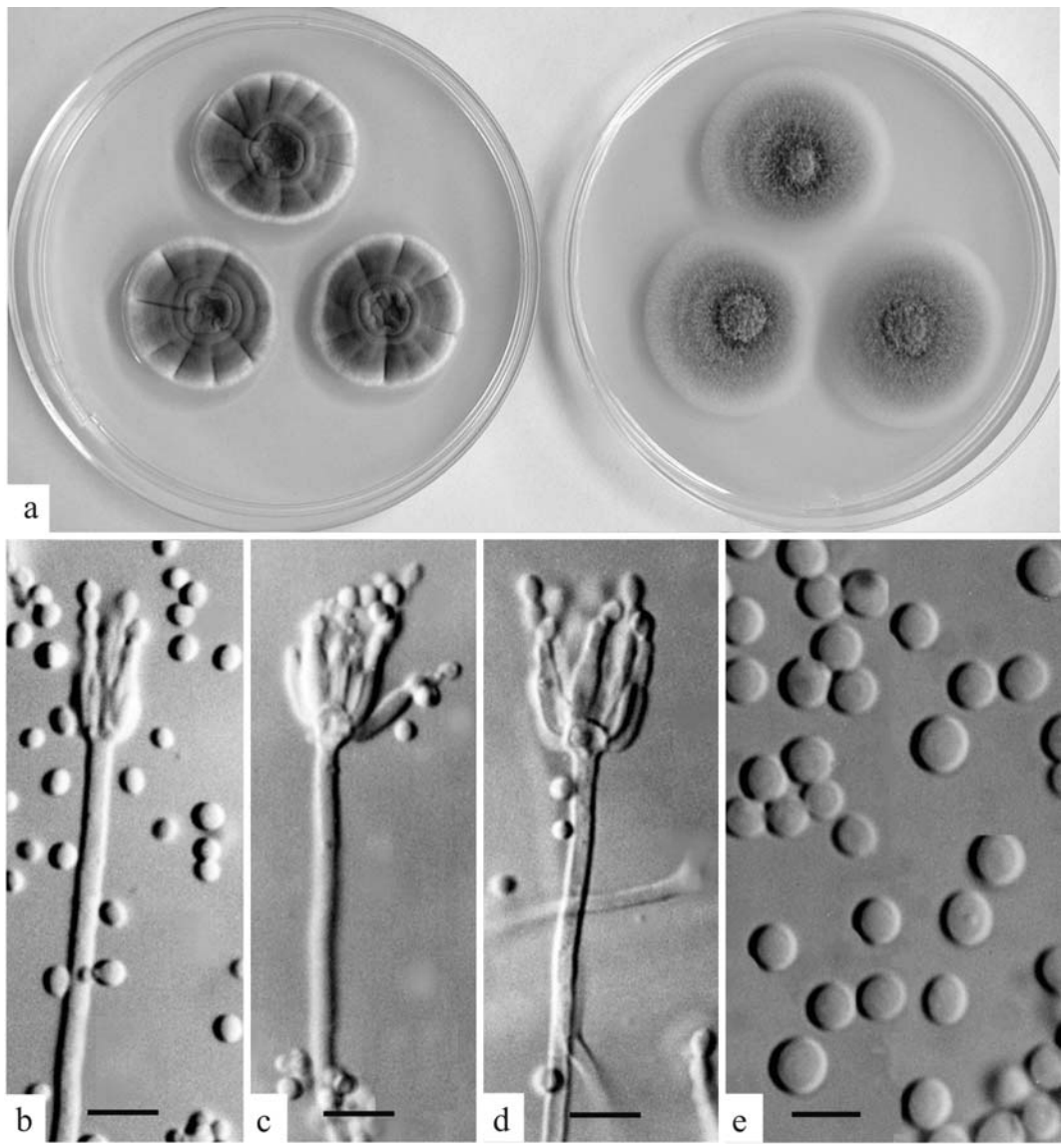


Fig. 7.17 *Penicillium decumbens* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 µm; **(e)** conidia, bar = 5 µm

Conidiophores borne from aerial hyphae, stipes short, 20–60(–100) μm long, with thin, smooth walls, monoverticillate, nonvesiculate to somewhat enlarged; phialides ampulliform, long and slender, 8–11(–14) μm long; conidia ellipsoidal, in some isolates also pyriform, smooth walled, 2.5–3.0(–4.0) μm long, borne in short, loose columns.

Distinctive features. *Penicillium decumbens* produces short, somewhat apically swollen, monoverticillate conidiophores from aerial hyphae, and smooth walled, distinctly ellipsoidal, dull green conidia. Colonies usually do not show any other colours. This is one of the few *Penicillium* species which typically grows at both 5 and 37°C.

Physiology. In the only recorded physiological study, *Penicillium decumbens* grew down to 0.87 but not 0.82 a_w at 20 and 30°C (Valero et al., 2007a).

Mycotoxins. No mycotoxins are known.

Ecology. A ubiquitous fungus, *Penicillium decumbens* has been isolated from a wide variety of foods. These include dried peas and beans (see Pitt and Hocking, 1997); several kinds of nuts (Pitt et al., 1998a); flour milling equipment (Hocking, unpublished), flour, rice, infant cereal preparations, soybeans, meat products and fresh vegetables (see Pitt and Hocking, 1997). This fungus was also isolated from Spanish grapes and dried vine fruits, although spoilage was not recorded (Valero et al., 2007a). Spoilage of food by this species appears to be unusual.

References. Pitt (1979b, 2000).

Penicillium glabrum (Wehmer) Westling

Fig. 7.18

Penicillium frequentans Westling

Colonies on CYA 40–50 mm diam, low, plane to radially sulcate; surface texture strictly velutinous; white mycelium sometimes visible at the centres; conidial production moderate to heavy, dull green to dark green (26-27E-F3-5); exudate produced centrally by some isolates, clear, yellow or brown; bright yellow soluble pigment sometimes produced; reverse varying from pale or greenish to bright yellow, deep orange, brown or occasionally reddish. Colonies on MEA 40–55 mm diam, low, strictly velutinous, plane or centrally umbonate; white mycelium visible at the centres only; conidial production moderate, coloured as on CYA; yellow soluble pigment sometimes produced; reverse sometimes pale, but more commonly strongly coloured, greenish, olive, yellow or brown. Colonies on G25N 17–24 mm diam, sulcate or wrinkled, velutinous; mycelium white. At 5°C, at least germination; usually microcolonies or colonies up to 4 mm diam formed. No growth at 37°C (Fig. 7.18).

Conidiophores borne from subsurface or surface mycelium or to a limited extent from aerial hyphae, stipes (25–)50–100(–200) μm long, with walls smooth to finely roughened, monoverticillate or occasionally with two metulae, commonly vesiculate up to 6 μm diam; phialides numerous, ampulliform, 8–12 μm long; conidia spherical to subspheroidal, 3.0–3.5 μm diam, with walls smooth or very finely roughened, borne in long well-defined columns.

Distinctive features. *Penicillium glabrum* grows rapidly at 25°C, producing dark green colonies with a strictly velutinous texture. Stipes are vesiculate; conidia are smooth walled and borne in long columns. This species is distinguishable from *P. spinulosum* only with difficulty: *P. glabrum* produces conidia with smooth to finely roughened walls, while those of *P. spinulosum* are finely roughened to distinctly spinulose (Pitt et al., 1990).

Taxonomy. The relationship of *Penicillium glabrum* with *P. spinulosum* and some less common species was considered in detail by Pitt et al. (1990). It was agreed that these two species were distinct, but very closely related.

Physiology. *Penicillium glabrum* is a psychrotroph, able to grow down to at least 0°C (Mislivec and Tuite, 1970b). Maximum temperatures for growth are near 30°C, not, as reported by Domsch et al. (1980), above 40°C. As it is closely related to *P. spinulosum*, *P. glabrum* is probably a xerophile.

Mycotoxins. Mycotoxins are not produced.

Ecology. *Penicillium glabrum* has caused spoilage of cheese and margarine (see Pitt and Hocking, 1997). It has been isolated frequently from a wide range of foodstuffs, in earlier literature under the name *P. frequentans*: from dried and concentrated products such as maize, peanuts, rice and jam; from fermented and cured meats; from fresh cabbage and yams (see Pitt and Hocking, 1997); from wheat, flour and other milling fractions (Hocking, unpublished) and canned carbonated beverage (our data and Ancasi et al., 2006). We have isolated *P. glabrum*

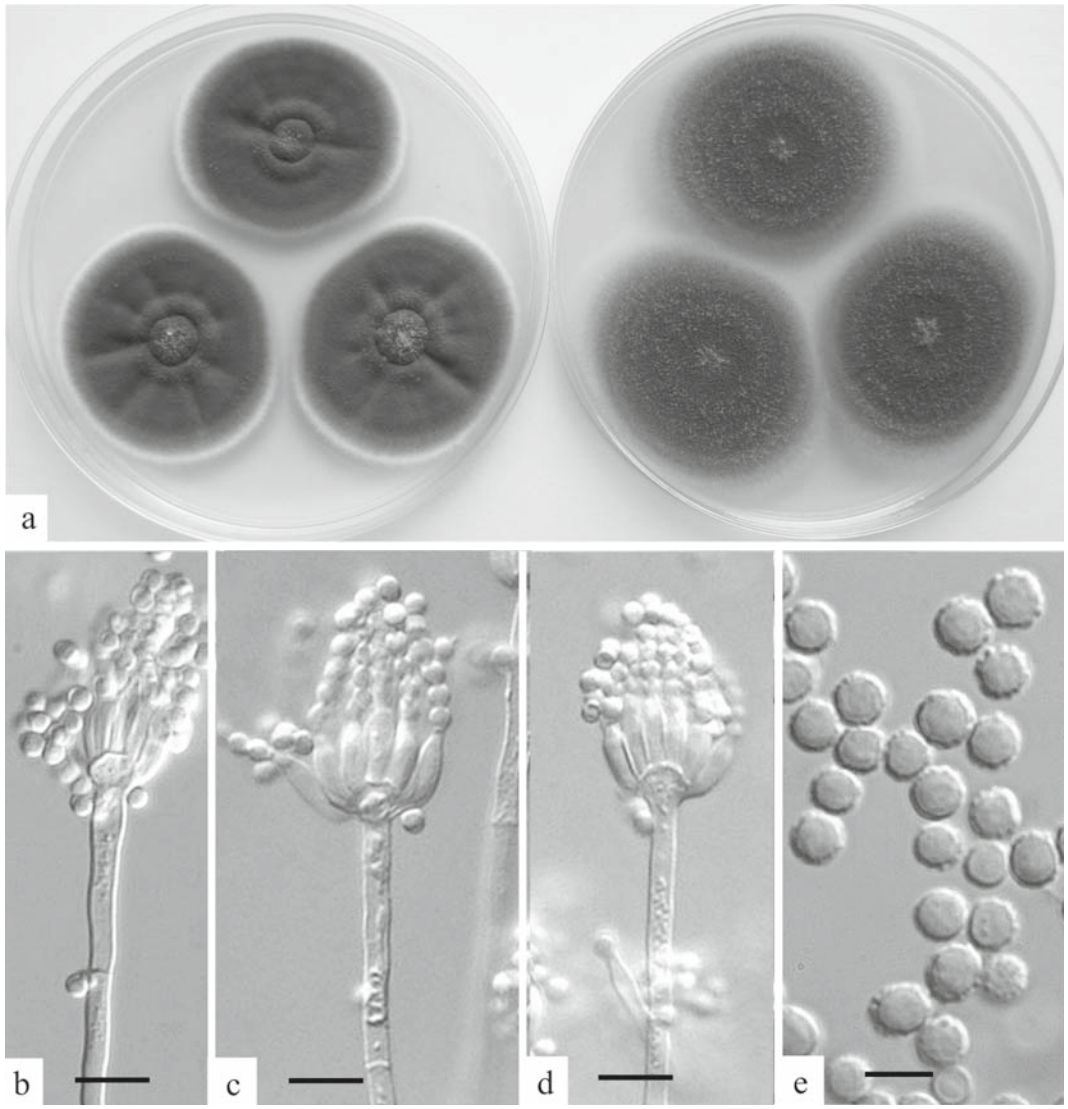


Fig. 7.18 *Penicillium glabrum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 µm; **(e)** conidia, bar = 5 µm

from bottled water, sports beverages, fruit juices, fruit purées and a variety of spoiled dairy products. *P. glabrum* has also been reported from chestnuts (Overy et al., 2003) and grapes (Serra et al., 2006).

Additional species. *Penicillium spinulosum* Thom closely resembles *P. glabrum*, the main difference being that *P. spinulosum* produces spinose conidia (Pitt et al., 1990). *P. spinulosum* is a xerophile, germinating down to 0.80 a_w at 22–25°C (Pelhate, 1968; Hocking and Pitt, 1979). Like *P. glabrum*, this is a psychrotroph and has not been reported to produce mycotoxins. *P. spinulosum* has been reported most frequently from wheat and flour and meat products (see Pitt and Hocking, 1997). We have isolated it from soybeans and peanuts in Southeast Asia (Pitt et al., 1998a).

References. Pitt (1979b, 2000); Domsch et al. (1980).

Penicillium implicatum Biourge Fig. 7.19

Colonies on CYA and MEA growing slowly, 15–20 mm diam or less, radially sulcate, strictly velutinous; mycelium low and dense, white or buff;

conidial production light to heavy, greyish green to dull green (25-26D-E4-5); pale to deep brown exudate sometimes produced on CYA; brown soluble pigment typically produced; reverse yellow, brown or reddish. Colonies on G25N 8–12 mm diam, velutinous; colours similar to those on CYA except reverse pale, olive or brown. At 5°C, usually no germination. At 37°C colonies of 5–10 mm diam usually produced, dense and velutinous; reverse brown to deep brown.

Conidiophores borne from subsurface or surface hyphae, stipes 30–100 µm long, with walls thin and smooth, monoverticillate, sometimes with two metulae, usually vesiculate up to 4–5 µm diam, but not exclusively so; phialides 8–11 µm long, slim, with short collula; conidia ellipsoidal to subspheroidal, 2.5–3.0 µm long, with thick, smooth or finely roughened walls, borne in loose columns.

Distinctive features. *Penicillium implicatum* produces slowly growing, dense colonies on the

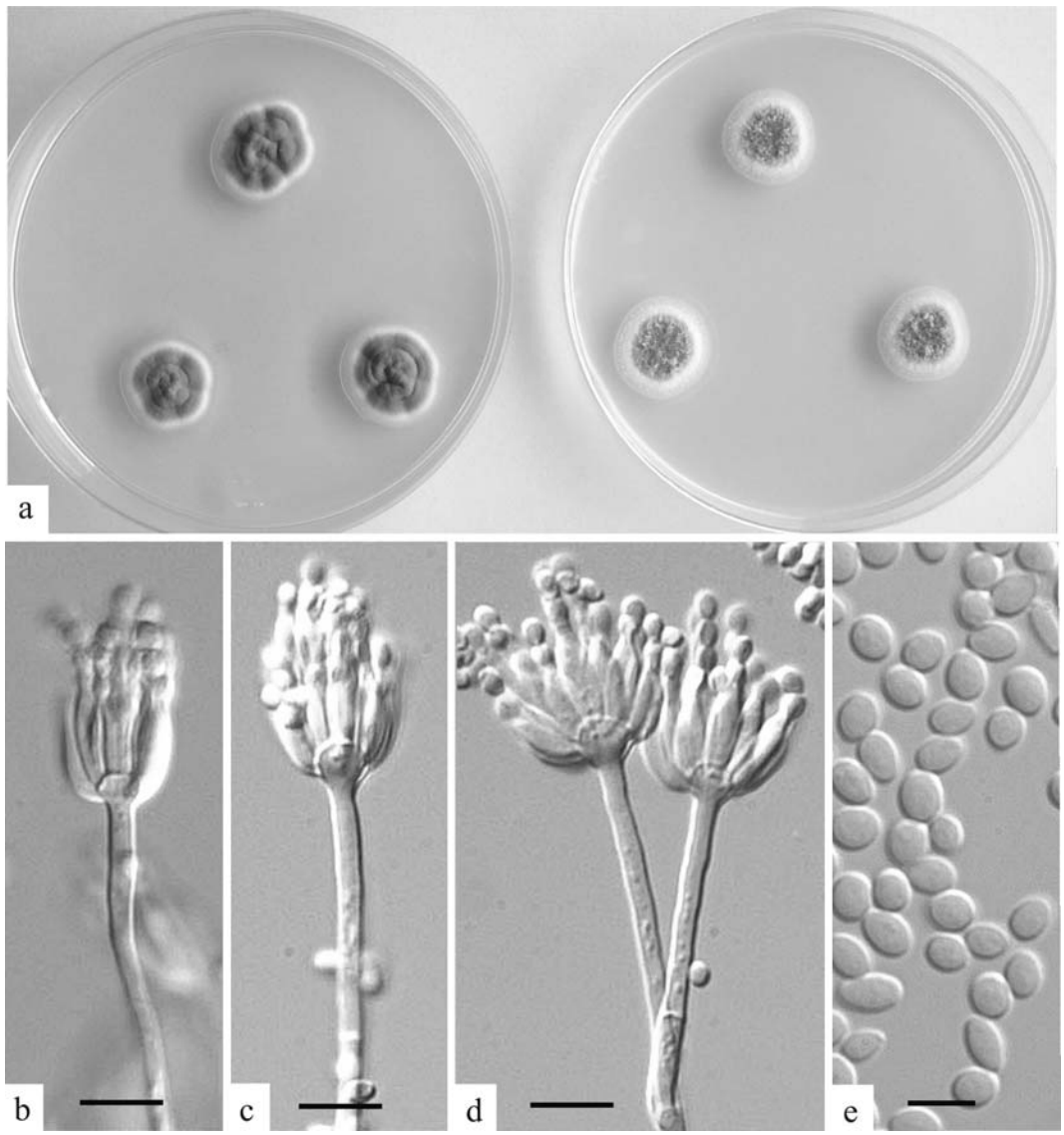


Fig. 7.19 *Penicillium implicatum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 µm; **(e)** conidia, bar = 5 µm

standard media; typical isolates grow slowly at 37°C. Conidiophores are relatively short and usually vesiculate, conidia are ellipsoidal and smooth or nearly so.

Physiology. This species is one of the most xerophilic of the Penicillia, being able to germinate at 0.78 a_w in 10 days (Hocking and Pitt, 1979). Conidiogenesis was unaffected by water activity (0.90 and 0.82) when grown on sterile maize grains, but fungal biomass increased at higher a_w (Cahagnier et al., 1993).

Mycotoxins. Mycotoxin production has not been reported.

Ecology. Perhaps because of its slow growth and xerophilic nature, *Penicillium implicatum* is readily overlooked in surveys. Pitt (1979b) regarded its basic habitat as soil and Nesci et al. (2006) have reported this species in agricultural soils where maize was cultivated. While not of common occurrence, it is a significant biodeteriogen (Raper and Thom, 1949: 203) and spoilage fungus in dried foods. Doupnik and Bell (1971) reported *P. implicatum* from spoiled pecans; it was also common in Indonesian kemiri nuts (1% of all nuts examined; Pitt et al., 1998a) and was found at lower levels in Thai cashews and Indonesian peanuts (Pitt et al., 1993, 1998a). It has been isolated mainly from cereals: maize, rice, wheat and flour (see Pitt and Hocking, 1997). It was relatively common in Australian flour and other milling fractions (Hocking, unpublished). It has also been reported from cashews and dried peas, meat products and frozen fruit pastries (see Pitt and Hocking, 1997).

References. Pitt (1979b, 2000).

Penicillium restrictum J.C. Gilman & E.V. Abbott Fig. 7.20

Colonies on CYA 18–25 mm diam, plane or lightly radially sulcate, typically deep and floccose; mycelium white; conidia absent or sparsely produced, pale grey to bluish grey; clear exudate occasionally present; reverse pale or light brown. Colonies on MEA 15–25 mm diam, plane or umbonate, texture variable, deeply floccose with sparse conidial production to low and funiculose with abundant conidia; mycelium white; conidia greenish grey; reverse pale to brown. On G25N, colonies 11–14 mm diam, plane, deeply floccose or less commonly mucoid; mycelium white; reverse pale. At 5°C, typically no germination; rarely a proportion of conidia with germ tubes. At 37°C, typically colonies 5–10 mm diam produced, sulcate or wrinkled, white or grey; rarely no growth.

Conidiophores borne from loose aerial hyphae or on MEA sometimes from rudimentary funicles, stipes very short, mostly 10–30 μm long, narrow, smooth walled, strictly monoverticillate, nonvesiculate; phialides ampulliform, short, (4–)6–7 μm; conidia spheroidal, ellipsoidal or less commonly pyriform, 2.0–3.0 μm long, finely to coarsely roughened, borne in short irregular chains.

Distinctive features. *Penicillium restrictum* grows relatively slowly on CYA and MEA at 25°C, and slowly at 37°C. Colonies are usually floccose, with white mycelium and sparsely produced grey conidia. Penicilli are very small.

Physiology. A minimum a_w for growth of 0.82 has been reported (Hocking and Pitt, 1979).

Mycotoxins. This species does not produce mycotoxins.

Ecology. *Penicillium restrictum* is a soil fungus and has not been reported to cause food spoilage. Wheat and flour are the foods from which it has been most frequently isolated (Kurata and Ichinoe, 1967; Basu and Mehrotra, 1976). Other reports have been from processed meats (Lofti et al., 1983), soybeans (Pitt et al., 1994) and poultry feeds (Magnoli et al., 1998).

References. Pitt (1979b, 2000).

Penicillium sclerotiorum J.F.H. Beyma Fig. 7.21

Colonies on CYA and MEA typically 30–40 mm diam, less commonly only 20–25 mm, wrinkled, low to moderately deep, dense, consisting of a layer of mycelium, white at the margins, becoming yellow to brilliant orange nearer the centres, usually enveloping abundant sclerotia and overlaid by scattered penicilli; conidia sparsely produced, greyish turquoise or greenish grey (24-26D2-3); exudate limited to abundant, pale, yellow, orange or orange red; yellow or brown soluble pigment usually produced; reverse orange yellow or orange to coffee coloured on CYA, on MEA similar or orange red. Colonies on

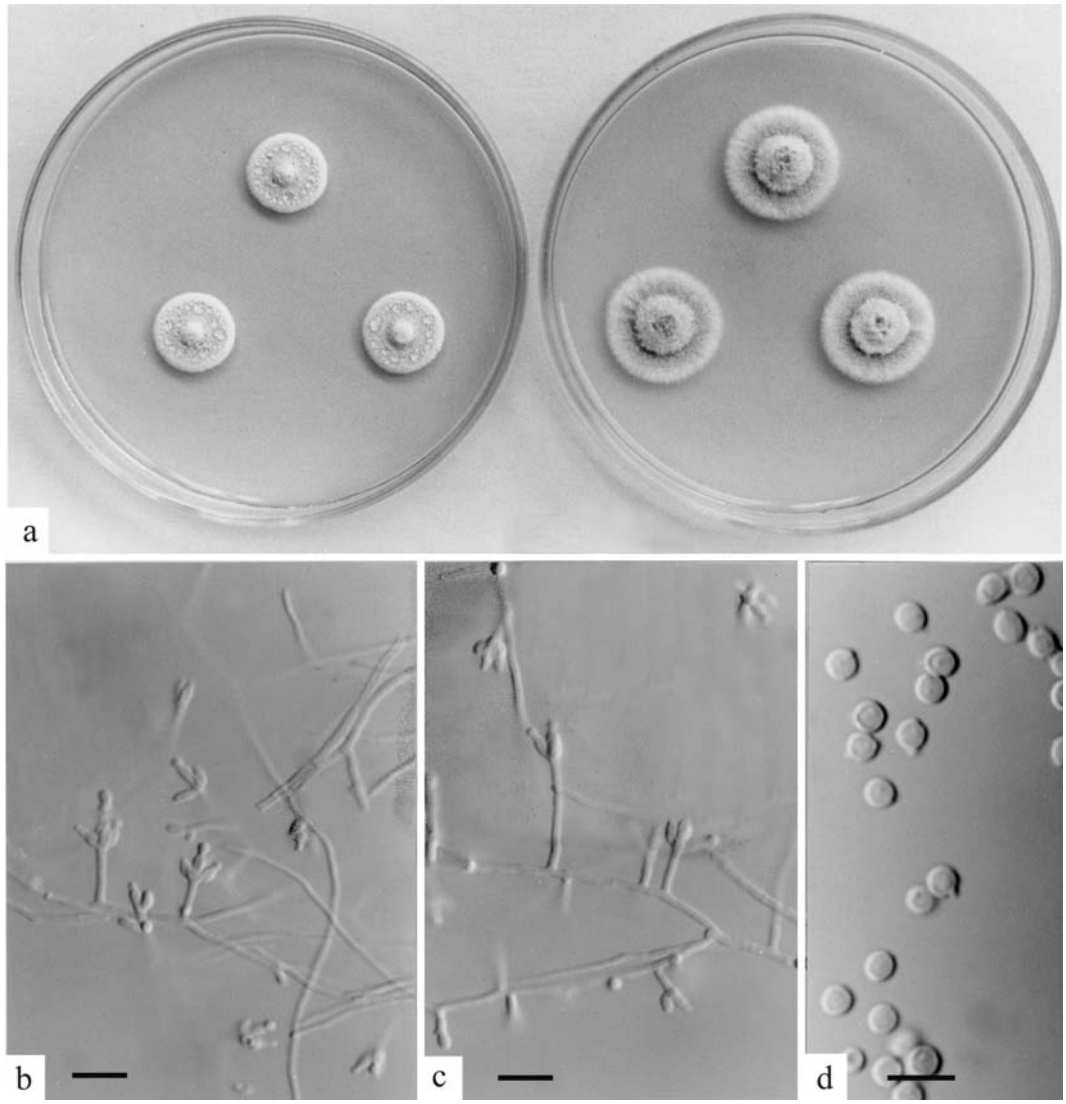


Fig. 7.20 *Penicillium restrictum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** penicilli, bars = 10 µm; **(d)** conidia, bar = 5 µm

G25N 13–18 mm diam, dense and wrinkled, generally in colours similar to those described above. Usually no germination at 5°C; occasionally germination observed. No growth at 37°C.

Sclerotia usually present in fresh isolates, pale, spherical or irregular, 200–400 µm long. Conidiophores borne from surface or subsurface hyphae, stipes 100–300 µm long, slender, with thin, smooth to finely roughened walls, strictly monoverticillate, vesiculate, 4–6 µm diam; phialides numerous, ampulliform, 7–9(–11) µm long; conidia ellipsoidal, 2.5–3.0 µm long, with smooth to finely roughened walls, borne in long and well-defined or irregular columns.

Distinctive features. Vivid orange to red colony colours, in both obverse and reverse, are striking characteristics which distinguish *Penicillium sclerotiorum* from other monoverticillate *Penicillium* species.

Physiology. Judged from its occurrence on a wide range of dried foods, *Penicillium sclerotiorum* is probably a xerophile. No physiological studies have been reported, however.

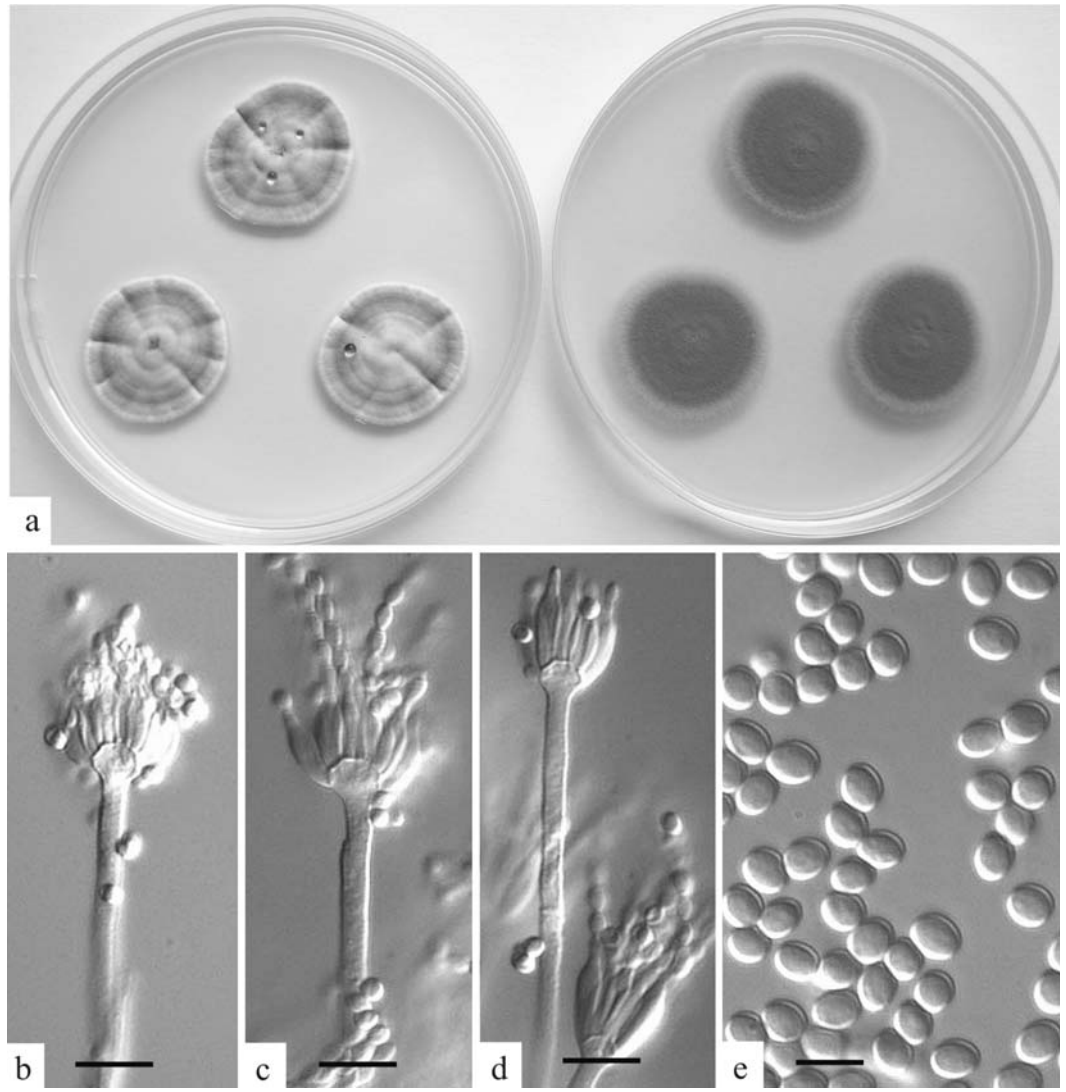


Fig. 7.21 *Penicillium sclerotiorum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 μm; **(e)** conidia, bar = 5 μm

Mycotoxins. Mycotoxin production has not been reported.

Ecology. With the exception of frozen fruit pastries (Kuehn and Gunderson, 1963), this species has been isolated from dried foods: wheat and flour; rice, maize, soybeans and jam (see Pitt and Hocking, 1997). It is not a common species, but we isolated it from peanuts, mung beans and soybeans in Southeast Asia (Pitt et al., 1998a). In the earlier literature, *Penicillium sclerotiorum* is often known as *P. multicolor*, a name not accepted by Pitt (1979b).

References. Pitt (1979b, 2000).

Penicillium thomii Maire — Fig. 7.22

Colonies on CYA 40–60 mm diam, radially sulcate, often lightly floccose, with white mycelium usually surrounding pale to pinkish brown sclerotia, in central areas overlaid by penicilli borne on long stipes; conidial production moderate, dull green (27D-E3); exudate abundant, clear; reverse buff to yellow, or centrally brown in sclerotigenic isolates. Colonies on MEA 40–55 mm diam, plane or centrally wrinkled, usually floccose; sclerotia sometimes present, borne in a layer near the colony

centre, coloured apricot (5B6) or paler; other characteristics similar to those on CYA, except reverse orange brown. Colonies on G25N usually 20–24 mm diam, radially sulcate or wrinkled, low and dense; mycelium white; conidia dark green; reverse buff, olive or brown. Germination always occurring at 5°C, usually colonies of 2–4 mm diam formed. No growth at 37°C.

Sclerotia produced by most isolates, ellipsoidal to irregular in shape, usually 250–350 μm long, rapidly becoming hard, pale at first, then pinkish brown, becoming apricot (5B6) on MEA. Conidiophores borne from surface or aerial hyphae, stipes 200–400 μm long, rough walled, strictly monoverticillate, vesiculate on CYA, less so on MEA; phialides large and crowded, 9–12 μm long, with long narrow collula; conidia ellipsoidal, commonly 3.5–4.0 μm long, finely to coarsely roughened, borne in long, rather irregular columns.

Distinctive features. Most isolates of *Penicillium thomii* produce large sclerotia, coloured a distinctive apricot or salmon shade. Colonies grow very rapidly at 25°C; stipes are long, vesiculate and rough walled; conidia are ellipsoidal and also rough walled.

Physiology. Judged from quite common occurrence in dried foods, rapid growth on G25N, and affinity with *Penicillium glabrum*, *P. thomii* is probably a xerophile. It grows rapidly at 5°C. Some isolates of *P. thomii* recovered from rotten grapes

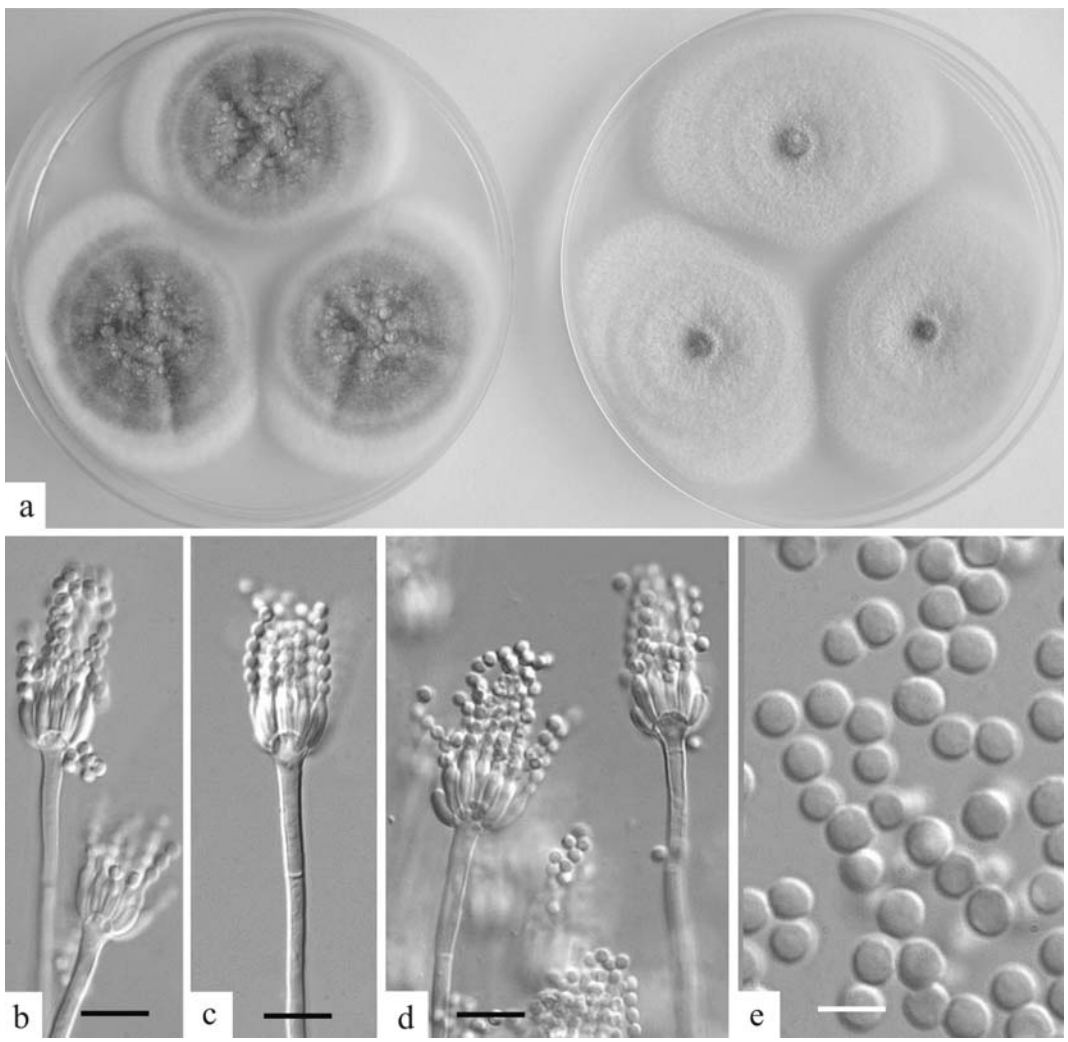


Fig. 7.22 *Penicillium thomii* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 μm; **(e)** conidia, bar = 5 μm

in French vineyards were able to synthesise 2-octen-1ol and 2-methylisoborneol, compounds responsible for mushroom and earthy smells, respectively, on malt and grape juice agar (La Guerche et al., 2006).

Mycotoxins. Mycotoxin production has not been reported for this species.

Ecology. Pitt (1979b) reported that *Penicillium thomii* is a common fungus, widespread in decaying materials as well as soil. In foods, most isolations have been reported from cereals, especially wheat and barley (see Pitt and Hocking, 1997). It occurred in almost 10% of Indonesian dried fish samples examined by Wheeler et al. (1986) and has also been isolated from meat products, peanuts, pistachios, miso (see Pitt and Hocking, 1997) and grapes (Bau et al., 2005). Under the names *P. parallelosporum* and *P. yezoense*, Sasaki reported this species as the cause of spoilage of butter (Pitt, 1979b: 186). We isolated *P. thomii* from spoiled Australian faba beans and at low levels from peanuts in Thailand and Indonesia (Pitt et al., 1993; 1998a).

References. Pitt (1979b, 2000).

7.7.2 Penicillium *subgenus* Furcatum *Pitt*

Penicillium subgenus *Furcatum* includes species which produce regularly or irregularly biverticillate penicilli, usually with 2–5 terminal metulae. Species in subgenus *Biverticillium* also produce biverticillate penicilli, but the two subgenera are distinguishable by several features. Species in subgenus *Furcatum* have phialides that are ampulliform, or at least have wide apical pores, and are distinctly shorter than their supporting metulae. Colonies on G25N always exceed 9 mm diam in 7 days at 25°C. A few species in this subgenus produce metulae in verticils of 5–9 (like species in subgenus *Biverticillium*), but are readily recognised as members of subgenus *Furcatum* by their relatively rapid growth on G25N and by a ratio of metula to phialide length greater than one.

Two quite different types of penicillus occur in subgenus *Furcatum*. Some species produce penicilli in which metulae are almost exclusively borne terminally, i.e. the penicilli characteristically consist of verticils of metulae (Fig. 7.15e). In contrast, a second group of species produces irregularly biverticillate penicilli, with metulae borne terminally, subterminally and lower down on the stipe (Fig. 7.15d). Most isolates classifiable in subgenus *Furcatum* can be readily assigned to one or other of these two groups, the recognition of which will greatly aid identification. In the key which follows, species have first been separated on penicillus morphology and then on growth rates.

Most species in subgenus *Furcatum* are soil fungi and are of relatively uncommon occurrence in foods. Some are included here because they are sometimes isolated from foods enumerated by dilution plating. However, their occurrence in foods is usually only as ubiquitous contaminants. There are some important exceptions of the species described here: *Penicillium citrinum*, *P. corylophilum*, *P. fellutanum* and *P. oxalicum* are important in spoilage of one kind of food or another.

Key to *Penicillium* subgenus *Furcatum* species included here

1	Penicilli predominantly terminal verticils of metulae	2
	Penicilli mostly irregular	7
2 (1)	Colonies on CYA at 25°C exceeding 35 mm diam	3
	Colonies on CYA at 25°C not exceeding 35 mm diam	5
3 (2)	Stipe walls usually rough	4
	Stipe walls smooth	*P. oxalicum*
4 (3)	No growth at 37°C, conidia 3 μm or less in diameter, smooth walled	*P. raistrickii* *P. novae-zeelandiae*
	Usually growth at 37°C, conidia up to 4 μm in diameter, rough walled	*P. simplicissimum*
5 (2)	Penicilli with verticils of 5–8 metulae	*P. paxilli*
	Penicilli with no more than 5 metulae	6

6 (5)	Colonies on MEA more than 25 mm diam; metulae often of unequal length	*P. corylophilum*
	Colonies on MEA less than 25 mm diam; metulae of equal length	*P. citrinum*
7 (1)	Growth at 37°C	*P. janthinellum*
	No growth at 37°C	8
8 (7)	Stipes and conidia smooth walled	*P. fellutanum* *P. waksmanii*
	Stipes or conidia rough walled	*P. janczewskii* *P. canescens*

Penicillium citrinum Thom — Fig. 7.23

Colonies on CYA 25–30 mm diam, radially sulcate, marginal areas velutinous, sometimes floccose centrally; mycelium white in peripheral areas, at the centres white to greyish orange (6B5-6); conidial production moderate, greyish turquoise (24C2-3); exudate clear, pale yellow or pale brown to reddish brown, only rarely absent; soluble pigment bright yellow or absent; reverse yellow, yellow brown, reddish brown or olive. Colonies on MEA 14–18 mm diam, rarely 22 mm, plane or radially sulcate, mycelium white to greyish orange (6B5-6); conidial formation moderate to heavy, grey blue at the margins, elsewhere dull green (26-27E3); reverse pale brown to deep yellow brown. Colonies on G25N 13–18 mm diam, radially sulcate, velutinous or sometimes floccose centrally; mycelium white; conidia often abundant, dull green; reverse pale, dull brown, yellow brown or olive. No germination at 5°C. At 37°C, colonies usually 8–10 mm diam of wrinkled white mycelium only.

Conidiophores borne from subsurface or surface hyphae, stipes 100–300 μm long, smooth walled, characteristically terminating in well defined verticils of 3–5 divergent metulae, less commonly with a divergent ramus, or metulae produced subterminally or along the stipe; metulae usually of uniform length, commonly apically enlarged or vesiculate, phialides ampulliform, 7–8(–12) μm long; conidia spherical to subspheroidal, 2.2–3.0 μm diam, with walls smooth or very finely roughened, typically borne in long well defined columns, one per metula, arranged in a characteristic whorl on each conidiophore.

Distinctive features. *Penicillium citrinum* is readily recognised by its penicilli, which consist of 3–5 divergent and usually vesiculate metulae, bearing long well defined columns of conidia. Colonies on CYA at 25°C are sometimes dominated by copious clear to yellow or brown exudate at the centres. On MEA growth is slower and usually dense, with heavy conidial production.

Physiology. This is a mesophilic species, with the minimum temperature for growth 5°C or slightly above, the maximum just above 37°C (Pitt, 1973) and the optimum 26–30°C (Domsch et al., 1980). At 25°C, the minimum a_w for growth has been reported as 0.80–0.84 (Galloway, 1935; Pitt and Christian, 1968; Hocking and Pitt, 1979). *Penicillium citrinum* grows over the pH range 2–10 (Wheeler et al., 1991).

Mycotoxins. *Penicillium citrinum* is the main producer of citrinin, a mycotoxin of moderate toxicity. Citrinin is a significant renal toxin to monogastric domestic animals, including pigs (Keblys et al., 2004) and dogs (Kogika et al., 1993; Krejci et al., 1996). The oral LD_{50} in mice is about 110 mg/kg (Scott, 1977). Domestic birds are also susceptible: citrinin causes watery diarrhoea, increased food consumption and reduced weight gain due to kidney degeneration in chickens (Mehdi et al., 1981; Glahn et al., 1989), ducklings and turkeys (Mehdi et al., 1984). Citrinin has also been reported to cause teratogenic effects in rats (Singh et al., 2007).

An impact of citrinin on human T cells was recently demonstrated (Wichmann et al., 2002, Tammer et al., 2007); however, the overall effect of citrinin on humans remains undocumented.

Penicillium citrinum produced citrinin in dry cured ham after 4 days incubation at 20°C, compared with only 2 days on YES medium (Bailly et al., 2005). After 16 days, significantly higher amounts of citrinin were produced on ham (1330 mg/kg) than on YES medium (86.9 mg/kg). However, pure citrinin extract added to the surface of cured ham was quickly lost or degraded, with more than 50% lost after only 6 h of incubation at 20°C and less than 15% remaining after 192 h (Bailly et al., 2005).

Ecology. *Penicillium citrinum* has been isolated from nearly every kind of food which has been surveyed for fungi. The most common sources are cereals, e.g. rice (Park et al., 2005a; Aziz et al.,

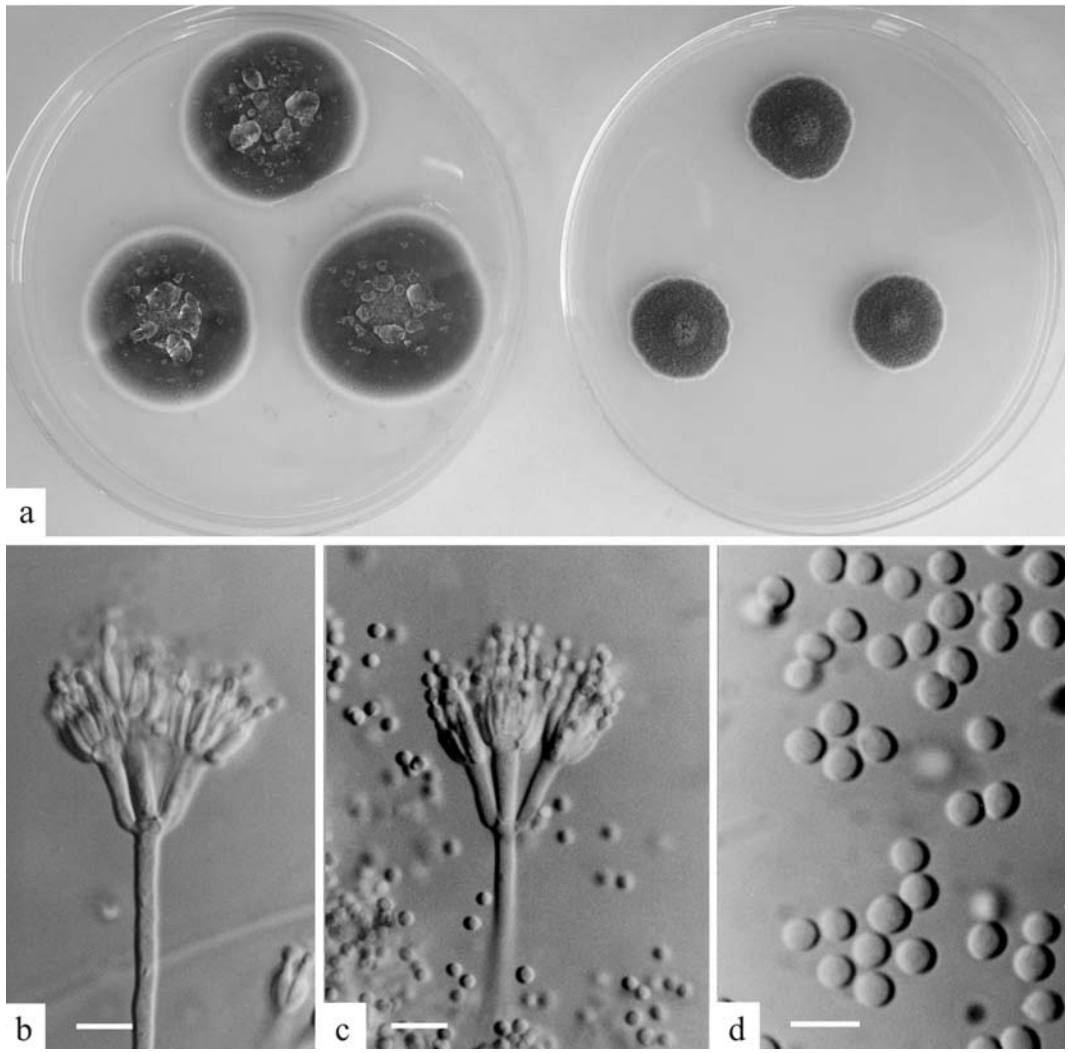


Fig. 7.23 *Penicillium citrinum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** penicilli, bars = 10 µm; **(d)** conidia, bar = 5 µm

2006), wheat and barley (Aziz et al., 2006), maize (Mazzani et al., 2004; Aziz et al., 2006), and milled grains and flour (Ogundare and Adetuyi, 2003; Hocking, unpublished; and also see Pitt and Hocking, 1997). Among other reported sources are nuts, blackgram in India, amaranth seeds in Argentina, cinnamon and soy sauce (see Pitt and Hocking, 1997). *Penicillium citrinum* has also been reported in fermented and cured meats (Tabuc et al., 2004), hams (Bailly et al., 2005), cocoa pulp (Ardhana and Fleet, 2003), young coconuts (Waje et al., 2005), soybeans (Aziz et al., 2006), wine grapes (Bau et al., 2005) and dried vine fruits (Romero et al., 2005), curd and cheese (Kumaresan et al., 2003), bottled mineral water (Cabral and Fernández Pinto, 2002) as well as coffee beans, dried beans and peppercorns (see Pitt and Hocking, 1997).

In Southeast Asia, *Penicillium citrinum* was isolated from maize, peanuts, copra soybeans, sorghum and cashews (Pitt et al., 1993, 1994). *P. citrinum* was very widespread in Indonesian food commodities: we isolated it from dried fish, sorghum, peanuts and kemiri nuts, pepper, coriander, maize and mung beans. Citrinin levels were never significant, however (see Pitt and Hocking, 1997).

Instances of food spoilage caused by *Penicillium citrinum* are rare, but this species is much more than a mere contaminant. Because of its mesophilic

nature, distribution is world wide and, in addition, its ability to grow down to 0.80 a_w helps to secure this species a niche in a very wide range of habitats.

References. Pitt (1979b, 2000); Domsch et al. (1980).

Penicillium corylophilum Dierckx Fig. 7.24

Colonies on CYA 25–35 mm diam, plane to deeply radially sulcate, low, moderately dense, strictly velutinous; mycelium white or rarely buff; conidial production light to moderate, dull green (25C-E3-4); clear exudate sometimes present; reverse pale, brownish or sometimes centrally dark grey. Colonies on MEA 30–45 mm diam, plane, low and relatively sparse, strictly velutinous; mycelium white or buff; conidia in moderate numbers, dull green (26-27D-E3); clear exudate occasionally present; reverse pale at the margins, but usually dull green to very dark green centrally. Colonies on G25N 10–16 mm diam, plane or centrally wrinkled, dense; mycelium white; colours similar to those on CYA; reverse pale. At 5°C, usually germination of conidia

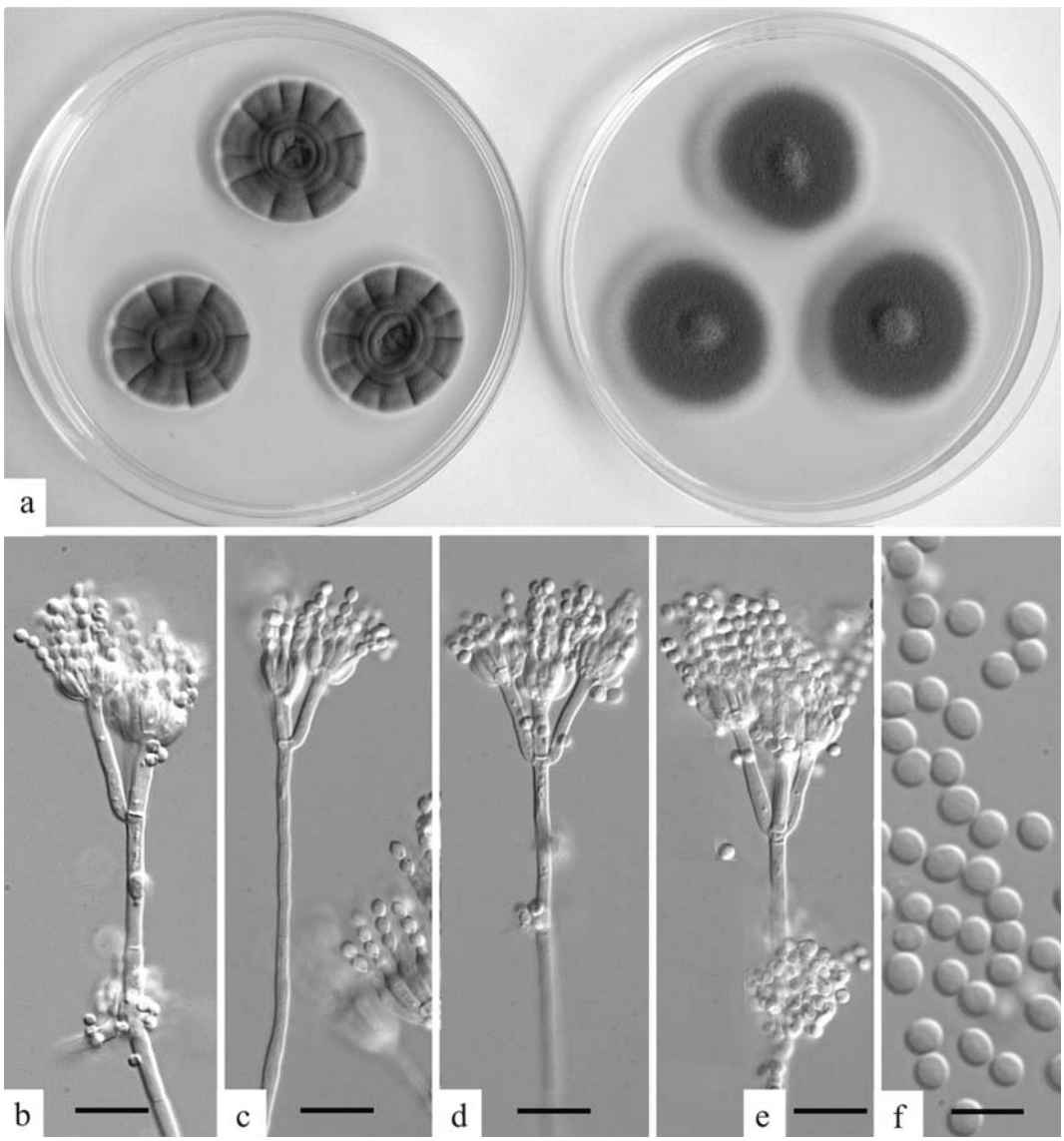


Fig. 7.24 *Penicillium corylophilum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d, e)** penicilli, bars = 10 μm; **(f)** conidia, bar = 5 μm

to formation of microcolonies; occasionally small macroscopic colonies produced. No growth at 37°C.

Conidiophores borne from subsurface hyphae, stipes 100–250 μm long, smooth walled; on CYA, penicilli usually verticils of 2–5 metulae, but sometimes with subterminal metulae or occasionally a ramus; on MEA usually less complex and frequently monoverticillate; in penicilli with two metulae the offset one often longer than the axial; phialides ampulliform, 7–11 μm long; conidia spherical to subspheroidal, commonly 2.5–3.0 μm diam, smooth walled, borne on CYA in disordered chains, on MEA sometimes in long columns.

Distinctive features. Relatively long divergent metulae often of unequal length are characteristic of *Penicillium corylophilum*. This species produces strictly velutinous colonies; on MEA growth is rapid and usually dark green in reverse.

Physiology. This is a xerophilic fungus: Hocking and Pitt (1979) reported germination at 0.80 a_w after 38 days at 25°C. Growth was observed at 0.85 a_w (Guynot et al., 2005). The addition of sorbic acid to fermented bakery products completely inhibited the growth of this fungus regardless of water activity (Guynot et al., 2005).

Mycotoxins. Mycotoxins are not known to be produced.

Ecology. *Penicillium corylophilum* has been reported to cause spoilage of high-fat foods, including rapeseed and rapeseed oil (Magan et al., 1993) and margarine (Hocking, 1994). In our experience, *P. corylophilum* occasionally causes spoilage of low a_w foods such as jams. It has been reported quite frequently from cereals: barley, paddy rice, wheat and flour (see Pitt and Hocking, 1997). It has also been isolated from salami (Cantoni et al., 2007), laban rayeb, a fermented dairy product (Ahmed and Abdel-Sater, 2003), peanuts, pecans, hazelnuts, soybeans and frozen fruit pastries (see Pitt and Hocking, 1997). In our laboratory, we have isolated *P. coryophilum* from a range of substrates including bottled water, fruit juices, thickened cream, cosmetics and petroleum products. *P. corylophilum* occurred at low levels in mung beans and soybeans in Thailand (Pitt et al., 1994), maize in Indonesia (Pitt et al., 1998a), and peanuts in the Philippines (our unpublished data)

References. Pitt (1979b, 2000); Domsch et al. (1980).

Penicillium fellutanum Biourge **Fig. 7.25**

Colonies on CYA 17–24 mm diam, very dense, radially sulcate, velutinous; mycelium white, usually visible only at the margins; formation of conidia light to heavy, coloured pale grey if sparse, but more commonly dark green (27-28F4); colourless exudate sometimes present; reverse pale. Colonies on MEA 14–18 mm diam, low and dense, radially sulcate, usually velutinous with a floccose central area, less commonly entirely floccose; conidia moderately abundant; wrinkled; colours similar to colonies on CYA. Colonies on G25N usually 12–16 mm diam, growth low and dense; reverse olive or yellow. No germination at 5°C and no growth at 37°C.

Conidiophores borne from aerial hyphae, characteristically of indeterminate form, sometimes terminating in well-defined penicilli with 2–4 metulae, sometimes bearing metulae in a random manner, with or without solitary phialides as well, less commonly giving the impression of monoverticillate penicilli borne perpendicular to fertile hyphae, but always with at least two terminal metulae; stipes smooth walled, of irregular and often indeterminate length; metulae usually long, terminating in well defined, thin walled vesicles; phialides ampulliform, 5–10 μm long; conidia ellipsoidal, 2.5–3.2 μm long, with surfaces finely to distinctly roughened, borne in long, irregular columns.

Distinctive features. A well defined species, *Penicillium fellutanum* is distinguished microscopically by conidiophores with irregularly located metulae of variable length, terminating in definite vesicles, and macroscopically by closely textured colonies, dark green conidia and failure to grow at either 5 or 37°C. Moreover growth on MEA is slower than on CYA and little faster than on G25N.

Physiology. *Penicillium fellutanum* is a slow growing xerophile. Snow (1949) reported a minimum a_w for germination of 0.80 at 25°C and, in good agreement, Hocking and Pitt (1979) reported germination at 0.78 a_w after 89 days. As noted above, *P. fellutanum* has a relatively narrow temperature range for growth, within the limits of 5 and 37°C.

Mycotoxins. Mycotoxins are not known to be produced.

Ecology. *Penicillium fellutanum* has been isolated from a wide variety of dried foods, which appear to

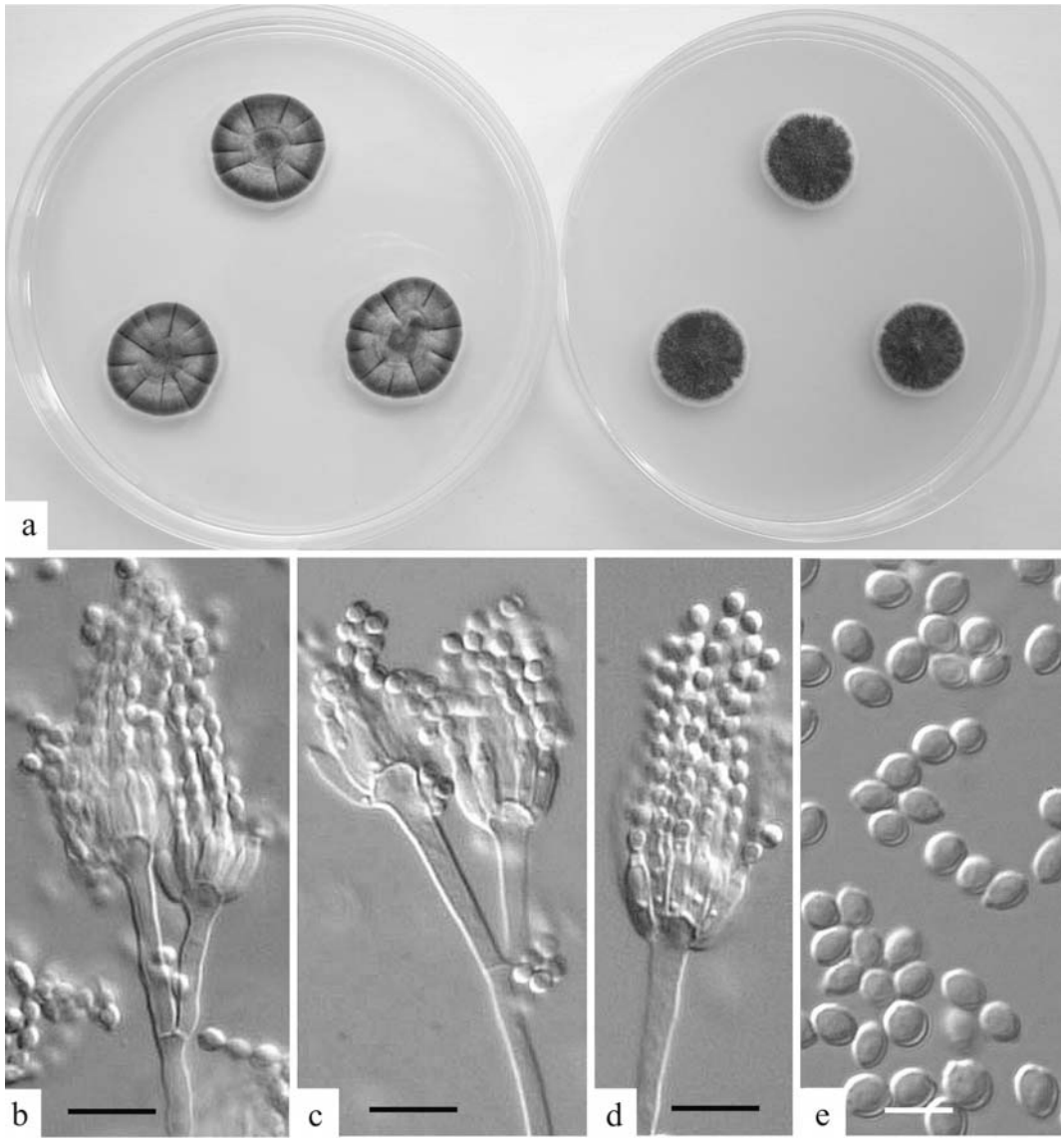


Fig. 7.25 *Penicillium fellutanum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 μm; **(e)** conidia, bar = 5 μm

be a major habitat, including barley, wheat and flour, nuts, beans and sultanas. It has been isolated also from frozen fruit pastries and miso (see Pitt and Hocking, 1997). We isolated *P. fellutanum* at low levels from Thai cashews, and Indonesian maize, peanuts and milled rice (Pitt et al., 1993, 1998a).

Additional species. *Penicillium waksmanii* K.M. Zalessky has much in common with *P. fellutanum*, including the variable and irregular penicillus structure and closely textured colonies. The principal distinctions are that *P. waksmanii* often grows much faster on MEA (20–35 mm), germinates at 5°C and produces greenish grey (25C2-26E3) spherical conidia. Mycotoxins are not produced. Habitats in foods appear to be similar to those of *P. fellutanum*, but *P. waksmanii* has been reported less frequently in the literature. Reports include ham during ripening (Spotti et al., 1989), peanuts (Pitt et al., 1993) and pistachios (Heperkan et al., 1994).

References. Pitt (1979b, 2000).

Penicillium janczewskii K.M. Zalessky

Fig. 7.26

Penicillium nigricans Bainier

Colonies on CYA 25–32 mm diam, radially sulcate, deep, dense to moderately floccose; mycelium usually white, in some isolates pale yellow; conidial formation variable, in lightly sporing isolates coloured greenish grey (25B2-3), but in those sporing more heavily much darker, dull green (28E3); clear exudate and brown soluble pigment sometimes produced; reverse coloured brown, dark brown, orange or deep reddish orange. Colonies on MEA 18–24 mm diam, less commonly 30 mm, plane or radially sulcate, moderately deep to deep, dense to floccose; mycelium white to pale yellow, occasionally pale orange or pinkish; conidial production moderate to heavy, marginal areas sometimes bluish but greenish grey (25-26D-E2) elsewhere; orange or yellow soluble pigment sometimes produced; reverse typically Salmon (6A4-5) or, in the presence of soluble pigment, dark orange (5A8). Colonies on G25N 15–20 mm diam, occasionally only 12 mm, typically closely radially sulcate, velutinous to floccose; mycelium white to pale yellow; reverse usually pale yellow to dull brown. At 5°C, typically germination by a proportion of conidia, less commonly general germination or microcolony formation. At 37°C, typically no growth, occasionally colonies up to 10 mm diam formed.

Conidiophores borne from aerial hyphae, stipes with thin smooth walls, commonly 50–200 μm long, but in some isolates much longer, and in the limit becoming indistinguishable from fertile hyphae bearing short conidiophores, characteristically bearing a terminal tetrad of divergent metulae, but frequently less regular in pattern, with intercalary rami and metulae commonly present, the latter appearing as short monoverticillate conidiophores; metulae 8–15 μm long, sometimes apically swollen; phialides ampulliform, 6–8 μm long, with short collula; conidia spherical, 2.5–3.5 μm diam, spinose, appearing olive brown, borne in short, poorly defined columns.

Distinctive features. Despite considerable isolate to isolate variation, *Penicillium janczewskii* usually can be recognised by spherical spinose conidia, and metulae which are often apically swollen and characteristically occur in tetrads. However, in some isolates the most characteristic feature of the conidiophores may appear to be a total lack of order in their structure. Colonies are floccose to a greater or lesser extent; conidial production is light to moderate; when moderate, conidial colour is dark green.

Taxonomy. Raper and Thom (1949: 325) used the name *Penicillium nigricans* for this taxon. However, *P. janczewskii* is the correct name for this species (Pitt, 1979b; Pitt and Samson, 1993), and it is now in general use.

Physiology. With an optimum temperature for growth near 25°C, *Penicillium janczewskii* grows weakly at 5°C and not above 33°C (Domsch et al., 1980). This species is among the most xerophilic Penicillia, germinating down to 0.78 a_w at 25°C (Hocking and Pitt, 1979).

Mycotoxins. *Penicillium janczewskii* has been reported to produce griseofulvin (El-Banna et al., 1987b; Nicoletti et al., 2007) and penitrem A (di Menna et al., 1986; Pitt and Leistner, 1991; Frisvad et al., 2006a). However, as this species is rarely if ever involved in food spoilage, significant mycotoxin production in the human food supply is unlikely.

Ecology. All the evidence indicates that *Penicillium janczewskii* is a soil fungus, present in foods only as a contaminant, as we have no record of it having caused food spoilage. It has been reported from barley, wheat, flour, peanuts, pecans, pistachios, soybeans, dried beans and meats (see Pitt and Hocking, 1997).

Additional species. *Penicillium canescens* Sopp is closely related to *P. janczewskii*, and in fact these species interface (Pitt, 1979b: 253). Both species grow at similar rates under the standard conditions and produce similar pigmentation, although *P. canescens* is often more strongly coloured. Microscopically, typical isolates are easily distinguished: *P. canescens* produces rough walled stipes and smooth conidia, while *P. janczewskii* has smooth stipes and rough walled conidia. Like *P. janczewskii*, *P. canescens* has been reported to produce griseofulvin (Nicoletti et al., 2007). This is a soil fungus, present in foods as a contaminant. *P. canescens* has been isolated from similar substrates to *P. janczewskii*, though less frequently, and also from rice, field peas (our data) and from cheese (Leistner and Pitt, 1977).

References. Pitt (1979b, 2000); Domsch et al. (1980).

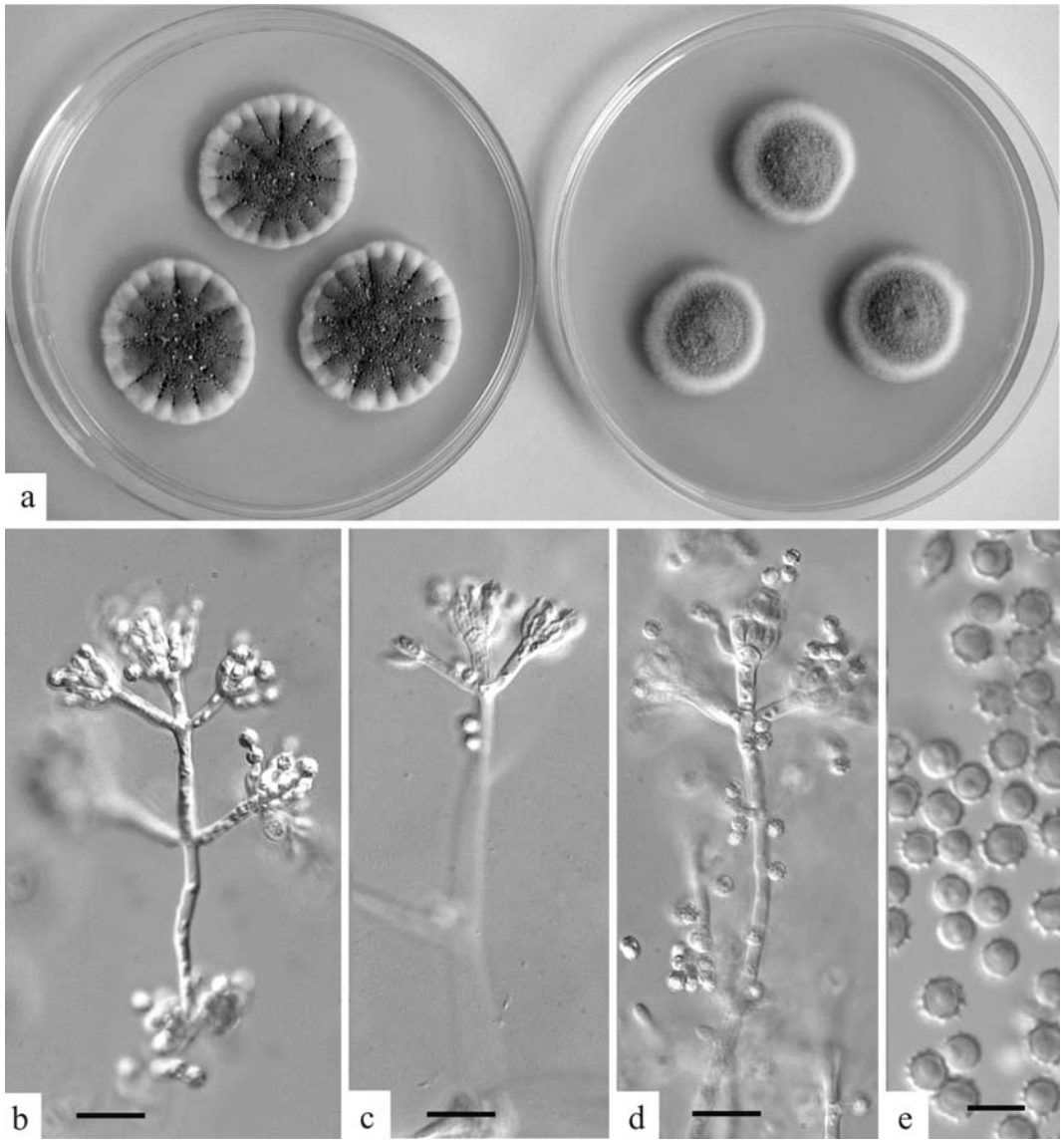


Fig. 7.26 *Penicillium janczewskii* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 μm; **(e)** conidia, bar = 5 μm

Penicillium janthinellum Biourge Fig. 7.27

Colonies on CYA 35–50 mm diam, radially sulcate or irregularly wrinkled, floccose, mycelium dense, coloured white, greyish, buff, pale yellow or pale pink, and overlaid by conidiophores, varying from inconspicuous to abundant; conidial production very light to moderate, in the latter case coloured greyish green to dull green (25-27C-D3-4), sometimes appearing more yellow or olive because of the coloured mycelium; limited amounts of clear to brown exudate and reddish brown soluble pigment sometimes produced; reverse colours variable, pale yellow or yellow brown to reddish brown, or occasionally brilliant dark green. Colonies on MEA usually 35–45 mm diam, floccose, lacking the density of colonies on CYA; mycelium white or buff; conidial colours similar to those on CYA; yellow or brown soluble pigment occasionally produced; reverse pale, brownish, deep brown, dark green or

quite commonly pink, centrally or in sectors, occasionally even bright red. Colonies on G25N 10–18 mm diam, typically plane, low or umbonate, velutinous to floccose; mycelium white or yellow; conidia grey green; reverse pale, yellow, brown or pinkish. Sometimes germination at 5°C. At 37°C, colonies 10–30 mm diam, dense and velutinous; clear exudate and brown or reddish soluble pigment sometimes produced; reverse pale, yellow, brown or reddish brown.

Conidiophores borne from surface or aerial hyphae, stipes smooth and slender, thin walled and easily bent, typically 200–400 µm long, but in some isolates also short, 30–70 µm, usually terminating in an irregular to regular verticil of 2–3 metulae, characteristically also with subterminal and intercalary metulae, the latter intergrading with short monoverticillate conidiophores, in some isolates long monoverticillate conidiophores present as well; phialides ampulliform, 7–11 µm long, typically

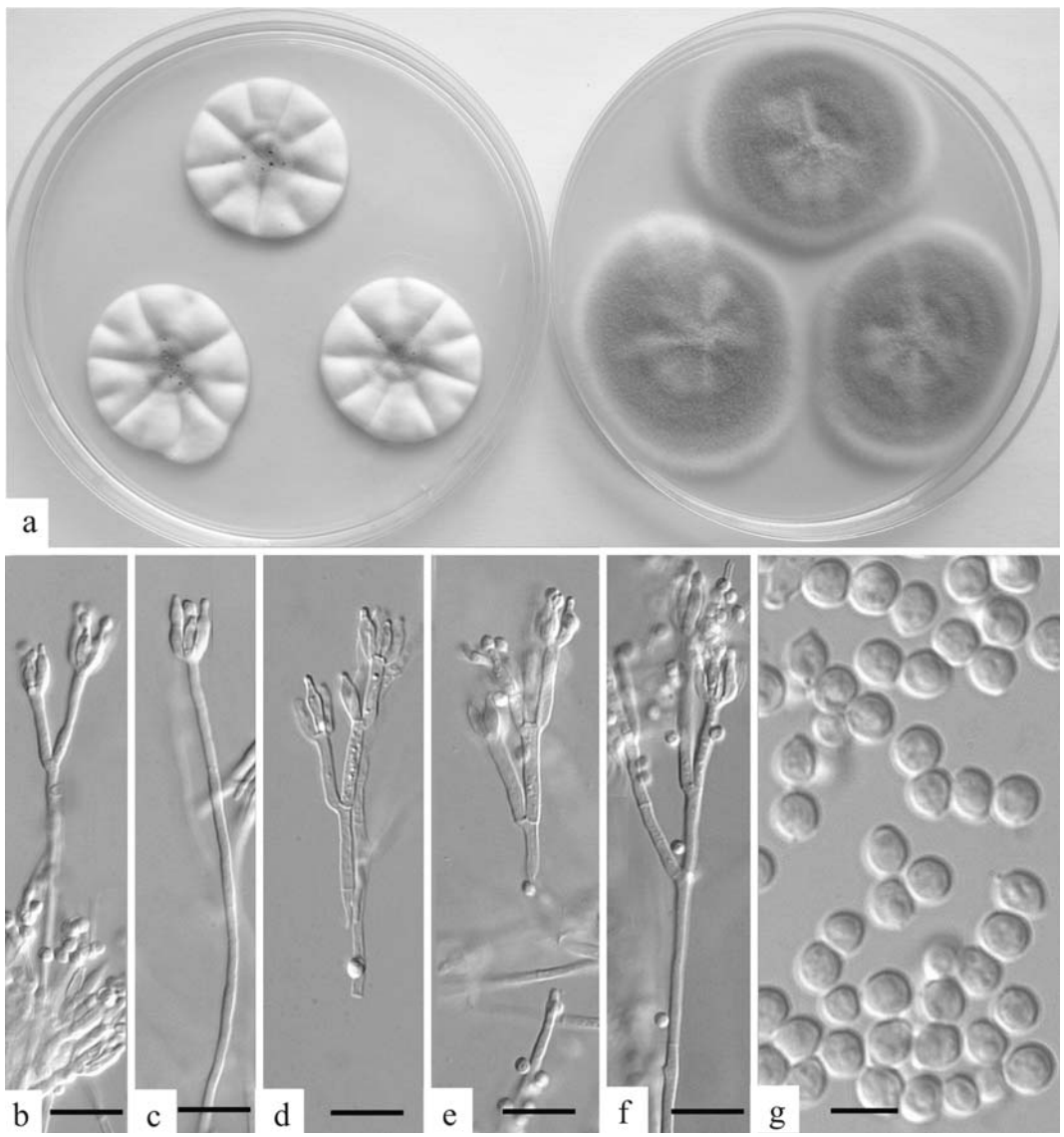


Fig. 7.27 *Penicillium janthinellum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d, e, f)** penicilli, bars = 10 µm; **(g)** conidia, bar = 5 µm

with long slender collula; conidia most often spherical, but sometimes short pyriform to ellipsoidal, 2.2–3.0 μm diam or long, with smooth to finely roughened walls, borne in short to moderately long disordered chains.

Distinctive features. *Penicillium janthinellum* is surely the most difficult of all Penicillia to define. Colonies on CYA and MEA at 25°C grow rapidly; growth typically occurs at 37°C; colonies are usually floccose and conidia rather sparsely produced. Penicilli, although usually biverticillate, are so irregular as to often appear monoverticillate. Stipes are delicate and smooth walled. Phialides characteristically have long slender necks and bear smooth to finely roughened conidia.

Physiology. Temperature requirements for this species are approximately known: minimum temperature for growth, above 5°C, optimum 25–30°C, good growth at 37°C (Pitt, 1973). No water relations studies have been reported, but this species is probably not a xerophile.

Mycotoxins. *Penicillium janthinellum* produces a series of tremorgenic toxins known as janthitrems (Gallagher et al., 1980). Closely related in chemical structure to the penitrems (de Jesus et al., 1984), janthitrems might be expected to show similar toxicities, however, toxicological data are not available. As *P. janthinellum* is uncommon in foods, these toxins are unlikely to be important in human health.

Ecology. As an ubiquitous soil fungus, the presence of *Penicillium janthinellum* on foods is adventitious. Isolations have been made occasionally from a wide variety of foods: dates and almonds, maize, dried beans, peanuts, pistachios, barley, fermented and cured meats, biltong and frozen fruit pastries (see Pitt and Hocking, 1997). There appear to be no records of food spoilage by this species.

References. Pitt (1979b, 2000); Domsch et al. (1980).

Penicillium oxalicum Currie & Thom

Fig. 7.28

Colonies on CYA 35–60 mm diam, plane or radially sulcate, velutinous or lightly floccose in central areas; mycelium usually inconspicuous, in floccose areas white or pale yellow, but the underlying surface growth coloured Salmon (6A4); conidial production typically very abundant, appearing as a continuous layer of long, closely packed chains under the low power microscope, and breaking off in masses if jarred, greyish green (25-26C3) at the margins, then dull green (27D3-28E4) or olive (1E3-4) towards the centres; exudate limited, clear, or absent; reverse pale to yellow, brown, orange or pinkish. Colonies on MEA variable in size, 20–50 mm diam, plane or lightly radially sulcate, strictly velutinous; conidia very abundant, forming readily detached masses; colours similar to those on CYA except reverse sometimes greenish. Colonies on G25N 12–16 mm diam, plane or wrinkled, velutinous; mycelium white or salmon; reverse pale, greenish, olive or salmon. At 5°C, germination by a proportion of conidia, or no germination. At 37°C, colonies 10–40 mm diam, deeply radially sulcate and often centrally wrinkled, velutinous; mycelium white; reverse olive or brown.

Conidiophores borne from surface mycelium, stipes mostly 200–400 μm long, with thin, smooth walls, characteristically terminating in verticils of 2–4 closely appressed metulae; metulae 15–25(–30) μm long; phialides acerose, 10–15(–20) μm long, with short collula; conidia ellipsoidal, very large, 3.5–5.0(–7) μm long, with walls smooth or rarely finely roughened, borne in long, closely packed columns.

Distinctive features. Of all the cosmopolitan Penicillia, this species is perhaps the most obviously distinctive. Colonies usually grow rapidly on CYA at 25 and 37°C, are strictly velutinous, and produce prodigious numbers of conidia. Under low magnifications, the conidia can be seen to lie in closely packed, readily fractured sheets, and to have a uniquely shiny, even silky, appearance. Under high magnification, the large penicilli and large smooth walled ellipsoidal conidia also are distinctive.

Physiology. Mislivec and Tuite (1970b) reported 8°C to be the minimum growth temperature for *Penicillium oxalicum*, with an optimum near 30°C. Judged from rapid growth at 37°C, the maximum temperature for growth is in excess of 40°C. The minimum a_w for germination has been reported as 0.86, both in glucose media at 23 and 30°C (Mislivec and Tuite, 1970b) and in NaCl media at 25°C (Hocking and Pitt, 1979). *P. oxalicum* was one of several *Penicillium* species associated with production of volatiles in mouldy wheat (Sinha et al., 1988).

Mycotoxins. Secalonic acids are dimeric xanthones produced by a range of taxonomically

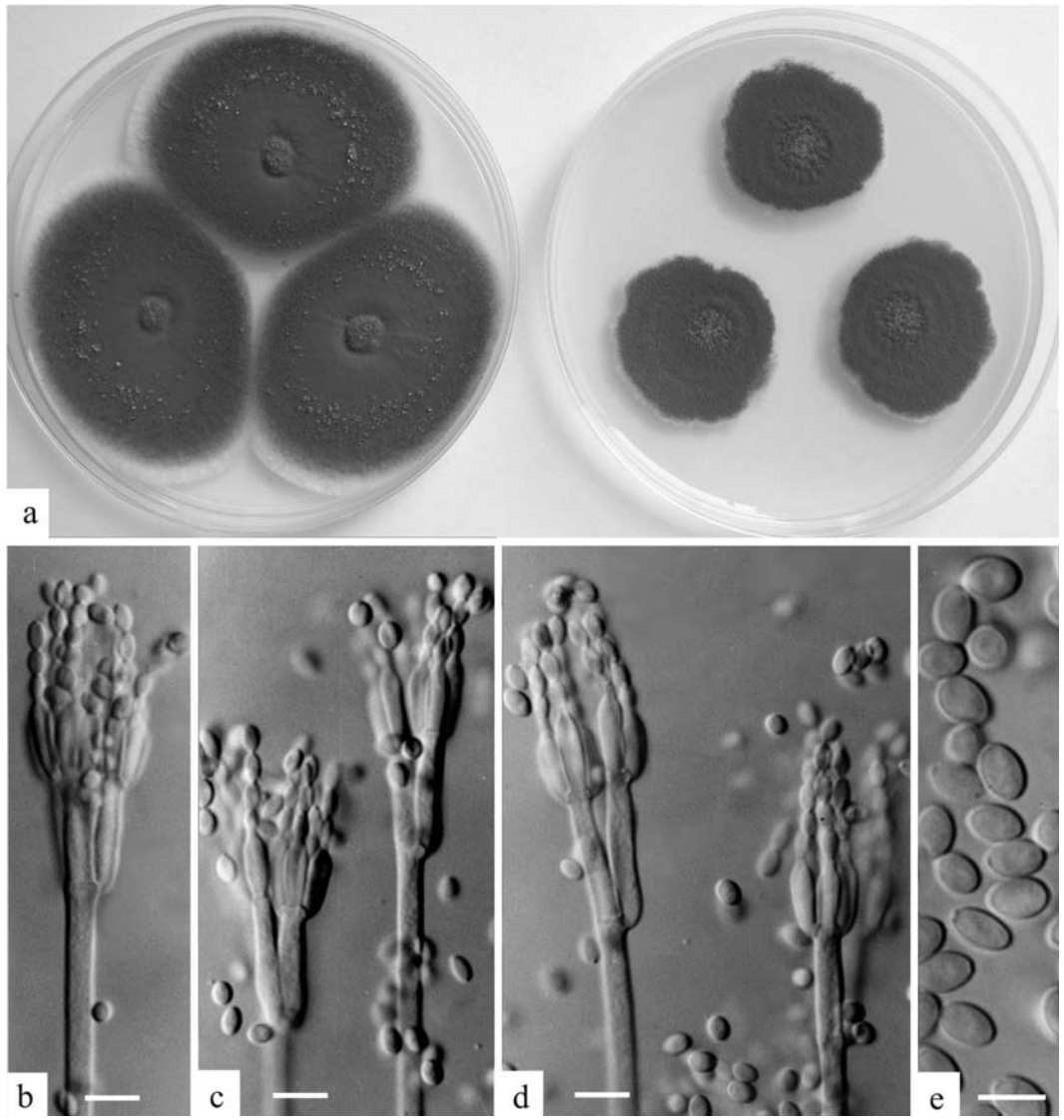


Fig. 7.28 *Penicillium oxalicum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 µm; **(e)** conidia, bar = 5 µm

distant fungi (Cole and Cox, 1981; Frisvad and Thrane, 2004). Secalonic acid D is produced as a major metabolite of *Penicillium oxalicum* and is known to induce cleft palate in animals and potentially in humans (Dhulipala et al., 2005; Reddy, 2005). It has been found in nature, in grain dusts, at levels of up to 4.5 mg/kg (Ehrlich et al., 1982) and was also reported from grain dust collected in Belgian cereal storage facilities (Tangni and Pussemier, 2007). The possibility that such levels can be toxic to grain handlers cannot be ignored.

Ecology. As a result of its rapid growth at 37°C, *Penicillium oxalicum* is widespread in tropical commodities and foods. We isolated it from Indonesian maize, red rice, paddy rice, cowpeas and sorghum, infecting 1% of all grains examined in each case (Pitt et al., 1998a) and at lower levels in peanuts, kemiri nuts, soybeans, mung beans, black pepper, coriander and milled rice in Thailand, Indonesia and/or the Philippines (Pitt et al., 1993, 1994, 1998a).

A major niche for *Penicillium oxalicum* is preharvest maize (see Pitt and Hocking, 1997). Koehler

(1938) suggested that entry of this species to ripening maize ears was through insect damage or wounds. *P. oxalicum* has also been recorded in stored maize, though at lower incidence (Amusa et al., 2005; Askun, 2006). This species is a pathogen on yams (Okigbo, 2003) and greenhouse cucumbers (Menzies et al., 2005), is considered to be the dominant fungus on cassava (Adegoke et al., 1993) and is of common occurrence on copra (Zohri and Saber, 1993) and cashew nuts (Freire and Kozakiewicz, 2005). It has also been reported from a wide variety of other foods: barley, wheat, flour, pecans, hazelnuts, walnuts, peppercorns, soybeans, spices, fermented sausages and biltong (see Pitt and Hocking, 1997).

The temperature and water relations of this species make it competitive with *Aspergillus flavus* except at very low a_w, and it occupies a similar range of habitats. It is not such a common cause of spoilage, however.

References. Pitt (1979b, 2000); Domsch et al. (1980).

Penicillium paxilli Bainier Fig. 7.29

Colonies on CYA 30–35 mm diam, occasionally only 25 mm, radially sulcate, velutinous to lightly floccose; mycelium white at the margins, sometimes centrally greyish orange; conidial production moderate, at the margins greyish turquoise (24D3-4), centrally dull green (25-26E3-4); clear to red brown exudate and red brown soluble pigment often produced; reverse pale to brownish orange. Colonies on MEA usually 25–30 mm diam, occasionally less, plane to deeply radially sulcate, velutinous or occasionally floccose centrally; mycelium white to buff; conidial production moderate to heavy, coloured as on CYA; exudate and soluble pigment produced occasionally, red brown; reverse pale to brownish orange. Colonies on G25N 16–20 mm diam, radially sulcate, deep, dense to floccose: mycelium white; exudate and soluble pigment occasionally produced, red brown; reverse pale to brownish orange or reddish brown. At 5°C usually no germination; less commonly germination or formation of microcolonies. At 37°C, typically no growth, occasionally colonies up to 10 mm diam formed.

Conidiophores borne from subsurface or surface hyphae, stipes usually 200–400 μm long but sometimes much longer, walls smooth to finely roughened, typically bearing terminal verticils of 5–8 closely appressed metulae, but in some isolates subterminal metulae or even a subterminal ramus also occurring; metulae 10–15 μm long, sometimes enlarging at the apices; phialides ampulliform, 7–9 μm long, with short collula; conidia subspheroidal, 2.2–3.0 μm diam, smooth walled, borne in disordered chains.

Distinctive features. *Penicillium paxilli* produces moderately sized, usually velutinous colonies, with terminal penicilli comprised of a crowded cluster of metulae. Stipes which are smooth or nearly so and the absence of sclerotia distinguish this species from *P. raistrickii* and *P. novae-zeelandiae*.

Physiology. Little is known about the physiology of this species. It grows poorly, if at all, at 5 and 37°C, and is probably not a xerophile.

Mycotoxins. *Penicillium paxilli* has been reliably reported to produce two toxic compounds: verruculogen and paxilline. Verruculogen, a tremorgenic toxin with the same active indole moiety as penitrem A, but of quite different structure (Cole, 1981a), is almost equally toxic (Cole et al., 1972). However, the absence of reports of natural occurrence means it may be regarded as a mycotoxin of little consequence in foods and feeds, although not because it lacks toxicity. See *P. simplicissimum* for further details.

Paxilline is also a tremorgen, but structurally quite different from the other tremorgens made by *Penicillium* species, and of lower toxicity. Again, this toxin has not been found from natural sources.

Paxilline and verruculogen were assessed for their genotoxicity to human lymphocytes and in a mammalian microsome assay. Verruculogen tested positive in the mammalian microsome assay and paxilline caused DNA damage in human lymphocytes (Sabater-Vilar et al., 2003a).

It is of interest that isolates of *Penicillium paxilli* producing verruculogen appear to come from Australia, while those producing paxilline are found in the United States (Cockrum et al., 1979).

Ecology. Like most other species in subgenus *Furcatum*, *Penicillium paxilli* is a soil fungus and its occurrence in foods is usually adventitious. However, it has been reported to cause spoilage of garlic (Roy et al., 1977). Although there are few other references in the literature, it is our

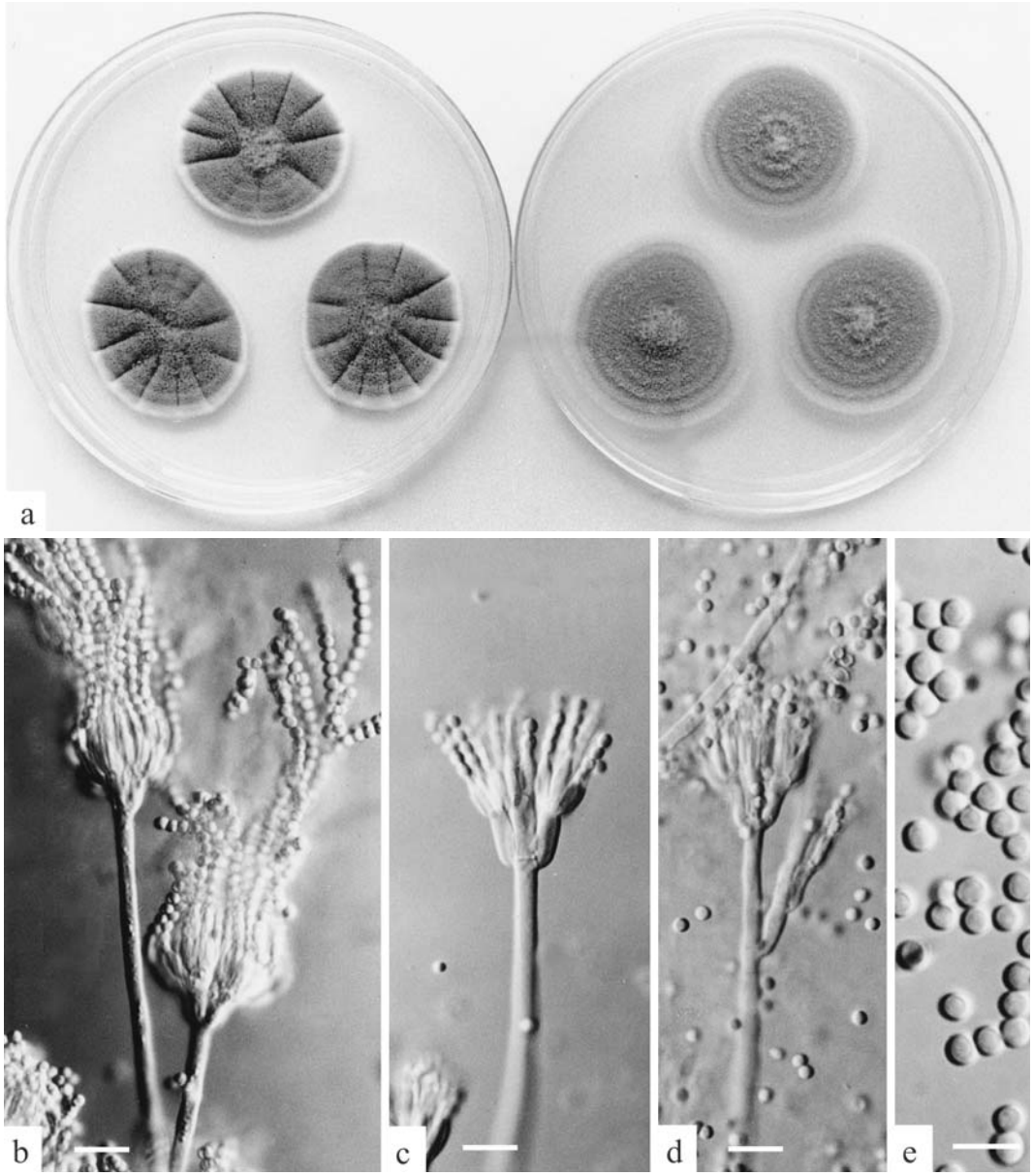


Fig. 7.29 *Penicillium paxilli* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 μm; **(e)** conidia, bar = 5 μm

experience that this species has a widespread though sparse distribution in foods. It has caused visual spoilage of cheese (our observations), and we isolated it at low levels from a wide range of Southeast Asian foods: peanuts from Thailand and dried fish, maize, paddy rice, peanuts, sorghum and soybeans from Indonesia (Wheeler et al., 1986; Pitt et al., 1993, 1998a).

References. Pitt (1979b, 2000).

Penicillium raistrickii G. Sm. Fig. 7.30

Colonies on CYA 35–45 mm diam, plane or nearly so, moderately dense to deeply floccose; mycelium usually white, sometimes centrally pale yellow (1-3A3); sclerotia usually abundant, enveloped by the mycelium; conidial formation often sparse and confined to central areas, sometimes moderate and then coloured dull green (26-27D3); clear exudate usually

present; reverse pale, yellow or yellow brown. Colonies on MEA usually 40–45 mm diam, occasionally as small as 30 mm, plane, sparse, velutinous to floccose; mycelium white to pastel yellow (3-4A3-4); sclerotia usually abundant, superficial or surrounded by wefts of mycelium; conidial production sparse, coloured as on CYA; exudate produced occasionally, red brown; reverse pale or yellow brown. Colonies on G25N 18–26 mm diam, plane or sulcate, often very deep and floccose at the centres; mycelium white; reverse pale. At 5°C, at least germination; usually colonies of 2–4 mm diam produced, of white mycelium. No growth at 37°C.

Sclerotia white or buff, rarely brown, 150–250 μm diam, texture firm to sclerotioid. Teleomorph not known. Conidiophores borne from subsurface mycelium and also from aerial hyphae adjacent to sclerotia, stipes usually 300–500(–800) μm long but from aerial hyphae sometimes much shorter, walls thick and roughened, bearing exclusively or

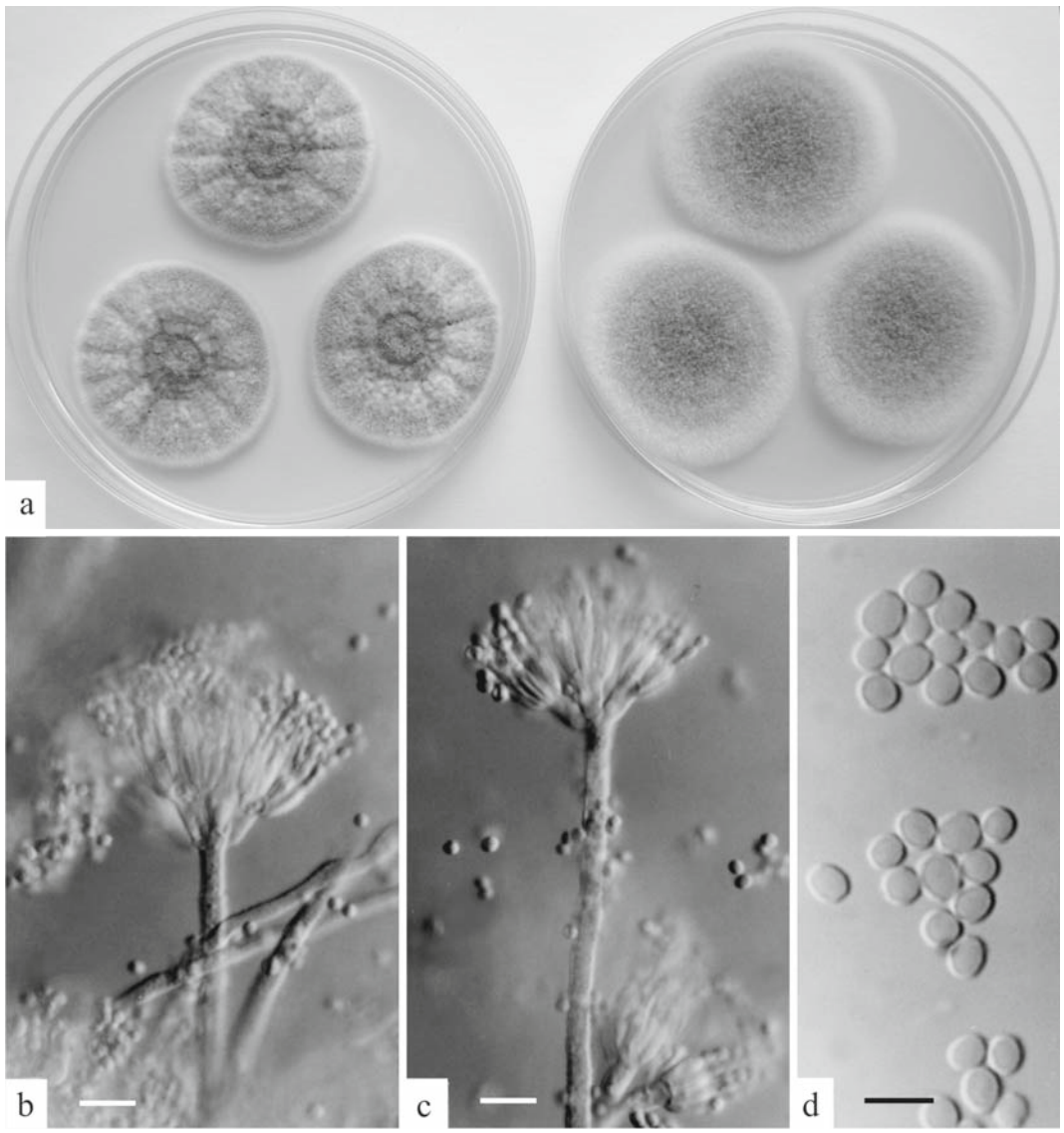


Fig. 7.30 *Penicillium raistrickii* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** penicilli, bars = 10 μm; **(d)** conidia, bar = 5 μm

predominantly terminal biverticillate penicilli, in some isolates accompanied by short subterminal rami; terminal metulae in divergent verticils of 3–5, apically inflated or clavate, of uniform appearance, 12–15 μm long, usually enlarging to 5–7 μm at the apices, sometimes rough walled; phialides acerose-ampulliform, 7–9 μm long, narrowing abruptly to short collula; conidia spherical to subspheroidal, 2.0–3.0 μm diam, smooth walled, borne in long, divergent columns.

Distinctive features. *Penicillium raistrickii* grows rapidly and produces long, rough walled stipes and terminal biverticillate penicilli with apically inflated metulae. Almost all isolates produce abundant pale to brown sclerotia.

Physiology. Little is known about the physiology of this species. Isolates are just capable of growth at 5°C, but not at 37°C. It is probably not a xerophile.

Mycotoxins. Griseofulvin is a valuable antibiotic, but is appreciably toxic, and must also be classed as a mycotoxin. *Penicillium raistrickii* is one species producing this compound. This species may also make other unknown tremorgenic toxins (Patterson et al., 1979).

Ecology. Basically a soil fungus, *Penicillium raistrickii* has a widespread though sparse distribution in foods. This species was isolated from contaminated capsicums (Martín et al., 2005). We isolated it at low levels from Indonesian dried fish (Wheeler et al., 1986) and from Thai maize (Pitt et al., 1993). Other isolates in our culture collection have come from a mandarin fruit, from flour and from peanuts.

Additional species. Perhaps a rarer species, *Penicillium novae-zeelandiae* J.F.H. Beyma appears to be very closely related to *P. raistrickii*. The major difference is that *P. novae-zeelandiae* characteristically produces irregular black sclerotia under the agar surface. What little is known of its physiology shows similarity to *P. raistrickii*. An isolate of *P. novae-zeelandiae* was found to produce an uncharacterised tremorgenic toxin (di Menna and Mantle, 1978). We have isolated *P. novae-zeelandiae* occasionally from a variety of foods, of note from "thread mould" spoilage of vacuum-packed cheese blocks (Hocking and Faedo, 1992), dried fruit and grape juice.

References. Pitt (1979b, 2000).

Penicillium simplicissimum (Oudem.) Thom
Fig. 7.31

Penicillium paraherquei S. Abe ex G. Sm.
Penicillium piscarium Westling
Penicillium pulvillorum Turfitt

Colonies on CYA typically 40–50 mm diam, radially sulcate; mycelium dense, white or occasionally buff; conidial production absent in some isolates, light to moderate in others, typically coloured greyish green to dull green (25C-D4) but in some isolates centrally or predominantly yellowish grey (2-3C2); sometimes clear exudate and occasionally red brown soluble pigment present; reverse typically pale, but sometimes in greyish yellow shades such as chamois or khaki (4B-D5). Colonies on MEA typically 40–50 mm diam, plane, up to 2–3 mm deep, velutinous to floccose; mycelium white; conidial production moderate to heavy, greyish green to dull green (26-29C-E3-4); reverse pale or sometimes yellow brown. Colonies on G25N 12–18 mm diam, plane, sulcate or wrinkled, velutinous to floccose; mycelium white; reverse pale, yellowish or olive. At 5°C, no germination to germination, occasional isolates forming microcolonies. At 37°C, colonies usually 20–30 mm diam, occasional isolates weaker, 5–10 mm or even absent, sulcate to deeply wrinkled, of white to pinkish mycelium, rarely with grey or greenish grey conidia present; reverse pale or brownish.

Sclerotia occasionally produced on CYA or MEA at 25°C, spheroidal or irregular, commonly 200–500 μm diam, brown, of soft texture. Teleomorph not known. Conidiophores borne from surface or aerial hyphae, stipes commonly 400–800 μm long, usually rough walled, bearing regular to irregular verticils of metulae and occasionally a well defined ramus, but only rarely short conidiophores or metulae in other than terminal or subterminal positions; metulae in verticils of 2–5, 12–20 μm or more long, rough walled; phialides ampulliform, mostly 7–9 μm long, narrowing abruptly to long collula; conidia variable, commonly ellipsoidal, but at times spherical, subspheroidal or pyriform, 2.5–4.0 μm long, with walls roughened or spinulose, borne in disordered chains.

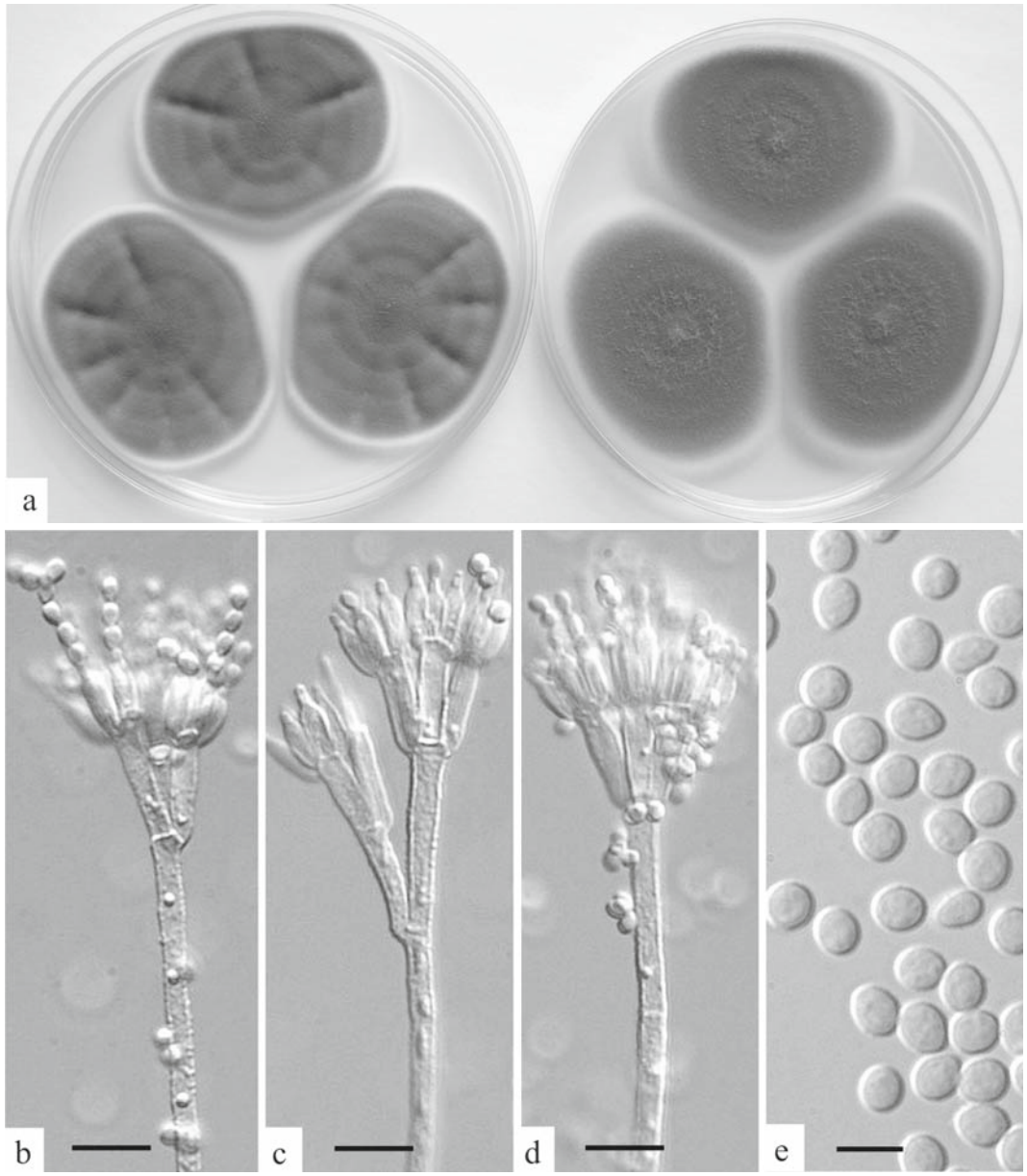


Fig. 7.31 *Penicillium simplicissimum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 µm; **(e)** conidia, bar = 5 µm

Distinctive features. Isolates of *Penicillium simplicissimum* grow rapidly at 25°C, forming comparatively deep colonies due to long conidiophores. The conidiophores are characteristic: stipes are long and coarse with rough walls and bear terminal or subterminal metulae and occasionally rami. Short conidiophores or intercalary metulae are uncommon.

Taxonomy. Controversy over the correct name and circumscription of this species (Stolk and Samson, 1983; Pitt, 2000; Frisvad et al., 1990b) has been resolved by maintaining the neotypification by Pitt (1979b), based on a herbarium specimen of Jensen's from Cornell University (Pitt and Samson, 1993). A recent taxonomic study of *Penicillium simplicissimum* and related species (Tuthill et al., 2001) concluded that *P. simplicissimum* encompasses several distinct species and that the name should be applied more narrowly within this group. However,

as all of those species are very similar, and it is not known which occur in foods, the concept of *P. simplicissimum* used here is the same as that of Pitt (1979b, 2000).

Physiology. Isolates of *Penicillium simplicissimum* from different parts of the world show variation in growth at higher temperatures. Tropical isolates grow strongly at 37°C; some from cooler regions of Australia grow at most weakly at this temperature. This probably reflects the report that *P. simplicissimum* actually includes several species (Tuthill et al., 2001). This species is not a xerophile, germinating down to 0.86 a_w only (Hocking and Pitt, 1979).

Mycotoxins. *Penicillium simplicissimum* has been reported to produce the mycotoxins verruculogen (Pitt, 1979c), fumitremorgen B (El-Banna et al., 1987b), penicillic acid and viridicatumtoxin (El-Banna et al., 1987b; Pitt and Leistner, 1991). Verruculogen, of quite different structure from the penitrems but containing the same active indole moiety (Cole, 1981b), is almost equally toxic (Cole et al., 1972, reported as *P. verruculosum*). Fumitremorgen B is closely related to verruculogen and can be expected to have a similar toxicity. However, natural production of these toxins by this species has not been shown, and their significance in human or animal health is doubtful. The importance of viridicatumtoxin is unknown (see *P. aethiopicum*).

In splitting *Penicillium simplicissimum* into several species, Tuthill et al. (2001) showed that fumitremorgens, verruculogen and viridicatumtoxin are produced by *P. brasilianum*, not *P. simplicissimum*. However, it is not yet clear which of these species occurs in foods.

This species appears to be resistant to formaldehyde. Isolates in our collection and at the International Mycological Institute, Egham, Surrey, UK, have come from 10% formalin solution or preserved specimens. Several IMI isolates have also come from polyvinyl chloride or polyester plastics.

Ecology. *Penicillium simplicissimum* appears to have its primary habitat in decaying vegetation, and perhaps soil. Its occurrence on foods is therefore not as a spoilage fungus, but as a contaminant. It has been reported from country cured hams (Monte et al., 1986), cashews, peanuts and mung beans (Pitt et al., 1993, 1994). Isolates in our collection have also come from spring water, sorghum malt and fermented sausage.

References. Pitt (1979b,c, 2000).

7.7.3 Penicillium *subgenus* Penicillium

(*Penicillium* Sect. *Asymmetrica* Raper and Thom)

In *Penicillium* subgenus *Penicillium*, penicilli are predominantly terminal terverticillate structures, i.e. phialides are always borne on metulae and the metulae usually are borne on well -defined terminal rami. Biverticillate and quaterverticillate penicilli are also produced by some species. Phialides are typically ampulliform (flask shaped), but are cylindroidal to acerose (needle shaped) in a few species.

Colonies on G25N normally exceed 18 mm diam, although they are consistently less in one species treated here, *P. digitatum*. Only *P. aethiopicum* and *P. chrysogenum* sometimes grow at 37°C. Growth at 5°C is always positive, and usually strong: most species produce macroscopic colonies in 7 days.

By far the most important subgenus of *Penicillium* with respect to food spoilage, subgenus *Penicillium* is also by far the most difficult taxonomically, both because there are numerous species and because apparent differences between species are small. For a number of years, the taxonomy of this subgenus remained controversial, with the systems of Raper and Thom (1949), Samson et al. (1976) and Pitt (1979b) all being commonly used. During the 1980s, students of *Penicillium* sought consensus on the classification of this subgenus. Secondary metabolite profiles (Frisvad, 1981; Frisvad and Filtenborg, 1983) and isoenzyme patterns (Cruickshank and Pitt, 1987) provided independent evidence about species concepts. Integration of classical morphology with these new approaches led to consensus for most species (Samson and Pitt, 1985). Later, a list of "Names in Current Use" (NCU) was established for *Penicillium* species (Pitt and Samson, 1993; Pitt et al., 2000). This now provides much needed stability. All names used here for species in this subgenus are protected by this NCU.

Recently, Samson and Frisvad (2004) have published a comprehensive revision of this subgenus and increased the number of included species from 23 to 58. Where these additions affect species included here they are discussed under the relevant species.

Identifications. It is usually relatively easy to decide whether an isolate belongs in subgenus *Penicillium*, but accurate identifications to species level require care and, often, acquired skill. In the

keys used here, the roughness or smoothness of stipes and small differences in colour will affect the disposition of an isolate. Perhaps the most important point to note in the keys which follow is that species which have perfectly smooth stipe walls under the light microscope at 400–600× magnification are distinguished from those in which some stipes, though not necessarily all, are finely to distinctly roughened. Roughening of stipe walls is often more readily seen in wet mounts made from cultures grown on MEA.

A second point to note is that judgment of colour should be made in daylight or artificial daylight conditions, i.e. in the latter case, under daylight type fluorescent tubes, not incandescent lamps. The use of the Methuen "Handbook of Colour" (Kornerup and Wanscher, 1978) will greatly assist colour differentiation of species in this subgenus. Some of the species accepted here are undoubtedly closely related and intermediate isolates will be encountered from time to time. Nevertheless, with care it should be possible to key out and recognise the majority of isolates from foods.

Neutral creatine sucrose agar. Creatine sucrose agar (CREA) was introduced as a new approach to the problem of identifying species in subgenus *Penicillium* (Frisvad, 1981, 1985). He pointed out that species in this subgenus fall into two groups: those with an affinity for proteinaceous foods and those which will grow vigorously on foods rich in carbohydrate. CREA was designed to permit differentiation between these two groups. It contained the organic base creatine as a sole source of nitrogen, sucrose as a source of carbon, and an indicator, bromocresol purple, to detect pH changes related to differential acid production (from sucrose) or base production (by liberation of ammonium ions from creatine). The concept was innovative and sound, but CREA was not easy to use in practice. A number of variations were introduced by Frisvad (1993), but without firm recommendations.

A medium based on CREA, but of initial pH neutral to bromocresol purple and with a change in sucrose to creatine ratio to provide more positive responses by some species, was developed by Pitt (1993a) and termed Neutral Creatine Sucrose agar (CSN). The formula is given in Appendix.

CSN has proved to be very useful in identification of species in *Penicillium* subgenus *Penicillium*, as eight different types of responses have been observed among common species (Table 7.2; modified from Pitt, 1993a). Reaction to CSN has been included in the descriptions and keys which follow. If only occasional isolates from this subgenus are seen, use of CSN is not essential. However, for the laboratory which frequently isolates species from this subgenus, CSN can be of great assistance. CSN is of little value outside this subgenus.

Use of Ehrlich reagent. In the quest for more rapid and reliable methods for identifying species in subgenus *Penicillium*, Lund (1995a) developed a simple test to distinguish among several species common in cheese and bread factories in Denmark. The test used Ehrlich reagent to visualise production of indole compounds as secondary metabolites. Ehrlich reagent is made by dissolving 4-dimethylaminobenzaldehyde (2 g) in 96% ethanol (85 ml) and adding 10 N HCl (15 ml).

To carry out the test, a cork borer (4 mm diameter) is used to cut an agar plug from a culture on CYA and then the plug is placed, mycelium side up, on a Petri dish lid. A strip (12 × 6 mm) of Whatman No. 1 filter paper is dipped in the Ehrlich reagent and then placed across the agar plug "like a propeller", with the agar plug in the centre of the strip, so the strip does not touch the Petri dish lid. The arrangement is then placed in an air stream in a fume hood and allowed to dry. A positive response is indicated by a violet ring in the filter paper, appearance after 2–6 min being a strong response, and after 7–10 min a weak one. Observation must be made within 10 min, as the ring fades after that time. Most isolates produce the colour on CYA after incubation for 7 days, however, a few require 14 days incubation. The test is of more value when positive than negative, therefore.

Species for which this test is positive include *Penicillium camemberti*, *P. commune, P. expansum* and *P. roqueforti* (Lund, 1995a). A few other species produce yellow colours with this test, including *P. crustosum* (Lund, 1995a).

Physiology. As noted above, species in subgenus *Penicillium* are very important in food spoilage. They are able to grow at low temperatures and quite low water activities and are of universal occurrence in cereals, refrigerated foods and

Table 7.2 Responses of common species of *Penicillium subgenus Penicillium* to Neutral Creatine Sucrose agar (CSN) after incubation for 7 days at 25°C[a]

Species	Colony growth	Colony diam (mm)	Medium reaction	Colony reverse
Penicillium aethiopicum	Moderate	15–20	Acid, margins sometimes neutral	Acid + brown
P. allii	Weak	15–20	Acid	Acid
P. aurantiogriseum	Moderate	15–25	Acid + brown	Acid + brown
P. brevicompactum	Weak	8–14(–20)	Neutral to weakly acid	Neutral (occasionally weakly acid)
P. camemberti	Strong	15–20	Alkaline, margins neutral	Alkaline
P. chrysogenum	Moderate to strong	12–18	Neutral or weakly acid	Neutral to weakly acid
P. commune	Strong	20–26	Alkaline, sometimes weakly acid	Alkaline
P. crustosum	Strong	25–30	Acid, occasionally alkaline	Alkaline, occasionally weak
P. digitatum	Weak	4–10	Neutral	Neutral
P. echinulatum	Strong	22–25	Alkaline	Alkaline
P. expansum	Strong	24–30	Acid	Acid + brown, occasionally alkaline
P. glandicola	Moderate	12–18	Weakly acid to acid	Acid + brown
P. griseofulvum	Weak	18–24	Neutral	Neutral
P. hirsutum	Strong	24–30	Acid	Acid (+ brown)
P. hordei	Strong	20–24	Acid	Acid + brown
P. italicum	Weak	10–20	Neutral	Neutral
P. nalgiovense	Weak	10–18	Neutral to weakly acid	Neutral to weakly acid
P. olsonii	Weak	6–14	Neutral	Neutral
P. roqueforti	Strong	25–40	Variable	Alkaline, occasionally neutral
P. solitum	Strong	18–22	Usually alkaline	Alkaline
P. ulaiense	Weak	4–8	Neutral	Neutral
P. verrucosum	Weak	10–15	Neutral	Neutral
P. viridicatum	Moderate to strong	15–22	Acid	Acid (+ brown)

[a] Acid, yellow; weakly acid, pale yellow; neutral, grey, sometimes with a reddish cast; weakly alkaline, grey violet; alkaline, violet or reddish violet. Brown responses are from naturally produced pigments.

many other environments. Controlling these fungi in many types of bulk stored food commodities relies on a combination of low water activity and low temperature: even marginal errors in these controls may sometimes lead to high losses.

Mycotoxins. With the possible exception of the fruit-rotting species *Penicillium digitatum*, *P. italicum*, *P. solitum* and *P. ulaiense*, all of the species under consideration here produce mycotoxins. Moreover, mycotoxin production appears to occur more consistently than in most other genera: a large majority of the isolates encountered from subgenus *Penicillium* will be mycotoxigenic. Fortunately, most of the toxins are believed to have relatively low potency or are not produced under conditions occurring in foods.

The question of species–mycotoxin associations for species in *Penicillium* subgenus *Penicillium*, considered a major problem when the first edition of this book was published, has been clarified. A comprehensive collaborative study, in which more than 1500 *Penicillium* isolates were critically examined both taxonomically and for mycotoxin production, greatly assisted this process (El-Banna et al., 1987b; Pitt and Leistner, 1991). When combined with data from Frisvad and Filtenborg (1989) and Samson and Frisvad (2004), this information has enabled recognition of the fact that species–mycotoxin associations in this genus are mostly quite specific. These data have been incorporated below.

Key to *Penicillium* subgenus *Penicillium* species included here

1	Conidia white or pale grey green	2
	Conidia more strongly coloured, blue, green or grey	3
2 (1)	Stipes often rough walled, conidia up to 5 μm diam; on CSN, reaction alkaline	*P. camemberti*
	Stipes consistently smooth walled, conidia not usually exceeding 3.5 μm diam; on CSN, reaction neutral to weakly acid	*P. nalgiovense*
3 (1)	Stipes on CYA and MEA smooth walled, or at most very finely roughened	4
	Stipes commonly rough walled, especially on MEA	16
4 (3)	Conidia borne as cylinders, with at least a proportion remaining so at maturity	5
	Conidia borne as ellipsoids or spheres and remaining so at maturity	7
5 (4)	Conidia olive, longer than 6 μm	*P. digitatum*
	Conidia green, shorter than 6 μm	6
6 (5)	Colonies on CYA and MEA exceeding 30 mm diam	*P. italicum*
	Colonies on CYA and MEA not exceeding 30 mm diam	*P. ulaiense* (*see P. italicum*)
7 (4)	Penicilli with 3 or more rami in large, compact terminal penicilli	*P. olsonii*
	Penicilli with 1–2 rami, penicillus structure variable	8
8 (7)	Penicilli complex or irregular, reverse on MEA strongly coloured, deep orange brown to brown, phialides with short, broad collula; on CSN, reaction neutral to weakly acid	*P. nalgiovense*
	Penicilli well defined, mostly terverticillate, reverse on MEA pale or dull yellow or brown, phialides with relatively long, narrow, collula; CSN response variable	9
9 (8)	Colonies on CYA exceeding 30 mm diam	10
	Colonies on CYA not exceeding 30 mm diam	14
10 (9)	Conidia grey blue	11
	Conidia grey green to green	12
11(10)	Colonies on CYA 35–45 mm diam, yellow exudate and soluble pigment often present, conidia ellipsoidal to subspheroidal; on CSN, medium response neutral to weakly acid	*P. chrysogenum*
	Colonies on CYA 30–38 mm diam, exudate clear to pale brown, soluble pigment sometimes present, brown to reddish brown, conidia spherical to subspheroidal; on CSN, medium response acid, reverse acid plus brown	*P. aurantiogriseum*
12(10)	Reverse on CYA pale to deep brown; colonies on CSN usually exceeding 24 mm diam	*P. expansum*
	Reverse on CYA pale, yellow or orange; colonies on CSN less than 24 mm diam	13
13(12)	Colonies dull green, conidia broadly ellipsoidal, reverse on CYA bright golden yellow; on CSN reverse acid plus brown	*P. aethiopicum*
	Colonies dark green, conidia mostly spherical to subspheroidal, reverse on CYA usually pale, sometimes orange or pale yellow; CSN reverse alkaline	*P. solitum*
14 (9)	Phialides commonly 4.5–6 μm long; on CSN, response neutral	*P. griseofulvum*
	Phialides exceeding 6 μm long; CSN response variable	15

15(14)	On CYA colonies dull green, penicilli very broad, metulae often apically inflated, conidia ellipsoidal; on CSN, reverse neutral to weakly acid	*P. brevicompactum*
	On CYA, colonies dark green, penicilli not very broad, metulae not usually apically inflated, conidia mostly spherical to subspheroidal; on CSN, reverse alkaline	*P. solitum*
16 (3)	Colonies on CYA exceeding 30 mm diam	17
	Colonies on CYA not exceeding 30 mm diam	24
17(16)	Conidia with walls finely roughened to rough or spinose	18
	Conidia smooth walled	19
18(17)	Mycelium inconspicuous, white, conidia dark green; growth on CSN strong, medium and reverse alkaline	*P. echinulatum* (see *P. crustosum*)
	Mycelium yellow, conidia dull green; on CSN, medium and reverse acid	*P. hordei* (see *P. hirsutum*)
19(17)	Growth on CSN strong, colonies more than 20 mm diam, medium and reverse alkaline	20
	Growth on CSN variable, medium and reverse acid, reverse sometimes also brown	22
20(19)	Colonies on CYA and MEA exceeding 40 mm diam, reverse on CYA and/or MEA often green to deep blue green; conidia up to 6 μm diam	*P. roqueforti*
	Colonies on CYA or MEA less than 40 mm diam, reverse pale or yellow to orange brown, conidia mostly 4 μm or less diam	21
21(20)	Colonies on CYA exceeding 35 mm diam and on MEA usually exceeding 30 mm diam; colonies on MEA often shedding masses of conidia when jarred	*P. crustosum*
	Colonies on CYA and MEA not exceeding 35 and 30 mm diam, respectively; conidia adhering to colonies on MEA	*P. commune*
22(19)	Colonies on CYA often exceeding 35 mm diam	23
	Colonies on CYA not exceeding 35 mm diam	*P. viridicatum*
23(22)	Exudate on CYA near maroon; mycelium often yellow; penicilli sometimes quaterverticillate	*P. hirsutum*
	Exudate on CYA pale yellow; mycelium white, penicilli terverticillate or somewhat irregular	*P. allii* (see *P. hirsutum*)
24(16)	Conidia mostly ellipsoidal, dark green	*P. glandicola* (see *P. brevicompactum*)
	Conidia near spherical, yellow green	25
25 (24)	Colonies on CYA and MEA exceeding 25 mm diam	*P. viridicatum*
	Colonies on CYA and MEA not exceeding 25 mm diam	*P. verrucosum*

Penicillium aethiopicum **Frisvad** **Fig. 7.32**

Colonies on CYA 32–40 mm diam, finely radially sulcate, deeply fasciculate; mycelium white, visible only at the margins; conidial production moderate to heavy, dull green (25-26E-F4); copious exudate usually present, clear to pale brown; reverse closely sulcate, golden yellow near mustard yellow or maize yellow (3-4B-C5-6). Colonies on MEA 32–40 mm diam, plane, low and dense, surface velutinous to granular; mycelium inconspicuous, white; conidial production moderate, dull green (26D-E4); reverse uncoloured to dull yellow brown. Colonies on G25N 22–24 mm diam, plane or sulcate, low to moderately deep, dense, usually fasciculate; mycelium white; sporulation moderate, dull green; soluble pigment

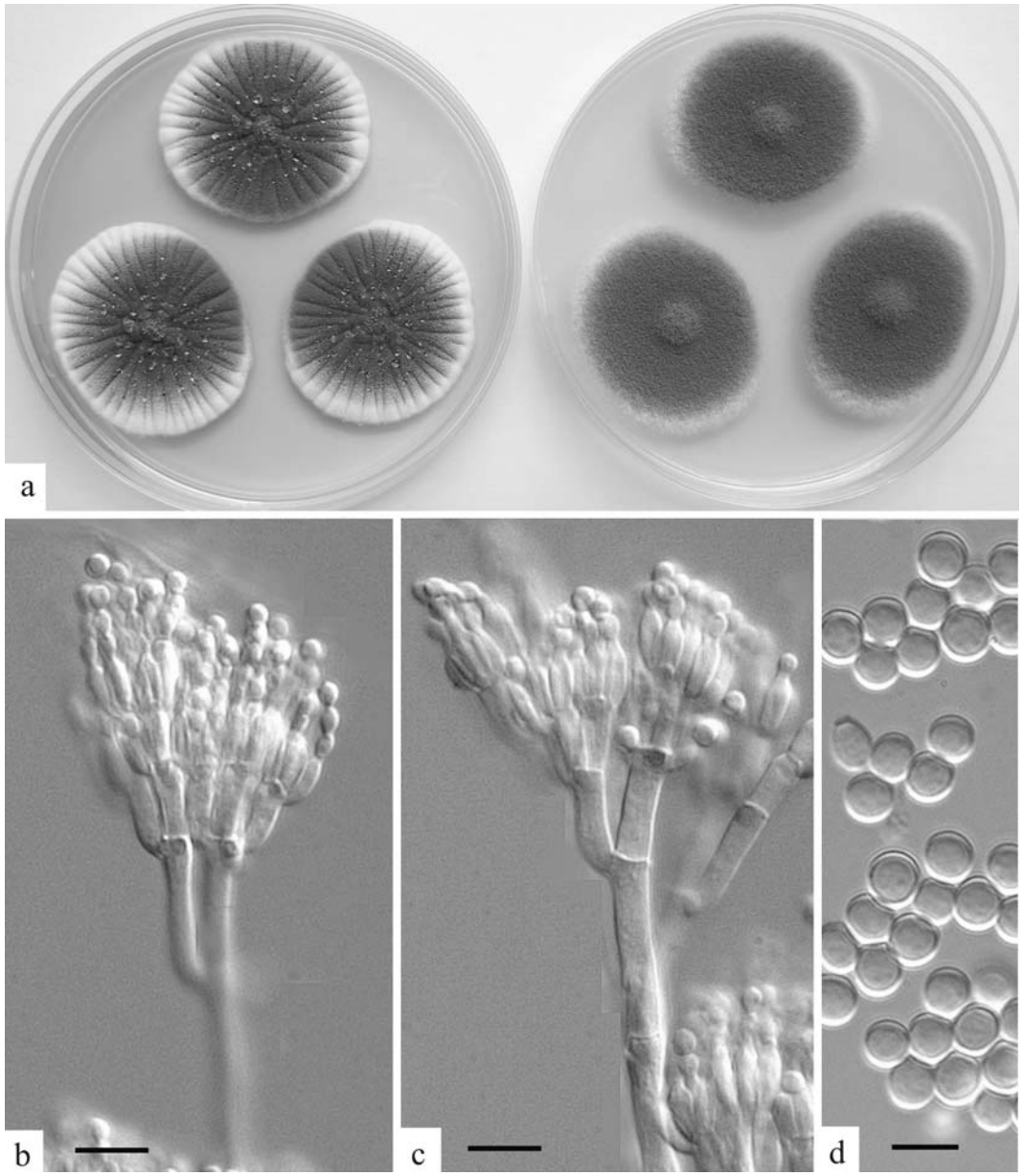


Fig. 7.32 *Penicillium aethiopicum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** penicilli, bars = 10 μm; **(d)** conidia, bar = 5 μm

sometimes produced, pale orange (5A3); reverse pale, bright yellow or pale orange. At 5°C, germination to colonies 3 mm diam. At 37°C, no growth to colonies 10 mm diam, of dense white mycelium.

On CSN, colonies 15–20 mm diam, growth moderate, medium reaction acid (pale yellow), sometimes neutral at the margins; reverse acid (yellow), plus brown.

Conidiophores borne singly or more commonly in fascicles, mostly from subsurface hyphae, stipes 200–400 μm long, or of indeterminate length in fascicles, with smooth or at most very finely roughened walls, typically bearing appressed, terminal terverticillate penicilli; phialides ampulliform, 7–9 μm long, with short collula; conidia broadly ellipsoidal, 3.2–3.8 μm long, smooth walled, borne in long, sometimes well defined, columns.

Distinctive features. *Penicillium aethiopicum* resembles *P. chrysogenum* in growth rates, including the ability to grow weakly at 37°C; *P. expansum*, in

producing fasciculate colonies; and both species, in producing conidiophores with smooth-walled stipes. The most obvious feature distinguishing *P. aethiopicum* from these other species is the formation of a closely sulcate golden yellow reverse on CYA in the absence of yellow soluble pigment. Penicilli are more compact and robust than those of *P. chrysogenum*, while brown pigmentation is much less than occurs in colonies of *P. expansum* on CYA. Growth on CSN resembles that of *P. chrysogenum*.

Physiology. No studies on physiology are known to us. The ability to grow at 37°C suggests a physiology similar to *Penicillium chrysogenum*.

Mycotoxins. *Penicillium aethiopicum* produces griseofulvin (see *P. griseofulvum*) and viridicatumtoxin (Frisvad and Filtenborg, 1989). The latter is a moderately toxic compound, with an LD_{50} of 70 mg/kg when injected into mice. Its oral toxicity is unknown and its practical significance unclear.

Ecology. *Penicillium aethiopicum* was originally described from barley from Ethiopia (Frisvad and Filtenborg, 1989). We found this species frequently in Southeast Asian commodities: in 16% of Indonesian kemiri nut samples, with a 4% infection rate in all nuts examined, in 18% of Indonesian cow pea samples, 9% of Indonesian peanut samples and 4% of Philippine maize samples. Incidence in some individual samples was high, up to 30–40% of all particles examined, providing a 1% infection level overall in each of these commodities. It was also present, at low levels, in Thai peanuts and cashews, Philippine soybeans and mung beans, and Indonesian milled rice, soybeans and mung beans (Pitt et al., 1993, 1994, 1998a).

References. Frisvad and Filtenborg (1989); Samson and Frisvad (2004).

Penicillium aurantiogriseum Dierckx

Fig. 7.33

Penicillium puberulum Bainier
Penicillium cyclopium Westling
Penicillium martensii Biourge
Penicillium aurantiovirens Biourge
Penicillium verrucosum var. *cyclopium* (Westling) Samson et al.

Colonies on CYA 30–37 mm diam, radially sulcate, moderately deep, texture smooth to granular; mycelium white, usually inconspicuous; conidial production moderate to heavy, greyish turquoise to dull green (24-25D-E3-4); exudate usually conspicuous, clear or pale brown; soluble pigment produced by some isolates, brown to reddish brown; reverse pale, light to brilliant orange, or reddish to violet brown. Colonies on MEA 24–35 mm diam, plane or rarely radially sulcate, low and relatively sparse, surface texture finely granular; mycelium usually subsurface, occasionally conspicuous and then bright yellow; conidial production usually moderate to heavy, greyish turquoise to dull green (24-25D-E4-5); soluble pigment sometimes produced, yellow brown to reddish brown; reverse pale, orange, or reddish brown. Colonies on G25N 18–24 mm diam, usually radially sulcate, moderately deep, dense, texture granular; reverse pale, yellow or brown. At 5°C, colonies 2–5 mm diam, of white mycelium. No growth at 37°C.

On CSN, colonies usually 15–25 mm diam, with moderately dense to dense growth, medium reaction acid (yellow); reverse acid (yellow) plus brown soluble pigment.

Conidiophores borne singly or in fascicles, mostly from subsurface hyphae, stipes 200–400 μm long, or of indeterminate length in fascicles, with walls smooth to finely roughened, bearing terminal terverticillate or less commonly biverticillate penicilli; phialides slender, ampulliform, mostly 7–10 μm long; conidia spherical to subspheroidal, less commonly ellipsoidal, 3.0–4.0 μm long, with smooth walls, mostly borne in long, well defined columns.

Distinctive features. *Penicillium aurantiogriseum* produces blue grey conidia on both CYA and MEA. Colonies on CYA grow relatively slowly (37 mm diam at most) and are dense and of granular texture. Colonies on MEA are relatively low and sparse, with distinctly granular margins. Stipes are usually smooth or nearly so. The response on CSN is distinctive: quite rapid growth, an acid reaction in medium and colony reverse, characteristically with brown colours also in the medium and beneath the colony.

Taxonomy. *Penicillium aurantiogriseum* as described here is a relatively broad species. It has essentially the same circumscription as given by Pitt et al. (1986) and Pitt (2000), but narrower than that described by Samson et al. (1976) or Pitt (1979b). More recently, several other species were segregated from *P. aurantiogriseum* on the basis of secondary metabolite, morphological and ecological characters (Lund and Frisvad, 1994; Samson and Frisvad, 2004).

We have maintained the concept of *Penicillium aurantiogriseum* described by Pitt et al. (1986) and Pitt (2000) as we believe that, with some practice, it

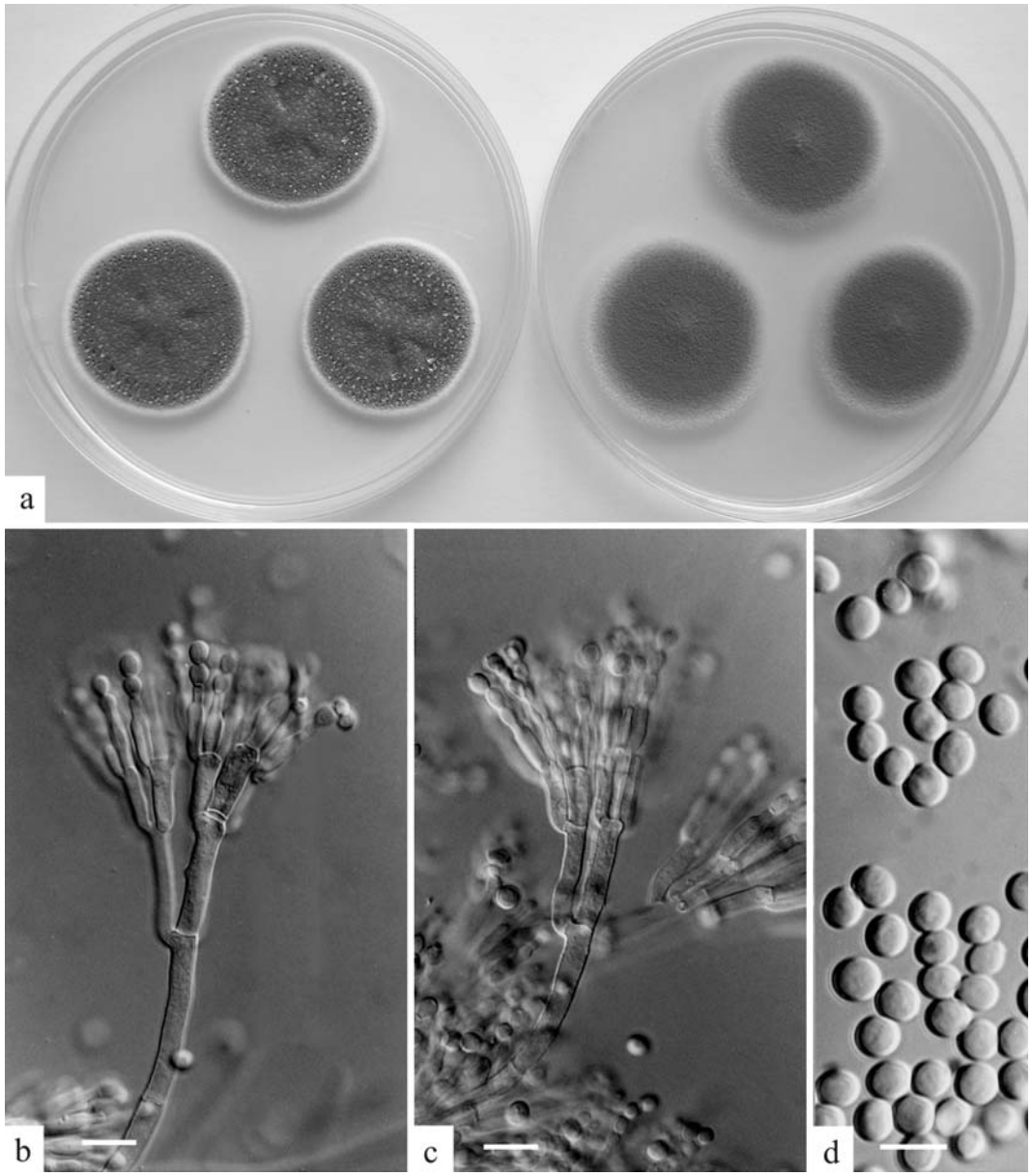


Fig. 7.33 *Penicillium aurantiogriseum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** penicilli, bars = 10 μm; **(d)** conidia, bar = 5 μm

can be distinguished purely on morphological criteria from other species described in this book. If further differentiation is required, see Lund and Frisvad (1994) or Samson and Frisvad (2004).

Physiology. *Penicillium aurantiogriseum* has a minimum temperature for growth near −2°C, an optimum near 23°C and a maximum near 30°C (Armolik and Dickson, 1956; Mislivec and Tuite, 1970b). The minimum a_w for growth is 0.81 (Mislivec and Tuite, 1970b). Growth at reduced a_w is little affected by pH or substrate (Hocking and Pitt, 1979). Growth was stimulated by carbon dioxide levels above 10% (Magan and Lacey, 1984b). Growth was observed in 30 and 50% CO_2 but not in atmospheres containing 70% CO_2 (Zardetto, 2005).

Considerable work has been carried out on the production of volatiles in stored grains by *Penicillium aurantiogriseum*. Major compounds reported include 1-propanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 1-octen-3-ol and 3-octanone on barley, wheat and synthetic media (Sinha et al., 1988; Wilkins and Scholl, 1989; Börjesson et al., 1990; Larsen and Frisvad, 1995).

The lipolytic activity of *Penicillium aurantiogriseum* was high, as judged by action on a medium containing 1% rapeseed oil (Magan et al., 1993). Lipase production was enhanced when a medium containing 0.5% yeast extract and 1% olive oil was supplemented with ammonium sulphate (Lima et al., 2003).

Mycotoxins. Isolates of *Penicillium aurantiogriseum* commonly produce penicillic acid, verrucosidin and nephrotoxic glyopeptides (Frisvad and Samson, 2004). Accumulation of penicillic acid by *P. aurantiogriseum* is favoured by low temperatures, in the range 1–10°C; production is faster at higher temperatures, but so is degradation (Ciegler and Kurtzman, 1970).

Reports that *Penicillium aurantiogriseum* isolates may also produce roquefortine C, xanthomegnin or viomellein (El-Banna et al., 1987b; Frisvad and Filtenborg, 1989) are now believed to be incorrect (Samson and Frisvad, 2004)

Ecology. Along with some other species in this subgenus, *Penicillium aurantiogriseum* is among the most commonly encountered fungi on earth. It is ubiquitous in maturing or drying crops, especially cereals and cereal products (see Pitt and Hocking, 1997). This association with cereals is very important: reports of *P. aurantiogriseum* from proteinaceous foods including meat and cheese are almost always in error (Frisvad and Filtenborg, 1989; Lund et al., 1995). It has also been isolated frequently from nuts (see Pitt and Hocking, 1997).

Penicillium aurantiogriseum has been reported to cause spoilage of a variety of stored fruits and vegetables, including apples, pears, strawberries, grapes, melons, tomatoes, cassava and potatoes (see Pitt and Hocking, 1997). Not all such reports have been authenticated: it is likely that related species are responsible for some of these cases. Other reported sources include cold stored eggs, frozen fruit pastries, spices, dried beans and peas, soybeans, dried fruit, health foods (see Pitt and Hocking, 1997) and luncheon meats (Ismail and Zaky, 1999).

We isolated *Penicillium aurantiogriseum* from a variety of Southeast Asian food commodities, including maize, peanuts, cashews, soybeans, mung beans and rice, but only at low levels (Pitt et al., 1993, 1994, 1998a).

References. Pitt (1979b, 2000); Samson et al. (1976) and Domsch et al. (1980), both under the name *Penicillium verrucosum* var. *cyclopium*; Samson et al. (1995); Samson and Frisvad (2004).

Penicillium brevicompactum Dierckx

Fig. 7.34

Penicillium stoloniferum Thom

Colonies on CYA 20–30 mm diam, radially sulcate, moderately deep, dense, texture typically velutinous; mycelium white; conidial formation light to moderate, dull green (25-28D-E3); exudate usually present in minute droplets, often deeply embedded, but sometimes copious, pale to deep reddish brown; soluble pigment usually produced, reddish brown; reverse sometimes pale but more usually yellowish to reddish brown. Colonies on MEA 12–22 mm diam, plane or less commonly radially sulcate, usually velutinous; mycelium white; conidial production moderate to heavy, dull green to dark green (27-29E-F4), rarely paler or more bluish; exudate occasionally present, clear to reddish brown; reverse pale or brown. Colonies on G25N 14–22 mm diam, plane or radially sulcate, texture granular; clear exudate and red brown soluble pigment sometimes produced; reverse pale, yellow or reddish brown. At 5°C, microcolonies to colonies up to 4 mm diam produced. No growth at 37°C.

Colonies on CSN 8–14 mm diam, occasionally larger, growth weak and sparse, medium response neutral to weakly acid (yellow); reverse neutral, occasionally weakly acid.

Conidiophores borne from surface mycelium, stipes usually broad, 500–800 μm long, smooth walled, characteristically bearing compact, broad terverticillate penicilli, usually less than 40 μm long and 40–50 μm across the phialide tips, with quaterverticillate and biverticillate penicilli usually evident also; rami short and broad, often bent away from the axis; metulae in divergent clusters, short and broad, 9–15 μm long, typically apically inflated; phialides in divergent verticils, ampulliform, 6–9 μm long; conidia ellipsoidal, 2.5–3.5 μm long, with walls smooth to very finely roughened, borne in divergent and disordered chains.

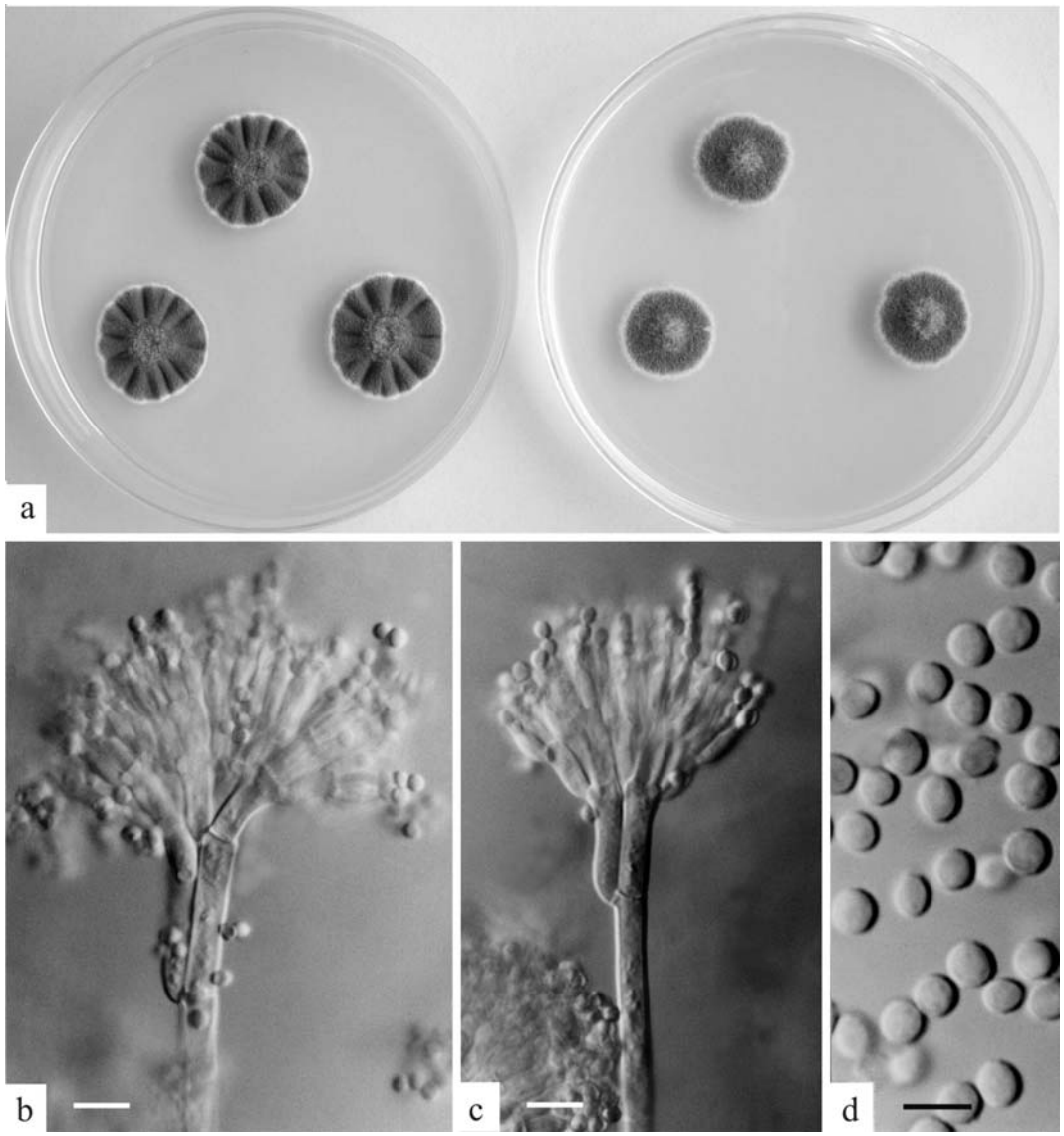


Fig. 7.34 *Penicillium brevicompactum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** penicilli, bars = 10 µm; **(d)** conidia, bar = 5 µm

Distinctive features. *Penicillium brevicompactum* produces compact (though not small) penicilli, often as wide as long. Metulae are short and broad, often apically inflated, fanning out so that the outermost phialides may point in almost diametrically opposed directions. As a result, conidial chains are borne over almost a hemisphere, in a shape suggesting *Aspergillus*.

Physiology. The minimum and maximum temperatures for growth of *Penicillium brevicompactum* are –2 and 30°C, respectively (Mislivec and Tuite, 1970b), with an optimum near 23°C. The minimum a_w for germination and growth is 0.78 at 25°C (Hocking and Pitt, 1979), categorising *P. brevicompactum* as one of the most xerophilic Penicillia.

Mycotoxins. This species produces mycophenolic acid (Frisvad and Filtenborg, 1989; Frisvad and Samson, 2004), a weakly toxic compound with an oral LD_{50} of 700 mg/kg in rats (Cole and Cox, 1981). It is now used as an immunosuppressant in

heart and kidney transplant patients (Bentley, 2000). The presence of mycophenolic acid in ginger due to infection by *P. brevicompactum* has been reported (Overy and Frisvad, 2005) but is unlikely to be of practical concern.

Ecology. Although less common than some other species in this subgenus, *Penicillium brevicompactum* is nevertheless of widespread occurrence especially, because of its xerophilic nature, in dried foods: beans, soybeans, pecans, pistachios and peanuts, health foods and peppercorns (see Pitt and Hocking, 1997). It has also been isolated from Brazilian cashew nuts (Freire et al., 1999), Brazil nuts and black and white pepper (Freire et al., 2000). It commonly occurs in European meat products, hams, biltong (see Pitt and Hocking, 1997) and salami (Cantoni et al., 2007). *P. brevicompactum* can also spoil refrigerated products, such as cheese (Kure and Skaar, 2000; Kure et al., 2001; Hayaloglu and Kirbag, 2007). It has also been reported in tap water in Portugal (Gonçalves et al., 2006). In our laboratory *P. brevicompactum* has been isolated from a range of substrates including spoiled margarine, dairy products, fruit purée, curry paste, sumac, bakery products and bottled water.

Penicillium brevicompactum can also behave as a weak pathogen having caused spoilage of stored apples, mushrooms, cassava, potato, pumpkin (see Pitt and Hocking, 1997), grapes (Bau et al., 2005, 2006; Patiño et al., 2007) yams (Aboagye-Nuamah et al., 2005) and lychees (our observations). This species has recently been isolated from fresh ginger, where it produced mycophenolic acid (Overy and Frisvad, 2005). This species was present at low levels in a variety of Southeast Asian commodities: rice, mung beans, soybeans, maize and peanuts (Pitt et al., 1993, 1994, 1998a).

Additional species. *Penicillium glandicola* (Oudem.) Seifert and Samson (synonym *P. granulatum* Bainier) grows at similar rates to *P. brevicompactum* under all standard conditions. *P. glandicola* is distinguished by a granular texture on CYA and MEA, with small coremia apparent at the margins; stipes, rami and metulae with rough walls; and dark green conidia. On CSN, colonies are 12–18 mm diam, and growth is moderate, with an acid (yellow) reaction in both medium and colony reverse; brown soluble pigment is usually also produced.

Hocking and Pitt (1979) reported 0.86 as the minimum a_w for germination and growth of *Penicillium glandicola*. This species has been reported to be one cause of taint in wine corks (Daly et al., 1984) and as one species producing 2,4,6 trichloroanisole, a cause of off flavour in coffee (Liardon et al., 1992).

Penicillium glandicola is one potential source of penitrem A, patulin and roquefortine C, which may be produced in silage (Frisvad and Samson, 2004). Natural occurrence of these toxins in foods due to this species has not been reported.

This is not a commonly occurring fungus, but it has been isolated from cereals sufficiently frequently to warrant mention here: from wheat, barley, maize and rice. Other sources include peanuts and meat products (see Pitt and Hocking, 1997).

References. Pitt (1979b, 2000); Domsch et al. (1980); Samson and Frisvad (2004).

Penicillium camemberti Thom **Fig. 7.35**

Penicillium candidum Roger
Penicillium caseicola Bainier

Colonies on CYA 25–35 mm diam, occasionally smaller, plane or lightly radially sulcate, convex, floccose; mycelium white; conidal production usually absent to light, pale grey green or in some isolates persistently white, occasionally heavier, greyish green (25-26C3); clear exudate sometimes present; reverse pale, yellow or weakly reddish brown. Colonies on MEA 25–40 mm diam, plane, similar to those on CYA, but without exudate. Colonies on G25N 18–22 mm diam, plane or lightly radially sulcate, similar to those on MEA. At 5°C, colonies commonly 3–6 mm diam. No growth at 37°C.

On CSN, colonies 15–20 mm diam, growing strongly, with medium reaction alkaline (purple), though often neutral near the margins; reverse alkaline.

Conidiophores borne from aerial hyphae, stipes 200–400 μm long, with smooth or roughened walls, typically bearing terminal terverticillate or quaterverticillate penicilli, sometimes irregular; phialides ampulliform, 10–12(–15) μm long, with long, wide collula; conidia subspheroidal to spherical, smooth walled, 3.5–5.0 μm long, borne in short, disordered chains.

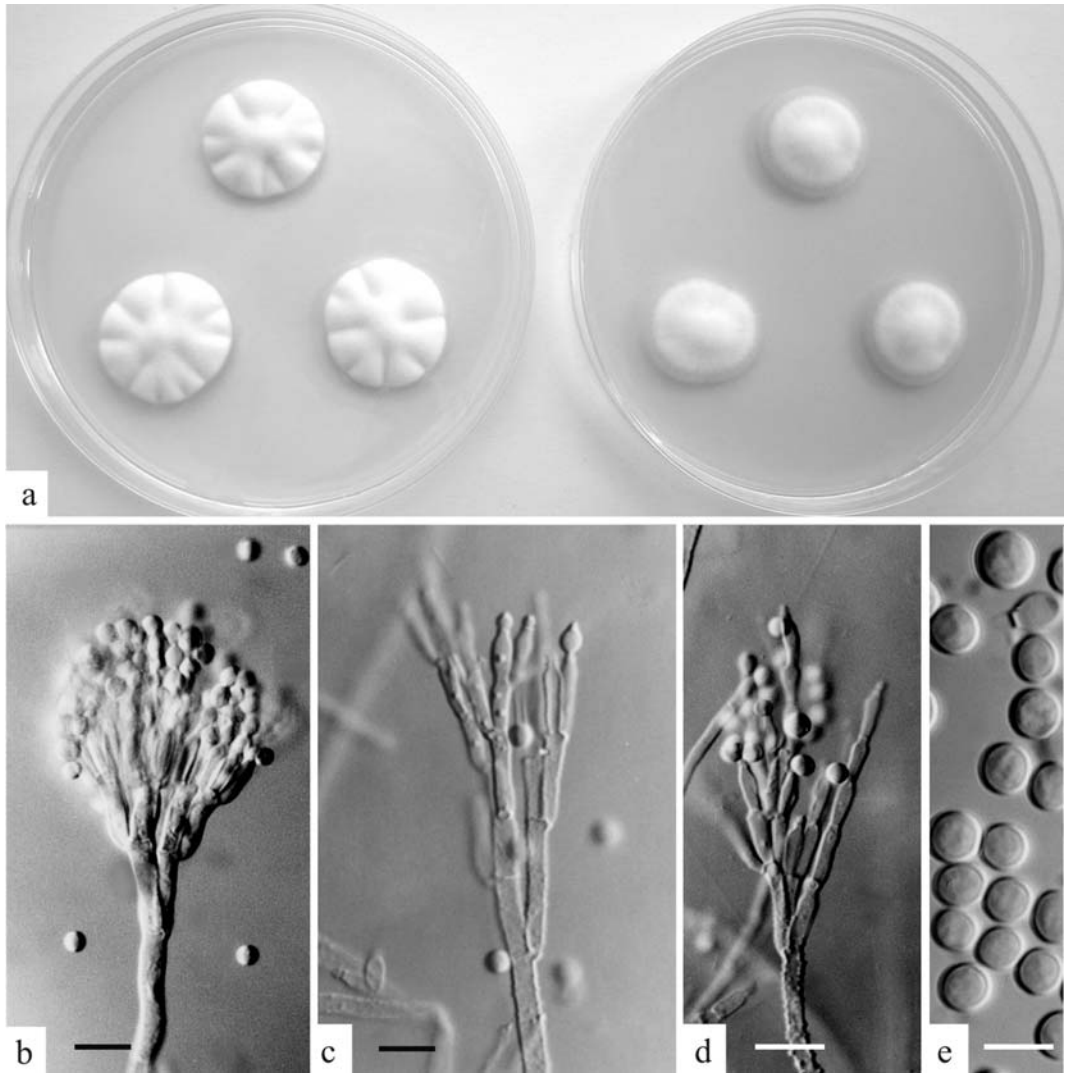


Fig. 7.35 *Penicillium camemberti* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 μm; **(e)** conidia, bar = 5 μm

Distinctive features. Apart from its unique habitat in cheeses and cheese factories, *Penicillium camemberti* is readily distinguished by its white, floccose colonies, sometimes coloured pale grey in age by tardily produced conidia. Penicilli are large and often irregular; conidia are white or grey, large and smooth walled. Strong growth occurs on CSN, with an alkaline response in medium and reverse. A violet reaction is produced with Ehrlich reagent (Lund, 1995a).

Taxonomy. Raper and Thom (1949) recognised two species used for the manufacture of white cheeses, *Penicillium camemberti* and *P. caseicola*. The two species were distinguished by conidial colour, as conidia of *P. caseicola* remained white in age. Later taxonomists concluded that the strains with white conidia are mutants of the grey green parent, which have been selected for properties desirable in cheese manufacture. *P. camemberti* is the earliest valid name for this species (Samson et al., 1977a; Pitt, 1979b). *P. camemberti* is correctly described as a domesticated species, derived from *P. commune* as the wild type (Pitt et al., 1986; Polonelli et al., 1987).

Physiology. *Penicillium camemberti* grows strongly at refrigeration temperatures. No other information is available.

Mycotoxins. *Penicillium camemberti* produces cyclopiazonic acid on synthetic media (Frisvad and Samson, 2004). Although most studies have reported that the toxin does not occur in cheese, not all agree. Cyclopiazonic acid is quite toxic (Purchase, 1971), is immunosuppressive in low doses (Hill et al., 1986) and has produced a mycotoxicosis in pigs (Lomax et al., 1984; Keblys et al., 2004). A role in "Turkey X" disease along with aflatoxin appears likely (Cole, 1986; Bradburn et al., 1994). Although no direct evidence of toxicity to humans has been reported, absence of toxicity in cheeses cannot be taken for granted, as searches for naturally non toxigenic stains have been unsuccessful (Leistner, 1990).

Ecology. *Penicillium camemberti* and its white mutant derivatives are used in the manufacture of soft cheeses, such as Camembert, Brie and Neufchatel, and are rarely found away from the local environment surrounding the manufacture of such cheeses. *P. camemberti* has occasionally been isolated from other sources: meats and pecans (see Pitt and Hocking, 1997).

References. Samson et al. (1977a); Pitt (1979b, 2000); Pitt et al. (1986); Samson and Frisvad (2004).

Penicillium chrysogenum Thom Fig. 7.36

Penicillium griseoroseum Dierckx (rejected name)
Penicillium notatum Westling
Penicillium meleagrinum Biourge

Colonies on CYA 35–45 mm diam, occasionally less, radially sulcate, usually low and velutinous; mycelium white to yellowish; conidial production light to moderate, greyish turquoise to dull green (24-25D-E3-4), in some isolates appearing more yellow green because of the presence of exudate; pale to brilliant yellow or yellow brown exudate and bright yellow soluble pigment usually produced; reverse usually brilliant yellow or yellow brown, but pale or red brown in the absence of soluble pigment. Colonies on MEA 25–40 mm diam, usually plane, low and velutinous, occasionally floccose centrally or somewhat granular; mycelium inconspicuous; conidial production moderate to heavy, greyish turquoise to dull green (24-26D3, 26-27E3-4); reverse pale, yellowish, yellow brown or reddish brown. Colonies on G25N 18–22 mm diam, usually radially sulcate and dense; reverse pale to bright yellow brown or reddish brown. At 5°C, at least microcolony formation; sometimes visible colonies up to 4 mm diam produced. At 37°C, response varying from no growth to colonies up to 5 mm diam.

On CSN, colonies mostly 12–18 mm diam, growth moderate to strong, medium neutral or weakly acid (yellow); reverse similar.

Conidiophores borne from surface or subsurface hyphae, stipes commonly 200–300 μm long, with thin smooth walls, penicilli typically terverticillate, with 1–2 rami, either terminal and appressed or sometimes subterminal and divergent, in that case appearing biverticillate; phialides ampulliform, 7–8(–10) μm long; conidia ellipsoidal to subspheroidal, 2.5–4.0 μm long, smooth walled, borne in long, irregular columns.

Distinctive features. *Penicillium chrysogenum* is usually a readily recognisable species: colonies grow rapidly on the standard media at 25°C, and on CYA produce blue-green conidia, and yellow exudate, soluble pigment and reverse; microscopically penicilli are terverticillate, smooth walled and rather delicate by comparison with those of *P. expansum* or *P. brevicompactum*. However, some isolates lack the yellow pigmentation. Growth on CSN is moderate to strong, with a neutral or weakly acid reaction in both medium and reverse.

Taxonomy. The name *Penicillium griseoroseum* predates *P. chrysogenum* (Cruickshank and Pitt, 1987; Frisvad and Filtenborg, 1989). To overcome this problem, *P. chrysogenum* was conserved under the provisions of the International Code of Botanical Nomenclature (Frisvad et al., 1990c; Kozakiewicz et al., 1992).

Physiology. A mesophilic species, *Penicillium chrysogenum* has a minimum temperature for growth of 4°C, an optimum at 23°C and a maximum at 37°C (Mislivec and Tuite, 1970b; Pitt, 1979b). Among the most xerophilic Penicillia, this species has been observed to germinate at 0.78 a_w by Hocking and Pitt (1979), at 0.79 a_w by Armolik and Dickson (1956) and 0.81 a_w by Mislivec and Tuite (1970b). The minimum inhibitory concentration of sorbic acid effective against *P. chrysogenum* was reported to be 1–2 mmol/l of undissociated acid across the pH range 4–6 (Skirdal and Eklund, 1993). Ethanol (4%, w/w) inhibited the germination of *P. chrysogenum* conidia on PDA (Dantigny et al., 2005).

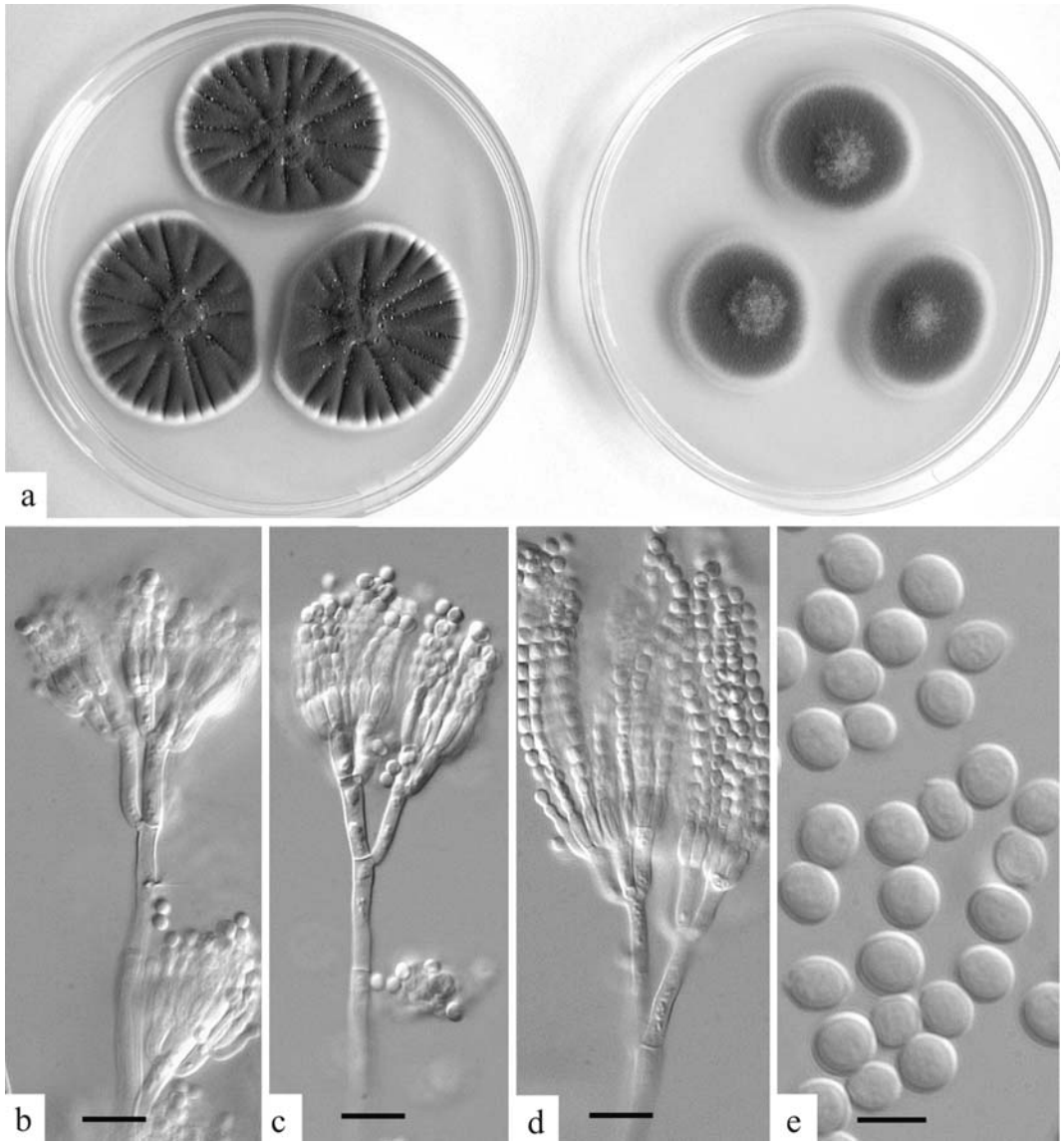


Fig. 7.36 *Penicillium chrysogenum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 µm; **(e)** conidia, bar = 5 µm

Penicillium chrysogenum is a major cause of tainting in foods transported in shipping containers. The taint is due to chloroanisole production from chlorinated phenols used to preserve timber in containers. *P. chrysogenum* was isolated from several such timber samples and shown to carry out this biochemical conversion (Hill et al., 1995).

Mycotoxins. *Penicillium chrysogenum* produces roquefortine C, PR toxin, penicillin and secalonic acids (Frisvad and Samson, 2004). Roquefortine C induced *in vivo* inflammatory responses in mouse lungs, following instillation with purified toxin. Mice subjected to high doses (12.5 nM/g of body weight of animal) of roquefortine C showed signs of trembling and lethargy until 24 h post instillation (Rand et al., 2005). However, *P. chrysogenum* does not appear to be a serious source of mycotoxins in foods.

Ecology. *Penicillium chrysogenum* is a ubiquitous fungus and occupies a very wide range of habitats. As a contaminant of foods, it is probably more

common even than *P. aurantiogriseum*. The original high penicillin producing strain of *P. chrysogenum* was isolated from a spoiled cantaloupe (Raper and Thom, 1949), and it has occasionally caused spoilage in stored grapes (Barkai-Golan, 1974) and carrots (Snowdon, 1991). Apart from these records, *P. chrysogenum* is not known as a pathogen. Few records report spoilage: that of margarine in Australia (Hocking, 1994) is an exception.

This species has been reported very commonly from cereals: for example rice, wheat, barley, maize, flour (see Pitt and Hocking, 1997; Lugauskas et al., 2006) and maize-based snack foods (see Pitt and Hocking, 1997). Other major sources have been luncheon meats (Ismail and Zaky, 1999; Mohamed and Hussein, 2004), Spanish ham (Ockerman et al., 2001), dry-cured ham (Rodríguez et al., 1998), cheese (Hayaloglu and Kirbag, 2007), dried fish, nuts and spices (see Pitt and Hocking, 1997). In our laboratory, we have isolated *P. chrysogenum* from spoiled bakery products, flavoured dairy products, cheese, margarine, lactose powder and pharmaceutical products.

Several authors have looked at the possibility of using *Penicillium chrysogenum* as a starter culture for fermented European meat production (e.g. El-Banna et al., 1987a; Philipp and Pedersen, 1988; Krotje, 1992) and a non toxigenic strain of *P. chrysogenum* (Pg222) has been used as a starter culture in the ripening of dry-cured ham (Martín et al., 2004, 2006).

References. Samson et al. (1977b); Pitt (1979b, 2000); Domsch et al. (1980); Samson and Frisvad (2004).

Penicillium commune Thom — Fig. 7.37

Penicillium lanosum Westling
Penicillium lanosogriseum Thom

Colonies on CYA 30–37 mm diam, radially sulcate, velutinous to floccose; mycelium white, usually inconspicuous; conidial production moderate, of variable colour, greyish turquoise to dull green (24-27D-F3-5); exudate usually present, clear to pale brown; reverse usually pale, occasionally yellow, brown or purple. Colonies on MEA 23–30 mm diam, plane or lightly sulcate, low and dense, surface velutinous or fasciculate; mycelium inconspicuous, white; conidial production moderate, dull green (26-27D-E3-4); reverse usually uncoloured. Colonies on G25N 18–22 mm diam, plane, sulcate or wrinkled, low to moderately deep, dense, usually fasciculate; mycelium white to yellowish; reverse pale to orange brown. At 5°C, at least microcolony formation; typically colonies of 2–4 mm diam formed. No growth at 37°C.

On CSN, colonies 20–26 mm diam, growth strong, medium reaction neutral to alkaline, less commonly weakly acid (pale yellow); reverse alkaline (purple).

Conidiophores borne singly or in fascicles, mostly from subsurface hyphae, stipes 200–400 μm long, or of indeterminate length in fascicles, with walls finely to conspicuously roughened, typically bearing terminal terverticillate penicilli; rami 1–2 per penicillus, phialides ampulliform, 9–11 μm long; conidia spherical, less commonly subspheroidal, 3.5–4.0(–5.0) μm diam, smooth walled, borne in disordered chains.

Distinctive features. *Penicillium commune* is similar to *P. aurantiogriseum* in many characteristics. However, *P. commune* produces grey green rather than grey blue conidia on MEA and growth of *P. commune* is often slower on that medium. Stipes are finely roughened to rough, only rarely smooth. *P. commune* is also distinguished by the ability to grow strongly on CSN and shows a positive reaction to the Ehrlich test (Lund, 1995a). Techniques for rapidly identifying this species in cheese factories were described by Lund (1996).

Immunological procedures suitable for detecting *Penicillium commune* in *P. camemberti* starter cultures were developed by Polonelli et al. (1987). Differentiation of *P. commune* from the closely related species *P. palitans* and *P. solitum* by secondary metabolite analysis was described by Lund (1995b).

Taxonomy. Regarded as a rare floccose species by Raper and Thom (1949), *Penicillium commune* was placed in synonymy with *P. puberulum* by Pitt (1979b). Pitt et al. (1986) showed that *P. puberulum* was a synonym of *P. aurantiogriseum*, so *P. commune* was revived as the earliest valid name for the isolates in question. *P. commune* is the wild type ancestor of *P. camemberti* (Pitt et al., 1986; Polonelli et al., 1987).

Physiology. Like most other species in *Penicillium* subgenus *Penicillium*, *P. commune* grows rapidly at refrigeration temperatures, has an optimum near 25°C and a maximum near 35°C. It is probably capable of growth below 0.85 a_w. *P. commune* failed to grow in an atmosphere of 20% CO_2 and less than

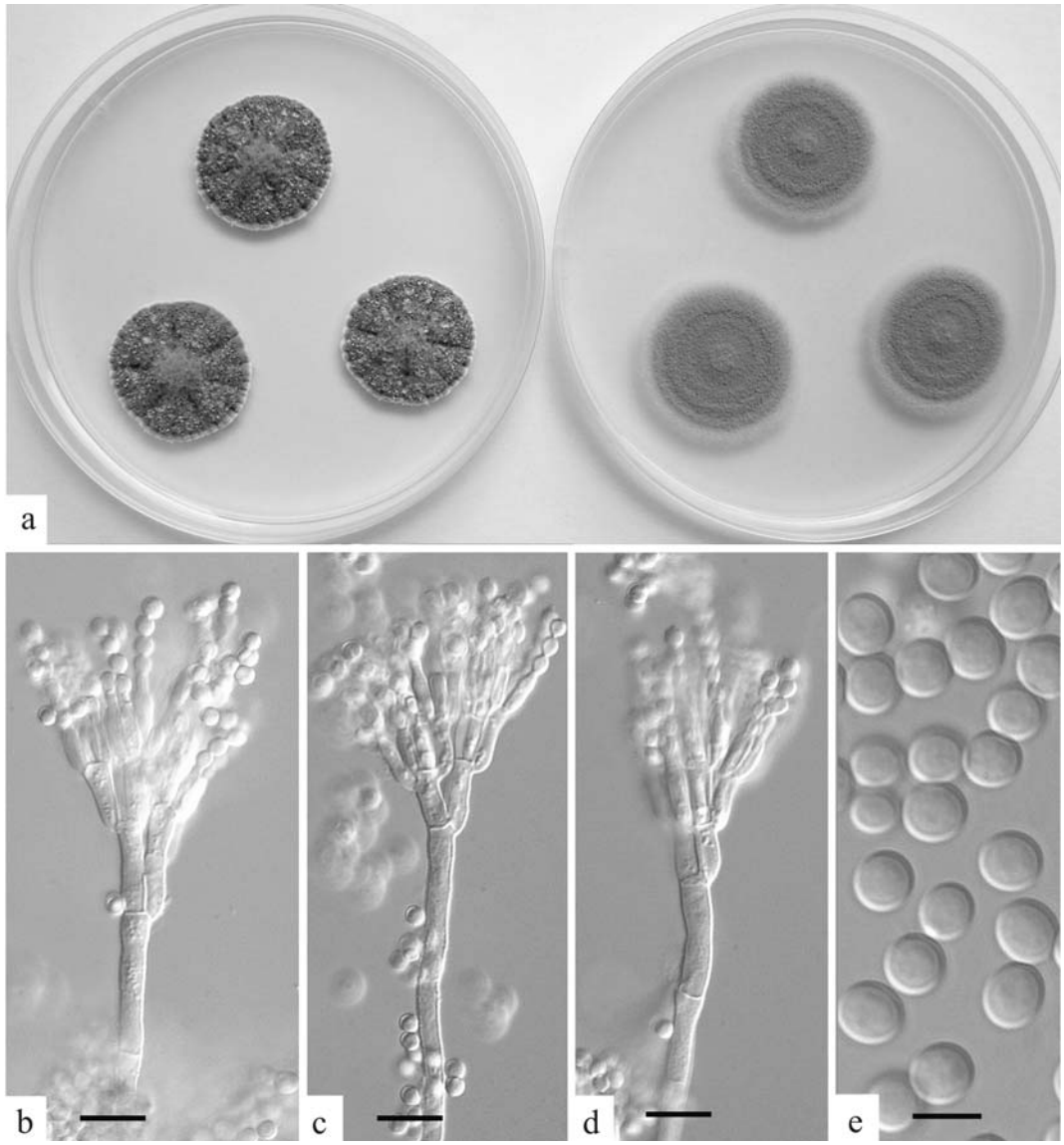


Fig. 7.37 *Penicillium commune* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 μm; **(e)** conidia, bar = 5 μm

0.5% O_2. However, it grew slowly in an atmosphere of 80% CO_2 and 20% O_2 (Taniwaki, 1995).

Mycotoxins. Most isolates of *Penicillium commune* produce cyclopiazonic acid (El-Banna et al., 1987b; Polonelli et al., 1987; Frisvad and Filtenborg, 1989), a toxin described in more detail under *P. camemberti*. A variety of other possibly toxic compounds may also be produced, including cyclopaldic acid, cyclopolic acid, cyclopiamine, palitantin and rugulovasines (Frisvad and Filtenborg, 1989; Lund, 1995b). *P. commune* produced cyclopiazonic acid, rugulovasines and viridicatin on cheese agar (Larsen et al., 2002).

Ecology. The primary habitat for *Penicillium commune* in foods is cheese, of which it is the principal cause of spoilage (Lund et al., 1995; Kure and Skaar, 2000, 2001, 2004; Hayaloglu and Kirbag, 2007). We have isolated it frequently from spoiled soft and hard cheeses in our laboratory, as well as from yoghurt, margarine, sour cream, lactose powder and cakes

containing high fat fillings. It was a major cause of "thread mould" spoilage of vacuum packed cheese blocks in Australia (Hocking and Faedo, 1992). The domesticated species *P. camemberti* probably had its origins during manufacture of cheeses over centuries.

Penicillium commune has also been implicated as a cause of "phenol defect" in Italian hams during ripening (Spotti et al., 1988). Other sources include apples (Amiri and Bompeix, 2005), pears and associated processing machinery (Sanderson and Spotts, 1995), flour (Dragoni et al., 1980c) and European sausages (López-Díaz et al., 2001; Papagiani et al., 2007). We isolated *P. commune* from maize, peanuts, soybeans and cowpeas in Southeast Asia, but always at low levels (Pitt et al., 1993, 1994, 1998a).

References. Pitt (1979b, 2000); Samson and Frisvad (2004).

Penicillium crustosum Thom Fig. 7.38

Colonies on CYA 30–40 mm diam, plane or less commonly radially sulcate, typically low with a velutinous or granular texture and surface appearing powdery, sometimes with small coremia at the margins or centres; mycelium inconspicuous, white; conidial production heavy over the entire colony area, coloured predominantly dull green (26-27D-E3-4) or slightly greyer (26D2), often greyish turquoise (24D3) in marginal areas; exudate clear to pale brown or occasionally deep brown; soluble pigment sometimes present, brown; reverse pale or more commonly yellow to orange brown, often intensely coloured at the margins. Colonies on MEA 25–40 mm diam, plane, usually low and velutinous; mycelium subsurface; conidia produced very abundantly, characteristically forming masses with a dry powdery appearance, breaking off in large numbers or in crusts when jarred, coloured dull green (26–27C-D3); reverse pale or yellow brown. Colonies on G25N 20–26 mm diam, finely radially sulcate, deep but dense; yellow or brown soluble pigment occasionally produced; reverse pale, yellow brown or brown. At 5°C, typically macroscopic colonies formed, 2–6 mm diam. No growth at 37°C.

On CSN, colonies 25–30 mm diam, showing strong growth, medium reaction acid (yellow) or occasionally alkaline (purple); colony reverse alkaline, occasionally weakly so.

Conidiophores mostly borne from subsurface hyphae, stipes commonly 200–400 μm long, with rough walls, bearing terminal penicilli, terverticillate to quaterverticillate; phialides ampulliform, 9–11 μm long; conidia spherical, less commonly subspheroidal or ellipsoidal, 3.0–4.0 μm diam, with smooth walls, borne in long parallel columns, on MEA adhering in masses.

Distinctive features. Colonies of *Penicillium crustosum* on CYA often show blue green margins, but mature conidia en masse are definitely grey green, not bright yellow green, blue green or blue. All conidiophore elements are large, and stipe walls are definitely rough. When grown on MEA for 7–10 days, nearly all isolates of *P. crustosum* produce enormous numbers of conidia which readily break loose when the Petri dish is jarred. This is a remarkably consistent and useful diagnostic character.

Isolates of *Penicillium crustosum* produced a yellow colour with the Ehrlich test (Lund, 1995a). Growth on CSN is strong, usually with an acid medium reaction and an alkaline colony reverse.

Physiology. Perhaps because of poor recognition, physiological studies on *Penicillium crustosum* are meagre. From our data obtained during growth studies, it can be inferred that it will grow down to ca. –2°C, has an optimum near 25°C and a maximum about 30°C. The pH limits for growth are from less than 2.2, optimally 4.5–9.0, to above 10.0 (Wheeler et al., 1991).

This species is an efficient degrader of sorbic acid, producing 1,3-pentadiene, which results in off-odours in many kinds of preserved foods (Kinderlerer and Hatton, 1990). Rancidity in oils due to ketone formation is also caused by growth of fungi including *Penicillium crustosum* (Kinderlerer and Hatton, 1991). It is also capable of producing chloroanisoles from chlorophenols used as preservatives, leading to taints in chickens from mouldy litter (Curtis et al., 1974), in goods packed in fibreboard cartons and in paper or jute sacks (Tindale et al., 1989).

Mycotoxins. *Penicillium crustosum* is the major producer of penitrem A, a powerful neurotoxin (Pitt, 1979c; El-Banna et al., 1987b; Frisvad et al., 2006a). Toxicity of penitrem A to animals is well documented (see Pitt and Hocking, 1997; Naude et al., 2002; Minervini et al., 2002) and it has recently been implicated in a tremor syndrome in humans (Lewis et al., 2005). All isolates of *P. crustosum*

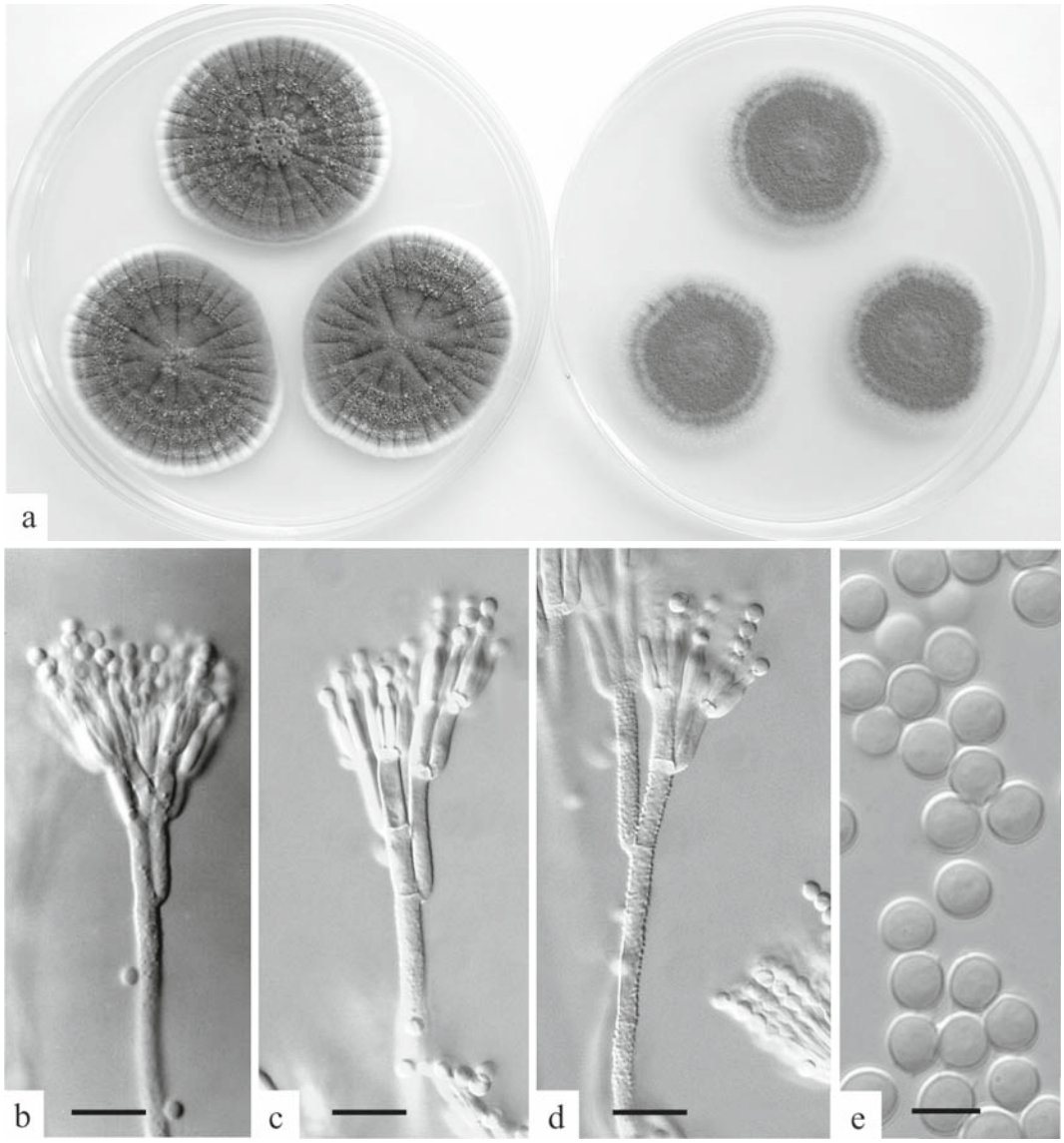


Fig. 7.38 *Penicillium crustosum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 μm; **(e)** conidia, bar = 5 μm

produce penitrem A at high levels (Sonjak et al., 2005; Frisvad et al., 2006a), so the presence of this species in foods (or feeds) is a warning signal (Pitt, 1979c; El-Banna et al., 1987a).

Penitrem A is produced only at high moisture levels, above about 0.92 a_w, with an optimum around 0.995 a_w (ICMSF, 1996b). This probably explains the relatively low number of reports of poisoning from a very toxic compound produced by a very common fungus.

Penicillium crustosum also produces roquefortine C and the cardiotoxin terrestric acid (Frisvad and Samson, 2004). All 120 strains of *P. crustosum* tested were able to produce roquefortine C, viridicatols, and terrestric acid as well as a range of other secondary metabolites (Sonjak et al., 2005). *P. crustosum* also produces thomitrems A and E (Rundberget et al., 2004).

Ecology. *Penicillium crustosum* is a ubiquitous spoilage fungus. It has, for example, been isolated

from the majority of cereal and animal feed samples examined by us over two decades. Because of confusion over species names, it was seldom mentioned in the earlier literature, but our examination of isolates published under a range of names indicates that *P. crustosum* has been responsible for spoilage of maize, processed meats, cheese, biscuits, cakes and fruit juices. It has been isolated as a weak pathogen from citrus fruits and melons (Snowdon, 1990) as well as apples and pears (Hee et al., 2002; Yun et al., 2006) and onions (in our laboratory). We have also isolated *P. crustosum* from spoiled dairy and jelly desserts, bread, rice product, fresh pistachios and from a soup can implicated in a human mycotoxicosis (Lewis et al., 2005). Other published sources include fresh cabbage, processed meats, hazelnuts, pistachios, peanuts, dried peas and amaranth grain (see Pitt and Hocking, 1997), cheese (Lund et al., 1995; Minervini et al., 2002; Hayaloglu and Kirbag, 2007) and chestnuts (Overy et al., 2003).

While not commonly isolated from Southeast Asian commodities, *Penicillium crustosum* occasionally occurred in high numbers. Ten percent of Philippine peanut samples contained this species, with up to 40% of individual nuts infected, resulting in infection of 1% of all kernels examined. Only one of 82 maize samples from Indonesia contained *P. crustosum,* but more than 50% of kernels in that sample were infected. Other sources were sorghum, rice, soybeans, mung beans and pepper, at levels of 1% or less total infected particles (Pitt et al., 1993, 1994, 1998a).

Additional species. *Penicillium echinulatum* Fassat. differs from *P. crustosum* by producing distinctly roughened conidia which are dark green en masse. Growth rates on the standard media are similar, but colonies of *P. echinulatum* usually produce copious clear to pale brown exudate, while conidia on MEA do not usually form crusts. Penicilli of the two species are similar. *P. echinulatum* grows strongly on CSN (22–25 mm diam). Colony reverses are usually alkaline, similar to *P. crustosum*, but unlike *P. crustosum*, an alkaline (violet) reaction is normally produced in the medium.

Penicillium echinulatum has been reported to produce territrems (Frisvad and Samson, 2004). *P. echinulatum* is not commonly isolated from foods but has been reported from pecans, processed meats, rice, katsuobushi (see Pitt and Hocking, 1997) and margarine (in our laboratory).

References. Pitt (1979b; 2000); Samson and Frisvad (2004).

Penicillium digitatum (Pers.: Fr.) Sacc.

Fig. 7.39

Colonies on CYA 35–55 mm diam, plane, surface texture velutinous to deeply floccose; mycelium white; conidial production moderate to heavy, greyish green to olive (1D-E3); reverse pale or brownish. Colonies on MEA of variable diameter, from 35 mm to greater than 70 mm, plane, relatively sparse, strictly velutinous; conidial production moderate, greenish olive or dull yellow green (30D4); reverse pale or brownish. Colonies on G25N 6–12 mm diam, plane, sparse, often mucoid; reverse pale or olive. At 5°C, at least germination, sometimes colonies up to 3 mm diam. No growth at 37°C.

On CSN, colonies 4–10 mm diam, growth weak, with medium pH remaining neutral and with no change in reverse colour.

Conidiophores borne from surface or aerial hyphae, stipes 70–150 μm long, with thin, smooth walls, bearing terminal penicilli, when best developed terverticillate but frequently biverticillate or irregular; phialides broadly ampulliform to cylindroidal, 10–15(–20) μm long, narrowing abruptly to large cylindroidal collula; conidia ellipsoidal to cylindroidal, 6–8(–15) μm long, smooth walled, borne in disordered chains.

Distinctive features. The production of conidia coloured yellow green to olive on all substrates and the close association with rotting fruit of *Citrus* species distinguish *Penicillium digitatum*. It is also distinctive microscopically: no other species of *Penicillium* consistently produces such large phialides or conidia.

Physiology. *Penicillium digitatum* can grow between 6–7°C and 37°C (Domsch et al., 1980). The minimum a_w for growth at 25°C is 0.90 (Hocking and Pitt, 1979; Plaza et al., 2003), but 0.95 a_w at 30°C and 0.99 at 5°C. No germination was recorded at 0.87 a_w or at 37°C (Plaza et al., 2003). The minimum inhibitory concentration of sorbic acid preventing growth of *P. digitatum* was 0.02–0.025% at pH 4.7 and 0.06–0.08% at pH 5.5 (Ray-Schroeder, 1983).

Mycotoxins. This species has not been reported to produce significant mycotoxins. However, about 70% of 24 isolates of *Penicillium digitatum* were

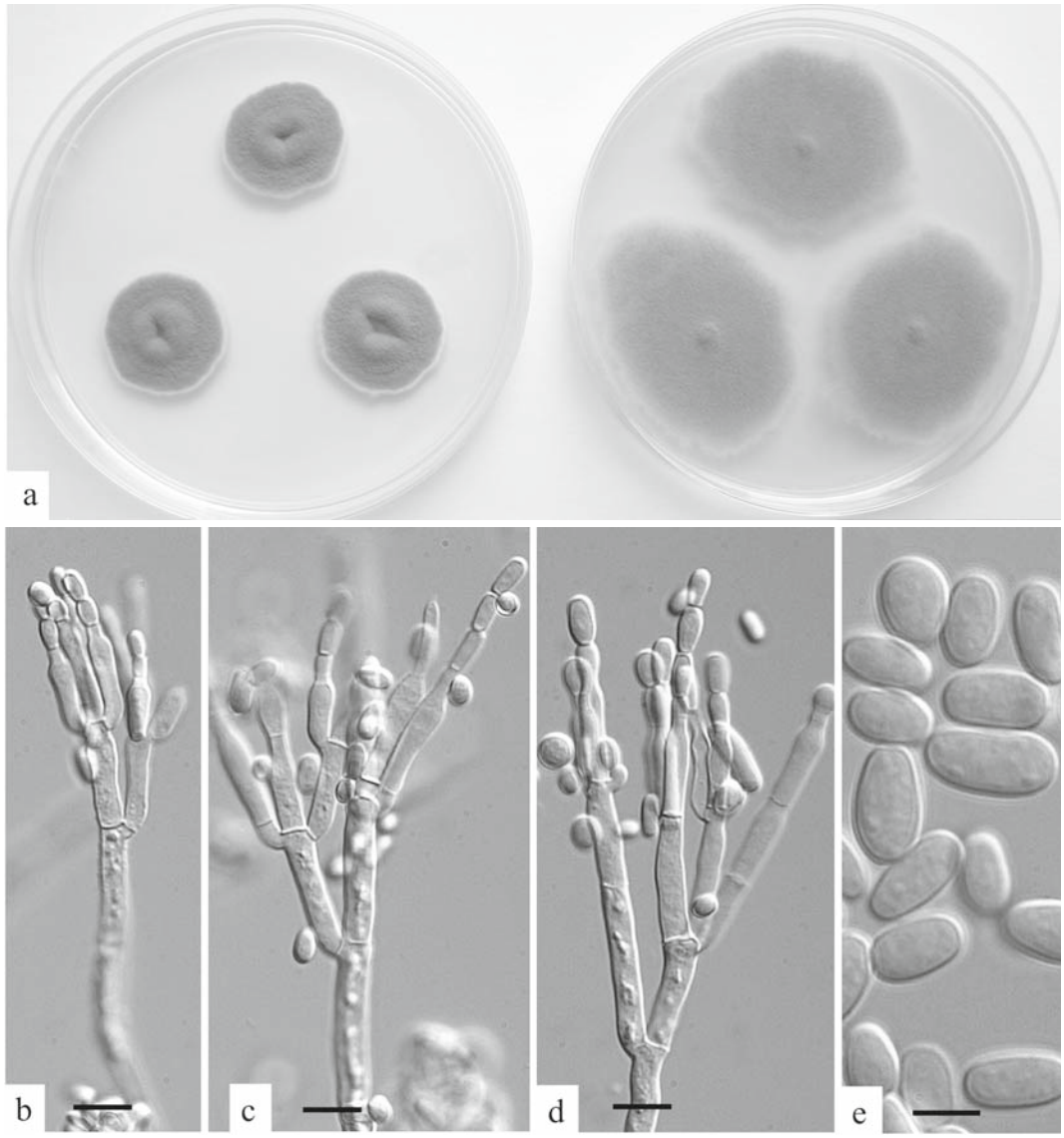


Fig. 7.39 *Penicillium digitatum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 µm; **(e)** conidia, bar = 5 µm

toxic to brine shrimp or chick embryos (Faid and Tantaoui-Elaraki, 1989).

Ecology. The cause of a destructive rot of *Citrus* fruits (Snowdon, 1990), *Penicillium digitatum* is universally distributed, with a preference for warmer climates (Domsch et al., 1980). Initial control involves reducing spore build up, in the orchard by removing fallen fruit and in the factory by removing culled fruit (Snowdon, 1990). Fungicidal sprays or dips provide the main control measure, but resistance to thiabendazole, benomyl and imazalil has developed in most countries to a greater or lesser degree (see Pitt and Hocking, 1997). Control of imazalil-resistant *P. digitatum* by pyrimethanil has been reported (Smilanick et al., 2006). Other control techniques include the use of essential oil vapours (Szczerbanik et al., 2007), UV light in conjunction with either a biocontrol agent or heating (D'Hallewin et al., 2005; Ben-Yehoshua et al., 2005), curing at elevated temperatures, which induces tissue healing (Plaza et al., 2004; Nunes et al., 2007) and vapour heat (Brown et al., 1991). Bacterial and yeast sprays are being

developed as biocontrol agents and their commercialisation is currently been examined (Torres et al., 2007).

It has been proposed that the acidification of host tissue by *Penicillium digitatum* enhances the pathogen's virulence and that pH may be an important regulator of gene expression in pathogenesis (Prusky et al., 2004).

Penicillium digitatum has occasionally been isolated from other food sources: hazelnuts and pistachio nuts (see Pitt and Hocking, 1997), kola nuts (Adebajo, 2000), black olives (Heperkan et al., 2006), rice, maize and meats (see Pitt and Hocking, 1997). We isolated this species at low levels from Southeast Asian peanuts, soybeans and sorghum (Pitt et al., 1993, 1994).

References. Pitt (1979b, 2000); Domsch et al. (1980); Samson and Frisvad (2004).

Penicillium expansum Thom Fig. 7.40

Colonies on CYA 30–40 mm diam, lightly radially sulcate, moderately deep to very deep, with surface typically tufted (coremial) in one or more annular bands, with adjacent areas velutinous to floccose; mycelium white; conidia produced in moderate numbers, dull green (27E3-4); exudate clear to pale orange brown; soluble pigment brownish orange near caramel (6C6); reverse pale to deep brown, often with areas of brownish orange (7C-E7-8). Colonies on MEA variable, ranging from 20 to 40 mm diam, plane, some isolates persistently velutinous, others at least partly coremial; mycelium often entirely subsurface; conidial production usually heavy, coloured as on CYA or slightly greyer (27C-D3-4); soluble pigment sometimes produced, coloured as on CYA; reverse pale or, in the presence of soluble pigment, orange brown. Colonies on G25N 17–22 mm diam, radially sulcate, dense, surface texture velutinous to granular; reddish brown soluble pigment sometimes produced; reverse pale, dull brown or reddish brown. At 5°C, occasionally only microcolonies, typically colonies of 2–4 mm diam formed. No growth at 37°C.

Growth on CSN strong, 24–30 mm diam, medium acid (yellow); reverse usually acid plus brown soluble pigment, occasionally alkaline (purple).

Conidiophores borne from surface or subsurface hyphae, singly, in fascicles, or in definite coremia, sometimes visible with the unaided eye, stipes 200–500 μm long, with smooth walls, bearing terminal penicilli, typically terverticillate, less commonly biverticillate; rami borne singly, phialides closely packed, ampulliform to almost cylindroidal, 8–11 μm long, with short collula; conidia ellipsoidal, 3.0–3.5 μm long, smooth walled, borne in long, densely packed, irregular chains.

Distinctive features. The most important features distinguishing *Penicillium expansum* are dull green conidia, often borne in coremia, smooth walled stipes on both CYA and MEA and, frequently, the presence of orange brown to brown exudate, soluble pigment and reverse colours. Isolates of *P. expansum* inoculated into apples or pears typically produce destructive rots. Growth on CSN is strong; medium and reverse colours are typically yellow. *P. expansum* produces a violet colour with Ehrlich reagent (Lund, 1995a).

Physiology. Like most other species in this subgenus, *Penicillium expansum* is a psychrophile: minimum temperatures for growth have been reported as –6°C (Brooks and Hansford, 1923), –3°C (Panasenko, 1967) and at most –2°C (Mislivec and Tuite, 1970b). Growth is quite strong at 0°C (Kuehn and Gunderson, 1963). The optimum temperature for this species is near 25°C and the maximum near 35°C (Panasenko, 1967). The effect of temperature on the germination time and growth rate of *Penicillium expansum* has been modelled by Baert et al. (2007a). They also provided excellent data for the optimum temperature for growth being 25°C. The minimum a_w for germination is 0.82–0.83 (Mislivec and Tuite, 1970b; Hocking and Pitt, 1979). Sodium chloride was more inhibitory than glucose, glycerol or sorbitol at a_w values between 0.98 and 0.89, with germination and growth at 15°C only slightly slower than at 25°C (Lahali et al., 2005). At 5°C, growth ceased at 0.91 a_w (NaCl and glucose) or 0.89 a_w (glycerol, sorbitol) but the incubation period was only 25 days (Lahali et al., 2005).

Penicillium expansum has a very low requirement for oxygen. Golding (1940a, 1945) showed that growth was virtually unaffected by levels of oxygen as low as 2.1%. When reduction in rates of growth did occur, it was at higher temperatures. Growth of *P. expansum* and some other fungi was stimulated by carbon dioxide concentrations up to 15% in air, but growth rates declined at higher CO_2 levels. Spores of *P. expansum* were inactivated by holding in an atmosphere of 13% CO_2 for 21 days, assisting the fumigation of fruit boxes (Cossentine et al., 2004).

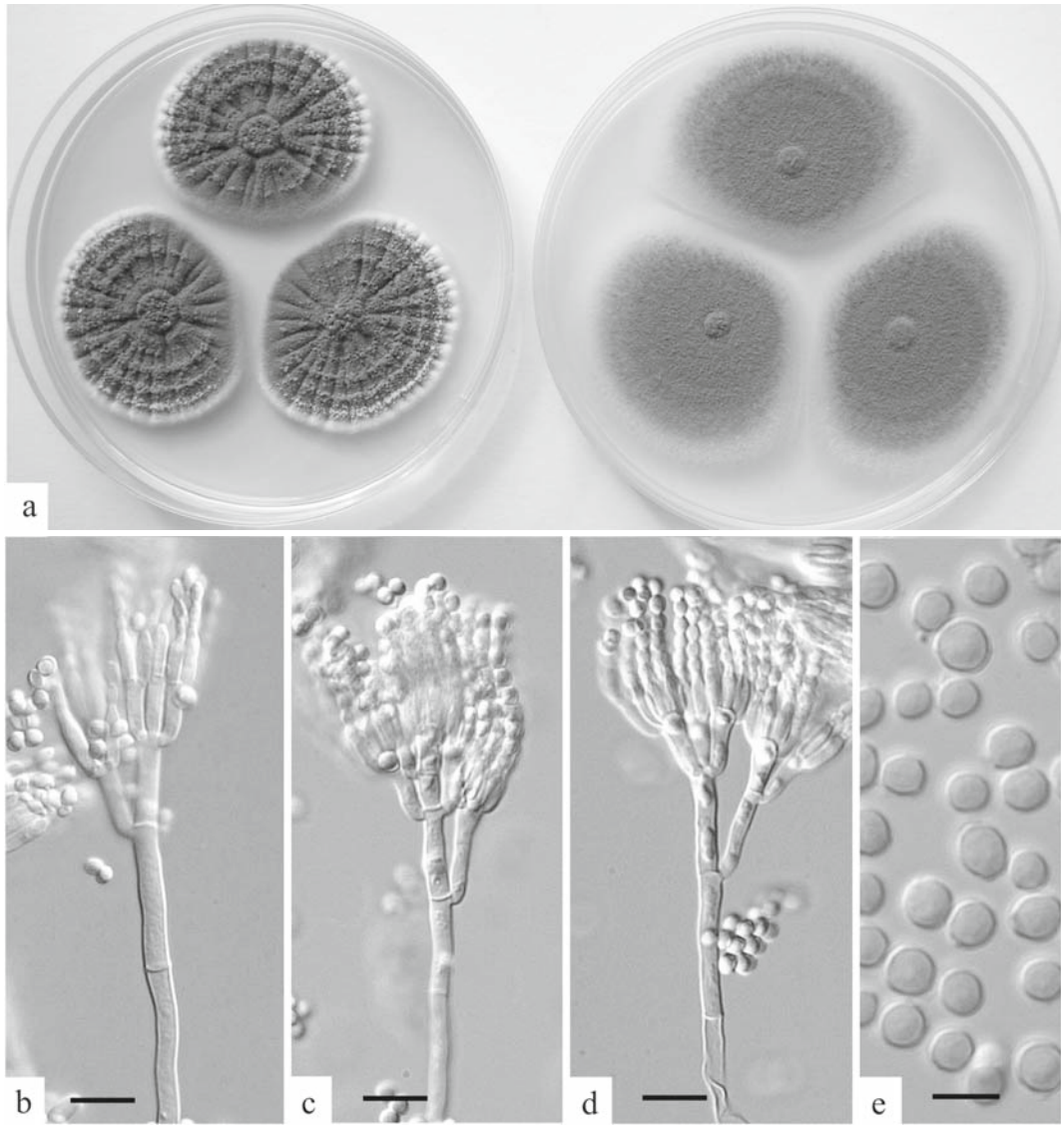


Fig. 7.40 *Penicillium expansum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 μm; **(e)** conidia, bar = 5 μm

Mycotoxins. *Penicillium expansum* is an important producer of patulin and citrinin (Harwig et al., 1973; Ciegler et al., 1977). Patulin is reported to affect renal cells (Heussner et al., 2006) and decrease sperm count in rats (Selmanoğlu, 2006). Patulin damaged the DNA of mammalian cells, but the effect could be negated by ascorbic acid (Alves et al., 2000). Patulin also caused oxidative stress response in mammalian cell lines, which can lead to cell changes during transformation and differentiation (Liu et al., 2007). However, patulin is still classified as a Group 3 compound by the International Agency for Research on Cancer (IARC) on the basis that cytotoxicity in animals does not constitute adequate evidence of human toxicity (IARC, 1993). Despite a lack of definite data on human toxicity, a number of countries have set an upper limit of 50 μg/l for patulin in apple juice and other apple products (FAO, 2003), with a limit of 10 μg/l for apple products destined for consumption by children (EU, 2004).

Penicillium expansum produces patulin as it rots apples and pears. Poor quality control, i.e. the use of

rotting fruit in juice or cider manufacture can result in high concentrations of patulin in juice (Brackett and Marth, 1979; Watkins et al., 1990). Levels of patulin ranging from 538 to 1822 μg/ml were detected in apple cider stored at 25°C and up to 396 μg/ml in cider stored at 4°C (McCallum et al., 2002). Aggressively pathogenic strains of *P. expansum* produced more patulin than less aggressive strains, and patulin synthesis seemed correlated with an ability to increase the acidity of cider, a characteristic of the virulent strains (McCallum et al., 2002). Patulin is important as an indicator of the use of poor quality raw materials in juice manufacture.

Patulin can be produced over the range 0–25°C at least, the optimum being at 25°C (Paster et al., 1995), but not at 31°C (Northolt et al., 1978). The minimum a_w for patulin production by *Penicillium expansum* is 0.95 at 25°C (Patterson and Damoglou, 1986). Patulin is produced over the narrow pH range of 3.2–3.8 in apple juice (Damoglou and Campbell, 1986). A modified atmosphere of 3% CO_2 and 2% O_2 completely inhibited patulin production at 25°C, but production occurred in atmospheres of 2% CO_2 and 10 or 20% O_2 (Paster et al., 1995). Patulin in quite stable in apple juice during storage (Damoglou and Campbell, 1986), and pasteurisation at 90°C for 10 seconds caused less than 20% reduction (Wheeler et al., 1987). No patulin was produced in apples stored under controlled atmospheres of either 2.5% O_2/3.9% CO_2 or 1.5% O_2/2.5% CO_2 at 1°C. Patulin was only detected in apples after further storage at 20°C (Morales et al., 2006, 2007a). It has been shown that *Penicillium expansum* invades apples through the stems during storage (Rosenberger et al., 2006).

Ecology. One of the oldest described *Penicillium* species, *P. expansum* has been established as the principal cause of spoilage of pome fruits throughout this century (Raper and Thom, 1949). Isolates of *P. expansum* come predominantly from rotting apples and pears (Snowdon, 1990). Control relies on sprays to prevent *Colletotrichum* infections which permit entry by *P. expansum*, prevention of damage to fruit, cool storage and rapid processing (Snowdon, 1990). A wide variety of other techniques have been advocated, including use of deoxyglucose as a control agent (Janisiewicz, 1994; El-Ghaouth et al., 1995), chlorine dioxide in wash waters (Roberts and Reymond, 1994; Okull et al., 2006) or wash water filtration (Spotts and Cervantes, 1993) and calcium chloride infiltration under increased temperature and pressure (Conway et al., 1988; Sams et al., 1993). Biological control using bacteria or yeasts has also been studied, and two commercial products, one based on *Candida oleophila* and the other on *Cryptococcus albidus* have been registered in the United States and South Africa (Janisiewicz and Korsten, 2002; Fravel, 2005). However, the efficacy of these products still depends on supplementation with low doses of synthetic fungicides to achieve large scale control (Droby et al., 2003). Other compounds including salicylic acid and cytokinin have been examined in conjunction with biocontrol yeasts to enhance the control of *P. expansum* in pears (Yu et al., 2007; Zheng et al., 2007b).

Penicillium expansum has been isolated from a wide range of other fruits including tomatoes, strawberries, avocados, mangoes, grapes (see Pitt and Hocking, 1997; Snowdon, 1990) indicating that it is a broad spectrum pathogen on fruits. Isolation from fresh vegetables has been uncommon: onions, carrots and cabbages (Lugauskas et al., 2005) have been reported.

Isolations from stored foods have been less frequent: in particular, *Penicillium expansum* appears to be much less common on cereals than some other species in this subgenus. Isolations have been reported from maize, wheat, rice and barley (Aziz et al., 2006) and a variety of retail cereal products (Aran and Eke, 1987). It is more widespread in other foods, especially meat and meat products (see Pitt and Hocking, 1997 and Cantoni et al., 2007). Other records include pecans and pistachios (see Pitt and Hocking, 1997), peanuts, (Aziz et al., 2006), olives (Kivanç and Akguel, 1990), dried beans, beans (see Pitt and Hocking, 1997), cheese (Hayaloglu and Kirbag, 2007) and margarine (Hocking, 1994), health foods, rapeseed, dried fish and frozen fruit pastries (see Pitt and Hocking, 1997). We have isolated *P. expansum* from spoiled cheese, fruit yoghurt, jellied fruit desserts, apple sauce and apple juice. Only low levels were found in Southeast Asian commodities (Pitt et al., 1993, 1994).

References. Pitt (1979b, 2000); Domsch et al. (1980); Samson and Frisvad (2004).

Penicillium griseofulvum Dierckx Fig. 7.41

Penicillium patulum Bainier
Penicillium urticae Bainier

Colonies on CYA 20–25 mm diam, occasionally 30 mm, finely radially sulcate, moderately deep, dense, surface texture granular; mycelium white; conidial production moderate to heavy, at the margins greyish green (26-27C3), centrally greenish grey (26-27C2); exudate usually present, clear to pale yellow; soluble pigment sometimes produced, reddish brown; reverse pale, dull yellow or brown. Colonies on MEA 15–25 mm diam, plane or rarely radially sulcate, moderately deep, of granular texture; mycelium usually inconspicuous, white; conidial production moderate to heavy, coloured as on CYA; reverse pale to brown. Colonies on G25N 16–22 mm diam, plane, low and velutinous at the margins, often floccose centrally; reverse pale. At 5°C, colonies up to 4 mm diam usually formed. No growth at 37°C.

On CSN, colonies 18–24 mm diam, growth moderate; medium and reverse reaction neutral.

Conidiophores borne in fascicles, with stipes of indeterminate length, often sinuous, smooth walled, brownish, terminating in distinctive penicilli, sometimes terverticillate, more commonly quaterverticillate and not infrequently with five or even more branch points between stipe and phialide; phialides closely packed, exceptionally short, 4.5–6.0 μm, abruptly tapering to short collula; conidia ellipsoidal, 3.0–3.5 μm long, smooth walled, borne in closely packed, disordered chains.

Distinctive features. *Penicillium griseofulvum* is unique in two respects: it produces very short phialides and it bears them on highly branched conidiophores. Other features include the production of grey colonies on CYA and MEA, with only weak greenish overtones, and a neutral reaction on CSN.

Physiology. Growth temperatures for *Penicillium griseofulvum* range from 4 to 35°C, with an optimum near 23°C (Mislivec and Tuite, 1970b). The minimum a_w for germination is 0.81 at 23°C and 0.83 at 16 or 30°C (Mislivec and Tuite, 1970b).

Mycotoxins. *Penicillium griseofulvum*, sometimes reported under its synonyms *P. patulum* and *P. urticae*, produces four mycotoxins, patulin, cyclopiazonic acid, roquefortine C and griseofulvin (Samson and Frisvad, 2004). Patulin and cyclopiazonic acid are moderately toxic compounds, with oral LD_{50} values in male rats and mice of 35 and 36 mg/kg, respectively. Griseofulvin, with an intravenous LD_{50} in rats of only 500 mg/kg, is sufficiently nontoxic to permit use as an antifungal antibiotic. Griseofulvin has been of great value in the treatment of cutaneous infections by dermatophytic fungi in both man and animals. However, as well as inhibiting invasive fungi, it also shows mammalian toxicity, acting as a mitotic blocker in the host's dividing cells, such as those of bone marrow, the intestinal lining, and tumours (see Vanden Bossche et al., 2003 and Woodward, 2005 for reviews).

When cyclopiazonic acid produced from *Penicillium griseofulvum* was fed to lactating ewes, most was excreted in milk within 10 days, the maximum concentration in milk (570 μg/kg) being ca. 10% of the ingested dose (5 mg/kg live weight). Appreciable amounts of this compound fed to chickens were also detected in eggs (Dorner et al., 1994).

Although one outbreak of cattle poisoning in Japan was attributed to *Penicillium griseofulvum* (synonym *P. maltum* M. Hori and T. Yamamoto) (Hori et al., 1954), recent literature provides no other example of animal toxicity due to this species. Cyclopiazonic acid was reported to be non-mutagenic to human liver cells and reduced the mutagenicity of aflatoxin B_1 (Sabater-Vilar et al., 2003b). It is unlikely that *P. griseofulvum* is a significant contributor to human illness.

Patulin was produced by *Penicillium griseofulvum* down to 0.88 a_w (Lötzsch and Trappe, 1978). Production occurred over the range 4–31°C at 0.99 a_w, and 8–31°C at 0.95 a_w (Northolt et al., 1978). Potato dextrose broth supplemented with manganese optimised patulin synthesis (Dombrink-Kurtzman and Blackurn, 2005).

Ecology. Although *Penicillium griseofulvum* can produce lesions when inoculated into apples and pears, it is not a common cause of spoilage of these fruits (Sanderson and Spotts, 1995). *P. griseofulvum* occurs quite commonly on cereals and nuts. Records from cereals include barley, maize, rice and wheat (Aziz et al., 2006), flour and bakery products (see Pitt and Hocking, 1997) and from nuts: peanuts (Aziz et al., 2006), pecans and pistachios (see Pitt and Hocking, 1997). Other sources include dried peas and beans,

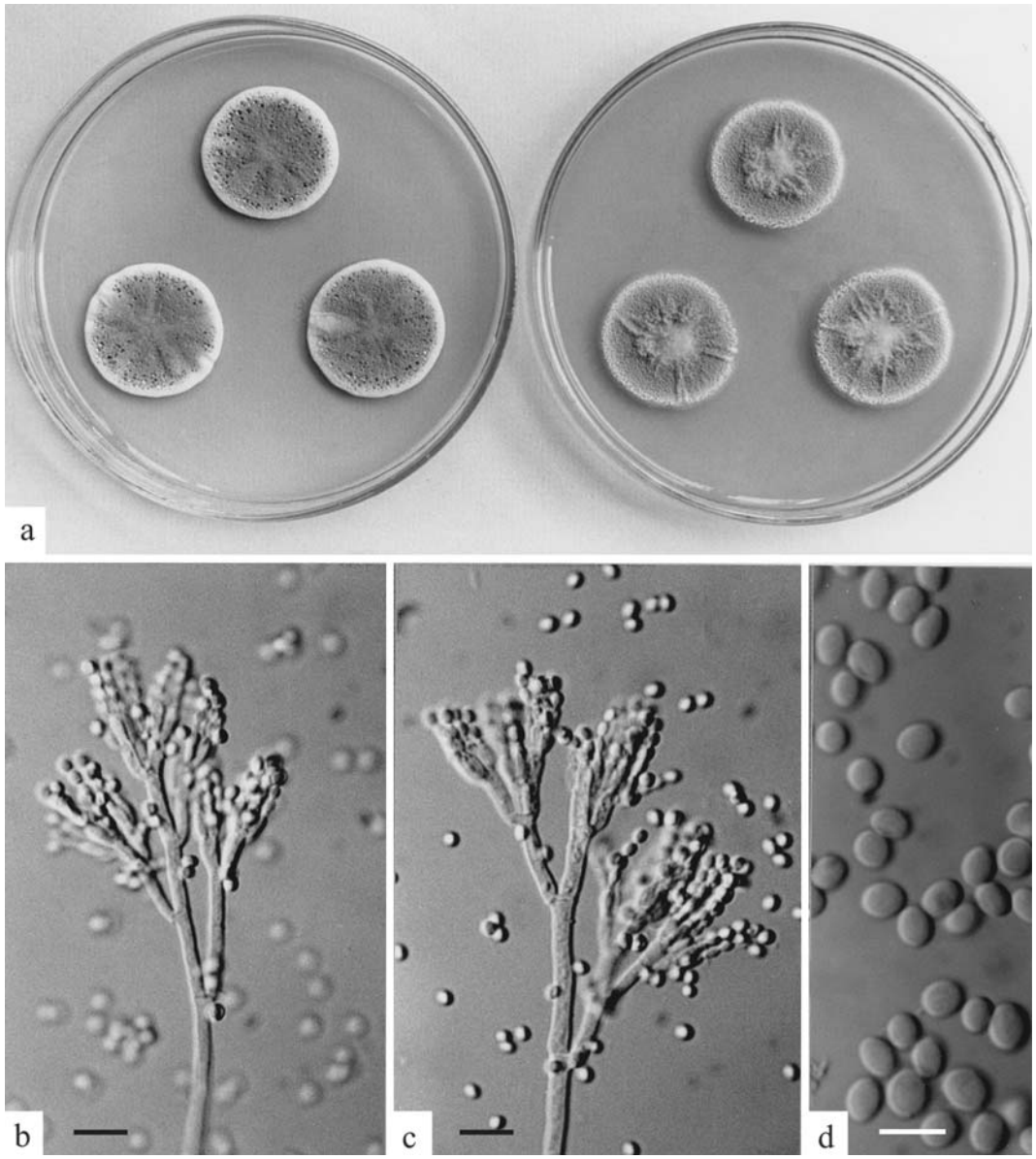


Fig. 7.41 *Penicillium griseofulvum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** penicilli, bars = 10 μm; **(d)** conidia, bar = 5 μm

meats, rapeseed, health foods and frozen fruit pastries (see Pitt and Hocking, 1997). *P. griseofulvum* occurs only rarely in tropical foods: we isolated it only from Thai and Philippine peanuts at low levels (Pitt et al., 1993, and our unpublished data).

References. Samson et al. (1976); Pitt (1979b, 2000); Domsch et al., 1980); Samson and Frisvad (2004).

Penicillium hirsutum Dierckx **Fig. 7.42**

Penicillium corymbiferum Westling
Penicillium verrucosum var. *corymbiferum* (Westling) Samson et al.

Colonies on CYA 30–40 mm diam, radially sulcate or less commonly plane, surface texture typically granular to coremial; mycelium white at the margins, white to bright yellow elsewhere; conidial

production moderate, greyish green to dull green near Spanish green or jade green (26-27D-E4-5); exudate usually violet brown near maroon (11E-F8) but occasionally lighter brown; soluble pigment typically produced, deep yellow to orange brown, occasionally reddish brown; reverse in similar colours. Colonies on MEA 25–35 mm diam, plane or rarely sulcate, surface texture usually granular to coremiform, less commonly velutinous to fasciculate; mycelium white to deep yellow; conidial production moderate, in colours similar to those on CYA; exudate produced by some isolates, deep red; reverse pale to deep yellow, green or orange brown. Colonies on G25N 18–22 mm diam, plane or sulcate, low to deeply floccose; mycelium white to yellow; yellow soluble pigment often produced; reverse bright yellow green or orange to brown. At 5°C, colonies of 2–5 mm diam usually produced. No growth at 37°C.

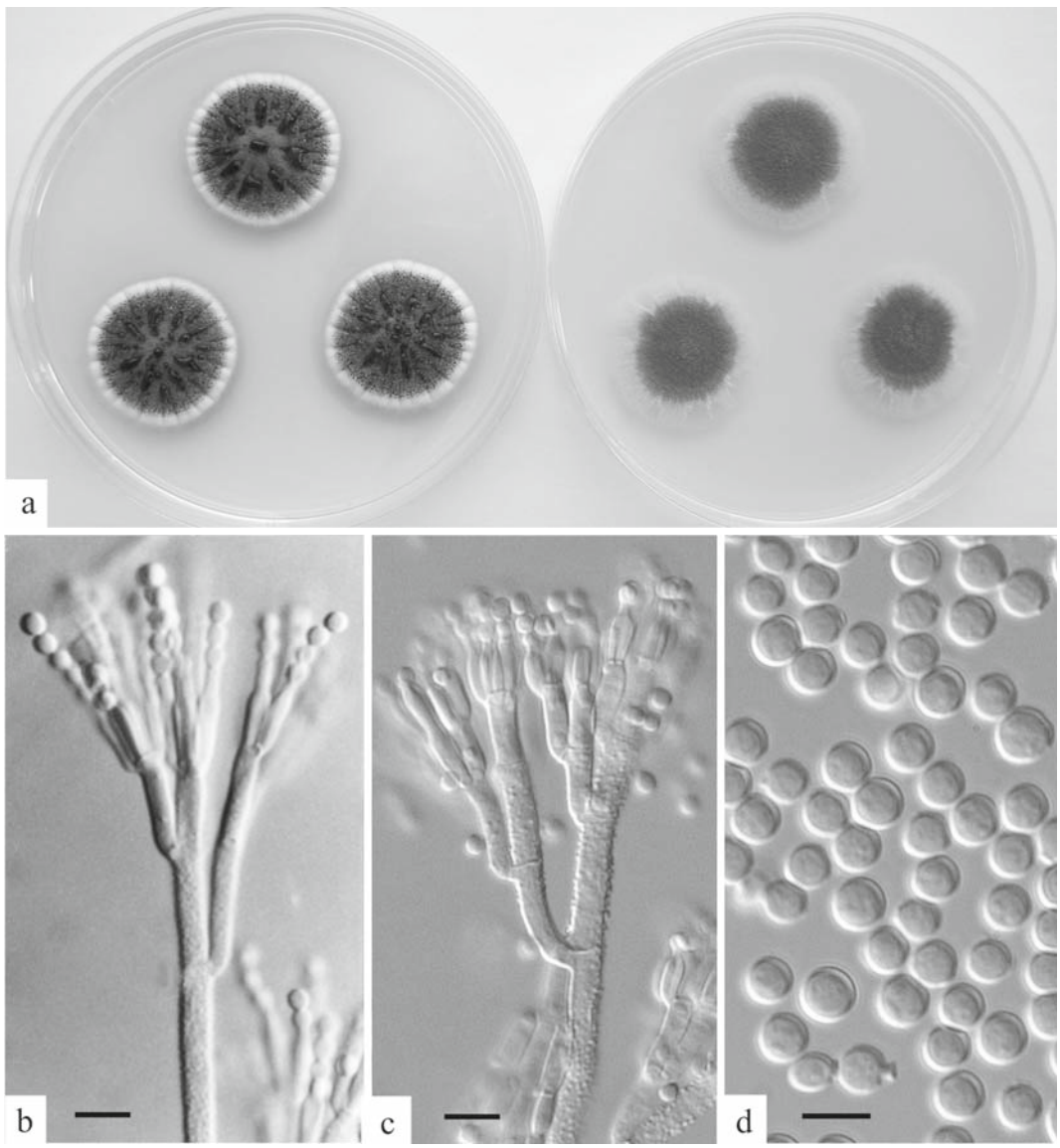


Fig. 7.42 *Penicillium hirsutum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** penicilli, bars = 10 µm; **(d)** conidia, bar = 5 µm

On CSN, colonies 24–30 mm diam, growth strong, medium reaction acid (yellow); reverse acid, sometimes with brown pigment also.

Conidiophores commonly borne from surface hyphae, with stipes 200–500 μm long, but also borne in fascicles and then of indeterminate length, typically with conspicuously roughened walls, bearing large terminal terverticillate to quaterverticillate penicilli; rami 1–2 per penicillus, typically with walls roughened; metulae usually rough walled; phialides ampulliform, smooth walled, 9–12 μm long, abruptly tapering to generally short collula; conidia spherical, 3.0–3.5 μm diam, less commonly ellipsoidal, 3.0–3.5 μm long, with walls smooth to very finely roughened, borne in disordered chains or irregular columns.

Distinctive features. On CYA, colonies of *Penicillium hirsutum* are usually deep and fasciculate to coremiform, exudate is reddish or violet brown, soluble pigment is deep yellow to orange brown and conidia are usually pure green, without blue or yellow tones. Conidiophores are frequently borne in fascicles, stipes are usually rough walled and penicilli are large. *P. hirsutum* grows strongly on CSN, and normally produces an acid reaction in both medium and colony reverse.

Taxonomy. Raper and Thom (1949: 544) called this taxon *Penicillium corymbiferum*. Samson et al. (1976) reduced this species to varietal status under *P. verrucosum*, but this change in rank was not accepted by Pitt (1979b), who took up the earlier name *P. hirsutum*. This has now been accepted (Frisvad and Filtenborg, 1989; Pitt and Samson, 1993).

Physiology. *Penicillium hirsutum* is capable of growth at particularly low temperatures, with a minimum of –5°C and relatively rapid growth at –1°C (Lowry and Gill, 1982). Overy et al. (2005a) reported growth at 0°C with an optimum at 20°C. It appears to be moderately xerophilic (Gill and Lowry, 1982).

Mycotoxins. This species produces roquefortine C (Frisvad and Filtenborg, 1989; Frisvad and Samson, 2004). The production of cyclopiazonic acid was reported by El-Banna et al. (1987b), but this was not confirmed by later studies (Frisvad, 1989; Frisvad and Filtenborg, 1989; Frisvad and Samson, 2004).

Ecology. Although an infrequently encountered species, *Penicillium hirsutum* is important as a cause of black spot in refrigerated meat (Gill et al., 1981). It has also caused spoilage of fresh asparagus (Saito et al., 2003), onion and garlic bulbs (Overy et al., 2005b) and refrigerated pear puree (Pitt, 1979b: 351). It has been reported from wheat, flour, rice and peanuts (see Pitt and Hocking, 1997). We isolated it infrequently from Indonesian soybeans (Pitt et al., 1998a).

Additional species. *Penicillium allii* Vincent and Pitt [synonym *P. hirsutum* var. *allii* (Vincent and Pitt) Frisvad] is similar to *P. hirsutum* in many respects. However, colonies are larger at 25°C (35–45 mm diam on both CYA and MEA) and growth does not occur at 5°C. Colonies are velutinous or minutely fasciculate. Stipes are very rough. Growth on CSN is weak, 15–20 mm diam, with an acid reaction in both medium and colony reverse. *P. allii* produces roquefortine C (Overy et al., 2005b). This species causes a destructive rot in garlic (Vincent and Pitt, 1989; Snowdon, 1991, as *P. corymbiferum*). We isolated it from 2% of all kemiri nuts examined from Indonesia (Pitt et al., 1998a), and at low levels from Philippine maize (our unpublished data).

Additional species. *Penicillium hordei* Stolk was distinguished from *P. hirsutum* by the production of yellow coremia on MEA, by stipes with walls less conspicuously roughened, and by smaller, rough-walled conidia (Stolk, 1969). Pitt (1979b) considered these taxa to be a single species, while *P. hordei* was treated as *P. hirsutum* var. *hordei* (Stolk) Frisvad by Frisvad and Filtenborg (1989). Ecological evidence (Stolk, 1969; Frisvad and Filtenborg, 1989) and differences in isoenzyme patterns (Cruickshank and Pitt, 1987) indicated that *P. hirsutum* and *P. hordei* are separate species, and this is now accepted (Pitt and Samson, 1993; Frisvad and Samson, 2004). *P. hordei* isolates always produce the minor toxins roquefortine C and terrestric acid (Frisvad and Filtenborg, 1989; Overy et al., 2005b). On CSN, growth is similar to that of *P. hirsutum*, colonies 20–24 mm diam, growth strong, medium reaction acid, reverse acid with deep brown pigments also. This species is of quite common occurrence in barley in Europe (Stolk, 1969) and also occurs on tomatoes and in spices (Frisvad and Filtenborg, 1989). *P. hordei* also causes blue rot in tulip bulbs (Overy et al., 2005b).

References. *Penicillium hirsutum*: Pitt (1979b, 2000); Samson and Frisvad (2004). *P. allii*: Vincent and Pitt (1989); Samson and Frisvad (2004). *P. hordei*: Stolk (1969); Samson and Frisvad (2004).

Penicillium italicum Wehmer **Fig. 7.43**

Colonies on CYA 30–40 mm diam, plane or radially sulcate, usually low and dense, velutinous to granular, some isolates with minute coremia at the margins; mycelium white; conidia abundant, greyish green (25-26C2-3); clear exudate and brown soluble pigment produced by some isolates; reverse usually brownish orange to greyish brown (7C7-F3). Colonies on MEA 35–55 mm diam, plane and sparse, usually strictly velutinous, sometimes with minute coremia at the margins; conidial formation moderate to heavy, coloured as on CYA; reverse typically chocolate brown (6E-F4). Colonies on G25N 12–17 mm diam, plane or sulcate; brown soluble pigment sometimes produced; reverse yellow brown to deep brown. At 5°C, microcolonies to colonies of 4 mm diam produced. No growth at 37°C.

On CSN, colonies 10–20 mm diam, growth weak, medium and colony reverse reaction neutral.

Sclerotia produced by some isolates, up to 300 μm diam, brown and soft. Teleomorph unknown. Conidiophores borne from surface or subsurface hyphae, stipes commonly 200–400 μm long, with thin, smooth walls, bearing large regular to irregular terminal terverticillate penicilli; rami 1–2 per penicillus, metulae often apically inflated; phialides 10–14 μm long, roughly cylindroidal in shape, then tapering abruptly to long cylindroidal collula;

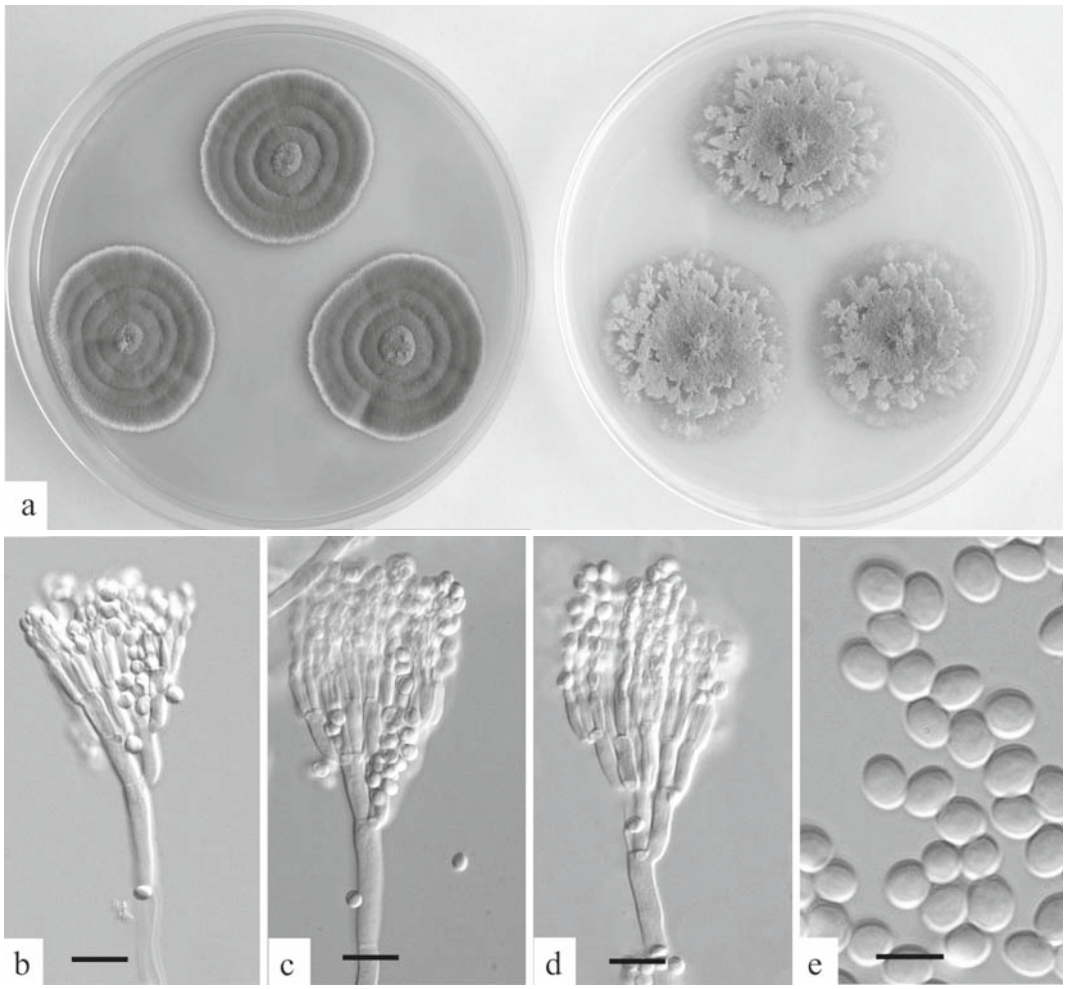


Fig. 7.43 *Penicillium italicum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 μm; **(e)** conidia, bar = 5 μm

conidia borne as cylinders, enlarging and rounding with maturation, ellipsoidal to short cylindroidal, 3.0–5.0 μm long, with smooth walls, borne in long, disordered chains.

Distinctive features. *Penicillium italicum* is readily recognised in nature as the cause of a destructive bluish grey rot on lemons or other *Citrus* fruit. In culture it forms relatively broad, grey green colonies with deep brown reverse colours, with conidia borne as cylinders, enlarging and rounding during maturation. It grows poorly on CSN, and the medium shows little or no change.

Physiology. According to Panasenko (1967), *Penicillium italicum* grows between –3°C and 32–34°C, with an optimum at 22–24°C; a minimum of 0°C was reported by Wyatt et al. (1995). The minimum a_w for germination at 10 and 25°C is 0.87(Panasenko, 1967; Plaza et al., 2003). The pH range for growth is 1.6–9.8 (Panasenko, 1967).

Mycotoxins. Although *Penicillium italicum* has been shown to exhibit toxicity in some biological assays (Faid and Tantaoui-Elaraki, 1989) and an isolate was somewhat toxic to ducklings (Kriek and Wehner, 1981), mammalian toxicity has not been shown.

Ecology. The primary habitat for *Penicillium italicum* is fruit of *Citrus* species, on which it produces a destructive rot of considerable economic importance (Snowdon, 1990). Control measures are essentially similar to those used against *P. digitatum*. *P. italicum* has also shown resistance to commonly used fungicides (Wild, 1983; Diaz-Borras et al., 1987; Davé et al., 1989). It has been reported only rarely from other foods: from avocados, tomatoes, sapodillas and rice (see Pitt and Hocking, 1997), meat (Papagianni et al., 2007), sausages, salami and cheese (Guillet et al., 2003) and fruit juices (Wyatt et al., 1995).

Additional species. *Penicillium ulaiense* H.M. Hsieh et al. is closely related genetically to *P. italicum* (Holmes et al., 1994; Frisvad and Samson, 2004). It differs by much slower growth on CYA (15–20 mm in 7 days at 25°C) and MEA (15–17 mm). Colony appearance is very similar, though reverse colours on CYA and MEA are yellowish orange, and coremium production more marked (Holmes et al., 1994; Frisvad and Samson, 2004). Colonies on CSN are 4–8 mm diam, growth weak and sparse, medium and reverse neutral. Like *P. italicum*, *P. ulaiense* is pathogenic on *Citrus* fruits, especially oranges and lemons. It has been isolated from most citrus growing areas around the world, including North and South America, southern Europe, Denmark, South Africa, Taiwan, Israel, Turkey and Australasia (Holmes et al., 1994; Carrillo, 1995; Frisvad and Samson, 2004). Most isolates are resistant to imazalil, the chemical of choice for control of *P. italicum*. *P. ulaiense* is a weaker pathogen than *P. italicum* (Holmes et al., 1994): they postulated that *P. ulaiense* is a rare species, but has developed imazalil resistance and become significant once *P. italicum* was controlled.

Penicillium ulaiense grows quite strongly at 5°C and reaches its optimum near 25°C; growth is weak at 30°C and absent at 33°C (Holmes et al., 1994). It is not known to produce mycotoxins and has not been found away from *Citrus* fruits or citrus packing houses.

References. *Penicillium italicum*: Samson et al. (1976); Pitt (1979b, 2000); Domsch et al. (1980); Samson and Frisvad (2004). *P. ulaiense*: Holmes et al. (1994); Samson and Frisvad (2004).

Penicillium nalgiovense Laxa **Fig. 7.44**

Colonies on CYA usually 28–35 mm diam, radially sulcate, dense and velutinous to quite floccose, conidial production light to heavy, white to dull green (25-26B-E3-5); clear exudate produced by most isolates; reverse pale to bolden or blonde (4-5B-E3-6). Colonies on MEA variable, 10–30 mm diam, plane, dense to somewhat floccose, usually heavily sporing, white to dull green (26-27D-E3-5); exudate and soluble pigment absent; reverse usually strongly coloured, in deep orange brown to brown shades (6C-E6-8). Colonies on G25N 14–22 mm diam, plane, dense to floccose, conidial production moderate to heavy, white to dull green, as on CYA; exudate and soluble pigment absent; reverse pale, yellow or golden brown. At 5°C, germination. No growth at 37°C.

Colonies on CSN usually 10–18 mm diam, of neutral to weakly acid reaction in both medium and colony reverse.

Conidiophores borne from surface hyphae, stipes commonly 150–400 μm long, or of indeterminate length, often branching irregularly from basal hyphae, smooth walled, bearing irregular penicilli; penicilli very variable within a single preparation, in

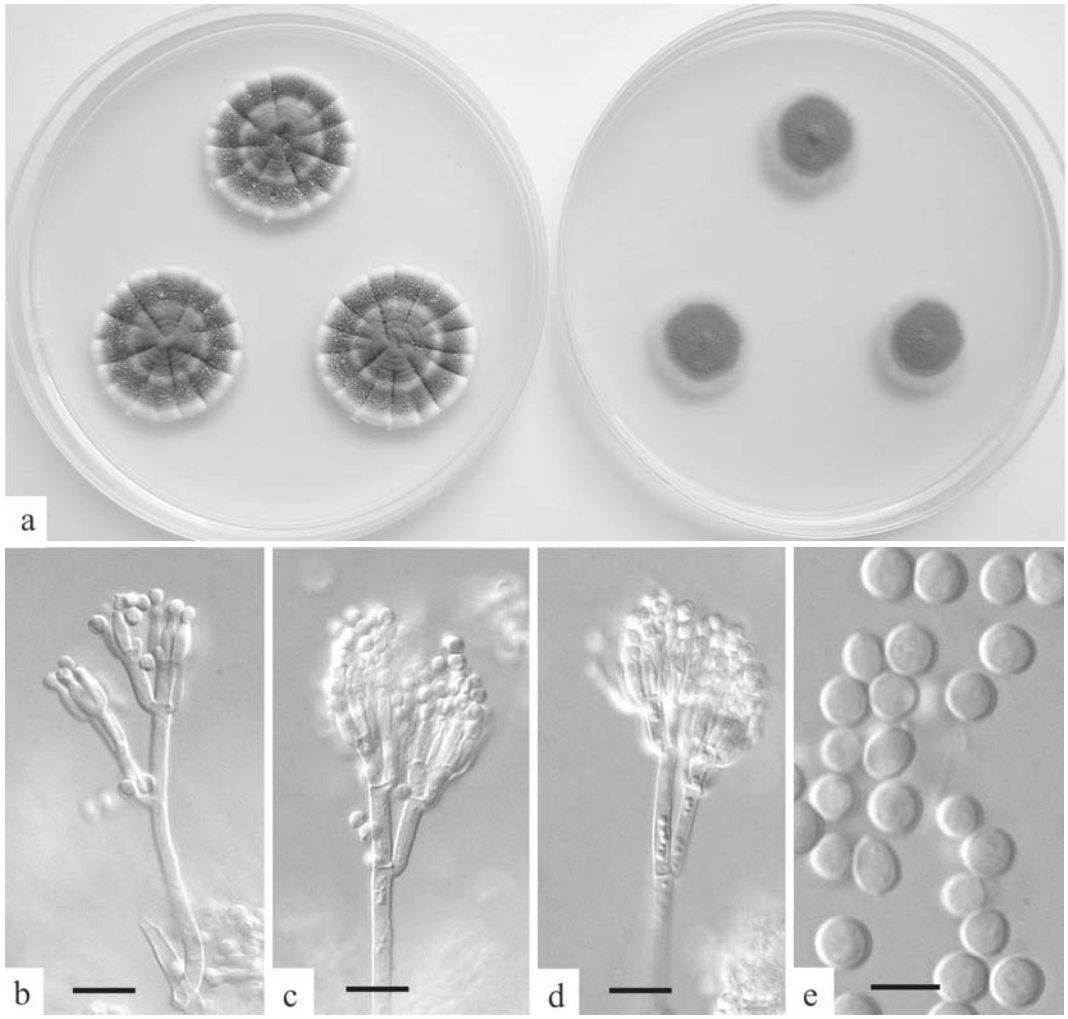


Fig. 7.44 *Penicillium nalgiovense* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 μm; **(e)** conidia, bar = 5 μm

the most characteristic form a compact terminal terverticillate structure with a divergent subterminal ramus, occasionally quaterverticillate or more complex, less commonly biverticillate, sometimes with intercalary irregular metulae or rami; phialides ampulliform to acerose or nearly cylindrical, 7–10 μm long, with short, broad collula; conidia spherical to subspheroidal, 2.8–3.5 μm diam, smooth walled, borne in short columns.

Distinctive features. Closely related to *Penicillium chrysogenum*, this species is distinguished by moderate growth, white to pale green sporulation, usually deep orange reverse colours on MEA, and complex, often irregularly branched penicilli. Growth on CSN is weak.

Taxonomy. For a long time, the name *Penicillium nalgiovense* has been applied to a mould used in the manufacture of fermented meats in Europe (Incze and Mihalyi, 1976; Dragoni and Marino, 1979). Based on the type isolate, from cheese, this species was synonymised with *P. jensenii* by Pitt (1979b). However, comparative studies of isolates from meats with the type of *P. nalgiovense* have shown that all belong to a single species (R.H. Cruickshank and J.I. Pitt, unpublished). It appears likely that *P. nalgiovense* is a domesticated form of the common spoilage species *P. chrysogenum*, with a major habitat in fermented meat products in Europe.

Isolates of *Penicillium nalgiovense* show substantial variation in morphology and the species can be

divided into six biotypes. These vary in rates of growth on MEA and colour and degree of sporulation on CYA and MEA (Fink-Gremmels et al., 1988).

Physiology. As with cheese starter cultures, much is known about the enzymatic and functional properties of strains of this species for use as starter cultures. Little has been published on the gross physiological properties of interest here. A close similarity to *Penicillium chrysogenum* is to be expected.

Mycotoxins. The possibility of mycotoxin production by *Penicillium nalgiovense* has been widely investigated. Most isolates show very low toxicity, and selection of non toxigenic strains has been successful (Leistner and Pitt, 1977; Fink-Gremmels et al., 1988; Hwang et al., 1993a; Andersen, 1995). Some isolates produce penicillin (Andersen and Frisvad, 1994; Papagianni et al., 2007) and nalgiovensin. However, the toxicity of the latter is unknown (Frisvad and Samson, 2004). Diaportins have been produced by isolates of *P. nalgiovense* on cheese agar (Larsen et al., 2002).

Ecology. The main ecological niche occupied by *Penicillium nalgiovense* is as a starter culture for fermented meat production in Europe. It is a reasonable postulate that this species evolved from *P. chrysogenum* during the millennia that such products have been made. Consult the review by Sunesen and Stahnke (2003) for the supply, use and safety of such starter cultures.

Other sources are rare: they include nuts (Sahin and Kalyoncuoglu, 1994) and cheese (Lund et al., 1995).

References. Fink-Gremmels et al. (1988); Samson et al. (1995); Samson and Frisvad (2004).

Penicillium olsonii Bainier & Sartory Fig. 7.45

Colonies on CYA 30–40 mm diam, plane or lightly sulcate, deep but velutinous; mycelium inconspicuous, white to pale brown; conidial production moderate to heavy over the whole colony area, greyish green to dull green (26-27C-E3); limited amounts of clear exudate sometimes produced; reverse pale yellow or yellow brown. Colonies on MEA 25–35 mm diam, plane and velutinous; mycelium inconspicuous except sometimes centrally, white to buff; conidial production moderate to heavy, greenish grey to greyish green (26-28C2-3); reverse pale or dull yellow brown. Colonies on G25N 22–28 mm diam, similar in morphology and colouration to colonies on CYA; reverse pale to yellow. At 5°C, germination of some conidia to formation of microcolonies. No growth at 37°C.

On CSN, colonies 6–14 mm diam, growth weak and sparse, no reaction in medium or colony reverse.

Conidiophores borne from subsurface hyphae, very large, stipes typically 500–2000 μm long, and 4.0–6.0 μm wide, but occasionally even larger, smooth walled, terminating in characteristic closely appressed multiramulate penicilli, usually terverticillate but sometimes quaterverticillate; rami commonly 2–3 but up to 5–6 per stipe; phialides ampulliform, 9–10 μm long, with short collula; conidia ellipsoidal, 3.0–4.0 μm long, with walls smooth or finely roughened, borne in disordered chains.

Distinctive features. Exceptionally large multiramulate terverticillate penicilli borne on long, wide stipes set *Penicillium olsonii* apart from other *Penicillium* species. Average penicilli probably contain some 200 phialides and numbers in excess of 300 are not impossible.

Taxonomy. *Penicillium olsonii* appears to be closely related to *P. brevicompactum* (Stolk and Samson, 1985; Cruickshank and Pitt, 1987) and the new species *P. astrolabium* isolated from grapes in Portugal (Serra and Peterson, 2007).

Physiology. No physiological studies are known.

Mycotoxins. No mycotoxins are produced (Frisvad and Filtenborg, 1989) but penicillin production has recently been reported (Papagianni et al., 2007).

Ecology. This was a very poorly recognised species until recently, so reports of its occurrence in foods are very limited. It is not a rare species, however. It comprised 8–15% of isolates from a large number of mould ripened sausages (Andersen, 1995; Papagianni et al., 2007). We have seen it occasionally from spoiled margarine in our laboratory. We isolated it from 20% of soybean samples from the Philippines; it was present on up to 34% of individual kernels in a sample, and on 3% of all seeds examined (our unpublished data). It occurred at low levels in maize, peanuts, cashews, sorghum, mung beans and copra from Thailand, from maize and paddy rice in the Philippines (our unpublished data) and from peanuts and mung beans in Indonesia (Pitt et al., 1993, 1994, 1998a).

References. Pitt (1979b, 2000); Samson and Frisvad (2004).

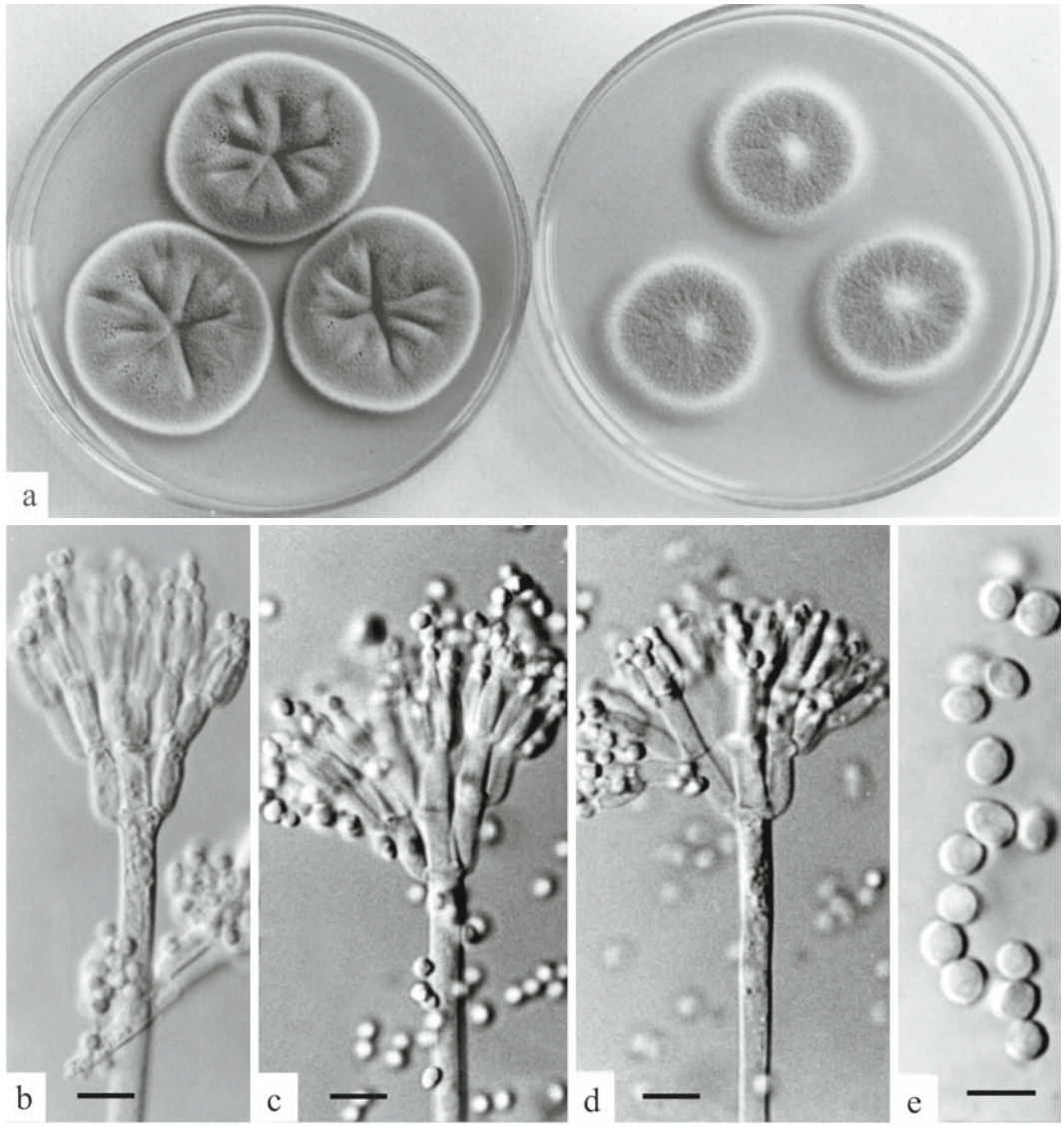


Fig. 7.45 *Penicillium olsonii* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 μm; **(e)** conidia, bar = 5 μm

Penicillium roqueforti Thom Fig. 7.46

Colonies on CYA and MEA growing rapidly, 40–70 mm diam, plane or lightly radially sulcate, low, strictly velutinous; mycelium inconspicuous, white; conidial production moderate to heavy, at the margins greyish turquoise (24C3-4), predominantly Dull Green (25-26E4) and sometimes centrally Olive Brown (4D3-4); reverse pale, brown, or green to deep blue green, almost black. Colonies on G25N usually 20–22 mm diam, but sometimes with spreading submerged margins and then up to 28 mm diam, plane or lightly radially sulcate, colours similar to those on CYA. At 5°C, colonies usually 2–5 mm diam. No growth at 37°C.

On CSN, colonies 25–40 mm diam, growth strong (though not deep), medium reaction variable, from acid (yellow) to alkaline (violet); reverse usually alkaline.

Conidiophores borne from subsurface hyphae, stipes 100–200 μm long, with walls characteristically very rough, bearing large terminal penicilli, typically

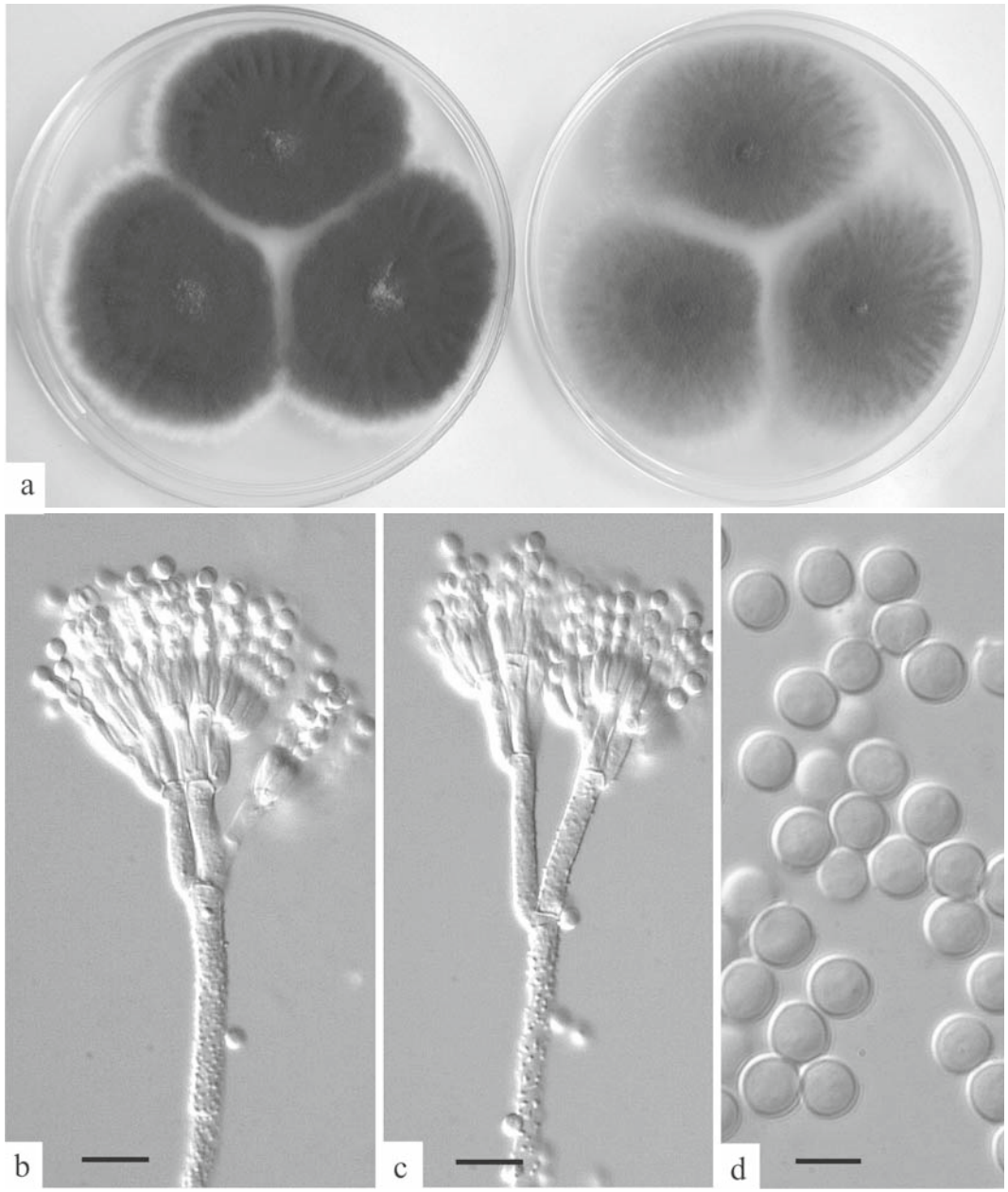


Fig. 7.46 *Penicillium roqueforti* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** penicilli, bars = 10 μm; **(d)** conidia, bar = 5 μm

terverticillate, occasionally quaterverticillate or rarely biverticillate, with elements appressed; rami rough walled; metulae occasionally rough walled; phialides ampulliform, commonly 8–10 μm long, with short collula; conidia spherical, 3.5–4.0(–6) μm diam, with thin, perfectly smooth walls, dark green, borne in long, closely packed, irregular to disordered columns.

Distinctive features. *Penicillium roqueforti* grows very rapidly and produces low and velutinous dark green colonies; stipes have very rough walls and conidia are large, spherical and smooth walled. Growth on CSN is rapid and colony reverse is usually alkaline.

Taxonomy. The species described as *Penicillium roqueforti* by Pitt (1979b, 2000) and Pitt and

Hocking (1997) comprises more than one taxon (Frisvad and Samson, 2004). Two varieties were recognised by Frisvad and Filtenborg (1989): *P. roqueforti* var. *roqueforti*, used in cheese manufacture, and *P. roqueforti* var. *carneum*, a spoilage fungus. These varieties differed in mycotoxin production and to some extent in ecology. On the basis of substantial differences in ITS DNA, Boysen et al. (1996) separated *P. roqueforti* into three species: *P. roqueforti*, *P. carneum* and *P. paneum*. Subsequent metabolic analyses have confirmed the existence of three separate species (Karlshøj and Larsen, 2005). These three species are difficult to distinguish morphologically. However, *P. roqueforti* isolates produce a deep green colour in the reverse on CYA, whereas the reverses of the other two species are yellow brown. *Penicillium roqueforti* and *P. carneum* produce a violet colour with the Ehrlich reaction, whereas *P. paneum* does not (Frisvad and Samson, 2004). *P. carneum* colonies on CYA produce a strong earthy smell, whereas those of *P. paneum* do not (Frisvad and Samson, 2004)

Physiology. *Penicillium roqueforti* appears to have the lowest oxygen requirement for growth of any *Penicillium* species. It grows normally down to 75 mmHg pressure, i.e. only 10% of standard atmospheric pressure (Golding, 1940a). Growth of *P. roqueforti* is little affected by oxygen concentrations down to 4.2% and is stimulated by carbon dioxide concentrations up to 15% in air (Golding, 1940b, 1945). In an atmosphere with less than 0.5% O_2, *P. roqueforti* grew in the presence of 20% but not 40% CO_2 at 25°C, and growth and sporulation also occurred in an atmosphere containing 20% O_2 and 80% CO_2 (Taniwaki, 1995). *P. roqueforti* grew on rye bread in a modified atmosphere comprising 0.03% O_2 and almost 100% CO_2 (Suhr and Nielsen, 2005). These properties are undoubtedly a major reason for the dominant growth of this species in ripening cheeses.

Like other species in this subgenus, *Penicillium roqueforti* is a psychrophile. It grows vigorously at refrigeration temperatures, but not above 35°C (Moreau, 1980). At a_w 0.89 and 0.92, conidia of *P. roqueforti* germinated at 25 and 30°C but not at 37°C or at lower a_w values (Gock et al., 2003). It is tolerant of alkali, the pH range for growth being 3–10 (Moreau, 1980). Furthermore it is highly tolerant of weak acid preservatives, being able to grow in the presence of 0.5% acetic acid, a property used as the basis for a selective medium (Engel and Teuber, 1978). Growth was stimulated in the presence of 0.3% propionic acid at 0.97 a_w (Suhr and Nielsen, 2004). Tolerance to sorbic acid has also been observed (Liewen and Marth, 1984; Bullerman, 1984 and our unpublished data), accompanied by degradation of the preservative (Finol et al., 1982) and development of a "kerosene" taint due to 1,3-pentadiene formation (Liewen and Marth, 1985; Daley et al., 1986). *P. roqueforti* also causes taints in bread due to production of a mixture of 2-methylisoborneol and various 8-carbon alcohols and ketones (Harris et al., 1986).

Mycotoxins. The cheese mould *Penicillium roqueforti* produces PR toxin in pure culture (Frisvad et al., 2006a; Nielsen et al., 2006). It is sometimes produced in very low levels in cheese (Scott, 1981; Teuber and Engel, 1983; Schoch et al., 1984), where the toxin is degraded (Chang et al., 1993; Siemens and Zawistowski, 1993). It was not detected in Tulum cheese, a traditional Turkish cheese (Erdogan and Sert, 2004) or blue cheese in Europe (Finoli et al., 2001). *P. roqueforti* also produces roquefortine C and mycophenolic acid (Frisvad and Samson, 2004; Nielsen et al., 2006), but these compounds have very low toxicity (Scott, 1981).

Ecology. Although best known for its role in the manufacture of Roquefort and related cheese types, *Penicillium roqueforti* is in fact a widely distributed spoilage fungus (though some reports may actually be *P. carneum* or *P. paneum*). Its ability to grow rapidly at refrigeration temperatures makes it a common cause of spoilage in cool stored foods, both commercial and domestic. Like *P. commune*, it is a common cause of cheese spoilage (see Pitt and Hocking, 1997; Finoli et al., 2001; Erdogan et al., 2003; Erdogan and Sert, 2004; Kure et al., 2004; Hayaloglu and Kirbag, 2007). One source can be raw milk (Frevel et al., 1985; Engel, 1986).

Penicillium roqueforti has caused a particular problem with spoilage of packaged rye bread in Europe (see Pitt and Hocking, 1997), due to preservative resistance mentioned above, but *P. paneum* may also be responsible for some of this spoilage. *P. roqueforti* is less frequently isolated from cereals than some other species in this subgenus, but has been reported from barley, rice, flour, baked goods and refrigerated dough products (see Pitt and Hocking, 1997). Frequent reports of *P. roqueforti* from meats and meat products (dried beef, salami and

cured meats, see Pitt and Hocking, 1997) are probably actually *P. carneum*. *P. roqueforti* has also been reported from peanuts, pecans, hazel nuts and walnuts, almonds and dried peas and fresh vegetables (see Pitt and Hocking, 1997). *P. roqueforti* is uncommon in the tropics: we isolated it at low levels in peanuts from the Philippines and Indonesia (Pitt et al., 1998a and our unpublished data).

Additional species. *Penicillium carneum* (Frisvad) Frisvad is almost indistinguishable from *P. roqueforti* using morphological criteria (see Taxonomy). *P. carneum* is associated with spoilage of meat products, but has also been isolated from silage, rye bread, water, beer, cheese, mouldy bakers yeast and cork (Frisvad and Samson, 2004). *P. carneum* produces patulin, penicillic acid (Frisvad and Filtenborg, 1989), penitrem A, mycophenolic acid and roquefortines (Frisvad and Samson, 2004; Nielsen et al., 2006). An isolate identified as *P. crustosum* involved in mycotoxicosis of a man who drank mouldy beer (Cole et al., 1983) was reidentified by Frisvad and Samson (2004) as *P. carneum*.

Additional species. *Penicillium paneum* Frisvad is very similar to *P. carneum* (see Taxonomy). It has been reported from mouldy rye bread, baker's yeast, silage and cassava chips (Frisvad and Samson, 2004). *P. paneum* produces patulin and roquefortine C (Frisvad and Samson, 2004; Nielsen et al., 2006).

References. *Penicillium roqueforti*: Samson et al. (1976); Pitt (1979b, 2000); Moreau (1980); Frisvad and Samson (2004). *P. carneum* and *P. paneum*: Boysen et al. (1996); Frisvad and Samson (2004); Karlshøj and Larsen (2005).

Penicillium solitum Westling — Fig. 7.47

Penicllium verrucosum var. *melanochlorum* Samson et al.
Penicillium melanochlorum (Samson et al.) Frisvad

Colonies on CYA 22–28 mm diam, usually lightly radially sulcate, less commonly plicate or plane; low to moderately deep, very dense, with surface texture velutinous or less commonly granular or fasciculate; mycelium white, usually visible only at the margins; conidia usually produced abundantly, dark bluish green or dark green (25-26E-F4-7); exudate usually absent or inconspicuous, occasionally abundant or even dominating colony appearance, clear; reverse usually pale or uncoloured, uncommonly yellow or brown, or light salmon to orange as on MEA. Colonies on MEA 20–28 mm diam, plane, low with velutinous to distinctly granular texture, or less commonly floccose; mycelium white or occasionally yellow, usually inconspicuous, but uncommonly visible over much of the colony surface; conidia abundant in most isolates, coloured as on CYA, rarely confined to central areas or absent; reverse characteristically greyish orange to brownish orange (5-6B-C4-6). Colonies on G25N usually 16–22 mm diam, closely resembling colonies on CYA; conidia dark green; reverse uncoloured to pale yellow. At 5°C, microcolonies or colonies up to 4 mm diam formed. No growth at 37°C.

On CSN, colonies 18–22 mm diam, growth strong, though not rapid; medium reaction usually alkaline (violet), but sometimes neutral or weakly acid (yellow); reverse alkaline.

Conidiophores borne singly or in definite fascicles, usually from subsurface hyphae, stipes commonly 150–250 μm long, but of indeterminate length when in fascicles, with walls smooth to finely roughened or, rarely, rough; penicilli predominantly terverticillate, but appreciable numbers biverticillate or quaterverticillate in some isolates; rami usually borne singly, sometimes rough walled; phialides ampulliform but tending to acerose or cylindroidal in some isolates, commonly 9–10 μm long, with short collula; conidia spherical to subspheroidal, uncommonly broadly ellipsoidal, 3.0–4.0(–4.5) μm diam or in length, with walls smooth to very finely roughened, borne in disordered chains.

Distinctive features. Conidial colour, dark bluish green to dark green, is the most useful feature distinguishing *Penicillium solitum* from other closely related species. On MEA, greyish to brownish orange reverse colours are a distinctive characteristic. Conidia are quite large. Growth on CSN is strong, though slow, and the colony reverse is alkaline. *P. solitum* can also be distinguished from *P. commune* by lack of reaction with the Ehrlich test (Lund, 1995a) and patterns of secondary metabolites (Lund, 1995b).

Taxonomy. Accepted by Raper and Thom (1949) as a poorly defined, floccose species, *Penicillium solitum* was ignored for nearly 40 years. Samson et al. (1976) included it as a synonym of *P. verrucosum* var. *cyclopium*, while Pitt (1979b) considered it to be a

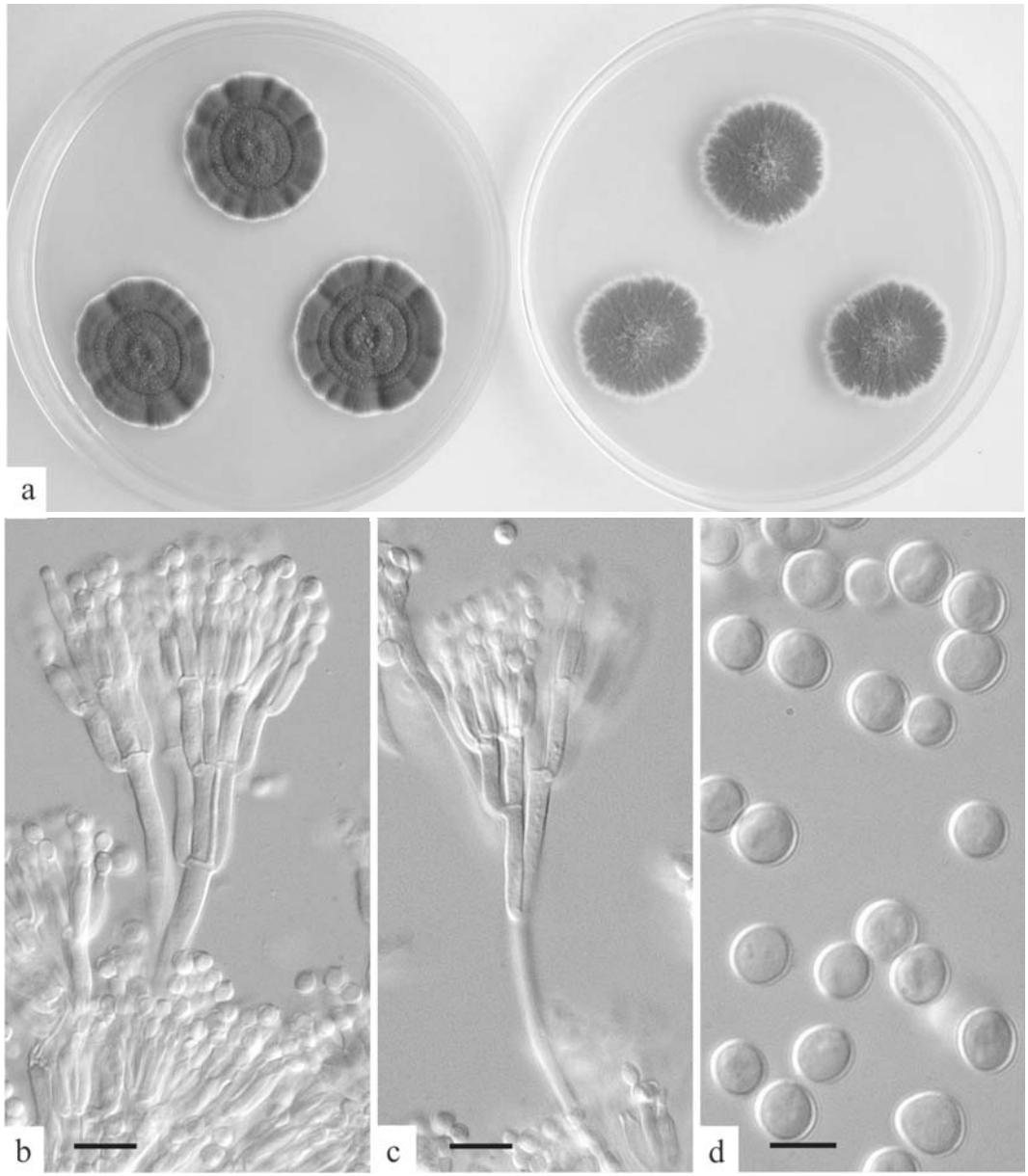


Fig. 7.47 *Penicillium solitum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** penicilli, bars = 10 µm; **(d)** conidia, bar = 5 µm

synonym of *P. aurantiogriseum*. Cruickshank and Pitt (1987) revived *P. solitum* on the basis of distinctive isoenzyme patterns and morphological examination of a number of fresh isolates. Complete descriptions were provided by Pitt (2000) and Pitt et al. (1991).

Physiology. Lack of recognition of this species until recently has meant that little information exists on its physiology. However, it is a typical member of *Penicillium* subgenus *Penicillium* in showing ability to grow at low temperatures and a_w and absence of growth at 37°C.

Mycotoxins. This species does not produce mycotoxins (Frisvad and Samson, 2004).

Ecology. *Penicillium solitum* is a significant pathogen of pomaceous fruit (Frisvad, 1981; Pitt et al., 1991; Sanderson and Spotts, 1995; Amiri and Bompeix, 2005). It is resistant to the fungicides used to control growth of *P. expansum* and so its role in

apple spoilage has increased (Pitt et al., 1991; Sanderson and Spotts, 1995). This species also causes spoilage of cheese (Hocking and Faedo, 1992; Lund et al., 1995; Kure et al., 2004) and has been reported from European sausages during manufacture (Andersen, 1995; Papagianni et al., 2007). Absence of other literature reports probably reflects lack of recognition, not rarity in foods. We isolated it, at a low frequency, from Thai cashews (Pitt et al., 1993), Indonesian peanuts (Pitt et al., 1998a) and Philippine peanuts, maize and mung beans (our unpublished data).

References. Pitt et al. (1991); Pitt (2000); Frisvad and Samson (2004).

Penicillium verrucosum Dierckx Fig. 7.48

Colonies on CYA 15–25 mm diam, usually closely sulcate, varying from low and velutinous to deep and fasciculate or floccose; mycelium white; conidial formation light to moderate, greyish green to dull green (26-27D-E4-5); clear to pale yellow exudate produced, copiously by some isolates; reverse yellow brown to deep brown. Colonies on MEA 12–15(–20) mm diam, sulcate, dense and velutinous or centrally floccose; mycelium white; conidial production moderate, coloured as on CYA; clear exudate occasionally produced; reverse dull brown or olive. Colonies on G25N 16–20 mm diam, plane or more commonly sulcate, velutinous to somewhat floccose; mycelium white; reverse pale to yellow. At 5°C, microcolony formation at least; often colonies of 2–4 mm diam produced. No growth at 37°C.

On CSN, colonies usually 10–15 mm diam, growth weak, mostly subsurface; reaction neutral in medium and reverse.

Conidiophores borne from subsurface or surface hyphae, stipes robust, 200–500 μm long, with walls finely to conspicuously roughened, bearing terminal penicilli; penicilli variable, some isolates producing compact terverticillate and quaterverticillate forms almost exclusively, others predominantly terverticillate and biverticillate, often with elements irregularly disposed; rami 1–2 per stipe, sometimes rough walled; phialides ampulliform, 7–9 μm long, narrowing abruptly to short collula; conidia usually spherical, 2.5–3.0 μm diam, less commonly subspheroidal to ellipsoidal, 3.0–3.5 μm long, with smooth walls, borne in disordered chains.

Distinctive features. *Penicillium verrucosum* is characterised by slow growth on CYA and especially on MEA, by conidia coloured relatively bright green and by the absence of other conspicuous pigmentation. Growth on CSN is weak, with no change in medium colour. *P. verrucosum* produces a red brown to terracotta reverse on YES agar (Frisvad and Samson, 2004).

Taxonomy. Raper and Thom (1949: 486) placed *Penicillium verrucosum* "in the series with" *P. viridicatum*, while neither recognising the species nor placing it in synonymy. Samson et al. (1976) considered *P. viridicatum* and *P. verrucosum* as synonyms, under the name *P. verrucosum* var. *verrucosum*. Frisvad and Samson (2004) placed this species in Section *Viridcata*, series *Verrucosa*, along with *P. nordicum* (see Additional species, below), while placing *P. viridicatum* in a separate series, *Viridicata*.

Physiology. *Penicillium verrucosum* grows from 0 to 31°C, with the optimum at 20°C. The minimum a_w for germination and growth is ca. 0.80 (Northolt et al., 1979; Cairns-Fuller et al., 2005; Pardo et al., 2006c). Growth and ochratoxin A production were significantly inhibited by 50% CO_2 at 0.90–0.995 a_w at 25°C (Cairns-Fuller et al., 2005). Growth occurs over the pH range 2.1–10.0 at least (Wheeler et al., 1991).

Mycotoxins. *Penicillium verrucosum* is the major source of ochratoxin A in cool temperate zone commodities, especially cereals. Ochratoxin A is also produced by several *Aspergillus* species, notably *A. carbonarius, A. westerdijkiae* and *A. steynii*. Those species are responsible for ochratoxin A production in warmer climates.

Penicillium verrucosum is endemic in cool temperate cereals, so it commonly occurs in foods for human consumption, especially breads and other cereal products. It also occurs in animal feeds and in animal tissue. Relatively high amounts are found in kidney and liver in comparison with muscle or fat. In high doses, it also appeared in eggs (JECFA, 2001).

Ochratoxin A is a chronic nephrotoxin, affecting kidney function in all animal species tested, causing damage to a variety of kidney tissues. Ochratoxin A also has immunosuppressive, teratogenic and genotoxic effects in animals and possibly humans. For reviews, see Bayman and Baker (2006) and Pfohl-Leszkowicz and Manderville (2007). Ochratoxin A is readily absorbed through the intestines, and once it enters the blood stream, has a long half life, up to 3

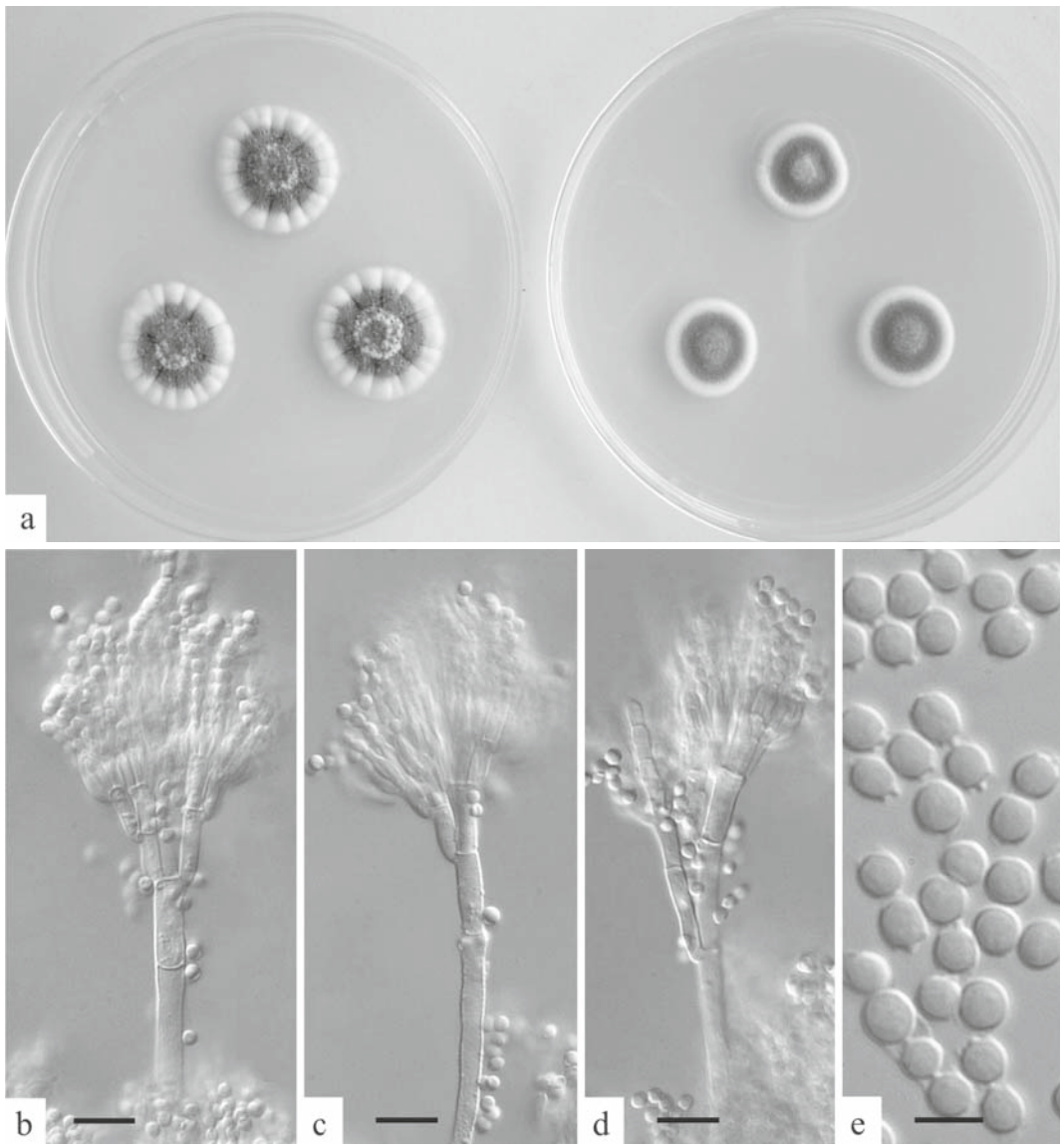


Fig. 7.48 *Penicillium verrucosum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 µm; **(e)** conidia, bar = 5 µm

weeks in monkeys. The result of this is that in areas where ochratoxin A is frequently part of the diet, the blood of healthy humans contains detectable amounts of ochratoxin A (JECFA, 2001). Because *P. verrucosum* has a restricted distribution, this phenomenon is confined almost entirely to Europe. Recent indications of ochratoxin A in human blood from other parts of the world is almost certainly due to the presence of ochratoxigenic *Aspergillus* species in foods.

Ochratoxin A also has carcinogenic properties, but the mechanism of carcinogenicity remains unknown. The carcinogenic effects in animals are considered to be of less importance than the nephrotoxicity (JECFA, 2001). Although ochratoxin A is demonstrably toxin to animals of all kinds, its effects in humans remain unclear (JECFA, 2001). The International Agency for Research on Cancer has classified ochratoxin A as a possible human carcinogen (Group 2B), based on sufficient evidence of carcinogenicity in experimental animal studies and inadequate evidence in humans. The target organ of toxicity in all mammalian species tested is the kidneys, in

which lesions can be produced by both acute and chronic exposure (IARC, 2002).

The majority of *P. verrucosum* isolates are ochratoxin A producers. Some *P. verrucosum* isolates also produce citrinin (Frisvad and Samson, 2004).

Ochratoxin A was once considered to be a possible cause of Balkan endemic nephropathy, a kidney disease with a high mortality rate in certain areas of Bulgaria, Yugoslavia and Romania. However, conclusive evidence of ochratoxin A involvement is lacking. It now seems likely that other toxins are responsible for this syndrome.

Ochratoxin A is produced over the whole temperature range for growth of *Penicillium verrucosum* (0–31°C) and down to 0.86 a_w (Northolt et al., 1979). The optimum conditions on a bread dough were reported to be 0.92 a_w at pH 5.6 (Patterson and Damoglou, 1986). Higher amounts of ochratoxin A were produced on wheat than other substrates including maize, peanuts, rapeseed and soybeans. Citrinin production was supported by both wheat and maize (Madhyastha et al., 1990). High concentrations of ochratoxin A were detected in exudate of *P. verrucosum* colonies on CYA (Gareis and Gareis, 2007). Ochratoxin A has been found in spoiled cheeses, at up to 7 mg/kg (Jarvis, 1983), and mouldy cheesecake, at up to 1 mg/kg (Piskorska-Pliszczynska and Borkowska-Opacka, 1984).

A study by Biffi et al. (2004) on the ochratoxin A levels in a range of cereal products, including commercial flours and other bakery products, revealed that although all samples analysed contained ochratoxin A, most were below the legal limits set by the Italian Ministry of Health of 3 μg/kg for cereal derivatives and 0.5 μg/kg for baby foods. In another investigation of rice and rice products in Europe, González et al. (2006) similarly found low levels of ochratoxin A in rice and other products from both organic and non-organic sources.

Ecology. *Penicillium verrucosum* is endemic in European and Canadian wheat, barley, rye and oats (Pohland et al., 1992; Lund and Frisvad, 2003; Samson and Frisvad, 2004; Lugauskas et al., 2006). It is a cold climate fungus, and is rare outside cool temperate zones, and away from cereals. It has also been isolated from cheese in Europe (Lund and Frisvad, 2003).

References. Pitt (1987, 2000; Frisvad and Samson, 2004).

Additional species. *Penicllium nordicum* Dragoni and Canatoni ex Ramirez is very similar in appearance to *P. verrucosum* but its ecology is quite different. *P. nordicum* is generally associated with high protein substrates such as meat products (Frisvad and Samson, 2004). This species (including reports as *P. viridicatum* or *P. verrucosum*) has been isolated quite frequently from meat products in Europe (Spotti et al., 1989, 1994; Andersen, 1995; Battilani et al., 2007), from Australian salami and prosciutto in our laboratory and from cheese (Hocking and Faedo, 1992; Lund et al., 1995). *P. nordicum* produces a cream yellow reverse on YES medium, in contrast to the red brown reverse produced by *P. verrucosum* (Frisvad and Samson, 2004). All strains of *P. nordicum* produce ochratoxin A (Frisvad and Samson, 2004), and this toxin can be produced in meat products (Spotti et al., 2001; Pietri et al., 2006; and our unpublished observations) (Fig. 7.49).

Penicillium viridicatum Westling **Fig. 7.49**

Penicillium olivinoviride Biourge
Penicillium aurantiogriseum var. *viridicatum* (Westling) Frisvad & Filtenborg

Colonies on CYA 28–32 mm diam, radially sulcate, dense, typically relatively low, velutinous, granular or less commonly floccose; mycelium usually inconspicuous, white; conidial production moderate to heavy, yellow green, usually near cactus green (27-29E4-5), but sometimes brighter (27D4-6) or greyer (26-27D-E2-3); exudate present, clear, pale yellow, pale brown or pale pink; soluble pigment sometimes produced, orange or reddish brown; reverse in shades of orange brown, from pale, near orange white (6A2) to bright or deep, near brownish orange (6B-D7). Colonies on MEA 25–30 mm diam, plane or occasionally radially sulcate, velutinous to granular or centrally floccose; mycelium white or brown; conidial formation moderate, in yellow green colours similar to those on CYA; reverse orange brown to yellow brown. Colonies on G25N 18–22 mm diam, plane or finely radially sulcate, dense, surface granular; conidial production blue grey, yellow green or brown; reverse pale, yellow or orange. At 5°C, macroscopic colonies formed, usually 2–5 mm diam. No growth at 37°C.

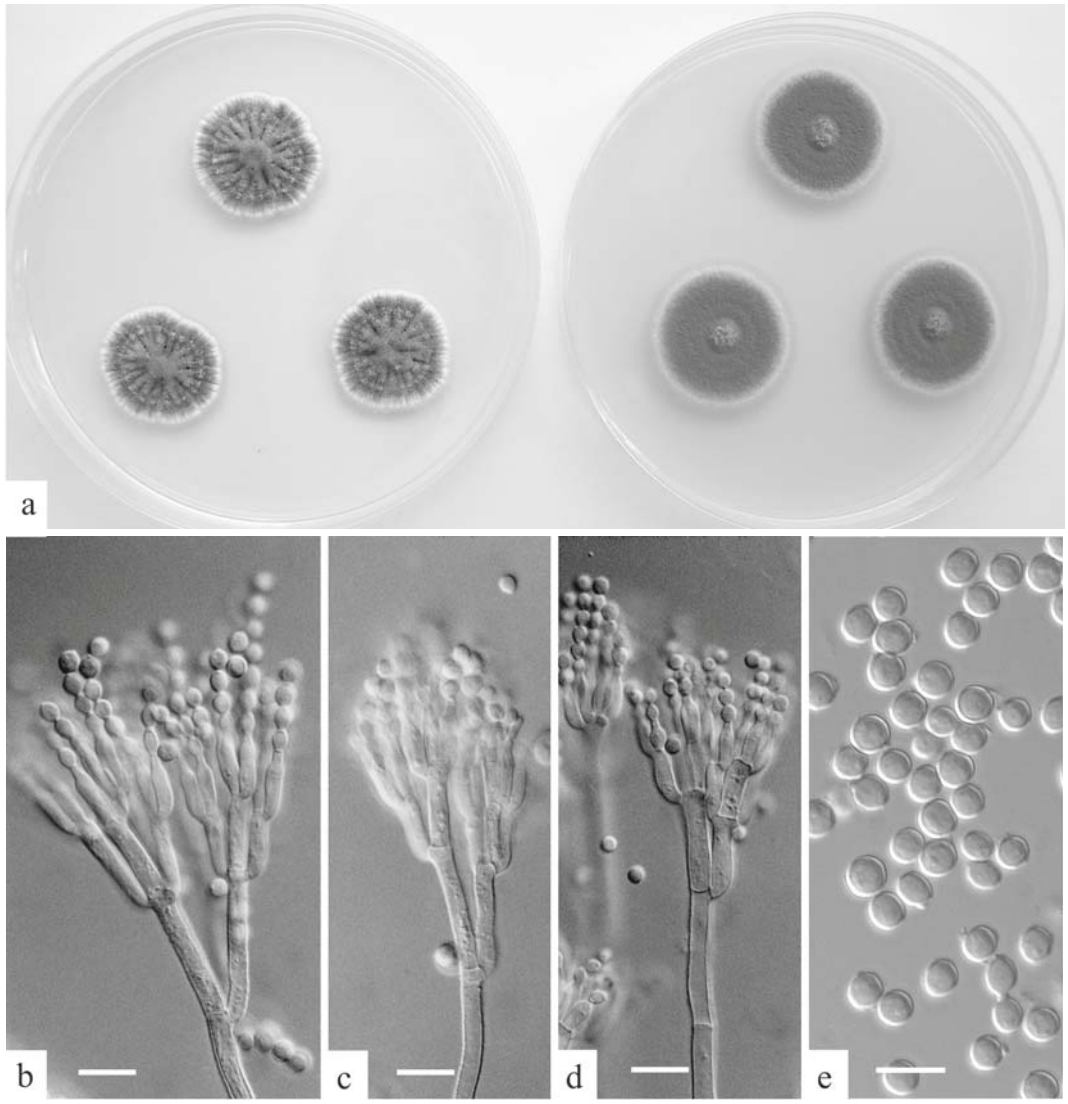


Fig. 7.49 *Penicillium viridicatum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 μm; **(e)** conidia, bar = 5 μm

On CSN, colonies usually 15–22 mm diam, growth moderate to strong; medium and colony reverse response usually acid (yellow), the reverse sometimes brown also.

Conidiophores borne from surface or subsurface hyphae, stipes commonly 200–300 μm long, usually with walls roughened, conspicuously so on MEA, bearing large, appressed penicilli, usually terverticillate but occasionally quaterverticillate; phialides ampulliform, 7–8(–10) μm long, usually with short collula; conidia subspheroidal to ellipsoidal, 3.0–4.0 μm long, with smooth or finely roughened walls, borne in disordered chains.

Distinctive features. On CYA, *Penicillium viridicatum* produces dense and compact colonies which grow moderately slowly; conidia are yellow green; stipes are rough walled. Unlike *P. verrucosum*, *P. viridicatum* grows moderately well on CSN, with an acid reaction in both medium and reverse. Like *P. verrucosum*, this species produces a distinctive violet brown reverse on DRYS (Frisvad, 1983) but a bright yellow reverse on YES, compared with red brown for *P. verrucosum* (Frisvad and Samson, 2004).

Taxonomy. *Penicillium viridicatum* was confused with *P. verrucosum* for a long time (Raper and Thom, 1949; Samson et al., 1976). Ciegler et al. (1973, 1981)

reported that *P. viridicatum* isolates produced two distinct classes of mycotoxins. The situation was clarified by Pitt (1987) who showed that anthraquinone toxins alone were produced by *P. viridicatum*. Frisvad and Filtenborg (1989) considered *P. viridicatum* a variety of *P. aurantiogriseum*, but these are separate species (Pitt and Samson, 1993; Frisvad and Samson, 2004).

Physiology. A psychrophile capable of growth down to at least –2°C, *Penicillium viridicatum* grows optimally near 23°C and has its maximum at 36°C (Mislivec and Tuite, 1970b). Its minimum a_w for growth is 0.80–0.81 at 23–25°C (Mislivec and Tuite, 1970b; Hocking and Pitt, 1979).

Mycotoxins. *Penicillium viridicatum* produces several naphthoquinones, notably xanthomegnin and viomellein capable of causing liver and kidney damage in mice, rats and pigs. Both compounds caused illness in young mice when administered at 450 mg/kg body weight (Carlton et al., 1973, 1976). Viomellein has been found in nature, in a Danish barley sample, at a level of 1 mg/kg (Hald et al., 1983). This level appears to be too low to be considered hazardous. Eleven of 19 samples of poor quality cereals and compounded feeds examined in the United Kingdom contained xanthomegnin and viomellein and a third related compound, vioxanthin. Levels of each up to 2 mg/kg were found, again a significant finding of naturally occurring mycotoxins, but below a clearly hazardous level (Scudamore et al., 1986). The role, if any, of these toxins in natural disease of animals remains unknown.

Ecology. The reported association of *Penicillium viridicatum* with European barley (Krogh et al., 1973) and Canadian wheat (Wallace et al., 1976) is incorrect: *P. verrucosum* is the species involved. *P. viridicatum* is probably rather rare, however, its occurrence in Canadian grain has been verified (Mills et al., 1995), and Frisvad and Samson (2004) report *P. viridicatum* from maize, wheat, barley, beans and peas from Europe, the United States, Canada, Ethiopia and Taiwan.

Penicillium viridicatum has been reported as weakly pathogenic, causing spoilage of grapes and melons in storage. This species has also been reported frequently from meats, dried fruit, spices, pasta, health foods, peanuts, pistachio nuts, almonds (see Pitt and Hocking, 1997) and cheese (Hayaloglu and Kirbag, 2007). We isolated *P. viridicatum* from Philippine maize, peanuts, milled rice, soybeans and black pepper and dehydrated infant food (our unpublished data).

References. Pitt (1979b, 2000); Samson et al. (1976) and Domsch et al. (1980), both under the name *Penicillium verrucosum* var. *verrucosum*; Frisvad and Samson (2004).

7.7.4 Penicillium *subgenus* Biverticillium *Dierckx*

(*Penicillium Sect. Biverticillata-Symmetrica* Thom)

In *Penicillium* subgenus *Biverticillium*, penicilli are characteristically biverticillate, though with sometimes a proportion terverticillate, and always terminal; metulae are numerous, in symmetrical appressed or divergent verticils, and of approximately equal length to phialides; phialides are typically acerose (shaped like a pine needle), with conical collula, tapering to narrow apical pores; conidia in species considered here are ellipsoidal to fusiform.

Colonies on CYA at 25°C commonly show yellow or red colours in mycelium, exudate, soluble pigment and/or reverse. Growth at 37°C commonly occurs. Growth at 5°C is confined to a single species considered here, *Penicillium rugulosum*. Growth on G25N is slow; colonies are not more than 10 mm diam in 7 days at 25°C.

Subgenus *Biverticillium* comprises a well circumscribed group of species with many characters in common, of which penicillus structure, phialide shape, colony pigmentation and growth rate on G25N are especially significant. The most readily recognised feature of isolates from this subgenus is the orderly, characteristically biverticillate penicillus. Only a few species in subgenus *Furcatum* produce penicilli which are superficially similar: however, such species grow more than 10 mm in 7 days on G25N and produce metulae which are consistently longer than phialides.

Where species in subgenus *Biverticillium* produce teleomorphs, they belong in *Talaromyces*. Recent molecular studies have shown that some species in subgenus *Biverticillium* have *Talaromyces* species as their nearest neighbours, indicating multiple losses of the teleomorph. In particular, *T. wortmannii* was shown to be very closely related to *Penicillium variabile*, and *T. flavus* with *P. minioluteum* and *P. funiculosum* (LoBuglio et al., 1993; Pitt, 1995).

Most species in subgenus *Biverticillium* are uncommon in foods. All species in this subgenus

have relatively high water requirements and appear to be associated primarily with soil or moist, decaying vegetation. Perhaps as a consequence, the six species considered here are all well known as biodeteriorative agents in situations where moisture is not a factor limiting growth.

Key to *Penicillium* subgenus *Biverticillium* species included here

1	Colonies on MEA exceeding 25 mm diam	2
	Colonies on MEA not exceeding 25 mm diam	4
2 (1)	Red soluble pigment produced by colonies on CYA	*P. purpurogenum*
	Soluble pigment on CYA pink or absent	3
3 (2)	Mycelium on CYA and MEA bright yellow; stipes more than 100 μm long	*P. pinophilum*
	Mycelium on CYA and MEA not bright yellow; stipes less than 100 μm long	*P. funiculosum*
4 (1)	Colonies on CYA deep and convex; the dominant colony colour bright yellow or orange mycelium	*P. islandicum*
	Colonies on CYA low and velutinous; the dominant colony colour green conidia	5
5 (4)	Colonies on CYA at 25°C not exceeding 12 mm diam; sometimes germination at 5°C	*P. rugulosum*
	Colonies on CYA at 25°C exceeding 12 mm diam; no germination at 5°C	*P. variabile*

Penicillium funiculosum Thom **Fig. 7.50**

Colonies on CYA 25–40 mm diam, plane, usually 2–5 mm deep, with conspicuous ropes of aerial hyphae (funicles), occasionally almost velutinous; mycelium salmon to peach (6-7A4-5) or brownish orange to brownish red (7-8C5-6); conidia moderately abundant, dull green (26-27C-D3-4); clear exudate and pink soluble pigment produced by some isolates; reverse pale, brown, or more commonly deeply pigmented, brownish red to red (8-10B-C8). Colonies on MEA 25–45 mm diam, usually conspicuously funiculose; mycelium usually white, less commonly salmon (6A4) to brownish red (8-9C6-7); conidial production moderate to heavy, coloured as on CYA; reverse pale, brown or orange to reddish brown. Colonies on G25N 3–8 mm diam, plane, usually funiculose; reverse pale to olive. No germination at 5°C. At 37°C, colonies 20–45 mm diam, usually similar to those on CYA at 25°C or more floccose, with reverse pale, brown or reddish brown.

Conidiophores borne from aerial hyphae, usually from well defined funicles, stipes short, commonly 25–40(–60) μm long, in uncommon isolates shorter or longer, with walls smooth to finely roughened, bearing terminal biverticillate penicilli, or occasionally more complex or irregular forms; metulae and phialides closely appressed, acerose, 9–11 μm long, phialides with gradually tapering collula; conidia small, cylindroidal to ellipsoidal, 2.2–3.0 μm long, smooth walled, borne in short columns.

Distinctive features. Colonies of *Penicillium funiculosum* often produce well defined ropes of hyphae (funicles) bearing short conidiophores at right angles. Growth at 37°C is rapid, similar to that on CYA at 25°C or more floccose; penicilli are closely appressed and conidia are pale greyish green.

Physiology. Mislivec and Tuite (1970b) reported that *Penicillium funiculosum* grows optimally at ca. 30°C, with a minimum near 8°C; the maximum growth temperature is near 42°C (Domsch et al., 1980). The minimum a_w for germination and growth is 0.90 at 23, 25 and 30°C (Mislivec and Tuite, 1970b; Hocking and Pitt, 1979). This species is very acid tolerant; we have isolated it from soils on media of pH 2.0.

Mycotoxins. *Penicillium funiculosum* strains toxic to day old cockerels were isolated from chestnuts (Wells and Payne, 1975) and pecans (Wells, 1980). The toxic compounds have not been characterised.

Ecology. *Penicillium funiculosum* has been found quite frequently in foods, including fruit, nuts and cereals. It is one cause of core rot in apples (Combrink et al., 1985; Vismer et al., 1996). *P. funiculosum* causes diseases in onions (Hussein et al., 1977) and blemishes in pineapples (Matos et al., 2005). It

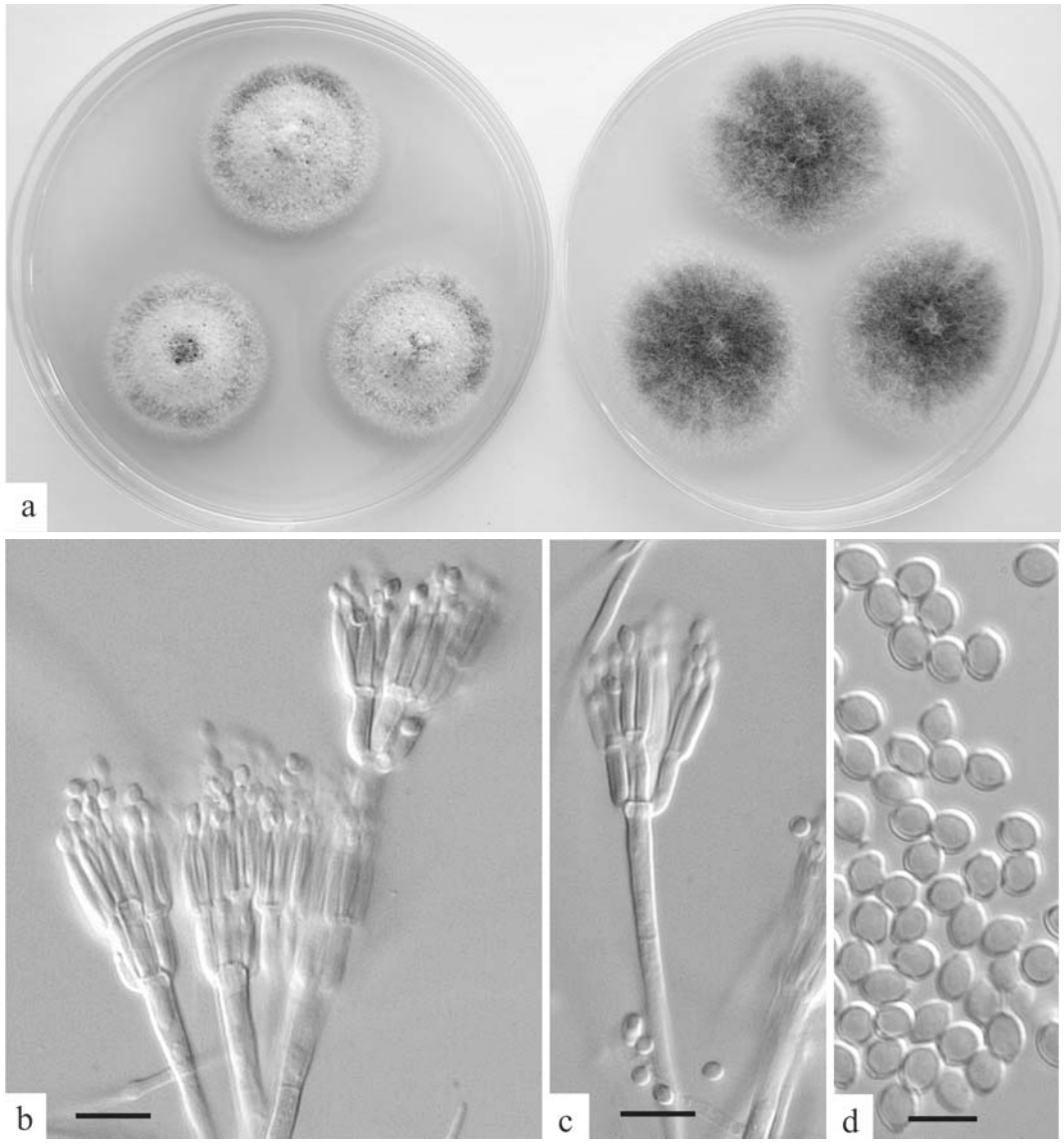


Fig. 7.50 *Penicillium funiculosum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** penicilli, bars = 10 μm; **(d)** conidia, bar = 5 μm

was reported as causing spoilage in pecans, rots in kola nuts and as being common in peanuts both before and after harvest (see Pitt and Hocking, 1997). This species also has a close association with maize (see Pitt and Hocking, 1997) and maize products (Ribeiro et al., 2003). It was present in 42% of maize samples we examined from Thailand, at levels up to 56% of kernels in a sample and in 4% of all kernels examined. Levels in Philippine and Indonesian maize were lower (Pitt et al., 1993, 1998a).

Isolations of *Penicillium funiculosum* have also been made from walnuts, wheat and flour, pasta, barley, soybeans, rice (see Pitt and Hocking, 1997), fresh cabbages (Lugauskas et al., 2005) and parboiled rice (Jayaraman and Kalyanasundaram, 1994). Other sources include dried peas, miso, biltong, a Nigerian spice, yam chips and carbonated soft drinks (see Pitt and Hocking, 1997).

As well as peanuts and maize, mentioned above, we isolated *Penicillium funiculosum* at low levels

from cashews and black rice in Thailand (Pitt et al., 1994).

References. Pitt (1979b, 2000); Domsch et al. (1980).

Penicillium islandicum Sopp Fig. 7.51

Colonies on CYA 17–22 mm diam, plane or centrally raised, velutinous to lightly floccose; mycelium intensely coloured, dominating the colony appearance, deep orange to brownish orange (6A-C8) or copper (7C8); conidia produced in moderate numbers, usually enveloped by the mycelium, but if conspicuous coloured greyish turquoise to greyish green (24-25D4); clear to pale yellow exudate sometimes produced; reverse very strongly coloured, orange to rust brown or burnt sienna (6-7B-E8), sometimes reddish brown (9D-E8). Colonies on MEA 17–22 mm diam, similar to those on CYA but with mycelium usually less dominant; conidial production heavy, greyish turquoise (24D5); reverse commonly with central areas brown to reddish brown (7-8B-E8). Colonies on G25N 4–9 mm diam, similar in appearance and colouration to those on CYA and MEA. No germination at 5°C. At 37°C, colonies 10–20 mm diam, deep and floccose; mycelium white or coloured as at 25°C; conidia absent to abundant, dark green near bottle green (26F5); reverse pale or as at 25°C.

Conidiophores borne from aerial hyphae, stipes 30–60 μm long, usually smooth walled, bearing terminal penicilli, typically biverticillate but not uncommonly bearing an appressed ramus; phialides acerose, 7–8 μm long, with abruptly narrowing collula; conidia broadly ellipsoidal to subspheroidal, mostly 3.0–3.5 μm long, with smooth heavy walls, borne in short, irregular to well defined columns.

Distinctive features. Among the most readily recognised *Penicillium* species, *P. islandicum* produces compact colonies with brilliant orange to brown mycelium and reverse on CYA. Conidia are usually blue and are smooth walled.

Physiology. The optimum temperature for growth of *Penicillium islandicum* has been reported as 31°C, with a minimum and maximum of 10 and 38°C, respectively (Domsch et al., 1980). However, a consistent ability to grow nearly as rapidly at 37°C as at 25°C indicates the maximum growth temperature may be nearer 42°C. The minimum a_w for growth at 31°C is 0.83 (Ayerst, 1969) and at 25°C, 0.86 (Hocking and Pitt, 1979). Growth occurs over the pH range 2.1–9.2 at least (Wheeler et al., 1991).

Mycotoxins. *Penicillium islandicum* produces at least four mycotoxins, unique to the species. Cyclochlorotine and islanditoxin are chlorine containing cyclic peptides which have the same toxic moiety, a pyrrolidine ring with two attached chlorine atoms and share a number of other physical and chemical properties (Scott, 1977). Both compounds are very toxic: cyclochlorotine has an oral LD_{50} in mice of 6.5 mg/kg, while that of islanditoxin by subcutaneous injection was 3 mg/kg. Fed to mice at the rate of 40 μg/day, cyclochlorotine caused liver cirrhosis, fibrosis and tumours (Uraguchi et al., 1972).

Luteoskyrin is a dimeric anthraquinone and erythroskyrin a heterocyclic red pigment. Both are liver and kidney toxins, though less acutely toxic than cyclochlorotine. Luteoskyrin is also carcinogenic.

The toxic "yellow rice" syndrome, described in Japan last century, has resulted in Japanese scientists taking a particular interest in *Penicillium islandicum*, a species which causes yellowing of rice (Saito et al., 1971a). However, the practical importance of the toxins produced by this species remains unclear. *P. islandicum* has not been commonly found growing in nor causing spoilage of foods.

Ecology. A marginal xerophile, *Penicillium islandicum* is more tolerant of low a_w than most other species in subgenus *Biverticillium*. This species is an active agent of spoilage in cereals stored a little above safe moisture contents. It occurs in soil, though not abundantly, and perhaps also is a weak animal pathogen (Pitt, 1979b; p 447). It appears to be more widespread in tropical than temperate regions.

Penicillium islandicum is often associated with rice and has been reported from Japan (Sakai et al., 2005) and South America (Tonon et al., 1997). Other reports include flour, peanuts, pecans and soybeans (see Pitt and Hocking, 1997), without mention of spoilage. We isolated *P. islandicum* from Southeast Asian commodities including maize from the Philippines (our unpublished data), peanuts from Indonesia and milled rice and mung beans from both countries (Pitt et al., 1998a). Levels were always low, without evidence of spoilage or toxin production.

References. Pitt (1979b, 2000); Domsch et al. (1980).

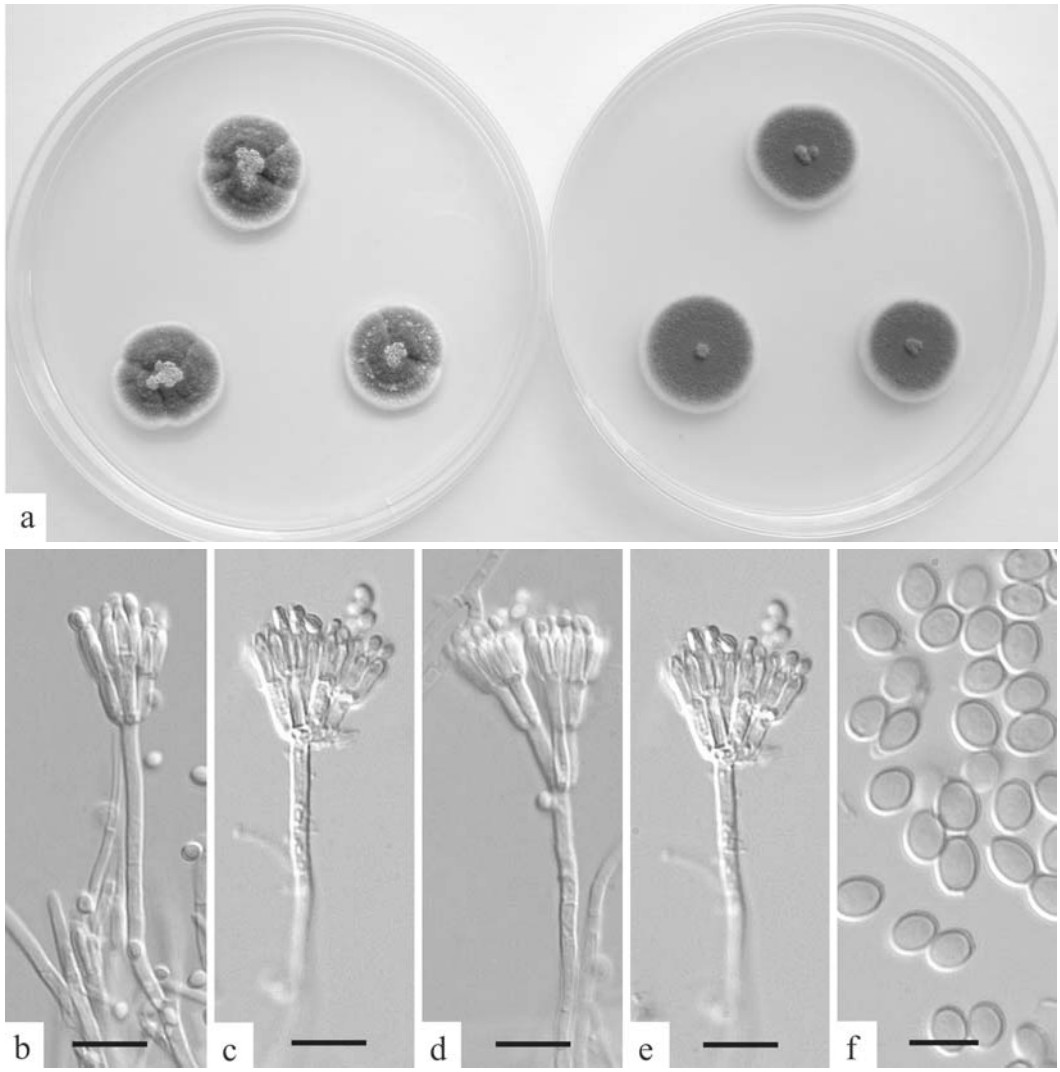


Fig. 7.51 *Penicillium islandicum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d, e)** penicilli, bars = 10 µm; **(f)** conidia, bar = 5 µm

Penicillium pinophilum Hedgcock Fig. 7.52

Colonies on CYA 20–30 mm diam, plane or less commonly sulcate, texture rather loose and floccose, often with tufts of rudimentary funicles also; mycelium white at the margins and also sometimes in superficial overlays, but predominantly slightly brighter than sulphur yellow (1A5), usually produced in a relatively dense overlay over most or all of the colony area, dominating colony appearance and masking conidial colour; conidial formation moderate, usually dark green (28F5) but often appearing lighter due to the yellow mycelium; exudate sometimes present in small amounts, clear to red; reverse usually strongly coloured, golden brown (5C-D7), sometimes masked by or mixed with deep red shades as well. Colonies on MEA 25–35 mm diam, plane, deep and rather floccose; characteristics similar to colonies on CYA, except for paler reverse colouration and absence of white mycelial overlays. Colonies on G25N 2–5 mm diam, rarely as large as 8 mm, low and sparse; mycelium white; reverse pale, brown or olive. No germination at 5°C. At 37°C colonies 20–40 mm diam, plane or lightly sulcate, sometimes low and velutinous and coloured yellow green, more commonly deeply

floccose with white mycelium predominant; conidial production absent to moderate, bluish to greenish grey; clear exudate usually present; reverse usually yellowish or reddish brown.

Sclerotia produced by some isolates, dark red to reddish brown, of variable size, composed of soft compacted hyphae, not of sclerotioid tissue. Teleomorph not known. Conidiophores borne from aerial hyphae, stipes 150–180 μm long, smooth walled, often with vesiculate apices, bearing terminal biverticillate penicilli; metulae in verticils of 8–12 or even 16, 9–12 μm long, forming a 60–90° angle and in the latter case 12–15 μm across the apices; phialides acerose, 8–10 μm long, with gradually tapering collula; conidia subspheroidal, rarely ellipsoidal, commonly 2.5–2.8 μm long, with walls relatively heavy and smooth to finely roughened, borne in short disordered chains or loose columns.

Distinctive features. *Penicillium pinophilum* is characterised by the production of bright yellow mycelium which dominates colony appearance on CYA and MEA. Conidia are subspheroidal to

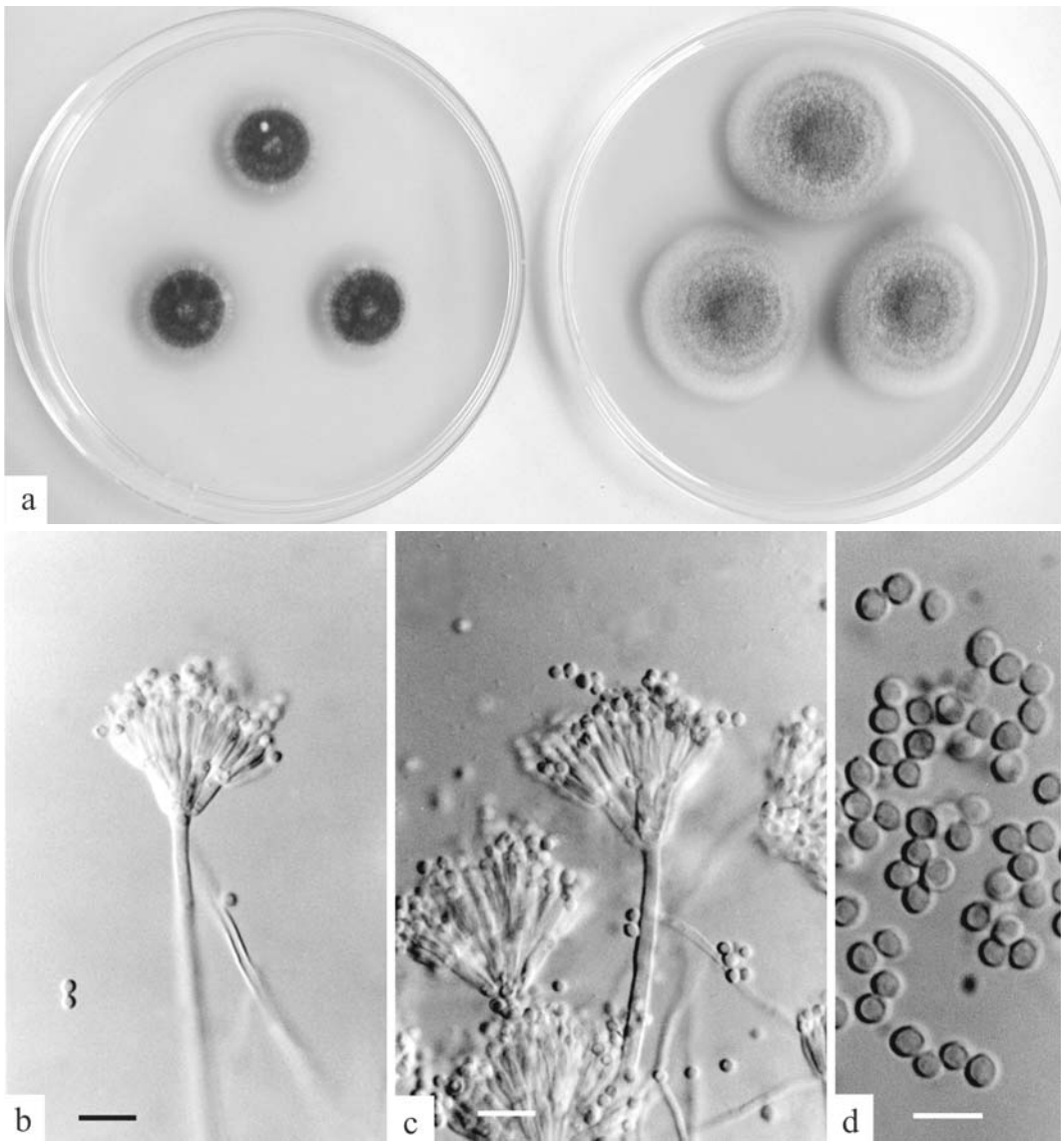


Fig. 7.52 *Penicillium pinophilum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c)** penicilli, bars = 10 μm; **(d)** conidia, bar = 5 μm

ellipsoidal, less than 3 μm in long axis, and with smooth to finely roughened walls.

Physiology. Little is known about the physiology of this species. It grows rather strongly at 37°C. From its taxonomic position, we can infer that it not a xerophile and is similar in most properties to *Penicillium funiculosum*.

Mycotoxins. Mycotoxin production has not been reported.

Ecology. Although *Penicillium pinophilum* has been reported from foods only occasionally, in our experience it is not rare. It was reported from fresh meat by Nassar and Ismail (1994) and it was found in low levels on Spanish grapes (Bau et al., 2005). We isolated it from Philippine peanuts, and in one sample 85% of kernels were infected: this was exceptional, however (our unpublished data). We also found it in maize from Thailand and the Philippines and peanuts from Indonesia (Pitt et al., 1993, 1998a).

References. Pitt (1979b, 2000).

Penicillium purpurogenum Stoll Fig. 7.53

Colonies on CYA 15–30 mm diam, plane or radially sulcate, dense, usually velutinous; mycelium bright yellow or red due to encrusted hyphae; conidial production moderate to heavy, dark green (25-27E-F5); exudate orange to red; soluble pigment vivid red (10A8); reverse dark red or purple, approaching black. Colonies on MEA 22–35 mm diam, plane, dense, velutinous; mycelium white to bright yellow; conidia abundantly produced, dark green (26-27E-G4-7); reverse usually pale, often brown or dull red centrally. Colonies on G25N microscopic to 6 mm diam, coloured as on CYA; reverse pale to deep brown. No germination at 5°C. At 37°C, colonies commonly 12–22 mm diam, usually similar to those on CYA at 25°C, occasionally lacking soluble red pigment.

Conidiophores borne from surface or aerial mycelium, stipes 70–300 μm long, smooth walled, bearing terminal biverticillate penicilli; penicilli narrow, metulae and phialides appressed, 10–14 μm long; conidia ellipsoidal, sometimes becoming subspheroidal at maturity, 3.0–3.5 μm long, with walls smooth, finely roughened or warty, borne in short irregular chains.

Distinctive features. An intense, rapidly diffusing red pigmentation on CYA at both 25 and 37°C is the most striking characteristic of *Penicillium purpurogenum*. In the (rare) absence of such pigmentation, other diagnostic features include conidia very dark green on both CYA and MEA; moderate growth at 37°C (15–22 mm in 7 days); narrow penicilli and heavy walled ellipsoidal to subspheroidal conidia.

Physiology. Minimum and optimum temperatures for growth by *Penicillium purpurogenum* are reported as 12 and 30°C (Mislivec and Tuite, 1970b); from our growth data, a maximum growth temperature near 40°C would be expected. Hocking and Pitt (1979) reported 0.84 as the minimum a_w for germination and growth of this species. Like *P. funiculosum*, *P. purpurogenum* is highly tolerant of acid and can be readily isolated on media of pH 2.0 (Pitt, unpublished).

Mycotoxins. The rubratoxins were originally reported from *Penicillium rubrum*, a species synonymised with *P. purpurogenum* by Pitt (1979b). Rubratoxins were originally suggested as causal agents of mouldy corn toxicosis, or haemorrhagic anaemia in chickens (Burnside et al., 1957; Forgacs et al., 1958). However, later studies (Wyatt and Hamilton, 1972) concluded that rubratoxins were not the cause of these syndromes. Recent taxonomic studies (International Commission on *Penicillium* and *Aspergillus*, unpublished) indicate that rubratoxins are produced by only three known fungal isolates which represent an undescribed species.

Ecology. A marginal xerophile and a recognised biodeteriogen (Pitt, 1981), *Penicillium purpurogenum* has been reported as a cause of spoilage in pineapples in India, pears and *Averrhoa bilimbi* fruit in Sri Lanka (see Pitt and Hocking, 1997). It has been isolated from a wide variety of other foodstuffs, but seldom as a cause of spoilage: from cassava (C.J. Rabie, unpublished), maize, rice, wheat, barley, peanuts, soya beans and kidney beans (Aziz et al., 2006), pecans and betel nuts (see Pitt and Hocking, 1997). It has also been found in processed meats (see Pitt and Hocking, 1997).

References. Pitt (1979b, 2000).

Penicillium rugulosum Thom Fig. 7.54

Penicillium tardum Thom

Colonies on CYA 4–8 mm or occasionally 12 mm diam, plane, low and dense, velutinous; mycelium usually inconspicuous, mainly white but yellow or

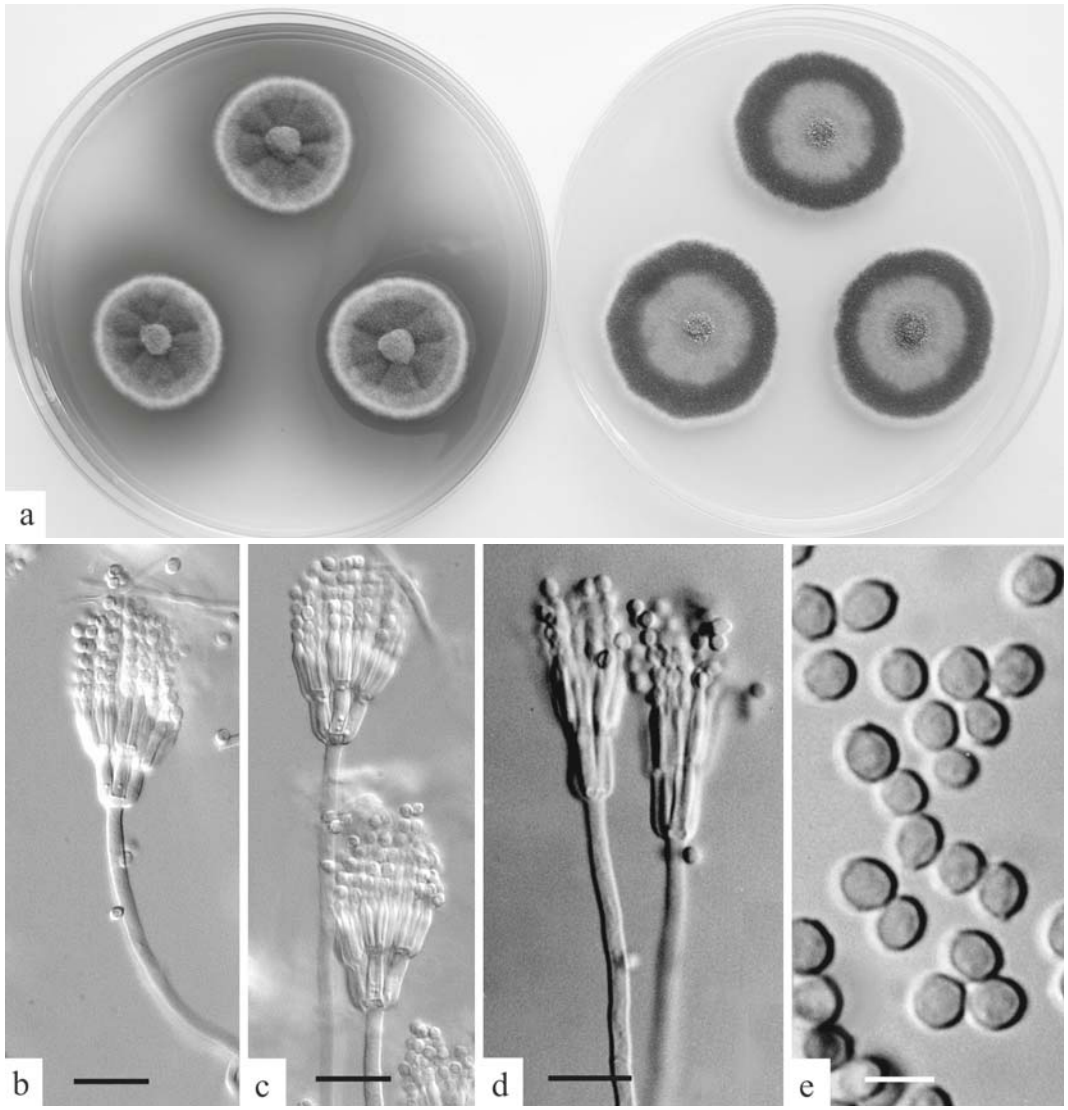


Fig. 7.53 *Penicillium purpurogenum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 µm; **(e)** conidia, bar = 5 µm

red encrusted hyphae often visible under magnification; conidia abundant, greenish grey to dark green (26-27E-F2-4); exudate and soluble pigment usually absent; reverse pale, dull olive or brown. Colonies on MEA 10–20 mm diam, similar to those on CYA except for more numerous and conspicuous yellow encrusted hyphae. Colonies on G25N 2–8 mm diam, velutinous; conidial production moderate, coloured as on CYA, other pigmentation absent. At 5°C, in some isolates germination of conidia, in others germination limited or absent. At 37°C, typically no growth, rarely colonies up to 4 mm diam formed (Fig. 7.54).

Conidiophores borne from surface or aerial hyphae, stipes 70–100 µm long, with thin, smooth walls; penicilli basically biverticillate, but more complex structures often present, for example rami in verticils of up to 4, or rami and metulae from a common origin; metulae appressed; phialides acerose, tending towards ampulliform or less commonly cylindroidal, 8–11 µm long, typically with collula tapering to narrow pores, but sometimes untapered or even enlarging at the apices; conidia ellipsoidal, 3.0–3.5 µm long, with heavy, smooth to rough walls, borne in short to quite long, irregular columns.

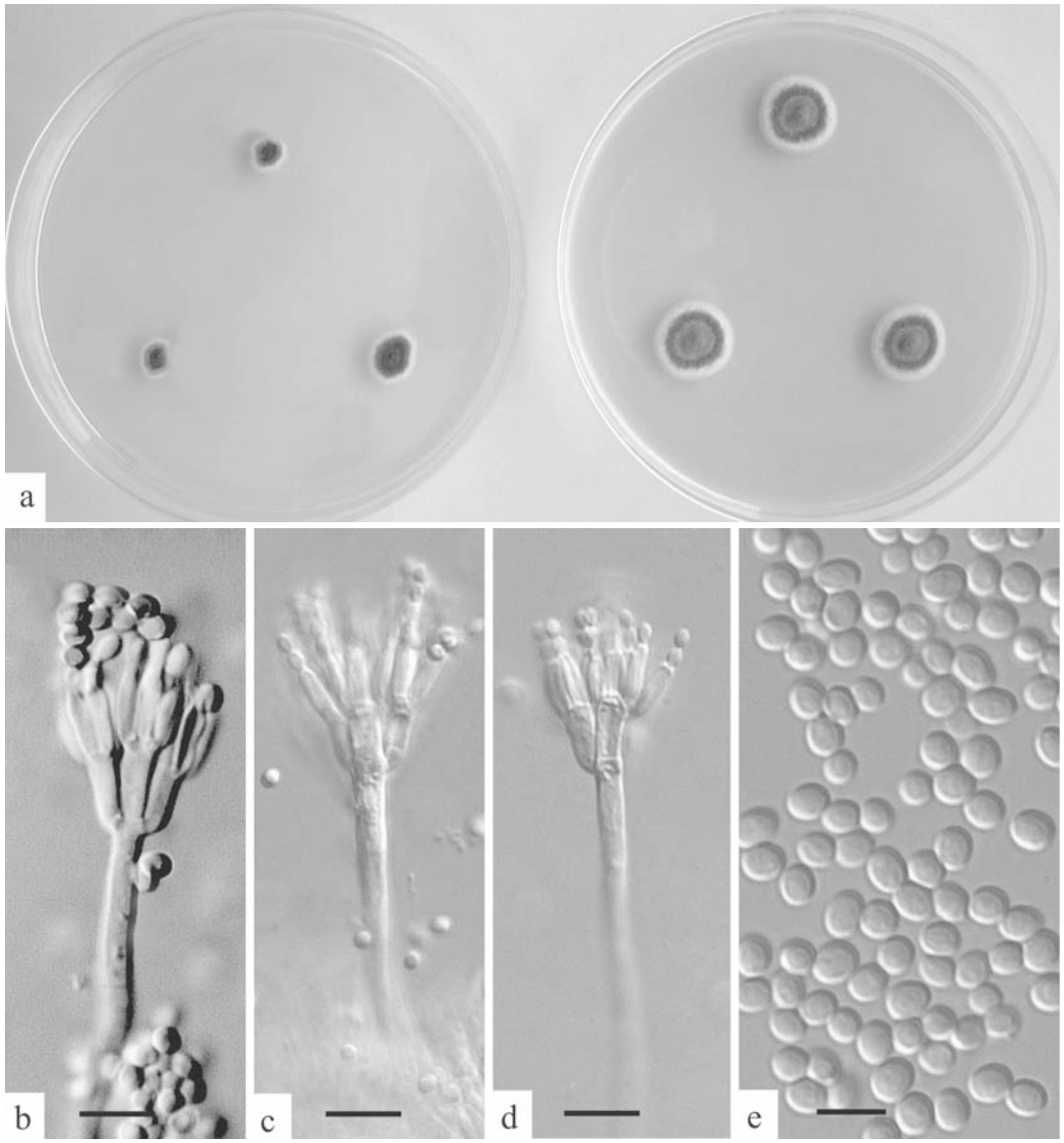


Fig. 7.54 *Penicillium rugulosum* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d)** penicilli, bars = 10 μm; **(e)** conidia, bar = 5 μm

Distinctive features. *Penicillium rugulosum* is characterised by very slow growth, velutinous colonies, and usually by germination at 5°C. Although this species clearly belongs to subgenus *Biverticillium*, penicilli may be of atypical structure at times, and may also bear ampulliform to cylindroidal phialides.

Physiology. From growth data (Pitt, 1973), this species grows between 5 and 37°C; the optimum would be expected to be near 25°C. Snow (1949) reported 0.86 a_w to be its minimum for germination.

Mycotoxins. Production of rugulosin by *Penicillium rugulosum* was reported by Yamazaki et al. (1971) and El-Banna et al. (1987b). Although clearly a toxic compound when given intraperitoneally, with an LD_{50} of 83 mg/kg in mice (Cole and Cox, 1981), rugulosin may be only a laboratory toxin. Disease due to rugulosin has not been reported.

Ecology. Because of its very slow growth, *Penicillium rugulosum* is easily overlooked in isolation procedures. It is probably a more commonly occurring species than records would suggest, and it appears to be widely distributed. This species can be a plant pathogen: it was described by Charles Thom in 1910 from rotting potato tubers, and was isolated by Barkai-Golan (1974) from decaying cold stored apples and the surface of fresh, healthy apples (Amiri and Bompeix, 2005). *P. rugulosum* has been reported quite frequently from dried and processed meats (see Pitt and Hocking, 1997). It has also been found in cheese (Hocking and Faedo, 1992; Lund et al., 1995), rice, maize, barley, wheat, peanuts, soybeans and kidney beans (Aziz et al., 2006), flour and pecans (see Pitt and Hocking, 1997). We isolated it at low levels from Thai cashews and Philippine soybeans (Pitt et al., 1994, 1998a).

References. Pitt (1979b, 2000).

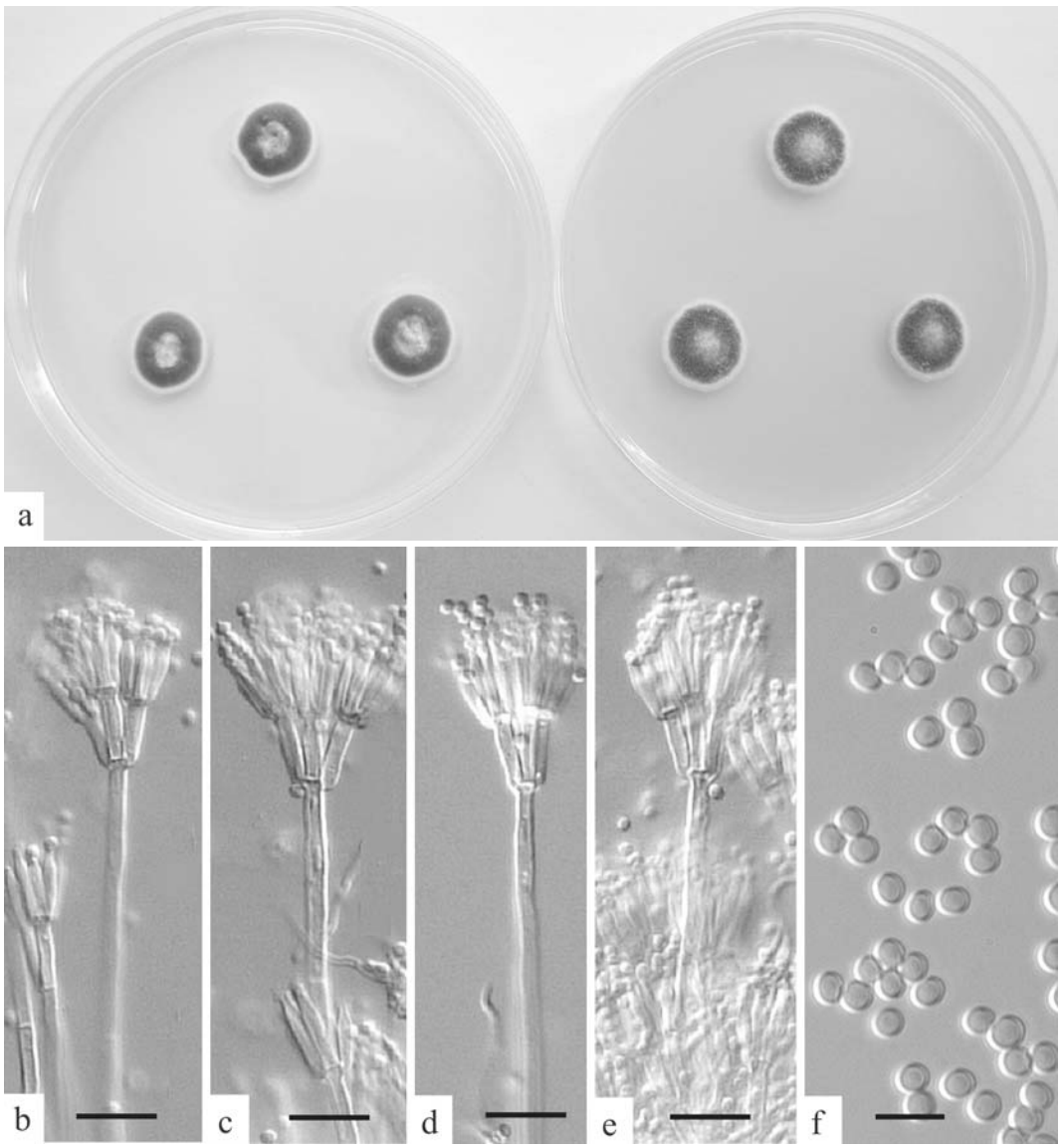


Fig. 7.55 *Penicillium variabile* **(a)** colonies on CYA and MEA, 7 d, 25°C; **(b, c, d, e)** penicilli, bars = 10 µm; **(f)** conidia, bar = 5 µm

Penicillium variabile Sopp **Fig. 7.55**

Colonies on CYA and MEA 15–22 mm diam, plane or irregularly furrowed, low and dense, velutinous; mycelium commonly sulphur yellow (1A4-5), usually conspicuous only at the margins and near colony centres; conidia moderately to abundantly produced, greenish grey (25-27D2); clear exudate occasionally produced; reverse on CYA deep orange to raw umber (5A-F8), on MEA more muted, usually deep orange to brownish yellow (5A-C8). Colonies on G25N microscopic to 9 mm diam, velutinous, sometimes heavily sporing; other colouration absent. No germination at 5°C. At 37°C, response variable, commonly no growth but sometimes colonies up to 5 mm diam produced.

Conidiophores borne mostly from surface hyphae, stipes commonly 100–200 μm long, but if from aerial growth much shorter, 10–30 μm long, smooth walled; penicilli usually biverticillate, sometimes with a subterminal ramus or with concurrent metulae and phialides, 8–12 μm long, appressed to quite divergent; conidia narrowly ellipsoidal, 3.0–4.0(–6.0) μm long, with walls smooth or faintly roughened, borne in closely packed but disordered chains.

Distinctive features. *Penicillium variabile* produces strictly velutinous colonies with abundant greenish grey conidia and with sulphur yellow mycelium conspicuous only in peripheral and central areas. Growth is poor to negative at 37°C. Unlike *P. rugulosum*, penicilli of *P. variabile* are typical of subgenus *Biverticillium* and conidia never germinate in 7 days at 5°C.

Physiology. Mislivec and Tuite (1970b) reported 12 and 30°C to be the minimum and optimal growth temperatures for *Penicillium variabile*; the maximum is near 37°C (Pitt, 1973). The minimum a_w for germination and growth is 0.86 (Hocking and Pitt, 1979).

Mycotoxins. Like *Penicillium rugulosum*, *P. variabile* produces rugulosin (Yamazaki et al., 1972; El-Banna et al., 1987b). This is probably not an important toxin, and there appear to be no records of *P. variabile* causing disease.

Ecology. Although the primary habitats of *Penicillium variabile*, like other species in this subgenus, would be expected to be soil and decaying vegetation, it has quite commonly been found in foods. Isolations have come principally from cereals: wheat and flour, maize, rice and barley (see Pitt and Hocking, 1997). Some other reported sources have been processed meats (Cantoni et al., 2007), peanuts (Pitt et al., 1998a), biltong, cheese, pecans, walnuts and betel nuts (see Pitt and Hocking, 1997).

References. Pitt (1979b, 2000); Domsch et al. (1980).

Chapter 8
Aspergillus and Related Teleomorphs

Thriving in, or at least tolerant of, elevated temperatures and reduced water activities, species of *Aspergillus* and its teleomorph *Eurotium* are the epitome of spoilage fungi. There are few kinds of foods, commodities and raw materials from which Aspergilli cannot be isolated consistently.

Aspergillus species compete with *Penicillium* and *Fusarium* species for dominance among the world's fungal flora. *Aspergillus* lacks the sheer numbers and diversity of *Penicillium* species but compensates by the ability to grow at higher temperatures or lower water activities or both. Aspergilli usually grow more rapidly than Penicillia, although they take longer to sporulate, and generally produce spores which are more resistant to light and chemicals. It is fair to say that *Aspergillus* species dominate spoilage in the tropics in the way *Penicillium* species do in temperate zones.

A small number of *Aspergillus* species are associated with plants, and here *Aspergillus* is more directly in competition with *Fusarium* than with *Penicillium*.

Aspergillus is a genus of Hyphomycetes characterised, in general terms, by the formation of conidiophores with large, heavy walled stipes and swollen apices, termed *vesicles*. Vesicles are usually roughly spherical, but are elongated or less conspicuously swollen in a few species. Vesicles bear crowded phialides, or metulae and phialides, which are characteristically all borne simultaneously (Fig. 8.1a). This character unequivocally distinguishes *Aspergillus* from *Penicillium* and the other genera grouped with it in Chapter 7. Phialide production in *Penicillium* and related genera is always successive, not simultaneous (Fig. 8.1b). Ready differentiation of these genera may be obtained by microscopic examination of developing conidiophores picked from near the colony margins. The presence of immature metulae or phialides all at the same stage of

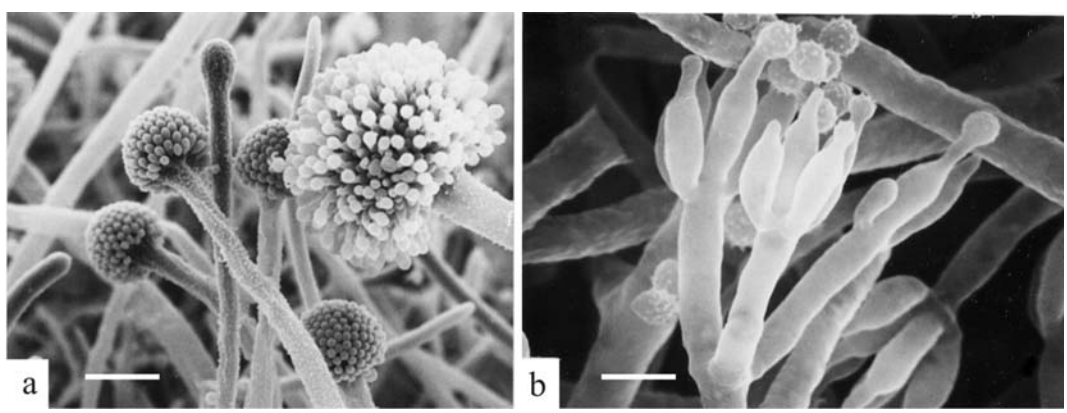


Fig. 8.1 Scanning electron micrographs: **(a)** *Aspergillus oryzae* showing simultaneous production of phialides, bar = 50 μm; **(b)** *Penicillium janczewskii* showing successive production of phialides, bar = 10 μm

J.I. Pitt, A.D. Hocking, *Fungi and Food Spoilage*, DOI 10.1007/978-0-387-92207-2_8,
© Springer Science+Business Media, LLC 2009

development indicates *Aspergillus*; structures with some phialides producing conidia while one or more others are still developing indicates *Penicillium* or a related genus.

Two other useful features are characteristic of most, but not all, *Aspergillus* species. First, stipes are usually formed from a short cell termed a *footcell* within a fertile hypha. Second, stipes are usually nonseptate, so that the vesicle, stipe and footcell all form a very large single cell. *Penicillium* stipes are usually septate and footcells are exceptional.

The authoritative text on *Aspergillus* for the past 40 years has been "The Genus *Aspergillus*" by Raper and Fennell (1965), and this monograph deserves its status. However, in using that monograph, the reader must be aware of the ways in which that classification is considered deficient or unacceptable in current systematics.

First, Raper and Fennell (1965) declined to give status to teleomorph names, an approach with some logic and merit (Raper, 1957), but which contravenes the Botanical Code. Never accepted by nomenclaturalists, that approach has lost favour among industrial mycologists as well. Second, Raper and Fennell (1965) referred to metulae and phialides as "primary and secondary sterigmata". The term sterigma has rightly been confined to Basidiomycete terminology in recent years (Kendrick, 1971). Third, subdivision of the genus was based on species "groups", i.e. species related to *Aspergillus ochraceus* were referred to as belonging to the "*Aspergillus ochraceus* group". The use of the term "group" in this way has no status under the Botanical Code and has been sharply criticised (Benjamin, 1966). Fourth, Raper and Fennell (1965) often failed to observe the rules of priority in accepting particular species names and, fifth, they failed to typify species. The failure to typify names by Thom and Raper and their coworkers over the years led to concept changes for some species, e.g. *Eurotium amstelodami* and *A. penicillioides*. For a discussion of these problems see Pitt (1989a) and Pitt and Samson (1990a).

The systematic difficulties arising from Raper and Fennell (1965) have been addressed by modern taxonomists. Subramanian (1971) and Malloch and Cain (1972) provided names for *Aspergillus* teleomorphs. Gams et al. (1985) developed a new classification of *Aspergillus* based on subgenera and series to replace the incorrect term "group". Gams and Samson (1985) provided typifications of *Aspergillus* and its associated teleomorphs, while Samson and Gams (1985) typified the species of *Aspergillus* accepted by Raper and Fennell (1965). Samson (1979) produced a useful compendium and taxonomic outline of the 90 species described between 1965 and that date. A guide to common *Aspergillus* species and *Aspergillus* teleomorphs which incorporated all of these advances was published by Klich (2002). Pitt and Samson (1993) produced a list of Names in Current Use (NCU) for the family Trichocomaceae, which includes *Aspergillus* and related teleomorphs. By this means the names used by Raper and Fennell (1965) were given *de facto* protection against earlier valid names. This was especially significant in *Aspergillus*, where a number of common names cannot be justified under the Botanical Code. One particularly important name, *A. niger*, was conserved under the provisions of the Botanical Code (Frisvad et al., 1990c; Kozakiewicz et al., 1992) because this name was known to be predated by *A. phoenicis* (Corda) Thom and *A. ficuum* (Reichardt) Hennings. The names used here are in all cases those given protected status by the NCU list.

Teleomorphs. As is the case with *Penicillium* and *Fusarium*, most *Aspergillus* species do not produce any known teleomorph. Those that do all form cleistothecia or reduced stromata resembling cleistothecia and are classified in eight Ascomycete genera. Three are significant in foods: *Eurotium*, *Neosartorya* and *Emericella*. Each of these genera is discussed below, in alphabetical order, before the strictly anamorphic species of *Aspergillus*. Rather than providing a separate key, diagnoses of the distinctions between these three genera are given below and then keyed in the general *Aspergillus* key.

Eurotium species produce bright yellow cleistothecia and pale yellow ascospores. Heads producing conidia are formed from phialides only. All species are xerophilic and grow more vigorously on G25N than on CYA or MEA. If a *Eurotium* isolate is entered in the key in Chapter 5 it will usually be recognised as a *Eurotium* in the general xerophile key in Chapter 9.

Neosartorya species also produce heads formed from phialides alone, but in this case cleistothecia are white and ascospores are uncoloured. Colonies grow rapidly on CYA and MEA at 25 and 37°C. Species are not xerophilic.

Emericella species produce heads with both metulae and phialides and white cleistothecia producing red or purple ascospores. The cleistothecia are surrounded by Hülle cells, which are thick walled roughly spherical

cells resembling chlamydoconidia. Growth patterns in *Emericella* species are similar to those of *Neosartorya*.

In *Emericella*, conidiophores of the *Aspergillus* anamorph are usually abundant, in *Neosartorya* they are often only visible through the stereomicroscope, while in *Eurotium* occurrence is variable. In all three genera the conidial colour is grey green.

Classification. Classification of *Aspergillus* into subgenera and sections relies primarily on four features: the presence of a teleomorph and its characteristics, the presence or absence of metulae, the arrangement of metulae or phialides on the vesicle, and colony colours. In species lacking teleomorphs, *Aspergillus* colony colours are dominated by conidial colour. These colours are consistently associated with particular species.

The subgeneric and sectional classification of Gams et al. (1985) adopted by Klich (2002) has been followed here. Colony diameters are a valuable additional character. In keeping with standardisation of media and growth conditions throughout this book, colony diameters at 5°C are incorporated in the descriptions which follow. However, it should be noted that very few *Aspergillus* or *Aspergillus* teleomorph species germinate at 5°C in 7 days, so if the user knows in advance that an isolate is an *Aspergillus* or related teleomorph, the 5°C plate is logically omitted. CY20S, the medium used here for *Eurotium* identification, can also be a useful identification medium for most *Aspergillus* species, but has not been included here. However, Table 8.1 provides figures for colony diameters on CY20S which may be of value to some users.

Key to *Aspergillus* species and teleomorphs included here

1	Colonies on CYA at 25 and 37°C both exceeding 35 mm diam	2
	Colonies on CYA at 25 or 37°C not exceeding 35 mm diam	12
2 (1)	Colonies black or grey	3
	Colonies white or coloured	6
3 (2)	Colonies black, exceeding 50 mm diam at 25°C	4
	Colonies grey, not exceeding 50 mm diam at 25°C	*A. ustus*
4 (3)	Vesicles bearing metulae and phialides	5
	Vesicles bearing phialides alone	*A. aculeatus* *A. japonicus*
5 (4)	Conidia less than 6 μm diam	*A. niger*
	Conidia more than 6 μm diam	*A. carbonarius*
6 (2)	Colonies white	Genus *Neosartorya*
	Colonies coloured	7
7 (6)	Colonies blue	*A. fumigatus*
	Colonies yellow, green or brown	8
8 (7)	Conidia dark green; developing cleistothecia present, surrounded by cells like chlamydoconidia (Hülle cells)	*Genus Emericella*
	Conidia yellow, yellow green or brown; developing cleistothecia not present	9
9 (8)	Conidia yellow green or yellow	10
	Conidia brown or olive brown	11
10 (9)	Conidia with relatively thin walls, smooth or finely roughened, spherical to broadly ellipsoidal; vesicles up to 50 μm diam, usually metulae present	*A. flavus* *A. oryzae* *A. nomius*
	Conidia with consistently rough, thick walls, spherical; vesicles not usually exceeding 30 μm diam; usually only a low proportion of heads with metulae	*A. parasiticus*

Key to *Aspergillus* species and teleomorphs included here (continued)

11 (9)	Colonies olive brown on CYA and MEA at 25°C; conidia 5–8 μm diam, rough walled; heads radiate	*A. tamarii*
	Colonies brown on all media; conidia less than 3 μm diam, smooth walled; heads developing into long columns	*A. terreus*
12 (1)	Colonies grey	*A. ustus*
	Colonies white or brightly coloured	13
13 (12)	Colonies white, off-white or cream	14
	Colonies coloured	15
14 (13)	Vesicles fertile over the entire area; metulae more than 10 μm long	*A. candidus*
	Vesicles fertile over the upper half to two-thirds; metulae less than 10 μm long	*A. niveus*
15 (13)	Developing yellow cleistothecia present, particularly in colonies on G25N	Genus *Eurotium*
	Developing cleistothecia not present	16
16 (15)	Conidia in shades of yellow, orange or brown	17
	Conidia green or blue	19
17 (16)	Colonies on CYA not exceeding 40 mm diam; conidia in yellow or orange shades	18
	Colonies on CYA exceeding 40 mm diam; conidia pale brown	*A. ochraceus* *A. westerdijkiae* *A. steynii*
18 (17)	Colonies greyish orange, conidia 2–3 μm diam, smooth walled	*A. flavipes*
	Colonies greyish yellow to olive, conidia 4–5 μm diam, with smooth or rough walls	*A. wentii*
19 (16)	Colonies on CYA exceeding 30 mm diam; vesicles more than 50 μm diam	*A. clavatus*
	Colonies on CYA not exceeding 30 mm diam; vesicles less than 25 μm diam	20
20 (19)	Colonies on CYA exceeding 15 mm diam; heads with metulae	21
	Colonies on CYA not exceeding 15 mm diam; heads with phialides only	22
21 (20)	Conidia green	*A. versicolor*
	Conidia blue	*A. sydowii*
22 (20)	Colonies on CYA and MEA 6 mm or more diam; conidia cylindrical to barrel shaped, borne in columns	*A. restrictus*
	Colonies on CYA and MEA not exceeding 6 mm diam; conidia subspheroidal to ellipsoidal, borne in radiate heads	*A. penicillioides*

Table 8.1 Colony diameters expected for *Aspergillus* species and teleomorphs

Species	CYA	MEA	G25N	37°C	CY20S
Emericella nidulans	40–50	35–60	10–15	50–70	40–50
Eurotium amstelodami	14–18	16–25	25–35	0–15	30–45
E. chevalieri	16–25	16–25	20–30	0–10	45–65
E. halophilicum	0	0	0–2	0	0
E. herbariorum	0–10	0–10	25–35	0	25–35
E. repens	10–20	10–20	25–45	0	45–65
E. rubrum	10–20	5–20	30–45	0	35–60
Neosartorya fischeri	50–70	60–70	8–12	60–70	60–70
Aspergillus aculeatus	65–70	45–70	15–20	5–30	65–70

Table 8.1 Colony diameters expected for *Aspergillus* species and teleomorphs (continued)

A. candidus	15–20	10–25	10–16	0–25	15–30
A. carbonarius	60–70	60–70	8–25	15–35	68–70
A. clavatus	30–45	30–45	8–12	10–30	30–45
A. flavipes	15–30	20–35	8–12	5–20	24–30
A. flavus	60–70	50–70	25–40	55–65	65–70
A. fumigatus	40–60	40–60	0–10	65–70	40–60
A. japonicus	45–70	65–70	15–20	20–50	65–70
A. niger	60–70	30–60	18–30	60–70	60–70
A. niveus	18–30	18–30	10–16	30–45	30–50
A. nomius	60–70	50–70	25–40	55–65	65–70
A. ochraceus	40–45	40–55	20–30	20–30	45–70
A. oryzae	55–70	60–70	20–40	40–65	60–70
A. parasiticus	50–70	50–65	20–40	65–70	65–70
A. penicillioides	0–5	0–5	8–15	0	0–10
A. restrictus	6–12	6–12	10–15	0	16–20
A. steynii	33–45	35–35	20–30	0	45–70
A. sydowii	18–30	16–25	15–20	0–10	25–30
A. tamarii	50–65	55–65	35–40	50–65	60–70
A. terreus	40–50	40–60	18–22	50–70	45–70
A. ustus	30–40	40–50	10–14	30–50	25–40
A. versicolor	15–25	12–25	10–18	0–10	15–30
A. wentii	25–35	22–30	30–45	0	50–70
A. westerdijkiae	50–55	40–47	20–30	0	45–70

8.1 Genus *Emericella* Berk.

As noted above, *Emericella* is an *Aspergillus* teleomorph characterised by the formation of white cleistothecia surrounded by Hülle cells (thick walled refractile cells like chlamydoconidia) and producing purple ascospores. Conidiophores usually have short brown stipes, bear both metulae and phialides and produce columns of dark green conidia.

Christensen and States (1982) accepted 29 species. Christensen and Raper (1978) and Christensen and States (1982) provided keys and descriptions to *Emericella* species and the related *Aspergillus* series. Many species are known primarily or solely from desert soils in the western United States and other parts of the world. Only one species is at all common in foods, *Emericella nidulans*.

Emericella nidulans (Eidam) Vuill. Fig. 8.2

Anamorph: *Aspergillus nidulans* (Eidam) G. Winter

Colonies on CYA 40–50 mm diam, plane, low, moderately dense to dense, sometimes with a floccose overlay; mycelium white; cleistothecia white, surrounded by white to buff or dull yellow Hülle cells; conidial heads sparse to quite dense, radiate when young, later forming long well defined columns, conidia coloured pale green or when dense dark green; exudate sometimes present, dull red to brown; violet soluble pigment sometimes produced; reverse sometimes pale, usually brightly coloured in shades of orange, orange brown, deep brown or violet brown. Colonies on MEA 35–60 mm diam, occasionally only 25 mm, sometimes low, plane and velutinous with heavy conidial formation and few cleistothecia, sometimes deeper and with abundant cleistothecia surrounded by dull yellow or buff Hülle cells; mycelium white; cleistothecia sometimes abundant, surrounded by Hülle cells, at other times sparsely produced; conidia dark green; reverse pale, brown or violet brown. Colonies on G25N 10–15 mm diam, low and dense; conidia pale green; reverse pale. No growth at 5°C. At 37°C, colonies 50–70 mm diam, low and sparse, usually predominantly cleistothecial, sometimes with areas of dark green conidia; reverse usually orange or brown.

Cleistothecia mostly 100–250 μm diam, white at first but at maturity dark red, maturing on CYA in 8–10 days, surrounded by heavy walled Hülle cells, 15–25 μm diam; ascospores red to purple, ellipsoidal, 4–6 μm long, smooth walled, usually ornamented with two conspicuous longitudinal flanges. Conidiophores borne from aerial hyphae, stipes 60–150 μm long, often sinuous, with smooth, brown walls and with conspicuous footcells; vesicles spathulate to pyriform, 8–12 μm wide, bearing metulae and phialides over the upper half or less, of similar

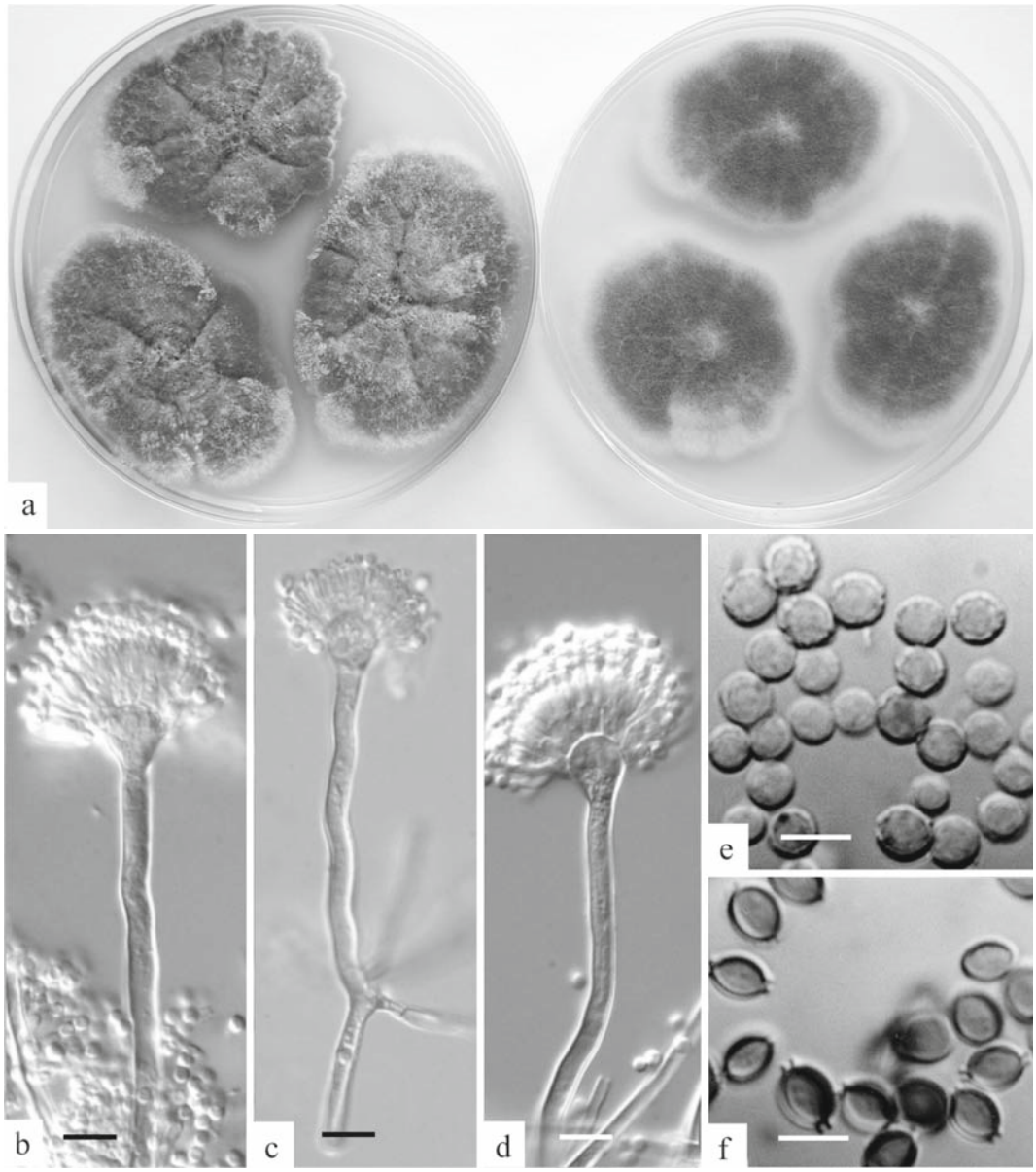


Fig. 8.2 *Emericella nidulans* **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b, c, d)** heads, bars = 10 μm; **(e)** conidia; and **(f)** ascospores, bars = 5 μm

size, 6–8 μm long; conidia spherical, green, 3.0–3.5 μm diam, with roughened walls, borne in radiate to loosely columnar heads.

Distinctive features. *Emericella nidulans* is distinguished by growth on CYA at least as fast at 37°C as at 25°C, developing cleistothecia surrounded by Hülle cells and sparse to abundant green conidia borne on metulae and phialides from diminutive brown walled stipes. Mature ascospores are red and have either two longitudinal flanges or no ornamentation. Other *Emericella* species show different ascospore ornamentation.

Taxonomy. The nomenclaturally correct name for the anamorph of *Emericella nidulans* is

Aspergillus nidulellus Samson and W. Gams (Samson and Gams, 1985). The commonly used name *A. nidulans* (Eidam) Winter was invalid because it was described inclusive of the teleomorph. However, *A. nidulans* has remained in common use and is very important to fungal geneticists, so conservation of *A. nidulans* was sought under the provisions of the Botanical Code (Kozakiewicz et al., 1992). This was rejected by the Special Committee on Fungi and Lichens. Nevertheless, the name *A. nidulans* was included in the *Aspergillus* NCU list (Pitt and Samson, 1993) and has been given protected status under the Botanical Code (Greuter et al., 1994). *A. nidulans* is used here as the appropriate name for the anamorph of *E. nidulans*.

Physiology. According to Panasenko (1967), *Emericella nidulans* is able to grow from 6–8 to 46–48°C, with an optimum at 35–37°C. Lacey (1980) gave a range of 10–51°C, so this species is a marginal thermophile. *E. nidulans* germinated at 0.82 a_w at 20°C (Snow, 1949), 0.80 a_w at 37°C, 0.81 a_w at 25°C, 0.83 a_w at 20°C and 0.90 a_w at 15°C (Ayerst, 1969), so this species is also a xerophile, a most unusual combination. The heat resistance of *E. nidulans* ascospores does not appear to have been studied; because of its thermophilic nature, that property might be expected to be high.

Mycotoxins. *Emericella nidulans* has been reported to produce sterigmatocystin, a mycotoxin more commonly associated with *Aspergillus versicolor* (Frisvad et al., 2006b) and emestrin (Terao et al., 1988, 1990). Emestrin is highly toxic, with an LD_{50} of 13 mg/kg when given to mice intraperitoneally (Terao et al., 1988). Toxicity due to the growth of *E. nidulans* has not been reported under practical conditions.

Ecology. Although not especially common in foods, *Emericella nidulans* has been isolated from a wide variety of sources. Cereals and cereal products have been the most common, including wheat, flour and bread, barley, rice and maize and sorghum (see Pitt and Hocking, 1997). Other sources include wine grapes (Bau et al., 2005), peanuts, hazelnuts, meat, soybeans, dried beans, peppercorns, chocolate and spices (see Pitt and Hocking, 1997). We isolated *E. nidulans* at low levels for Thai soybeans, sorghum and cassava (Pitt et al., 1994).

References. Raper and Fennell (1965), as *A. nidulans*; Christensen and Raper (1978); Domsch et al. (1980); Christensen and States (1982); Klich (2002).

8.2 Genus *Eurotium* Link: Fr.

Eurotium is a very well defined and very well-known genus of Ascomycetes, characterised by the formation of barely macroscopic yellow cleistothecia with smooth, cellular walls. The *Aspergillus* anamorphs are also characteristic: vesicles bear uncrowded phialides and the phialides produce dull green, spinose conidia in loosely radiate heads.

All species of *Eurotium* are xerophilic and often do not develop characteristic fruiting structures on high water activity media such as CYA and MEA. Incorporation of 20% sucrose into Czapek agar greatly assisted identification of *Eurotium* species (Thom and Raper, 1941). This medium, of about 0.98 a_w, produces suboptimal growth of all *Eurotium* species but permits development of both anamorphs and teleomorphs plus mycelial colours which greatly aid species identification. Media of lower a_w, such as G25N, usually produce rampant growth which is of less value in determinative taxonomy. For compatibility with CYA, we introduced Czapek yeast extract agar with 20% sucrose (CY20S) for *Eurotium* taxonomy (Pitt and Hocking, 1985). This medium has proved to be of considerable value in general *Aspergillus* taxonomy (Klich, 2002), but its use here is confined to *Eurotium* identification.

When an isolate has been identified as a *Eurotium* by using the standard media and incubation conditions, and growth data recorded as usual, the culture should be inoculated onto CY20S agar. After incubation for 7 days at 25°C, diameters and colours should be recorded and the cultures returned to the incubator. At intervals, wet mounts of cleistothecia from the central area of a colony should be made, stained with lactofuchsin and examined with the 40× objective. Identification can be completed when mature ascospores are produced, which will be within 14 days for the majority of isolates. Ascospores are mature when they are readily liberated from asci and do not take up stain immediately. Conidia are usually present in wet mounts also and may be distinguished from ascospores by their uniformly spinose walls and the fact that lactofuchsin stains them quite readily. Unstained conidia are green under the microscope while mature ascospores are faintly yellow and are more refractile. Ascospore dimensions should be measured under the 100× objective and

ornamentation noted, i.e. smooth or rough walls and presence or absence of a longitudinal furrow, ridges or flanges.

Nomenclaturally correct names were introduced for all common *Eurotium* anamorphs by Samson and Gams (1985), but they are rarely used. In general, use of the *Eurotium* names for these species is more logical, as the teleomorphs are often essential for identification and are normally present in Petri dish cultures on CY20S. As described below, differences exist among the anamorphs of *Eurotium* species, but these are not usually considered in species identification procedures.

About 20 *Eurotium* species are known (Raper and Fennell, 1965; Blaser, 1975). Descriptions and synonymy for seven species are given by Pitt (1985). Four of those are exceedingly common in all kinds of environments where just sufficient moisture exists to support fungal growth. The three others are encountered less frequently, and the remainder are curiosities known only from three isolates or less.

The four common species, *E. amstelodami*, *E. chevalieri*, *E. repens* and *E. rubrum*, are treated here together with *E. herbariorum*, which resembles *E. rubrum*, and *E. cristatum*, for which *E. amstelodami* is sometimes mistaken.

Key to *Eurotium* species included here

1	No growth on CYA, MEA or CY20S; on MY50G cleistothecia white	*E. halophilicum* (see *E. rubrum*)
	Growth on CY20S; usually growth on CYA and MEA	2
2 (1)	Ascospores with conspicuous ridges or flanges	3
	Ascospores without conspicuous ridges or flanges	5
3 (2)	Colonies coloured only yellow, from cleistothecia, and green, from conidial heads; ascospores with two prominent, irregular, broad, longitudinal ridges	4
	Colonies with conspicuous yellow to orange sterile hyphae; ascospores like pulley wheels, with two prominent, narrow, longitudinal flanges	*E. chevalieri*
4 (3)	Ascospores with rough walls, not exceeding 5 μm long overall	*E. amstelodami*
	Ascospores with walls smooth or very finely spiky, up to 6 μm long overall	*E. cristatum* (see *E. amstelodami*)
5 (2)	Colonies with yellow or orange sterile hyphae; ascospores smooth walled and with just a trace of a longitudinal furrow	*E. repens*
	Colonies with orange to reddish hyphae, in age becoming red brown; ascospores with a fine but distinct longitudinal furrow flanked by two low, minutely roughened ridges	6
6 (5)	Ascospores up to 6 μm long, asci maturing within 14 days	*E. rubrum*
	Ascospores frequently exceeding 6 μm long, ascospores usually not maturing within 14 days	*E. herbariorum* (see *E. rubrum*)

Eurotium amstelodami L. Mangin **Fig. 8.3**

Aspergillus amstelodami (L. Mangin) Thom & Church (invalid name, includes teleomorph)

Anamorph: *Aspergillus vitis* Novobr.

Colonies on CYA 14–18 mm diam and on MEA 16–25 mm diam, low and dense, plane; mycelium inconspicuous, white or yellow; abortive yellow cleistothecia conspicuous centrally in patches or sectors, surrounded by well formed bright to dark green conidial heads; reverse pale or occasionally dark green. Colonies on G25N 25–35 mm diam, plane, deep and floccose, with appearance usually uniformly dull green from layers of conidial heads; mycelium inconspicuous, white; yellow cleistothecia sometimes visible; conidial heads abundant, dull green; reverse yellow under cleistothecia, or uncoloured. No growth on CYA at 5°C. On CYA at 37°C, colonies up to 15 mm diam sometimes formed.

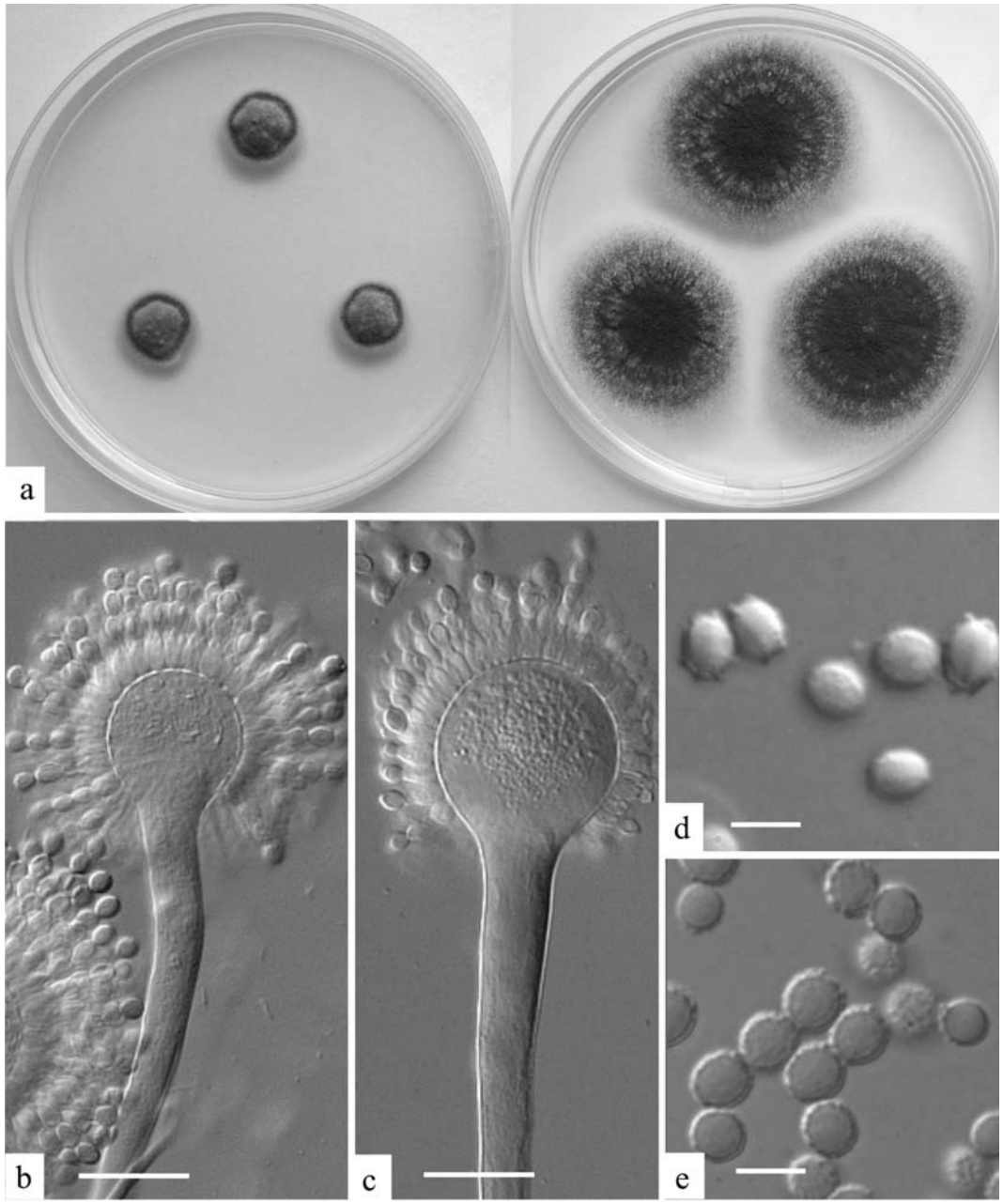


Fig. 8.3 *Eurotium amstelodami* **(a)** colonies on CYA and CY20S, 7 days, 25°C; **(b, c)** heads, bars = 20 μm; **(d)** ascospores; and **(e)** conidia, bars = 5 μm

Colonies on CY20S usually 30–45 mm diam, plane, low and velutinous, usually with a layer of yellow cleistothecia near the agar surface surmounted by a dense layer of radiate dull green conidial heads; reverse uncoloured, i.e. yellow beneath cleistothecia and pale grey green under conidial areas.

Cleistothecia bright yellow, mostly spherical, 75–150 μm diam, usually without surrounding sterile hyphae; maturing in 9–12 days on CY20S and 12–14 days on G25N; ascospores yellow, ellipsoidal, 4.5–5.0 μm long, with rough walls and with two conspicuous, often sinuous, longitudinal ridges of

low, irregular height and spacing. Conidiophores borne from aerial hyphae, stipes relatively short, 250–400 μm long, vesicles spherical to spathulate, 18–30 μm diam on CY20S, 35–40 μm on G25N, fertile over the upper two thirds to three quarters, bearing phialides only; phialides 5–8 μm long; conidia spherical to subspheroidal, 4.0–5.0 μm diam, with densely spinulose walls, borne in radiate to loosely columnar heads.

Distinctive features. The principal diagnostic feature of *Eurotium amstelodami* is its conspicuous ascospore ornamentation: wide, irregular ridges or flanges and rough walls. Mycelium remains inconspicuous; colony colours consist only of yellow from the cleistothecia and dull green from the conidia, in both obverse and reverse.

Taxonomy. Pitt (1985) pointed out that Mangin's original concept of this species differed from later usage (Thom and Raper, 1941; Raper and Fennell, 1965), including the neotypification by Blaser (1975). Acceptance of the NCU list for the family Trichocomaceae (Pitt and Samson, 1993) has legitimised the modern concept. The correct anamorph name for this species, *Aspergillus vitis*, is seldom used (Pitt and Samson, 1993).

Physiology. The optimal temperature for growth of *Eurotium amstelodami* is 33–35°C (Domsch et al., 1980), with a maximum at 43–46°C (Blaser, 1975). It was reported to grow down to 0.70 a_w at 25°C by Armolik and Dickson (1956). Spore germination was noted after 120 days, but not below 0.75 a_w within 100 days by Wheeler and Hocking (1988). Scott (1957) showed that the optimal a_w for growth was near 0.96 regardless of controlling solute, but the maximum growth rate in sucrose media was twice as fast as in glycerol. Faster growth was observed on media controlled with NaCl than on glycerol based media, and maximum growth rates occurred at 0.90–0.93 a_w (Avari and Allsopp, 1983).

In a medium of pH 3.8 and 0.98 a_w, 80–85% of ascospores of *Eurotium amstelodami* survived heating at 60°C for 10 min; 1–3% survived a similar treatment at 70°C; and 0.2% at 75°C. Only 0.3% of conidia of *Aspergillus vitis* survived heating for 10 min at 60°C (Pitt and Christian, 1970). Tolerance of sorbate was developed after 21 transfers through increasing concentrations, rising to 0.07% at pH 5.5 (Viñas et al., 1990).

In an atmosphere of 1% O_2, *Eurotium amstelodami* grew at 50% of the rate in air (Hocking, 1990). This species has been reported as one source of chloroanisoles which may cause off odours in food carried in shipping containers (Hill et al., 1995) and of isoprene, a cause of off odour in a bakery product (Berenguer et al., 1991). Ketonic rancidity in coconut has also been caused by *E. amstelodami* (Kinderlerer, 1984a; Kinderlerer and Kellard, 1984). It showed the highest proteolytic activity on meat of any *Eurotium* species (Binzel, 1980) and was relatively resistant to propionic acid (Müller et al., 1981), but lipase activity was low (Magan et al., 1993). Marín et al. (2003) reported *N*-acetyl-β-D-glucosaminidase activity at 0.90 a_w at pH 6 and at 0.85 a_w at pH 7.5. Conidia of *E. amstelodami* were found to survive in 30% (w/v) NaCl solution over a period of 14 weeks (Butinar et al., 2005b).

Mycotoxins. Isolates of *Eurotium amstelodami* from hams were toxic to chick embryos and when injected into mice (Resurreccion and Koehler, 1977). However, the possible toxins involved have not been identified (Frisvad and Samson, 1991), and it is generally assumed that this is a benign fungus, like most *Eurotium* species.

Ecology. In Australia, *E. amstelodami* is less common than the other *Eurotium* species described here; however, literature reports indicate that elsewhere it is a ubiquitous foodborne species, usually associated with stored goods. This species has been reported to spoil rapeseed due to heat damage, Japanese traditional noodles and cheese (see Pitt and Hocking, 1997). *E. amstelodami* has been reported as one cause of gushing in beer (Gyllang and Martinson, 1976). As with other *Eurotium* species, cereals are a major substrate, including wheat, flour, refrigerated dough, bread and bakery products, rice and rice bran, barley, maize, cereal flakes and corn snacks (see Pitt and Hocking, 1997). Other sources include grapes and raisins (Valero et al., 2007a), mixed feeds, cereals and legume feeds (Accensi et al., 2004), pasta (Halt et al., 2004), peanuts, hazelnuts and walnuts, pistachios, soybeans, sunflower seeds, meat products, smoked bacon, biltong, dried salt fish and jam (see Pitt and Hocking, 1997).

Eurotium amstelodami was less common in Southeast Asian commodities than *E. chevalieri* or *E. rubrum*. Nevertheless, we isolated it at 1% or more of all particles examined in maize, peanuts, soybeans,

cashews, copra, paddy rice, mung beans, sorghum and peppercorns. Percentages of samples infected were often relatively low, usually 8–15%, but rates of infection in infected samples were often high, up to 40–60%. This indicates some samples becoming infected during storage (Pitt et al., 1993, 1994, 1998a).

Additional species. *Eurotium cristatum* (Raper and Fennell) Malloch and Cain, anamorph *Aspergillus cristatellus* Kozak., synonym *E. chevalieri* var. *intermedius* Thom and Raper (invalid name): in culture this species resembles *E. amstelodami* and is often mistaken for it. However, ascospores are larger, up to 6 μm long including crests, and have walls ornamented with discrete, very fine spikes. Unlike *E. amstelodami,* colonies have bright yellow hyphae surrounding the cleistothecia. No physiological studies are known, and mycotoxin production is also unknown. We have isolated this species uncommonly, from heated strawberry puree and Philippine peanuts.

References. *Eurotium amstelodami*: Raper and Fennell (1965), as *Aspergillus amstelodami*; Blaser (1975); Domsch et al. (1980); Pitt (1985); Klich (2002). *E. cristatum*: Blaser (1975); Pitt (1985).

Eurotium chevalieri L. Mangin Fig. 8.4

Anamorph: *Aspergillus chevalieri* L. Mangin

Colonies on CYA 16–25 mm diam, low and dense, plane or lightly sulcate; mycelium bright yellow, often darker centrally, enveloping abundant abortive yellow cleistothecia and overlaid by sparse to abundant greyish green conidial heads; yellow brown soluble pigment sometimes produced; reverse pale to orange or deep brown. Colonies on MEA 16–25 mm diam, plane, dense to floccose; mycelium white to yellow, overall colours and characteristics as on CYA; yellow soluble pigment sometimes produced; reverse pale, olive, orange or brown. Colonies on G25N 20–30 mm diam, usually very deep and floccose, with pale to bright yellow mycelium enveloping abundant developing cleistothecia and overlaid by sparse to abundant greyish green conidial heads, occasionally development more sparse and producing few cleistothecia; reverse usually pale, sometimes yellow or dull green. No growth on CYA at 5°C. Usually no growth on CYA at 37°C, occasionally colonies up to 10 mm diam formed.

Colonies on CY20S 45–65 mm diam, plane, low and dense; colony character varying with production of conidial heads, in isolates with sparse heads, mycelium white at the margins, then bright to deep yellow or orange centrally, enveloping abundant cleistothecia, in isolates with heavy conidial production these elements more or less obscured and colony appearance dominated by conidia, dull green; reverse pale, yellow, orange brown or dull green.

Cleistothecia bright yellow, spherical, 100–140 μm diam, enveloped in yellow to orange vegetative hyphae, maturing at colony centres in 8–10 days on CY20S and 12–14 days on G25N; ascospores yellow, ellipsoidal, shaped like pulley wheels, 4.5–5.0 μm long, smooth walled, with two prominent, parallel, narrow, sometimes sinuous, longitudinal flanges. Conidiophores borne from aerial hyphae, stipes mostly 400–700 μm long, thin walled, already collapsing in 7 days, broadening to vesicles 25–35 μm diam, fertile over the whole area, bearing phialides only; phialides ampulliform, 5–8 μm long; conidia on CY20S and G25N ellipsoidal or doliiform (barrel shaped), 4.0–5.5 μm long, with spinose walls.

Distinctive features. As with other *Eurotium* species, the ascospore is the prime distinguishing feature: in *E. chevalieri* these are characteristically shaped like minute pulley wheels. Colonies grow quite well on CYA and MEA, but sporulate poorly; colonies on CY20S produce conspicuous yellow to orange hyphae, while on CYA and MEA they can be brown.

Taxonomy. This taxon was originally described as a holomorph species, so the name "*chevalieri*" is valid for both the *Eurotium* and the *Aspergillus* morphs (Pitt and Samson, 1993).

Physiology. The optimum temperature for growth of *Eurotium chevalieri* is 30–35°C (Domsch et al., 1980), with a maximum at 40–43°C (Blaser, 1975). Ayerst (1969) obtained growth down to 0.71 a_w at 33°C; Pitt and Christian (1968) reported a minimum of 0.74 a_w at 25°C on a medium of pH 3.8. Germination of ascospores was little affected by glucose or glycerol as solute, but was slower in NaCl. Media of pH 4 or 6.5 did not affect germination. Maximum growth rates were much higher on glucose/fructose or NaCl media than in the presence of glycerol; again pH had little effect. The optimum a_w for growth was 0.94–0.95 (Pitt and Hocking, 1977). *E. chevalieri* was able to grow in the presence of 2,000 ppm potassium sorbate, following a 20 day

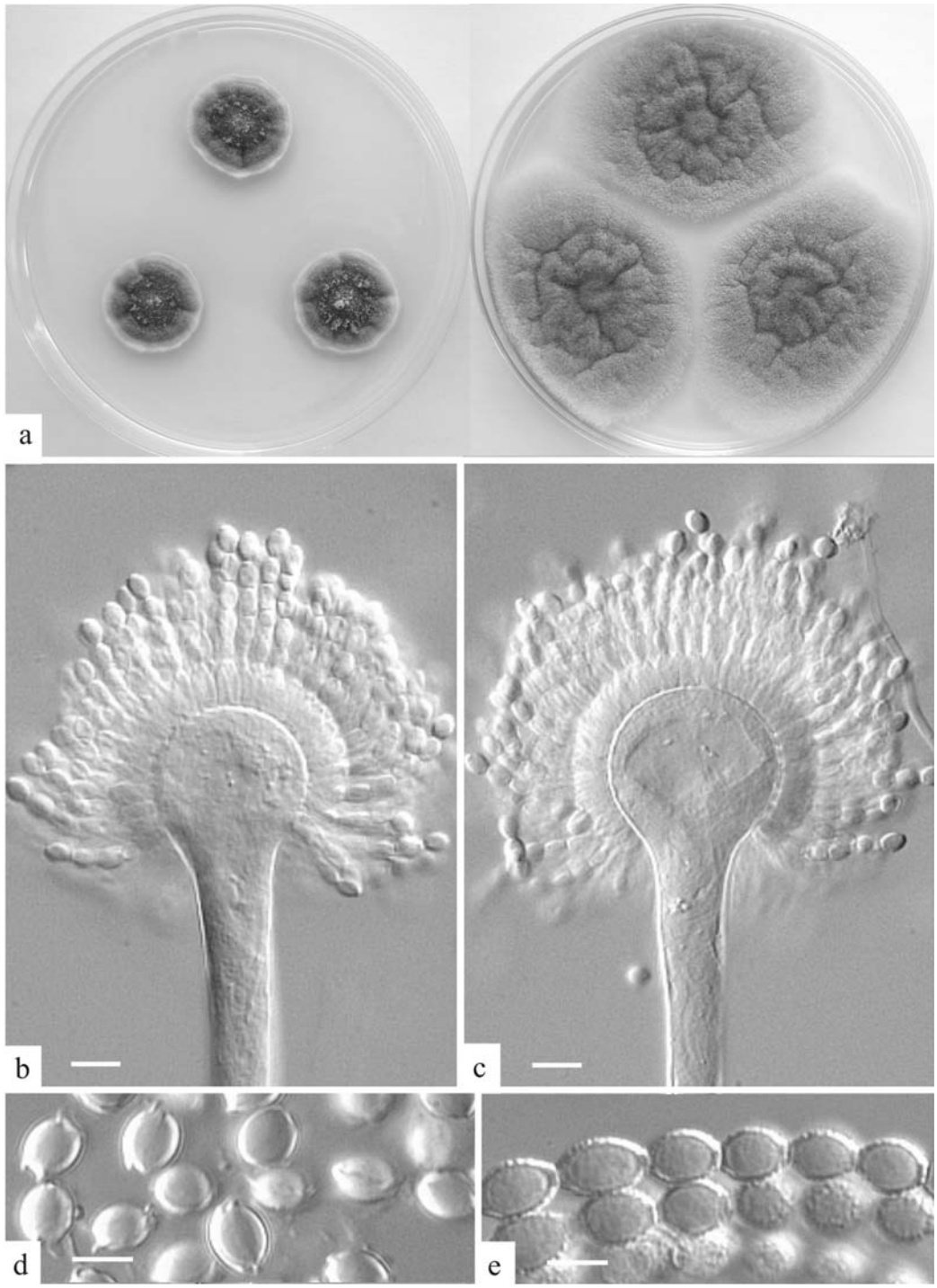


Fig. 8.4 *Eurotium chevalieri* **(a)** colonies on CYA and CY20S, 7 days, 25°C; **(b, c)** heads, bars = 10 μm; **(d)** ascospores; and **(e)** conidia, bars = 5 μm

lag period, at 0.85 a_w and pH 6.0. No growth was recorded at lower pH and water activity values (e.g. 0.80 a_w and pH 5.5) in the presence of preservative (Char et al., 2006).

Eurotium chevalieri was the most heat resistant xerophilic fungus studied by Pitt and Christian (1970): 18–25% of ascospores survived heating at 70°C for 10 min in a medium of 0.98 a_w and pH 3.8, and up to 0.5% a similar treatment at 80°C. The decimal reduction line had an F_{80} of 3.3 min with a high *z* value, 12.8C°, under these conditions. However, conidia of *Aspergillus equitis* were not especially heat resistant: only 0.1% survived heating for 10 min at 60°C.

This species has been reported as one source of isoprene, a cause of off-odour in a bakery product (Berenguer et al., 1991). It is also a cause of ketonic rancidity in coconut (Kinderlerer, 1984a; Kinderlerer and Kellard, 1984). High proteolytic activity on meat (Binzel, 1980) and moderate lipolytic activity on vegetable oils (Kuku, 1980) have been reported.

Mycotoxins. *Eurotium chevalieri* has been reported to produce toxic compounds, identified as echinulin and neoechinulin, which cause feed refusal in swine (Vesonder et al., 1988). However, other tests for toxicity of *E. chevalieri* have been negative (Frisvad and Samson, 1991; Adebajo and Oyesiku, 1994).

Ecology. *Eurotium chevalieri* must rank as one of the most common spoilage fungi on earth, especially in warmer regions. It has been reported to cause spoilage of high moisture prunes, pecans, Japanese traditional noodles, a semisoft baked cookie and, in our laboratory, cheese spread, chick peas and faba beans (see Pitt and Hocking, 1997). Like the other common species in this genus, it has been isolated from a great variety of foods, especially cereals, including wheat and flour, rice, rice flour and rice bran, maize and cereal flakes. It is also common in processed and dried meats and nuts: peanuts, pecan nuts, hazelnuts and walnuts and coconut. Other sources include filled chocolates, dried beans and peas, soybeans, sunflower seeds, cocoa beans, spices, dried salt and cured fish (see Pitt and Hocking, 1997). It has recently been isolated from evaporated milk products in Argentina (Char et al., 2005) and black pepper in Brazil (Gatti et al., 2003). Many other habitats no doubt exist.

This species was commonly isolated in our study of Southeast Asian commodities, particularly from peanuts and maize from Thailand, Indonesia and the Philippines. *E. chevalieri* infections in other nuts and oilseeds were also heavy (Pitt et al. 1993, 1994, 1998a). Infections in beans were usually much lower, except for soybean samples from the Philippines (Pitt et al., 1993, 1994, 1998a). Rice was less affected: no more than 20% of samples were infected, and overall infection rates in milled rice were only 2–4% (Pitt et al., 1993, 1994, 1998a).

References. Raper and Fennell (1965), as *Aspergillus chevalieri*; Blaser (1975); Domsch et al. (1980); Pitt (1985); Klich (2002).

Eurotium repens de Bary **Fig. 8.5**

Aspergillus repens (Corda) de Bary
Eurotium pseudoglaucum (Blochwitz) Malloch & Cain
Aspergillus pseudoglaucus Blochwitz

Anamorph: *Aspergillus reptans* Samson & W. Gams

Colonies on CYA and MEA 10–20 mm diam, plane, deep and dense, mycelium white, yellow or orange, either enmeshing abortive yellow cleistothecia or surmounted by dull green to dull blue conidial heads, or both, depending on isolate; reverse pale, dull yellow, green or orange, less commonly bright yellow or orange. Colonies on G25N usually 25–45 mm diam, plane, deep and floccose, sometimes reaching the Petri dish lid; mycelium white to bright yellow or orange, usually enmeshing many layers of developing bright yellow cleistothecia and overlaid by sparse dull green conidial heads, in uncommon isolates cleistothecia and yellow hyphae inconspicuous and dull green conidial heads dominant; reverse pale, brilliant yellow to orange, or orange brown. No growth on CYA at 5 or 37°C.

Colonies on CY20S 45–65 mm diam, plane, low or somewhat floccose, broader but much less luxuriant than on G25N; mycelium white to yellow or orange, overall colour varying from yellow with scattered dull green areas in predominantly cleistothecial isolates to dull green or bluish green in those with conidial heads dominant; reverse dull green or bright yellow to orange or both, at maturity dull yellow brown.

Cleistothecia on CY20S or G25N borne from and enveloped in sterile yellow to orange hyphae, bright yellow, spherical, 75–100(–125) μm diam, maturing at colony centres in 7–10 days; ascospores yellow, ellipsoidal, 5.0–5.5 μm long, without ridges

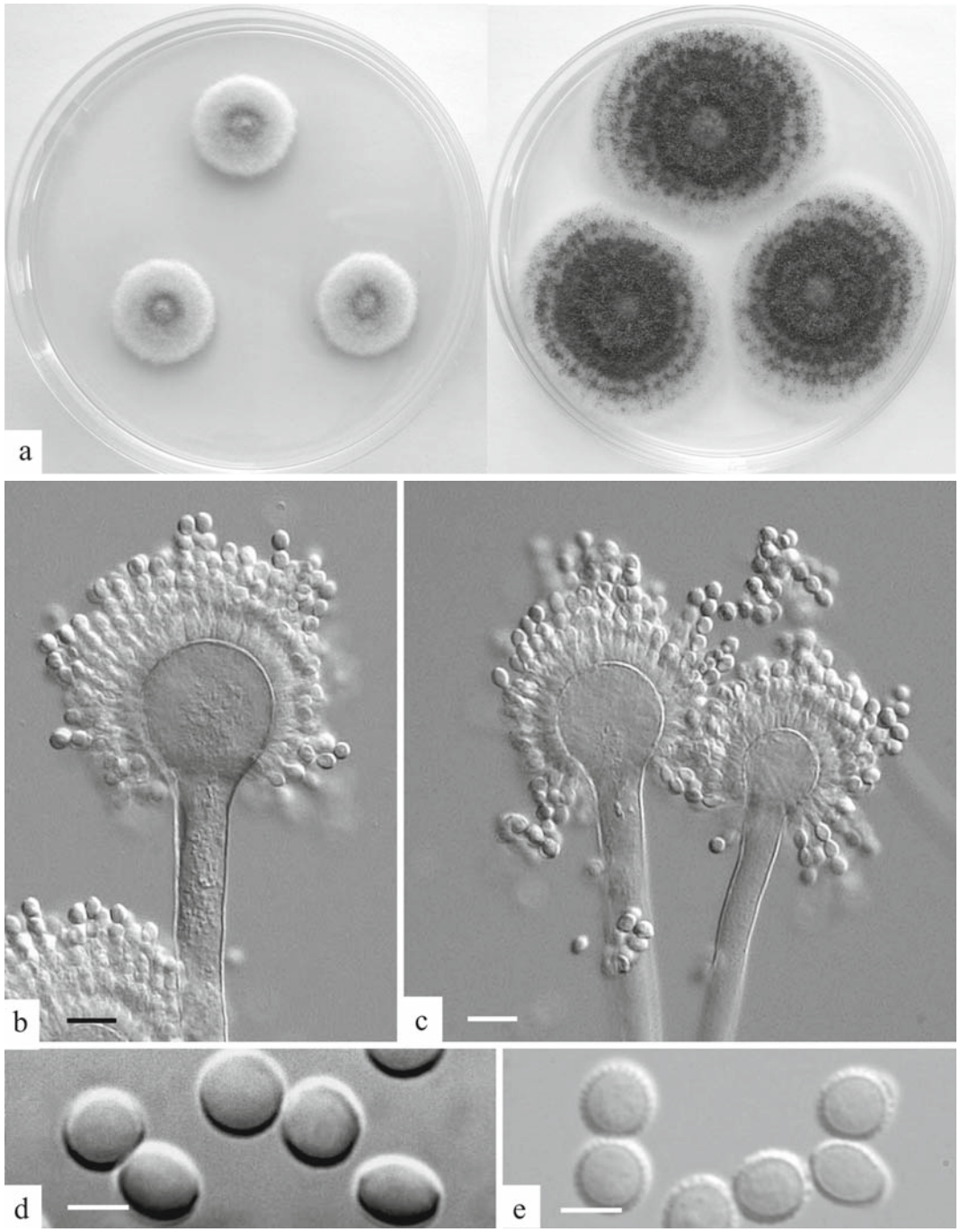


Fig. 8.5 *Eurotium repens* **(a)** colonies on CYA and CY20S, 7 days, 25°C; **(b, c)** heads, bars = 10 μm; **(d)** ascospores; and **(e)** conidia, bars = 5 μm

or flanges and with no more than a trace of a longitudinal furrow, smooth walled, borne in loosely radiate heads. Conidiophores borne from aerial hyphae, stipes mostly 500–1,000 μm long, broadening to vesicles 15–30 μm diam, fertile over the whole area, bearing phialides only; phialides ampulliform, 7–10 μm long; conidia on CY20S spherical to subspheroidal, 5–7 μm diam, on

G25N ellipsoidal to pyriform, 7–10 μm long, with densely spinulose to spinose walls.

Distinctive features. The prime distinction of *Eurotium repens* from other *Eurotium* species is the production of ascospores without ridges or flanges, usually without a longitudinal furrow, and smooth walls. Hyphal and reverse colours on CYA and MEA are yellow to orange, never red.

Taxonomy. Samson and Gams (1985) provided a correct anamorph name for this species, *Aspergillus reptans*, but it is rarely used.

Physiology. *Eurotium repens* grows between 4–5°C and 38–40°C, with an optimum at 25–27°C (Panasenko, 1967; Gonzalez et al., 1988). The minimum a_w for germination of *E. repens* was reported as 0.72 on media of neutral pH, at temperatures of 20–25°C (Snow, 1949; Armolik and Dickson, 1956; Magan and Lacey, 1984a). Andrews and Pitt (1987) reported that the minimum a_w for germination in NaCl and glycerol media were 0.83 and 0.72 a_w, with optima at 0.95 and 0.91 a_w, respectively. In media controlled by glucose/fructose, the minimum a_w for germination was much lower, 0.69 a_w (Andrews and Pitt, 1987). The influence of pH from 4.0–6.5 was slight (Avari and Allsopp, 1983), with pH 4.5–5.5 being optimal (Gock et al., 2003).

Eurotium repens ascospores are tolerant of elevated temperatures and survived heating to 60 and 70°C for 10 min; however, they did not recover after heating at 75°C for 10 min (Pitt and Christian, 1970).

Eurotium repens has been reported as one source of chloroanisoles which may cause off-odours in food carried in shipping containers (Hill et al., 1995). It is one of a group of species producing odorous compounds including 3-methyl-1-butanol, 1-octen-3-ol and 3-octanone in grain, useful in quality monitoring systems (Sinha et al., 1985).

Mycotoxins. Mycotoxins are not known to be produced by this species (Frisvad and Samson, 1991).

Ecology. *Eurotium repens* is a very common species. It has been reported to cause spoilage of cheese, cheesecake, pecans, maize and traditional Japanese noodles. It is very common in stored grains, for example, wheat, barley, maize and rice. *E. repens* is of common occurrence on processed and dried meat or fish, for example salami, meat products, ripening and ripened hams, biltong, dried salted fish and katsuobushi. Other sources include nuts, sunflower seed and red pepper powder (see Pitt and Hocking, 1997).

In our laboratory, *Eurotium repens* has been isolated from spoiled prunes, salami, strawberry puree, bread, cake, nuts, cheese and other products. It was relatively uncommon in Southeast Asian commodities by comparison with *E. chevalieri* or *E. amstelodami*, exceeding 1% infection overall only in copra from Thailand (3%) and kemiri nuts from Indonesia (2%) (Pitt et al., 1993, 1994, 1998a).

Eurotium repens has found use in food manufacture, as a starter culture in the manufacture of katsuobushi from bonito (Dimici and Wada, 1994) and of fish sauce from fish meal (Hayakawa et al., 1993).

References. Raper and Fennell (1965) as *Aspergillus repens*; Blaser (1975); Domsch et al. (1980); Pitt (1985); Klich (2002).

Eurotium rubrum Jos. König et al. Fig. 8.6

Aspergillus sejunctus Bainier & Sartory (invalid name, includes teleomorph)
Aspergillus ruber (Jos. König et al.) Thom & Church (invalid name, includes teleomorph)

Anamorph: *Aspergillus rubrobrunneus* Samson & W. Gams

Colonies on CYA 10–20 mm diam, plane, deep, usually dense and velutinous, sometimes floccose; mycelium yellow to bright orange; conidiophores and developing cleistothecia usually present, but often poorly formed; reverse pale yellow to orange brown. Colonies on MEA usually 10–20 mm diam, sometimes only 5–8 mm, similar to those on CYA, but sometimes less deep and with more conspicuous orange hyphae. Colonies on G25N 30–45 mm diam, plane, often floccose and with hyphal strands sometimes reaching the Petri dish lid, consisting of cleistothecia in layers supported and surrounded by relatively sparse orange to dark orange hyphae; conidial heads usually rare, above or within the cleistothecial layer, dull green; reverse yellow, brown or reddish orange. No growth on CYA at 5 or 37°C.

Colonies on CY20S at 7 days 35–60 mm diam, plane or lightly sulcate, usually low, dense and velutinous; mycelium conspicuous, at the margins yellow, becoming orange or more reddish elsewhere, enveloping abundant yellow cleistothecia and surmounted by sparse to abundant dull green heads; reverse in colours similar to the mycelium, though rarely so bright, or deep yellow brown; in

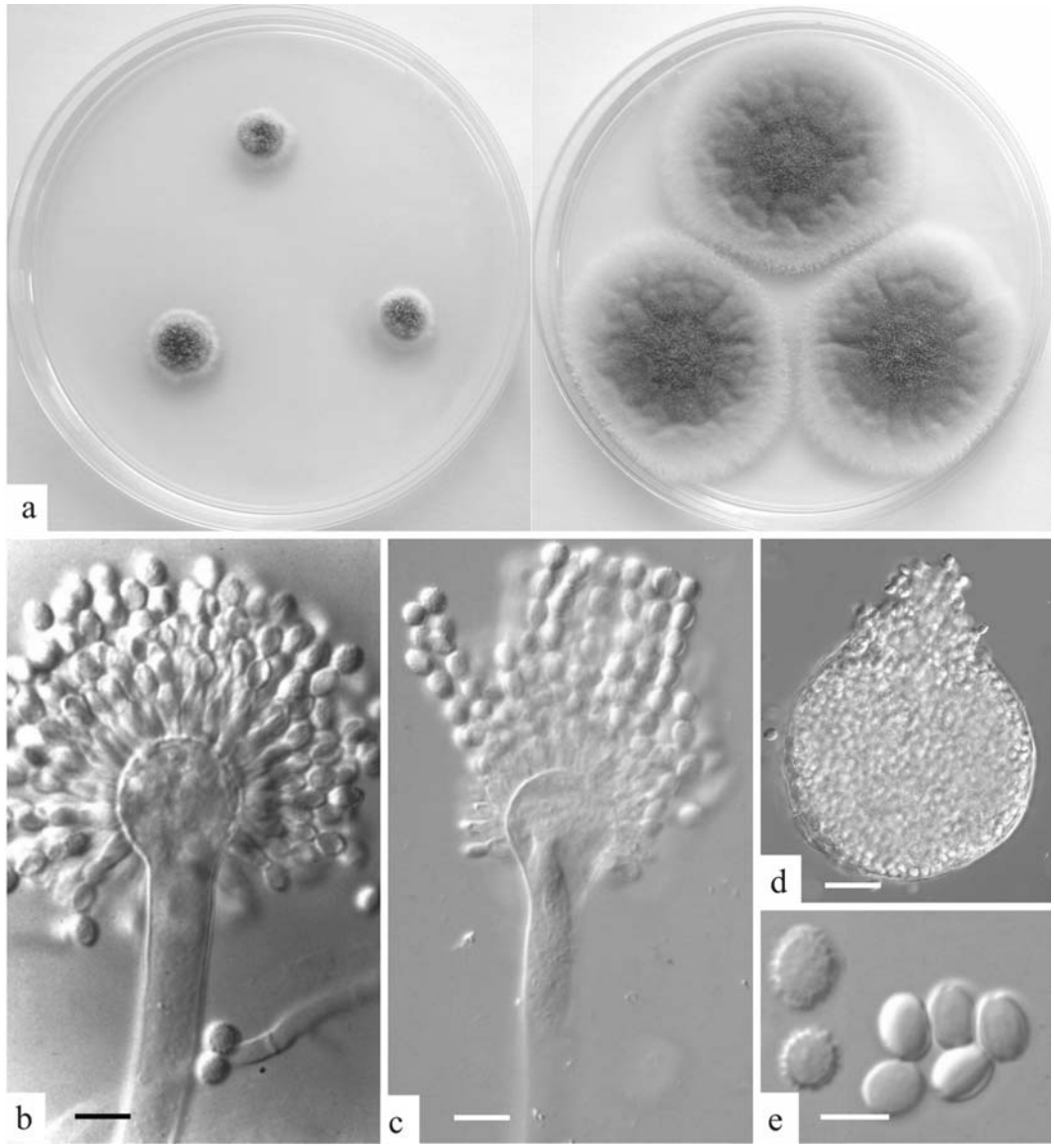


Fig. 8.6 *Eurotium rubrum* **(a)** colonies on CYA and CY20S, 7 days, 25°C; **(b, c)** heads, bars = 10 μm; **(d)** mature cleistothecium releasing ascospores, bar = 25 μm; and **(e)** conidia (spiny) and ascospores, bar = 5 μm

age, 10 days or more, developing bright to brick red brown hyphal colours over the whole plate or in patches, not under densely conidial areas or, rarely, remaining orange; reverse darkening over time to deep brown or almost black.

Cleistothecia on CY20S or G25N borne from and enveloped in sterile orange to red hyphae, spherical, yellow, 80–150 μm diam, maturing in 9–12 days; ascospores ellipsoidal, yellow, 5.0–6.0 μm long, with a shallow longitudinal furrow flanked by low, usually minutely roughened ridges, otherwise with smooth walls. Conidiophores borne from aerial hyphae, stipes mostly 300–700 μm long, terminating in spherical vesicles, 20–35 μm wide, fertile over the upper two-thirds, bearing phialides only; phialides 7–9 μm long; conidia subspheroidal to ellipsoidal, less commonly spherical or pyriform, generally 6–7 μm long, larger in occasional isolates, with spinose walls, borne in loosely radiate heads.

Distinctive features. Colonies of *Eurotium rubrum* on CY20S usually show areas of brilliant red or rusty colours after 10 days or more incubation.

Ascospores are 5.0–6.0 μm long, rarely otherwise, and show a definite longitudinal furrow and low, minutely roughened ridges.

Taxonomy. The commonly used anamorph name *Aspergillus ruber* and *A. sejunctus*, which predates it, are both invalid because they include the teleomorph. Samson and Gams (1985) provided a correct anamorph name, *A. rubrobrunneus,* but it is rarely used.

Physiology. Growth temperatures for *Eurotium rubrum* are probably similar to those reported for *E. repens*: minimum ca. 5°C, optimum 25–27°C and maximum near 40°C (Panasenko, 1967). The minimum a_w for germination has been reported as 0.70 a_w (Snow, 1949; Gock et al., 2003) and 0.72 a_w (Armolik and Dickson, 1956), at 25°C, or 0.72 at 20°C and 0.73 at 25°C (Wheeler et al., 1988b). On a medium of pH 3.8, germination occurred down to 0.75 a_w after 98 days at 25°C (Pitt and Christian, 1968). Maximum growth was achieved at 30°C at 0.92 a_w (Gock et al., 2003). *E. rubrum* was able to grow on media with up to 20% (w/v) NaCl (0.87 a_w) and germination occurred at 25% (w/v) NaCl (0.82 a_w), although mycelial growth did not follow (Butinar et al., 2005b). Growth rates were similar in glycerol at pH 4.0 and 6.5, and NaCl at pH 6.5, over a wide a_w range; growth in NaCl at pH 4.0 was much slower (Wheeler et al., 1988b). The optimum a_w for growth was ca. 0.94 and varied little over all conditions tested (Avari and Allsopp, 1983). At higher temperatures on glucose/fructose media of pH 6.5, the minimum a_w was 0.76 at 30°C and 0.79 at 37°C (Wheeler et al., 1988b). On NaCl-based media, the minimum a_w for germination was 0.86–0.88 a_w from 20–34°C; at 37°C it was 0.91 a_w, indicating temperature had little effect (Wheeler et al., 1988b). *E. rubrum* was a highly competitive species over a wide range of a_w and temperature on glucose based media, but not in NaCl based media (Wheeler and Hocking, 1993).

Eurotium rubrum was relatively tolerant of modified atmospheres, producing visible colonies in 65% CO_2 + 1% O_2 at 0.90 a_w in 15 days. At lower a_w values, growth was inhibited: at 0.82 a_w and 50% CO_2 + 1% O_2, visible growth occurred after 35 days, and at 0.78 a_w, growth occurred in 20% CO_2 + 1% O_2 after 25 days. Growth did not occur in higher concentrations of CO_2 at these a_w values (our unpublished data).

A high percentage (80–100%) of ascospores of *Eurotium rubrum* survived heating at 60°C for 10 min, at a_w 0.98 and pH 3.8; 0.5% or less survived 10 min at 70°C and there were no survivors after 10 min at 75°C. Conidia of *Aspergillus rubrobrunneus* were much less heat resistant: only 8% survived 10 min at 50°C, 3% 10 min at 60°C and none at 70°C under the same conditions (Pitt and Christian, 1970).

This species developed tolerance to sorbate after 21 transfers through increasing concentrations, rising to 0.07% at pH 5.5 (Viñas et al., 1990).

Mycotoxins. Several reports have been published which indicate that *Eurotium rubrum* produces a range of toxic compounds. However, well documented confirmation of toxicity is still lacking (Frisvad and Samson, 1991). Production of echinulin and flavoglaucin by *E. rubrum* was not correlated with pH or water activity. Further, metabolite production was enhanced on media containing 0.3% propionate (Suhr and Nielsen, 2004).

Ecology. A very widely distributed xerophilic fungus, *Eurotium rubrum*, appears to be somewhat more common in warmer regions. It has caused spoilage of dried and high moisture prunes, coconut and pancake syrup. It has been reported from a wide range of cereals (Lugauskas et al., 2006), especially wheat, maize and rice. It is also common on nuts, meat products and dried salt fish. Other reports have been from sunflower seeds, sesame seeds, cocoa beans, palm kernels, dried vegetables and jam (see Pitt and Hocking, 1997). It has frequently spoiled fruit cakes in Australia (our observations). *E. rubrum* has been isolated from salterns in the Adriatic (Butinar et al., 2005b).

Along with *Eurotium repens, E. rubrum* has found use in food manufacture, as a starter culture in the manufacture of katsuobushi from bonito (Dimici and Wada, 1994).

Eurotium rubrum was readily isolated from most food commodities we examined from Southeast Asia. It was particularly common in peanuts, kemiri nuts, cow peas and red kidney beans, mung beans, soybeans and maize kernels, with high levels in some soybeans. Levels in milled rice were low, however (Pitt et al., 1993, 1994, 1998a).

Additional species. *Eurotium herbariorum* Link [anamorph *Aspergillus glaucus* Link, synonym *A. manginii* Raper and Thom (invalid name)] resembles *E. rubrum* in many features. It is distinguished by the following: (i) colonies on CYA and MEA at 25°C do not exceed 10 mm diam in 7 days and are sometimes absent; (ii) colonies on CY20S and

G25N rarely exceed 35 mm diam in 7 days; (iii) cleistothecia on CY20S and G25N develop slowly, with ascospores usually taking more than 14 days to mature; and (iv) while ascospores are similar in appearance to those of *E. rubrum*, they are larger, commonly 6–7(–8) μm long.

Taxonomic confusion exists because some authors have synonymised *Eurotium rubrum* with *E. herbariorum* or *E. repens* with *E. herbariorum*. In our opinion these three species are distinct, although closely related.

Eurotium herbariorum is a vigorous xerophile. When grown on media of pH 3.8 containing glucose/fructose as the controlling solute, ascospores of *E. herbariorum* germinated at 0.74 a_w after 19 days, the shortest lag time of any of the common species. Conidia of *Aspergillus glaucus* germinated at 0.75 a_w in 14 days (Pitt and Christian, 1968). When heated in 5°Brix grape juice, *E. herbariorum* ascospores showed a D_{70} of 2.5 min and a *z* value of 9.1C°; in 65°Brix concentrate, the D_{70} was 5.2 min and *z* value 7.1C° (Splittstoesser et al., 1989).

Eurotium herbariorum is able to grow in low oxygen concentrations, producing visible colonies at 25°C in 0.5% O_2 at 0.82 a_w within 60 days and at 0.78 a_w within 75 days. It also tolerated elevated CO_2 concentrations at reduced a_w: at 0.82 a_w producing visible colonies in 20 days in 35% CO_2 + 1% O_2 and 34 days in 50% CO_2 + 1% O_2. At 0.78 a_w, visible growth occurred in 35% CO_2 + 1% O_2 only after 95 days, and no growth occurred in 50% CO_2 + 1% O_2 (our unpublished data).

Although a far less common species than *Eurotium rubrum*, *E. herbariorum* is nevertheless widespread. Spoilage by *E. herbariorum* has occurred in French and Australian prunes and European cheese (see Pitt and Hocking, 1997). It has also been recorded from maize, meat products, rice, sardines and miso and spices (see Pitt and Hocking, 1997). We isolated it from samples of sorghum and peanuts from Indonesia and from one of velvet beans (30% infection). Low levels were present in coriander from Indonesia and soybeans and mung beans from Thailand (Pitt et al., 1993, 1994, 1998a).

Additional species. *Eurotium halophilicum* C.M. Chr. et al. is distinguished by the production of white cleistothecia and by inability to grow on CYA, MEA or CY20S, even after 14 days incubation. On MY50G, after 14 days, colonies are 15–20 mm diam, consisting almost entirely of cleistothecia surrounded by a weft of white mycelium (Hocking and Pitt, 1988). *Aspergillus* heads, characteristic of those from *Eurotium* species, are formed only on concentrated media, below 0.85 a_w, and bear pyriform, rough walled conidia 8–11 μm long. It was originally described as a halophile (Christensen et al., 1959), but in fact is an extreme xerophile, failing to germinate above 0.94 a_w, with growth occurring optimally between 0.85 and 0.80 a_w. The minimum recorded germination occurred at 0.68 a_w after only 38 days incubation at 25°C (Andrews and Pitt, 1987). Germination occurred on saturated NaCl based media (0.75 a_w) in a similar time.

This is undoubtedly a rare species, but is of interest because it will not be isolated on any medium designed for the recovery of *Eurotium* species. It is also very difficult to recognise because its cleistothecia are white and it produces its anamorph only at very low a_w. In our laboratory we isolated it from long-stored cardamom seeds on MY50G, the medium usually reserved for *Xeromyces bisporus* and xerophilic *Chrysosporium* species (Hocking and Pitt, 1988).

References. Raper and Fennell (1965), *Eurotium rubrum* under *Aspergillus ruber* and *E. herbariorum* under *A. manginii*; Pitt (1985), *E. rubrum* and *E. herbariorum*; *E. herbariorum*, Klich (2002); *E. halophilicum*, Hocking and Pitt (1988).

8.3 Genus *Neosartorya* Malloch and Cain

Neosartorya produces cleistothecia with cellular walls like *Eurotium*; however, the walls and ascospores are colourless or white, not yellow. Like those of *Eurotium*, *Neosartorya* anamorphs produce vesicles bearing phialides only, but vesicles are small and pyriform, enlarging towards the apices. Twelve species and varieties are currently accepted (Kirk et al., 2001), which mainly inhabit soil and decaying vegetation. All are thermoduric or thermophilic, none are xerophiles. The well known human pathogen *Aspergillus fumigatus* is closely related (Girardin et al., 1995; Geiser et al., 1998), and occasional pathogenicity to humans has been reported. *Neosartorya* isolates should be handled with care.

From the viewpoint of the food technologist, the main importance of *Neosartorya* species is the very high heat resistance of their ascospores. *Neosartorya fischeri*, described below, is the only *Neosartorya* species significant in foods.

***Neosatorya fischeri* (Wehmer) Malloch & Cain** **Fig. 8.7**

Aspergillus fischeri Wehmer (invalid name, includes teleomorph)

Anamorph: *Aspergillus fischerianus* Samson & W. Gams

Colonies on CYA 50–60 mm diam, occasionally larger, plane, sparse to moderately dense, surface texture floccose; mycelium white to pale yellow, enveloping abundant developing cleistothecia; conidial heads usually small and sparsely produced, above the layer of cleistothecia, grey green; reverse pale to

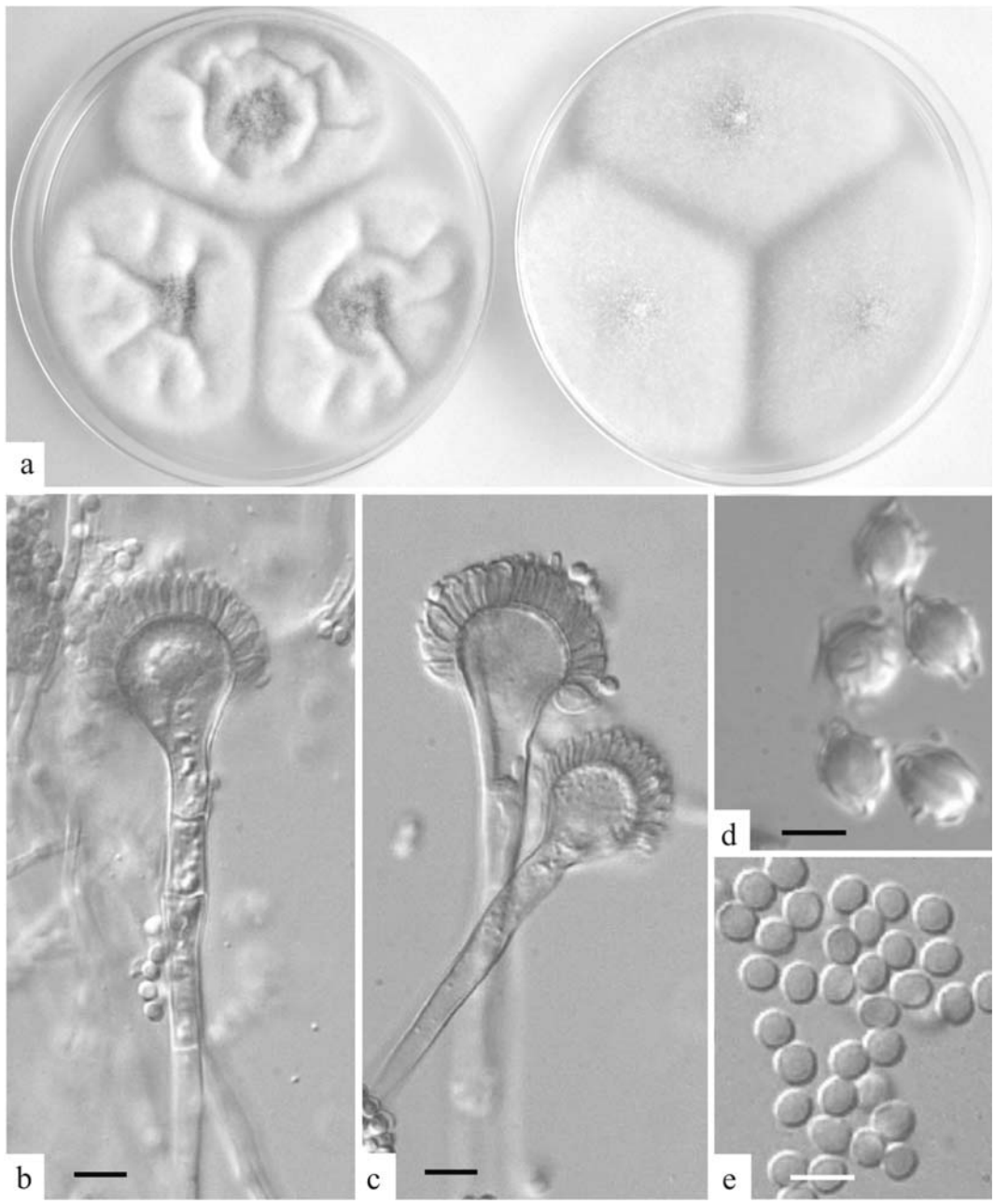


Fig. 8.7 *Neosartorya fischeri* **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b, c)** heads, bars = 10 μm; **(d)** ascospores; and **(e)** conidia, bars = 5 μm

pinkish brown, sometimes yellow centrally. Colonies on MEA 60 mm or more diam, usually covering the whole Petri dish, low and sparse; mycelium white to pale yellow, surrounding layers of developing white cleistothecia; conidial heads small and sparse, grey green; reverse pale, pale brown or dull yellow. Colonies on G25N 8–12 mm diam, of sparse to dense white mycelium; reverse pale to dull yellow. No growth at 5°C. At 37°C, colonies covering the whole Petri dish, plane or sulcate, of white or grey mycelium and abundant conspicuous white cleistothecia; reverse pale to pinkish brown.

Cleistothecia 150–400 μm diam, with a definite wall of flattened cells with hyphae attached, white to cream, maturing in 9–12 days at 25°C, slightly faster at 37°C; ascospores ellipsoidal, 7–8 μm long overall, including two prominent, sinuous longitudinal flanges, other irregular ridges sometimes present as well. Conidiophores borne from aerial mycelium, stipes usually 300–500 μm long, with thin, colourless, smooth walls, enlarging terminally to pyriform vesicles; vesicles 12–18 μm diam, fertile over the upper half, bearing phialides only; phialides 5–6 μm long, with short necks; conidia subspheroidal to ellipsoidal, 2.5–3.0 μm long, smooth walled, borne in radiate to columnar heads.

Distinctive features. Colonies of *Neosartorya fischeri* spread rapidly at both 25 and 37°C and are white; white cleistothecia and inconspicuous grey green *Aspergillus* heads are produced.

Taxonomy. Earlier literature discussed *Neosartorya fischeri* under its invalid anamorph name *Aspergillus fischeri*. However, the teleomorph is produced under most growth conditions and, from the food technologist's point of view, is the morph responsible for this species' most important property, high heat resistance. *N. fischeri* is thus the appropriate name for this species when it is found in foods. Samson and Gams (1985) provided a valid anamorph name, *A. fischerianus*.

Two varieties of *Neosartorya fischeri* were accepted by Raper and Fennell (1965), distinguished by differences in ascospore ornamentation. These varieties were raised to species status by Kozakiewicz (1989), as *N. glabra* (Fennell and Raper) Kozakiewicz and *N. spinosa* (Fennell and Raper) Kozakiewicz, again based on the differences in ascospore ornamentation. Other differences between these taxa appear to be very small. So, in agreement with Samson et al. (1990), we have not followed Kozakiewicz (1989) here, but maintained *N. fischeri* as a species with well defined varieties. It is worth noting that only *N. fischeri* var. *glabra* and *N. fischeri* var. *spinosa* normally are found in foods. That fact suggests ecological differences between these taxa, and if that is confirmed, may provide a basis for maintaining separation at species level. Molecular studies have shown a very close relationship between *N. fischeri* var. *glabra* and *N. fischeri* var. *spinosa* and between *N. fischeri* var. *fischeri* and *A. fumigatus* (Girardin et al., 1995).

Physiology. Ascospores of this species rank with those of *Byssochlamys* as the most heat resistant fungal spores known. Kavanagh et al. (1963) reported that ascospores of an isolate more recently identified as *Neosartorya fischeri* withstood boiling in distilled water for 60 min. They reported that spore age, pH and sugar concentration affected heat resistance. McEvoy and Stuart (1970) heated ascospores of *N. fischeri* in distilled water: they reported 100% survival after 20 min at 80°C and 0.002% survival after 5 min at 100°C. This degree of heat resistance is comparable with that of many bacterial spores and is higher than that of *Byssochlamys fulva* ascospores. More detailed studies of the heat resistance of *N. fischeri* ascospores indicate D_{88} values of 1.2–7.5 min (Quintavalla and Spotti, 1993) or of 4.2–16.2 min (Beuchat, 1986) or a D_{88} value of 1.4 min and a z value of 5.6C° (Scott and Bernard, 1987). A mathematical model for the combined effect of a_w, pH and redox potential on the heat resistance of *N. fischeri* has been published (Reichart and Mohacsi-Farkas, 1994). Salomão et al. (2007) reported that citric acid had a greater influence than malic acid on the thermal resistance of ascospores of *N. fisheri*. Ninety day old ascospores, isolated from tomatoes, were successfully killed by a heat treatment of 115°C for 30 s (Pacheco and De Massaguer, 2004).

High pressure treatments (600–900 MPa) for 20 min at 20°C in apricot nectar reduced counts of *Neosartorya fischeri* 100 fold. At 50°C, inactivation required less than 4 min at 800 MPa and at 60°C, 1–2 min at 700 MPa. Pressure resistance in distilled

water was lower (Maggi et al., 1994). Pressure resistance of ascospores increases with age: ascospores that were 3 weeks old were relatively sensitive to pressure treatment (600 MPa for 10 min), with pressure resistance increasing as ascospores matured, at least up to 15 weeks (the oldest ascospores tested). Ascospores of *N. fischeri* var. *spinosa* that were 9–15 weeks old were activated by a pressure treatment of 600 MPa for 10 min (Chapman et al., 2007).

Outbreaks of spoilage due to this species seem to be rare. In view of its exceptional heat resistance, we speculated that this may be due to an inability to grow at low oxygen tensions. However, growth has been reported in O_2 levels as low as 0.1% at 25°C (Nielsen et al., 1989).

Mycotoxins. The production of fumitremorgens A and C and verruculogen by this species has been confirmed (Beuchat et al., 1988; Nielsen et al., 1988; Frisvad and Samson, 1991). However, although these compounds are highly toxic, this is unlikely to have practical significance, as *Neosartorya fischeri* has only rarely been reported as growing in foods.

Ecology. *Neosartorya fischeri* ascospores have been isolated from canned strawberries, which had been processed at 100°C for 12 min (Kavanagh et al., 1963). Isolation of *N. fischeri* from pasteurised fruit juices and fruit powders has been reported several times, but only occasionally from spoiled product (see Pitt and Hocking, 1997).

On several occasions we have isolated *Neosartorya fischeri* from pasteurised fruit juices, strawberry purees and syrups, and a sports drink, but have only seen actual spoilage in canned strawberry puree. Heat treated fresh fruit frequently yield isolates of this species (Spotti et al., 1992, and our observations).

Neosartorya fischeri has been reported only rarely from foods which have not been heat treated or processed: from pistachios, soybeans and fresh mangoes (see Pitt and Hocking, 1997). Contamination from soil rather than fungal growth appears probable. *N. fischeri* has been reported from a case of pulmonary aspergillosis in a liver transplant patient (Gori et al., 1998).

References. Raper and Fennell (1965); Domsch et al. (1980); Hocking and Pitt (1984); Klich (2002).

8.4 Genus *Aspergillus* Fr.: Fr.

Aspergillus is among the best known and most frequently recognised fungal genera on earth. Species of *Aspergillus* are among the most economically important fungi, on the positive side being very widely used for synthesis of chemicals, for biosynthetic transformations and enzyme production. On the negative side, they are of great importance in food spoilage and they produce the aflatoxins, the most important of all mycotoxins.

Current taxonomies recognise about 185 *Aspergillus* species (Kirk et al., 2001). Perhaps 30 of these are well defined and usually readily distinguished. Peripheral to these central, common species are a large number of variations on each theme, often with the status of species, but which are seldom encountered and are little more than ephemeral variants. Placement of an unknown isolate in the central species is sufficient identification in all but the most detailed investigations.

Twenty four species are described below. Together with the 8 teleomorph species described earlier in this chapter, these 32 represent the vast majority of Aspergilli which will be isolated from foods. These species are all keyed out in the general key at the beginning of this chapter, and the *Aspergillus* species are described in alphabetical order.

Aspergillus aculeatus Iizuka **Fig. 8.8**

Aspergillus japonicus var. *aculeatus* (Iizuka) Al-Musallam

Colonies on CYA 65–70 mm diam or more, plane, velutinous, sometimes with a central floccose overlay; mycelium white; conidial heads red brown to black; pinkish sclerotia sometimes present; exudate and soluble pigment absent; reverse drab yellow to brown. Colonies on MEA 45–70 mm diam, plane, velutinous, mycelium white; conidial heads rather sparse, reddish brown or black; reverse uncoloured or yellowish. Colonies on G25N 15–20 mm diam, plane, sparse, velutinous, similar to those on CYA; reverse pale. No growth at 5°C. At 37°C, colonies 5–30 mm diam, consistently less than half of the diameter on CYA at 25°C.

Conidiophores borne from subsurface or surface hyphae, stipes 500–2,000 μm long, smooth walled, sometimes brown; vesicles 25–60 μm diam, bearing phialides only, covering three quarters of the vesicle

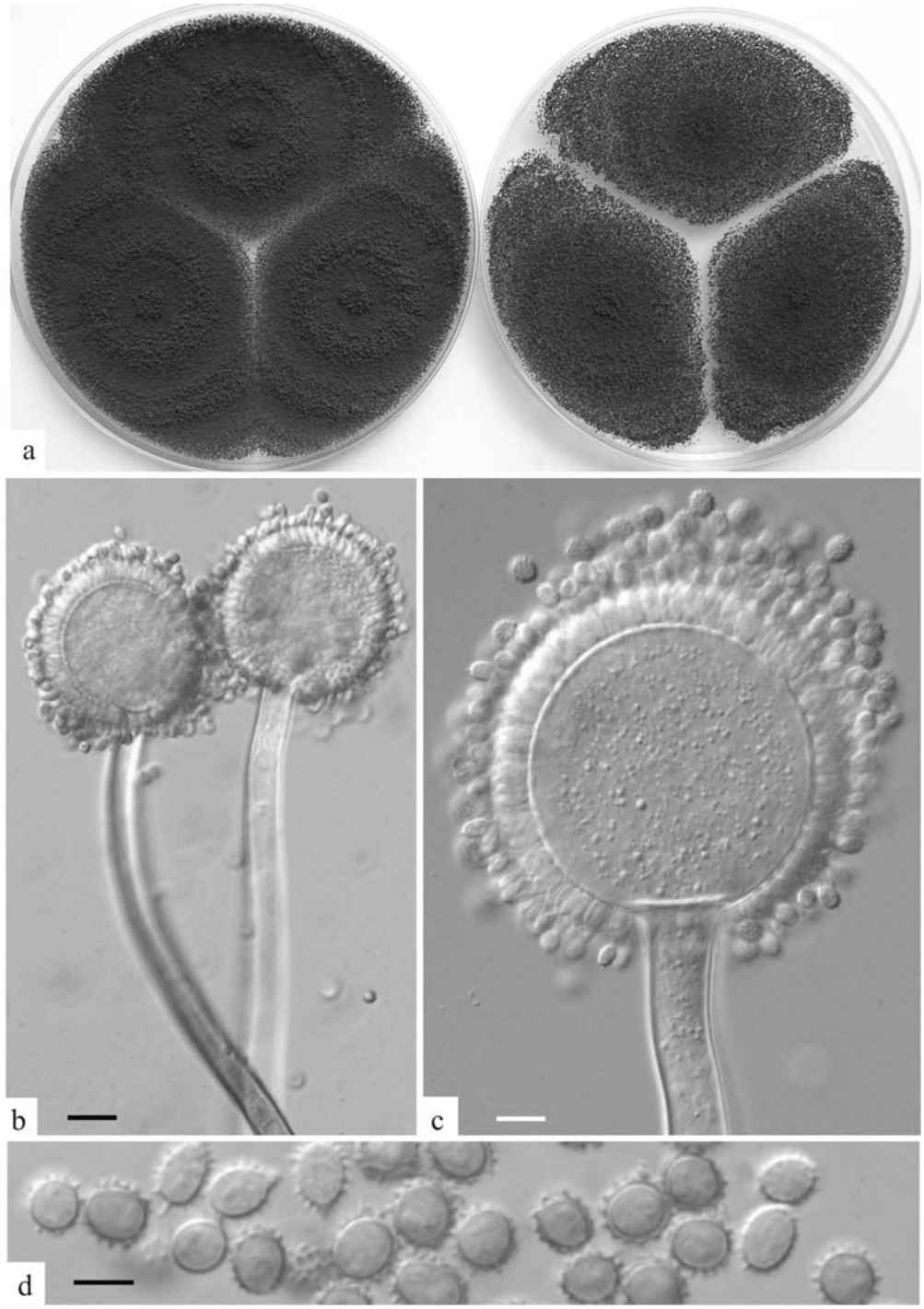


Fig. 8.8 *Aspergillus aculeatus* Iizuka: **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b)** heads, bar = 20 μm; **(c)** head showing the presence of phialides only, bar = 10 μm; **(d)** conidia, bar = 5 μm

surface or more, 5–9 μm long; conidia ellipsoidal, sometimes subspheroidal, 4–5 μm long, spinose, borne in radiate heads, in age splitting into columns.

Distinctive features. Red brown to black colonies indicate a close relationship with *Aspergillus niger*; however, vesicles of *A. aculeatus* bear phialides only, unlike those of *A. niger*. *A. japonicus* also produces phialides only, but *A. aculeatus* is distinguished from it by the production of larger vesicles and ellipsoidal conidia.

Taxonomy. *Aspergillus aculeatus* and *A. japonicus* are very closely related species, and *A. aculeatus* has sometimes been considered to be a variety of *A. japonicus* (Al-Musallam, 1980). These two species have morphological differences (Raper and Fennell, 1965) and differences in secondary metabolites and RFLP profiles that correlate with each other (Pařenicová et al., 2001). However, a study to correlate the morphological differences with these other parameters has not been carried out. ITS sequences of the two species are essentially identical (Pařenicová et al., 2001).

From morphological identifications based on the work of Raper and Fennell (1965), there is general agreement that *Aspergillus aculeatus* is the species commonly found in foods (Pitt and Hocking, 1997; Leong et al., 2004; Bau et al., 2005), so *A. aculeatus* is given priority here.

If the two species are ever formally combined, *Aspergillus japonicus* has priority as it was described much earlier.

Physiology. At 0.97 a_w *Aspergillus aculeatus* grew at 20, 30 and 40°C. At a lower water activity (0.92), it grew better at 30°C than at 20°C, and no growth was recorded at water activity values below 0.92 (0.87 or 0.82 a_w) (Valero et al., 2007a). It has a high pectinolytic and cellulolytic enzyme activity (Adisa, 1989), and a fructosyltransferase with thermal and pH stability has recently been characterised (Ghazi et al., 2007).

Mycotoxins. This species produces secalonic acid D (Frisvad et al., 2006a) and some other minor compounds (Frisvad and Samson, 1991). Secalonic acid D has significant animal toxicity (Ciegler et al., 1980), but a role in human or animal disease, especially from this species, has not been shown.

Ecology. *Aspergillus aculeatus* causes a postharvest dry rot of tomatoes (Fajola, 1979). It is commonly associated with grapes (Leong et al., 2004; Bau et al., 2005; Martínez-Culebras and Ramón, 2007; Valero et al., 2007a) and is one of the causes of bunch rot (Jarvis and Traquair, 1984). We isolated it in low numbers from Philippine and Indonesian peanuts and soybeans from the Philippines (Pitt et al., 1998a and unpublished).

Additional species. *Aspergillus japonicus* Saito closely resembles *A. aculeatus* (see Taxonomy). *A. japonicus* sometimes produces larger colonies on CYA at 37°C, up to 50 mm diam. Vesicles are smaller, usually less than 30 μm diam, and conidia are spherical. It does not produce secalonic acid D (Frisvad and Samson, 1991). The two species would be expected to have a similar physiology.

Aspergillus japonicus has been isolated from Nigerian maize (Aja-Nwachukwu and Emejuaiwe, 1994).

References. Raper and Fennell (1965); Al-Musallam (1980), both as *A. japonicus* var. *aculeatus* and *A. japonicus* var. *japonicus*; Klich (2002), *A. japonicus* only.

Aspergillus candidus Link Fig. 8.9

Colonies on CYA 15–20 mm diam, plane, low to moderately deep, dense, surface texture granular to floccose; mycelium white; conidial heads densely packed, persistently pure white to off-white; sometimes small amounts of clear exudate produced; reverse pale or yellow orange. Colonies on MEA 10–25 mm diam, similar to those on CYA except reverse dull brown. Colonies on G25N 10–16 mm diam, similar to those on CYA but conidial production often sparse and reverse pale or sometimes yellow. No growth at 5°C. At 37°C, colonies usually 20–25 mm diam, occasionally larger or absent, typically centrally umbonate and radially sulcate, velutinous or centrally floccose, with most characters similar to those on CYA at 25°C.

Conidiophores borne from surface or aerial hyphae, stipes usually 200–500(–1,000) μm long, with colourless, smooth to quite roughened walls; vesicles varying with isolate, 10–40 μm diam, bearing metulae and phialides over the entire surface, but numbers of metulae limited on the smallest heads; metulae variable, (5–)15–20(–30) μm long; phialides 5–9 μm long; conidia mostly spherical,

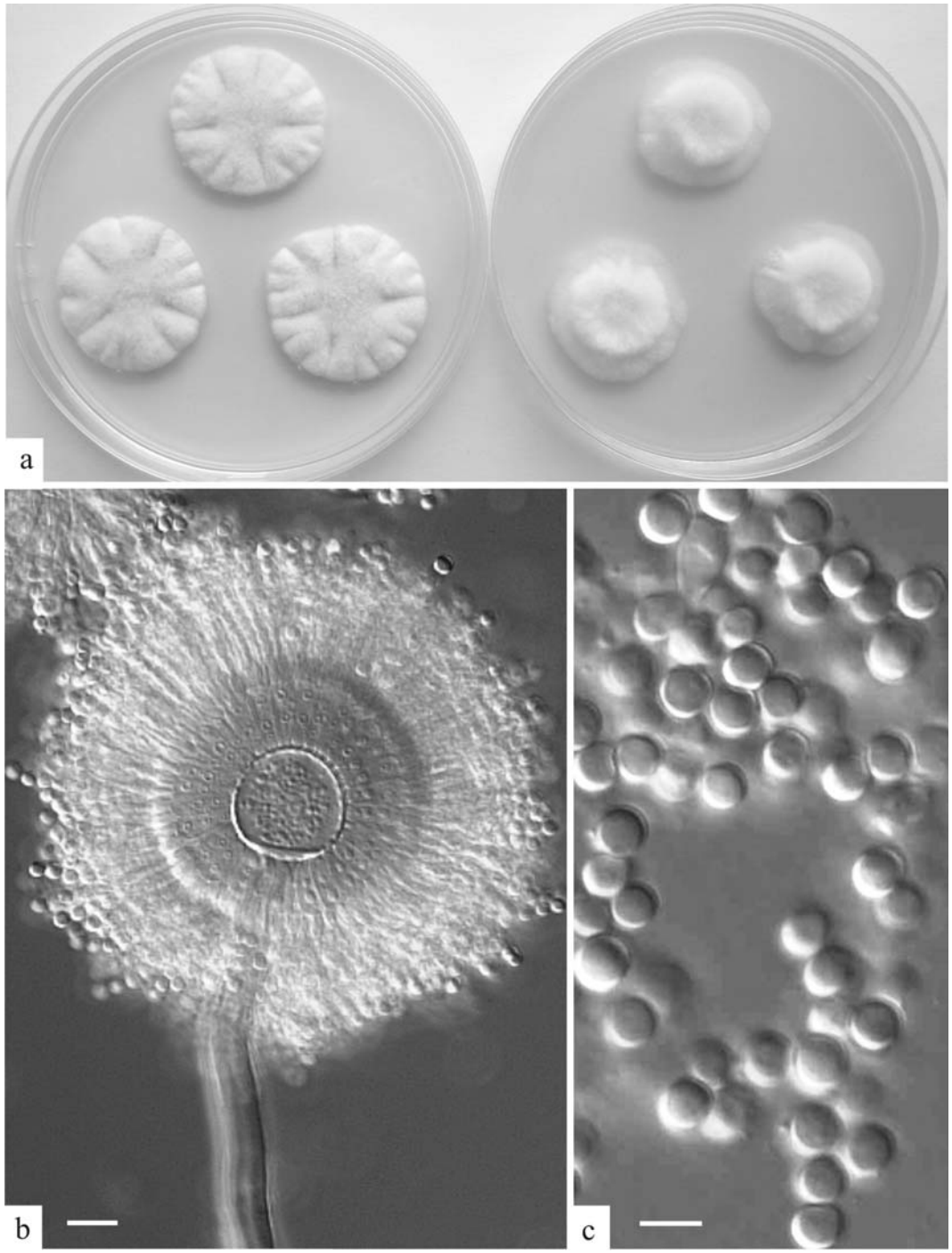


Fig. 8.9 *Aspergillus candidus* **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b)** head, bar = 10 μm; **(c)** conidia, bar = 5 μm

2.5–3.5 μm diam, with smooth walls, borne in radiate heads.

Distinctive features. The only species of *Aspergillus* with persistently white conidia, *A. candidus* is readily distinguished from all species other than *A. niveus*. It differs from *A. niveus* by producing vesicles fertile over the entire area and metulae usually more than 10 μm long.

Physiology. The growth rates reported for this species vary considerably from optimum temperatures of 20–24 to 45–50°C, minimum temperatures ranging from 3–4 to 11–13°C and a maximum of 41–42 to 50–55°C (see Pitt and Hocking, 1997). We have encountered a group of isolates from tropical dried fish, clearly identifiable as *Aspergillus candidus*, unable to grow at 37°C. These figures are surprisingly conflicting for what appears to be a well defined species.

The minimum a_w for growth of *Aspergillus candidus* was reported as 0.75, by both Galloway (1935) and Ayerst (1969), after 14 days incubation at 25°C and ca. 30 days at 30°C, respectively. The optimum a_w for growth was greater than 0.98 (Ayerst, 1969). Conidia of *A. candidus* showed 100% survival after heating for 10 min at 50°C, but no survival after 10 min at 60°C (Pitt and Christian, 1970).

This species is more tolerant of low O_2 than most *Aspergillus* species, being capable of growth in 0.45% O_2. More than 15% CO_2 was required to halve the growth rate in air (Magan and Lacey, 1984b). Tolerance to propionic acid used as a grain preservative is also high (Müller et al., 1981).

Mycotoxins. *A. candidus* produces a range of secondary metabolites (Frisvad and Samson, 1991), but of these only kojic acid has even marginal toxicity.

Ecology. *Aspergillus candidus* is widespread in foods. A xerophile, it is of common occurrence in stored cereals, where it may cause loss of germinability (Sinha and Wallace, 1977). It has been recorded from freshly harvested wheat, wheat during drying and in storage, cereal products, flour and refrigerated dough bran, bread, stored and mouldy maize (see Pitt and Hocking, 1997 and Lugauskas et al., 2006), milled rice (see Pitt and Hocking, 1997 and Taligoola et al., 2004), polished rice (Park et al., 2005a), yellow rice (Phillips et al., 1988), barley (see Pitt and Hocking, 1997 and Lugauskas et al., 2006) and cereal flakes (Weidenbörner and Kunz, 1994).

Aspergillus candidus is also a major species in nuts, including peanuts, hazelnuts and walnuts and pecans. *A. candidus* frequently occurs on salamis and other processed meats. Other sources include dried fish, cheesecake, soybeans, faba beans, sorghum, rapeseed and sunflower seed, mango powder, and health foods (see Pitt and Hocking, 1997).

Aspergillus candidus was present in many samples of commodities we examined from Southeast Asia, but usually at low levels (Pitt et al., 1993, 1994). The exception was Indonesian milled rice, where *A. candidus* was present in high levels (occasionally up to 100% infection) in 56% of samples (Pitt et al., 1998a). On the positive side, *A. candidus* has been recommended as a starter culture of low toxicity for processed meat manufacture (Grazia et al., 1986; Spotti et al., 1994).

Aspergillus candidus has been reported from a wide range of human infections, including invasive aspergillosis and ear infections. It has also been isolated from birds (De Hoog et al., 2000).

References. Raper and Fennell (1965); Domsch et al. (1980); Klich (2002).

Aspergillus carbonarius (Bainier) Thom Fig. 8.10

Aspergillus pulchellus (Speg.) Thom and Church
Aspergillus fonsecaeus Thom and Raper

Colonies on CYA 60 mm or more diam, consisting of surface or subsurface white mycelium, surmounted by a layer of conidiophores; near margins individual conidial heads visible with the naked eye, more or less covering central areas, jet black; reverse colourless to pale yellow. Colonies on MEA 60 mm or more diam, similar to those on CYA but less dense; mycelium subsurface and inconspicuous; conidial heads usually individually visible over the entire colony, jet black; reverse uncoloured. Colonies on G25N 8–25 mm diam, of sparse white surface or subsurface mycelium surmounted by sparse black conidial heads; reverse uncoloured. No germination at 5°C. At 37°C, colonies 15–35 mm diam, of dense white to yellow mycelium; margins irregular; sporulation sparse, with visible individual heads, to very dense, jet black; reverse pale, pale brown, or grey to black.

Conidiophores borne from surface or subsurface hyphae, 2.0–3.0 mm long, with heavy, smooth walls, uncoloured or grey; vesicles spherical or oblate, usually 60–90 μm diam, bearing closely packed metulae and phialides over the whole surface; metulae 12–18 μm long; phialides 9–12 (–15) μm long; conidia spherical or near, 6–7(–8) μm diam, black, with walls conspicuously roughened or sometimes spiky, borne in large, radiate heads.

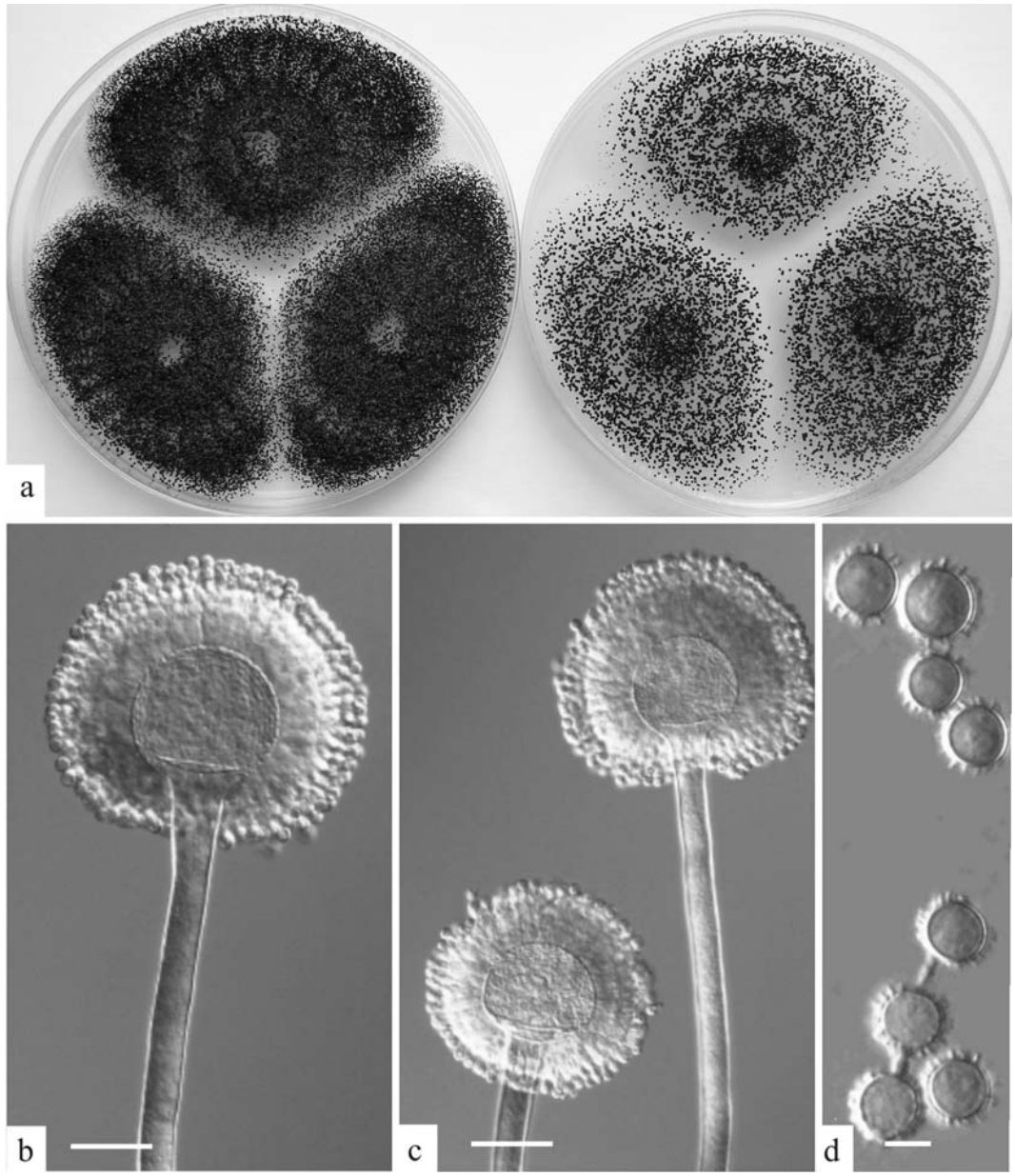


Fig. 8.10 *Aspergillus carbonarius* **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b, c)** heads, bars = 40 μm; **(d)** conidia, bar = 5 μm

Distinctive features. *Aspergillus carbonarius* is distinguished from related black *Aspergillus* species by producing conidia more than 6 μm in diameter. Colonies are jet black, and all heads produce metulae.

Physiology. *Aspergillus carbonarius* can grow at 10°C but not 7–8°C, has an optimum near 30°C and maximum near 41°C (Bellí et al., 2004; Mitchell et al., 2004; Leong et al., 2006b; Marín et al., 2006; Romero et al., 2007). The optimum a_w for growth is 0.96–0.98 (Mitchell et al., 2004; Leong et al., 2006b), with a minimum near 0.85 a_w at 25–30°C (Romero et al., 2007). No growth was observed at 0.82 a_w in a glycerol based medium over the temperature range 15–35°C (Romero et al., 2007). *A. carbonarius* grows over a wide pH range (2–10) (Esteban et al., 2005). Low levels (100 mg/l) of sodium metabisulphite, which is widely

used in the wine industry to treat grape juice before fermentation, stimulated growth of *A. carbonarius*, and high levels (720–1,000 mg/l) were required to inhibit growth by 90% (Pateraki et al., 2007).

Aspergillus carbonarius is very tolerant of elevated levels of CO_2. Compared with growth in air, 25% CO_2 + 1% O_2 stimulated growth, and an atmosphere of 50% CO_2 + 1% O_2 resulted in growth rates only slightly slower than the air control on grape juice medium at 0.985–0.93 a_w (Pateraki et al., 2007). Conidia of *A. carbonarius* were more resistant to ultraviolet radiation than *A. niger*, but more sensitive than *Alternaria alternata* (Valero et al., 2007b). *A. carbonarius* conidia dried onto filter papers survived well at low a_w (0.4 a_w) and low temperature (1°C), with little loss of viability after 618 days storage (Leong et al., 2006e).

Mycotoxins. Ochratoxin A production by *Aspergillus carbonarius* was first reported by Horie (1995), Téren et al. (1996) and Wicklow et al. (1996). Since then, *A. carbonarius* has been recognised as the primary source of ochratoxin A contamination in grapes and grape products throughout the world (Battilani and Pietri, 2002; Cabañes et al., 2002; Sage et al., 2002; Abarca et al., 2003; and many subsequent papers). *A. carbonarius* is also a potential source of ochratoxin A in coffee. It has been reported from coffee beans in Brazil (Taniwaki et al., 2003; Magnani et al., 2005), Ivory Coast (Kouadio et al., 2006), Uganda (Ngabirano et al., 2001), Thailand (Joosten et al., 2001) and Vietnam (Leong et al., 2007). Infection occurs postharvest, and ochratoxin A may be formed if beans are not dried efficiently (Taniwaki et al., 2003).

Where grapes are further processed to make dried vine fruits, the black Aspergilli continue to grow, as in most producing countries grapes are dried in the sun without preservatives. Dried grapes (raisins, sultanas) can therefore readily contain unacceptable levels of ochratoxin A (Lombaert et al., 2004; Iamanaka et al., 2005; Jørgensen, 2005; Meyvaci et al., 2005).

Aspergillus carbonarius produces ochratoxin A optimally at cooler temperatures: 15°C and 0.95–0.97 a_w or 20°C and 0.98–0.99 a_w. Little or no ochratoxin A is formed at temperatures above 35°C. The lower a_w limit for ochratoxin A production is near 0.92 (Mitchell et al., 2004; Esteban et al., 2004; Bellí et al., 2005; Marín et al., 2006; Leong et al., 2006b). Ochratoxin A is produced over the pH range 2–10 at 15 and 30°C on CYA and YES media, respectively (Esteban et al., 2005).

Sodium metabisulphite inhibits ochratoxin A production, but at high a_w (0.985), at least 650–700 mg/l is required for 90% inhibition (Pateraki et al., 2007). Modified atmosphere storage (25 or 50% CO_2) has little effect on ochratoxin formation, with 25% CO_2 resulting in stimulation of toxin production (Pateraki et al., 2007). Application of fungicides to grapes can reduce fungal growth and ochratoxin production, but some fungicides appear to stimulate ochratoxin production (Bellí et al., 2006).

Ochratoxin A is largely removed during the wine making process as it is bound to solid fractions such as wine lees and sediment. Use of some fining agents can also reduce ochratoxin levels in wine. Red wines retain slightly more ochratoxin A than white wines but, overall, the carryover from grapes into finished wine is between 1 and 8% (Leong et al., 2006c,d; Fernandes et al., 2007).

Ecology. The primary food related habitat for *A. carbonarius* is grapes and the vineyard environment. *A. carbonarius* was first reported as an agent of Aspergillus bunch rot in India by Gupta (1956), well before the problem of ochratoxin A contamination of grape products was recognised. Since the late 1990s, there has been a concerted effort to understand the ecology of *A. carbonarius* and ochratoxin A formation in both wine grapes and dried grapes. *A. carbonarius* has been reported from grapes in Australia, South America and countries around the Mediterranean rim including Italy, Spain, Portugal, France, Greece, Turkey, Tunisia, Morocco, Lebanon and Israel (see Battilani et al., 2006 and references therein; El-Khoury et al., 2006). *A. carbonarius* can be isolated from vineyard soil and vine remnants (dried berries, bunch stems, dead canes, etc). It occurs on grapes from berry set to harvest, but populations increase from veraison onwards. *A carbonarius* is a saprophyte rather than a pathogen, so predisposing factors such as insect damage, infections with powdery mildew or *Botrytis*, or berry splitting due to rain or storm damage are needed to initiate Aspergillus bunch rot which generally occurs near harvest when sugar content of berries is highest (Leong et al., 2006a). *A. carbonarius* occurs on grapes during drying and can develop to high numbers on fruit that has been rain affected before harvest (Leong et al.,

2004; 2006a). *A. carbonarius* has been reported from dried vine fruits from many countries including Spain (Abarca et al., 2003), Greece (Tjamos et al., 2004), Australia (Leong et al., 2004), Brazil (Iamanaka et al., 2005) and Argentina (Romero et al., 2005).

Populations of *Aspergillus carbonarius* in vineyards can be reduced by vineyard management practices such as irrigation, pruning to improve air flow through vines, use of cover crops between rows and appropriate fungicide applications (Pollastro et al., 2005; Bellí et al., 2006; Leong et al., 2006a). Biocontrol of black Aspergilli on grapes using epiphytic yeasts has been proposed (Bleve et al., 2006).

Other reported sources of *Aspergillus carbonarius* are figs (Doster et al., 1996), peanuts and maize (Magnoli et al., 2006a, b), bee pollen (Gonzalez et al., 2005), paprika (Almela et al., 2007), red bay berries (Li et al., 2002) and fermenting cocoa beans (Mounjouenpou et al., 2008). In our laboratory we have isolated *A. carbonarius* from dried vine fruits, walnuts and margarine. *A. carbonarius* isolated from Greek tannery wastes reduced tannins by 78%, significantly reducing the BOD of the effluent (Marakis, 1995).

References. Raper and Fennell (1965); Klich (2002).

Aspergillus clavatus Desm. Fig. 8.11

Colonies on CYA and MEA 30–45 mm diam, plane, of sparse surface mycelium surmounted by regular or irregular clusters of positively phototropic conidiophores up to 3 mm long, growing vertically if incubated in darkness; mycelium white; conidiophores readily visible under the stereomicroscope, with stout stipes and heads like match heads, with spore chains coloured greyish turquoise; clear exudate sometimes present in minute droplets; sometimes faint brown soluble pigment produced; reverse pale. Colonies on G25N 8–12 mm diam, of sparse, floccose, white mycelium and small, scattered conidiophores; reverse pale. No growth at 5°C. At 37°C, colonies 10–30 mm diam, of white mycelium, with small blue grey heads; sometimes yellow brown soluble pigment produced; reverse yellow green.

Conidiophores borne from subsurface or surface hyphae, stipes 1.5–3.0 mm long, with thick, smooth walls; vesicles narrow ellipsoids up to 250 × 70 μm, fertile over the whole area, bearing phialides only; phialides very closely packed, mostly 7–8 μm long; conidia ellipsoidal, 3.0–4.5 μm long, smooth walled, borne in radiate heads, each splitting into two or more ordered columns in age.

Distinctive features. Long ellipsoidal vesicles, heads with phialides only and grey blue conidia set *Aspergillus clavatus* apart from other species.

Taxonomy. The relationship of *Aspergillus clavatus* with closely related species was studied by Varga et al. (2003).

Physiology. Panasenko (1967) reported that *Aspergillus clavatus* had an optimal growth temperature near 25°C, a minimum of 5–6°C and a maximum of 42°C. Similar figures were reported by Northolt et al. (1978). The minimum a_w permitting growth was reported as 0.88 a_w by Panasenko (1967) and graphed as near 0.87 a_w by Northolt et al. (1978).

Mycotoxins. This species produces patulin which has the potential to cause ill-thrift in animals and may have harmful effects in man. Patulin is discussed under *Penicillium expansum*. *Aspergillus clavatus* produces a number of other mycotoxins of marginal importance including kojic acid, cytochalasin E, tremorgenic tryptoquivalines and glyantrypine (Varga et al., 2003; Sabater-Vilar et al., 2004).

Patulin was produced by *Aspergillus clavatus* at 0.99 a_w but not 0.95 a_w on laboratory media (Northolt et al., 1978).

Ecology. *A. clavatus* is of particularly common occurrence in barley during malting and can build to unacceptably high levels if malting temperatures are elevated or spontaneous heating occurs (Flannigan et al., 1984; Flannigan, 1986; Sabater-Vilar et al., 2004). In extreme cases blue green mats of *A. clavatus* may form on grain during malting (Shlosberg et al., 1991). Under these conditions it can be allergenic and is reported to be the cause of "malt workers' lung" (Riddle et al., 1968; Flannigan, 1986; Sabater-Vilar et al., 2004).

Aspergillus clavatus is mostly associated with cereals and has been reported from wheat, flour, bread, rice and maize (see Pitt and Hocking, 1997). Other sources include red peppers, pecan nuts, health foods, biltong and salted dried fish (see Pitt and Hocking, 1997).

In Southeast Asian food commodities, *Aspergillus clavatus* occurred at low levels in maize from Thailand and the Philippines; peanuts, kemiri nuts and pepper from Indonesia; and milled rice, soybeans and mung beans from the Philippines. Levels were slightly higher in copra from Thailand (Pitt et al., 1993, 1994, 1998a and unpublished).

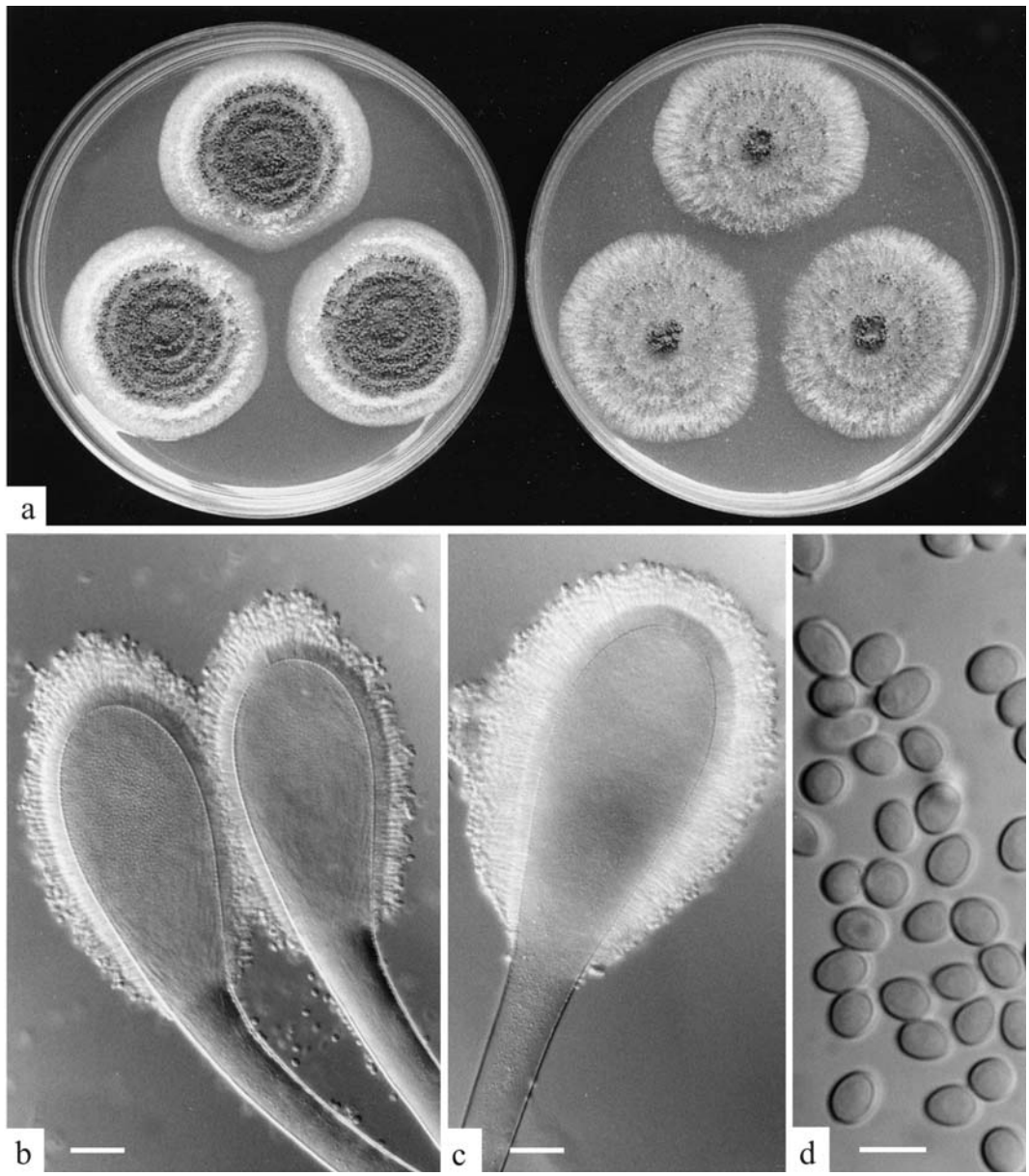


Fig. 8.11 *Aspergillus clavatus* **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b, c)** heads, bars = 25 μm; **(d)** conidia, bar = 5 μm

References. Raper and Fennell (1965); Domsch et al. (1980); Klich (2002).

Aspergillus flavipes (Bainier & Sartory) Thom & Church Fig. 8.12

Teleomorph: *Fennellia flavipes* B.J. Wiley & E.G. Simmons

Colonies on CYA 15–30 mm diam, deep but velutinous, lightly radially sulcate, occasionally floccose centrally; mycelium white to yellow; conidial heads borne uniformly over the colony surface, pale greyish orange; clear exudate sometimes present; reverse pale, pale yellow or light to yellowish brown. Colonies on MEA 20–35 mm diam, plane, velutinous or sometimes slightly granular; mycelium white, inconspicuous; conidial production light, pale orange brown; reverse greyish yellow to golden brown. Colonies on G25N 8–12 mm diam, plane, dense to floccose, white to greyish orange; reverse brown. No growth at 5°C. At 37°C, colonies 5–20 mm diam.

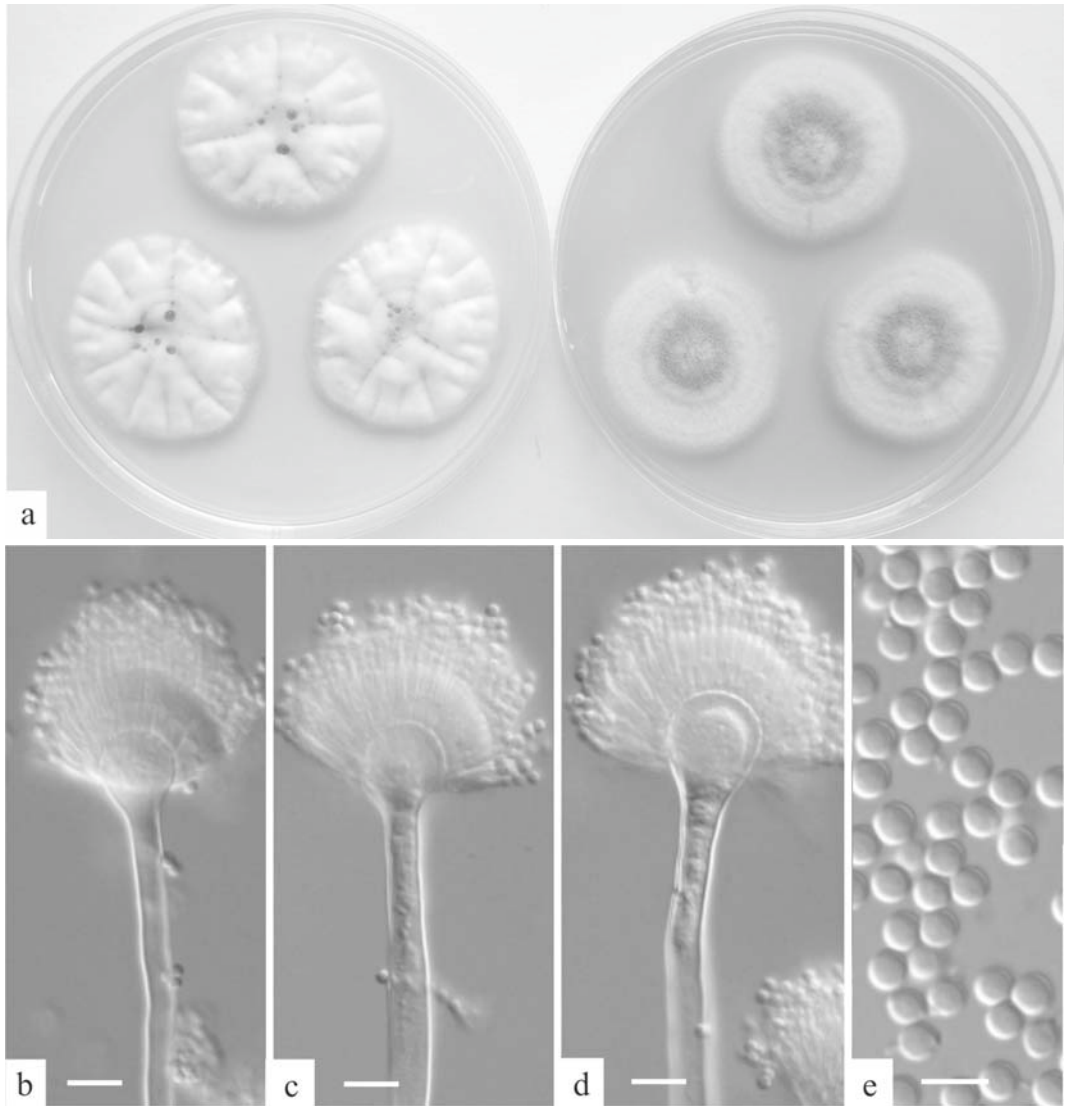


Fig. 8.12 *Aspergillus flavipes* **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b, c, d)** heads, bars = 10 μm; **(e)** conidia, bar = 5 μm

Cleistothecia *fide* Wiley and Simmons (1973) yellow, embedded in discrete clumps of rounded to elongate Hülle cells; ascospores maturing in about 3 weeks, hyaline, subspheroidal, 6.4–8.0 × 5.6–6.4 μm, smooth walled, with an inconspicuous longitudinal groove. Conidiophores borne from surface or aerial hyphae, stipes 150–600 μm long, uncoloured to pale brown, walls smooth to slightly roughened, broadening gradually into spathulate to subspheroidal vesicles mostly 10–20 μm diam, bearing metulae and phialides over the upper half to two thirds; metulae and phialides 5–7 μm long; conidia spherical, 2.0–3.0 μm diam, smooth walled, borne in radiate to loosely columnar heads.

Distinctive features. This species is distinguished by pale greyish orange colonies with metulae and phialides bearing small, smooth walled, spherical conidia.

Taxonomy. *Aspergillus flavipes* occasionally produces a teleomorph, described by Wiley and Fennell (1973) and transferred to *Fennellia* by Wiley and Simmons (1973).

Physiology. This species grows from 6–7 to 38–40°C, with an optimum near 26–28°C (Domsch et al., 1980). It is capable of growth in at least 25% (w/v) NaCl, corresponding to 0.82 a_w (Tresner and Hayes, 1971).

Mycotoxins. This species produces a variety of secondary metabolites (Frisvad and Samson, 1991) including lovastatin (Valera et al., 2005), but no known mycotoxins.

Ecology. *Aspergillus flavipes* is common in tropical soils (Domsch et al., 1980), but uncommon in foods. It has been reported to cause spoilage of a variety of tropical fruit and cassava. It has been reported in wheat from France and we isolated it at low levels from cassava, peanuts and coriander in Southeast Asia (Pitt and Hocking, 1997).

References. Raper and Fennell (1965); Wiley and Fennell (1973); Domsch et al. (1980); Klich (2002).

Aspergillus flavus Link **Fig 8.13**

Colonies on CYA 60–70 mm diam, plane, sparse to moderately dense, velutinous in marginal areas at least, often floccose centrally, sometimes deeply so; mycelium only conspicuous in floccose areas, white; conidial heads usually borne uniformly over the whole colony, but sparse or absent in areas of

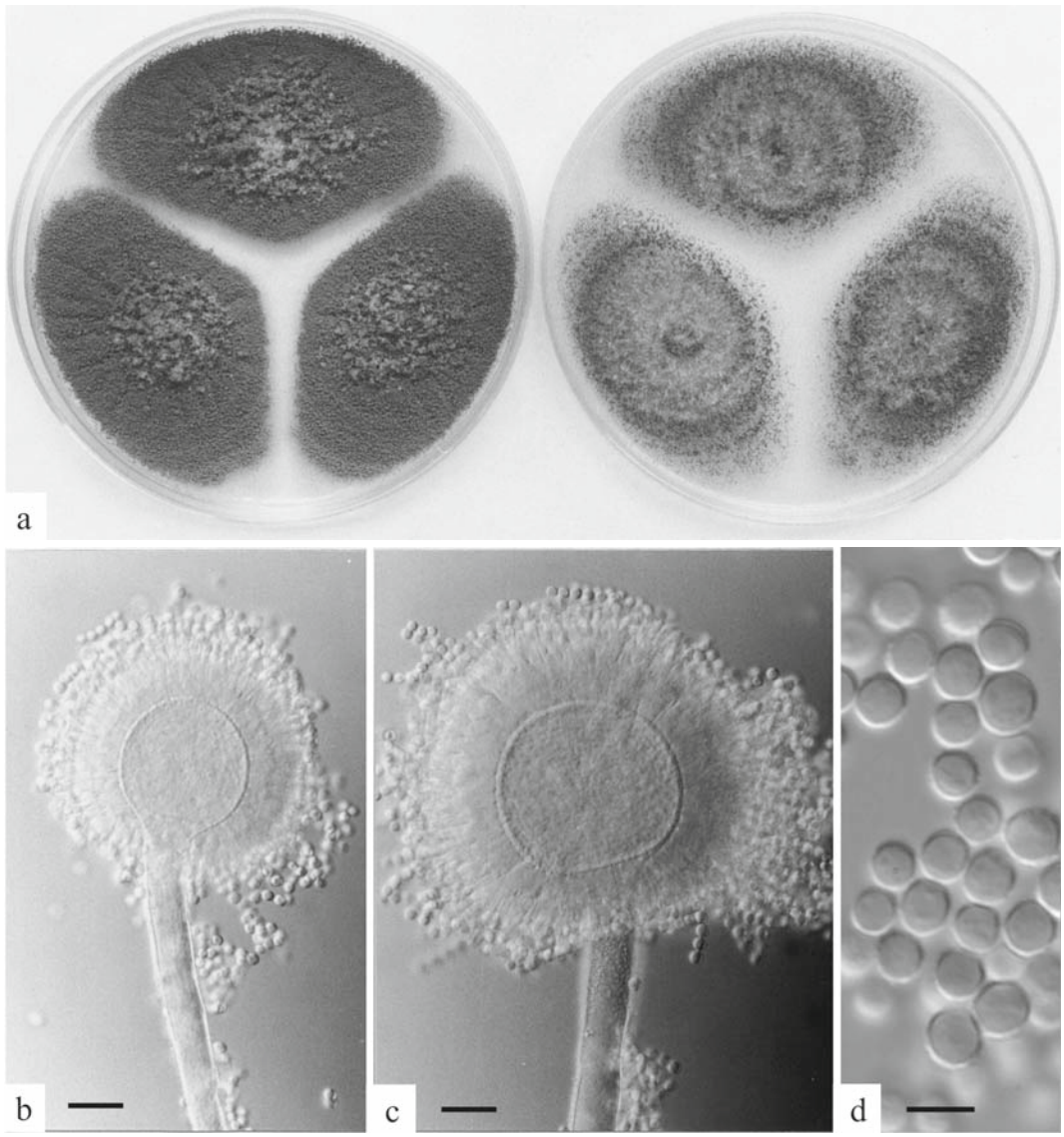


Fig. 8.13 *Aspergillus flavus* **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b, c)** heads, bars = 20 μm; **(d)** conidia, bar = 5 μm

floccose growth or sclerotial production, characteristically greyish green, yellow green or olive yellow, but sometimes pure yellow, then becoming greenish in age; sclerotia produced by about 50% of isolates, at first white, becoming deep reddish brown, density varying from inconspicuous to dominating colony appearance and almost entirely suppressing conidial production; exudate sometimes produced, clear, or reddish brown near sclerotia; reverse uncoloured or brown to reddish brown beneath sclerotia. Colonies on MEA 50–70 mm diam, similar to those on CYA although usually less dense. Colonies on G25N 25–40 mm diam, similar to those on CYA or more deeply floccose and with little conidial production, reverse pale to orange or salmon. No growth at 5°C. At 37°C, colonies usually 55–65 mm diam, similar to those on CYA at 25°C, but more velutinous, with olive conidia, and sometimes with more abundant sclerotia.

Sclerotia produced by some isolates, at first white, rapidly becoming hard and reddish brown to black, spherical, usually 400–800 μm diam, but less than 400 μm in "S" strains (Cotty, 1989). Teleomorph not known. Conidiophores borne from subsurface or surface hyphae, stipes 400 μm to 1 mm or more long, colourless or pale brown, rough walled; vesicles spherical, 20–50 μm diam, fertile over three quarters of the surface, typically bearing both metulae and phialides, but in some isolates a proportion or even a majority of heads with phialides alone; metulae and phialides of similar size, 7–10 μm long; conidia spherical to subspheroidal, usually 3.5–5.0 μm diam, with relatively thin walls, finely roughened or, rarely, smooth.

Distinctive features. *Aspergillus flavus* and *A. parasiticus* are distinguished by their rapid growth at both 25 and 37°C and their bright yellow green (or less commonly yellow) conidial colours. The definitive difference between the two species is that *A. flavus* produces conidia which are rather variable in shape and size, have relatively thin walls and range from smooth to moderately rough, the majority being finely rough. In contrast, conidia of *A. parasiticus* are spherical and have relatively thick, rough walls. In addition, vesicles of *A. flavus* are larger, up to 50 μm in diameter, and usually bear metulae, while vesicles of *A. parasiticus* rarely exceed 30 μm in diameter and metulae are uncommon.

Taxonomy. Klich and Pitt (1985, 1988) showed that metulae production, the principal character used by Raper and Fennell (1965) for distinguishing *Aspergillus flavus* from *A. parasiticus*, was not a satisfactory criterion. Some *A. flavus* isolates produce very few metulae. Careful study showed several differences between the two species, the most useful of which are mentioned above. The criteria used here provide a 100% correlation with mycotoxin production (see below).

Kurtzman et al. (1986b) reduced *Aspergillus parasiticus* to the status of subspecies, as *A. flavus* subspecies *parasiticus* (Speare) Kurtzman et al. The domesticated species *A. oryzae* and *A. sojae* were reduced to the status of variety. Kurtzman et al. (1986b) based these changes on a DNA hybridisation study using only a single strain from each species. Apart from doubt over the validity of this work, or the meaning of DNA hybridisation percentages in these fungi, compelling reasons exist for maintaining distinct species names for these taxa, as has been done here. First, *A. flavus* differs reliably from *A. parasiticus* in the morphological features noted above. Second, some isolates of *A. flavus* produce B aflatoxins and some form cyclopiazonic acid, whereas *A. parasiticus* isolates all produce both B and G aflatoxins and do not form cyclopiazonic acid. These differences are of both scientific and commercial importance. Third, much evidence suggests that *A. parasiticus* has a more limited geographical range than *A. flavus*. Fourth, while *A. flavus* and *A. parasiticus* are both recognised as producers of carcinogenic mycotoxins, *A. oryzae* and *A. sojae* are widely used in food fermentations. It is of great practical importance to the food industry and to regulatory authorities everywhere that the naturally occurring highly toxigenic species have names quite distinct from those used in food fermentations. On this latter ground alone, maintenance of all four species names is a matter of necessity. All four species names are protected in the NCU list (Pitt and Samson, 1993).

Physiology. A problem in assessing physiological data for *Aspergillus flavus* and related species has been inaccurate identification. Most physiological studies reported on *A. flavus* up to 1990 in fact dealt with *A. parasiticus* or *A. nomius* (Pitt, 1993b). Fortunately, differences among these species do not appear to be great, but some data below cannot

with certainty be identified as relating to *A. flavus*. Reported growth temperatures for *A. flavus* show some variation: a minimum near 10–12°C, a maximum near 43–48°C and an optimum near 33°C appear to be most frequently mentioned (see Pitt and Hocking, 1997). Differences in water activities permitting growth have also been rather variable: from a low of 0.78 a_w at 33°C (Ayerst, 1969) to 0.84 at 25°C (Pitt and Hocking, 1977). Pitt and Miscamble (1995) reported a minimum of 0.82 at 25°C, 0.81 at 30°C and 0.80 at 37°C. Data from that paper were used to provide a predictive model for *A. flavus* growth in relation to a_w and temperature (Gibson et al., 1994). High temperatures (41°C) decreased germination rates of *A. flavus* by 45% compared with germination at 30°C (Araujo and Rodrigues, 2004). Growth of *A. flavus* occurred over the pH range 2.1–11.2 (the entire range examined) at 25, 30 and 37°C, with optimal growth over a broad range from pH 3.4–10 (Wheeler et al., 1991) and a peak near 7.5 (Olutiola, 1976). The use of acetic acid to adjust the pH of growth media to 4.5 reduced the germination of *A. flavus* conidia, whereas lactic and hydrochloric acids had no effect on germination (Araujo and Rodrigues, 2004).

The heat resistance of *A. flavus* has been studied under various conditions by several authors. The most reliable figures indicate a D_{45} value of more than 160 h, a D_{50} of 16 h, a D_{52} of 40–45 min and a D_{60} of 1 min, at neutral pH and high a_w, with *z* values from 3.3 to 4.1C° (Beuchat, 1981b,c; Doyle and Marth, 1975a; ICMSF, 1996a).

The addition of phosphine, used to control insects, to grain at 0.80 or 0.86 a_w reduced growth of *Aspergillus flavus* while having little effect on the survival of conidia (Hocking and Banks, 1991).

After 42–48 h incubation on AFPA (Pitt et al., 1983), colonies of *Aspergillus flavus* and *A. parasiticus* exhibit a brilliant orange yellow reverse colouration (see Chapter 4).

Mycotoxins. *Aspergillus flavus* is the main source of aflatoxins, the most important mycotoxins in the world's food supplies. Aflatoxins are produced in nature by *A. flavus, A. parasiticus* and a number of other species, including *A. nomius*, which are of little practical importance in foods. The four major naturally produced aflatoxins are known as aflatoxins B_1, B_2, G_1 and G_2. "B" and "G" refer to the blue and green fluorescent colours, respectively, produced under UV light on thin layer chromatography plates, while the subscript numbers 1 and 2 indicate major and minor compounds, respectively. Aflatoxins are both acutely and chronically toxic to animals, including man. They have long been know to produce four distinct effects: acute liver damage, liver cirrhosis, induction of tumours and teratogenic effects (Stoloff, 1977). However more recent information indicates that the consequences of prolonged aflatoxin exposure are more widespread, including immunosuppression and interference with protein uptake (Williams et al., 2004).

Outbreaks of acute aflatoxin poisoning are not common, but are a recurrent public health problem. A 1974 outbreak of hepatitis that affected 400 Indian people, of whom 100 died, was traced to maize heavily contaminated with *Aspergillus flavus* and containing up to 15 mg/kg of aflatoxins (Krishnamachari et al., 1975). Consumption of toxins by some of the affected adults was calculated to be 2–6 mg in a single day. It can be concluded that the acute lethal dose for adult humans is of the order of 10 mg.

In 2004, one of the largest and most severe outbreaks of aflatoxicosis occurred in eastern Kenya, where 317 people fell ill and 125 died. This was followed by a second outbreak in 2005 during which a further 25 people died (Strosnider et al., 2006). A case study conducted soon after the first outbreak revealed that mouldy maize was the source of aflatoxin contamination (Azziz-Baumgartner et al., 2005).

Aflatoxin B_1 (AFB_1) has been demonstrated, in a variety of animal species, to be the most potent liver carcinogen known. Aflatoxin B_1 has been linked to human hepatocellular carcinoma in several regions of Africa and Southeast Asia (Wogan, 1992; Wang and Groopman, 1999). In consequence, AFB_1 is regarded as a category I human carcinogen by the International Agency for Research on Cancer (IARC, 1993; Rastogi et al., 2006). Kuiper-Goodman (1991) concluded that between 5 and 9 μg/kg body weight/day of AFB_1 intake had a 50% probability of increasing the incidence of primary liver cancer in a population by a factor of 1 case per 10,000 population. Based on those figures and aflatoxin levels in Indonesian diets (Pitt and Hocking, 1996), Lubulwa and Davis (1994) estimated that the minimum numbers of deaths in Indonesia from aflatoxins in peanuts and maize exceeded 22,000 per annum.

A strong synergy is observed between aflatoxin and hepatitis B (HBV) virus in the aetiology of liver cancer (Williams et al., 2004). The relative risk of cancer for HBV antigen positive patients rises from about 5 to about 60 when combined with aflatoxin exposure. In some areas where HBV and aflatoxin co-occur, hepatomas are the predominant cancer and may be a predominant cause of death (Williams et al., 2004). This known carcinogenicity is so high that every effort must be made to monitor levels in human foods and to reduce them as far as possible.

When aflatoxin B_1 and B_2 are ingested by lactating cows, a proportion (ca. 1.5%; Frobish et al., 1986) is hydroxylated and excreted in the milk as aflatoxins M_1 and M_2, compounds of lower toxicity than the parent molecules. Children consume large quantities of milk and are also susceptible to the long term ill effects of aflatoxins, so concern soon mounted about the possible presence of aflatoxin M_1 in milk, and studies in many countries reported positives (van Egmond, 1989). As a result stringent limits have been imposed on aflatoxin levels in feeds for dairy animals (van Egmond, 1989). A recent survey of UHT and pasteurised milk in Brazil indicated a high incidence (80%) of aflatoxin M_1 in samples analysed; however, the concentration of aflatoxin M_1 was below the 500 ng/l permitted limit (Garrido et al., 2003).

Residues in other animal products are generally low. In particular, ruminant animals detoxify aflatoxins quite efficiently. Levels in eggs and meat from monogastric animals may be higher, but in general toxicity to the animal limits feeding of excessively contaminated feed, so minimising residues (Stoloff, 1977).

It is believed that constant exposure to aflatoxins through contaminated foods leads to chronic diseases which also have a considerable impact on human health (Williams et al., 2004).

Kwashiorkor, also known as protein energy malnutrition, is a leading cause of disease and death in children under 5 years of age in Northern Africa and elsewhere and it was Hendrickse et al. (1982) who first proposed a link with aflatoxin intake. Kwashiorkor was among the 10 most common admissions to one paediatric ward in Tanzania and had the third highest mortality rate (43%) after AIDS and septicaemia (Immerzeel et al., 2006). It is now established that chronic exposure to aflatoxins is correlated with the disease, in which hepatic metabolism is impaired (Fuhrman et al., 2004; Müller and Krawinkel, 2005). Hatem et al. (2005) reported the presence of aflatoxins in 80% of sera from infants with kwashiorkor.

Aflatoxins have also been reported to be potent immunotoxic agent in both animals and humans and may be responsible for greatly increasing susceptibility to many kinds of disease agents in countries where aflatoxin ingestion is common (Wogan, 1992; Wang and Groopman, 1999; Williams et al., 2004).

Cyclopiazonic acid (CPA) is produced by some strains of *Aspergillus flavus*. Its role in human health remains unclear, but evidence for its involvement in animal disease is strong (Cole, 1986). Occurrence at low levels in maize has been reported (Lee and Hagler, 1991; Giorni et al., 2007).

The universal occurrence of *Aspergillus flavus* and aflatoxins in commodities such as peanuts, maize and cottonseed has prompted a great deal of work on causes, course and prevention, work too voluminous to report in detail here (for reviews see Diener et al., 1987; Payne, 1992). Earlier studies assumed that invasion was primarily a function of inadequate drying or improper storage, but more recent work has shown that, at least in developed countries, invasion before harvest is more important and is dependent primarily on plant stress induced by drought and/or high soil temperatures (Sanders et al., 1981; Cole et al., 1982). The problem can be overcome most effectively by irrigation, but unfortunately this is not a practical solution in many peanut growing regions. Preharvest invasion also occurs in maize, cottonseed, pistachios and figs (see Pitt and Hocking, 1997).

In peanuts, reduction in aflatoxin levels during processing is accomplished by colour sorting of individual kernels after shelling. The process was developed originally to reject commercially unacceptable discoloured nuts, regardless of cause: but as fungal growth is a prime cause of discolouration, the process is also an effective nondestructive means of removing most aflatoxin-containing nuts. Maize and fig samples are screened for the presence of aflatoxin by the examination of cracked kernels or fruit by ultraviolet light (see Pitt and Hocking, 1997). No effective nonchemical testing techniques

exist for cottonseed or pistachios and, as with other commodities, nondestructive chemical assays are not available.

The use of biological control, based on competitive exclusion, has been developed for peanuts and cottonseed (Pitt and Hocking, 2006; Dorner and Horn, 2007). This involves establishing high populations of nontoxigenic strains of *Aspergillus flavus* or *A. parasiticus* in the soil which then compete with naturally occurring aflatoxigenic strains, particularly when crops become stressed and susceptible to invasion. Dorner and Horn (2007) reported that treatment with a nontoxigenic *A. flavus* strain alone was more effective than a single strain of *A. parasiticus* and equally effective as a mixture of both strains in controlling toxigenic *A. flavus* in peanut crops.

Counts of *Aspergillus flavus* in spices are often high, and concern has been expressed over the possible occurrence of aflatoxins in these commodities. Although the quantities of spices used in most foods are small, limiting any direct hazard, spices are used in the preparation of many types of foods, so high levels of *A. flavus* in spices are undesirable. Sterilisation of them by chemicals or irradiation is now widely practised. The presence of *A. flavus* in spices such as pepper may result in infections in hospital patients (De Bock et al., 1989).

It has been suggested that the presence of aflatoxins in *Aspergillus flavus* conidia is a cause for concern. Individual spores can contain high concentrations: up to 1,000 mg/kg, an incredibly high concentration in comparison with foods (Wicklow and Shotwell, 1983). However, the minute weight of individual spores means that actual intake levels from spores are very low. Aflatoxins are readily absorbed into the body from spores ingested into the lungs, with detrimental effects on alveolar macrophage function (Miller, 1994b; Williams et al., 2004). Aflatoxins can also be absorbed through the skin. Experiments in mice showed that continued dermal exposure to AFB_1, even at low doses, might induce tumors (Rastogi et al., 2006).

Foods prepared from maize as grits or cakes, etc., such as are consumed in Southeastern United States and other areas, often contain significant aflatoxin levels (Stoloff and Friedman, 1976). In a recent survey of breakfast and infant cereals in Canada, 50% of samples contained detectable levels of aflatoxins. However, only 4% of the breakfast samples and 1% of infant cereals had AFB_1 levels exceeding the 0.1 μg/kg maximum limit set by the European Union (Tam et al., 2006). Flour (made from seed, pulses or cereal) and starch samples purchased from traditional bazaars and markets in Turkey showed that more than 60% of samples contained aflatoxins (Baydar et al., 2005). Samples of food products purchased from Japanese retail outlets containing maize, peanuts, buckwheat (flour and noodles), rice or sesame oil did not contain aflatoxins. However, 10 of the 21 samples of peanut butter tested were contaminated with high levels of AFB_1 (Sugita-Konishi et al., 2006).

From the few studies of the effects of physical factors on aflatoxin production, it appears that *Aspergillus flavus* can produce aflatoxins over the range 13–37°C, and above 0.82 a_w, with optima perhaps 16–31°C and 0.95–0.99 a_w (ICMSF, 1996a). Aflatoxin production by *A. flavus* in agricultural soils appears to be correlated with latitude, aflatoxigenic strains being more prevalent in the southern peanut growing regions of the United States. It appears that dry, hot conditions favour the presence of toxigenic strains of *A. flavus* (Horn, 2003). We have made similar observations: *A. flavus* strains from New Zealand produce aflatoxins less frequently and at lower concentrations than strains from Australia or Southeast Asia (our unpublished data). The influence of crop species has also been raised as a factor in selecting for toxigenic *A. flavus* in agricultural soils (Horn, 2003). Additional studies are still needed to provide a more accurate picture.

Ecology. The quest for knowledge about potential aflatoxin problems means that *Aspergillus flavus* has been sought in every conceivable kind of foodstuff. *A. flavus* has become the most widely reported foodborne fungus, reflecting its economic importance and relative ease of recognition as much as its ubiquity. It is especially abundant in the tropics, and it has a particular affinity for nuts and oilseeds as substrates.

Aspergillus flavus was prevalent in 97% of peanuts examined in Southeast Asia. Maize samples from Indonesia and the Philippines also contained high levels of *A. flavus* contamination. Other Southeast Asian commodities found to harbour *A. flavus* included kemiri nuts, peppercorns, velvet and talo beans, copra, cassava, sorghum, paddy rice and soybeans (see Pitt and Hocking, 1997).

Aspergillus flavus occurs in most types of nuts from time to time and is capable of causing spoilage and/or producing aflatoxins (see Pitt and Hocking, 1997). Reports include tree nuts, pistachios (Bayman et al., 2002a), halva containing pistachios (Var et al., 2007), hazelnuts and walnuts (Bayman et al., 2002a; Gürses, 2006), coconut (Onifade and Jeff-Agboola, 2003), copra (Srinivasulu et al., 2003), pecans, and kola nuts (see Pitt and Hocking, 1997).

Cereals are also a common source of *Aspergillus flavus*. Maize and maize products are a particular problem (see Pitt and Hocking, 1997; Sekiyama et al., 2005; García and Heredia, 2006; Giorni et al., 2007). Insects may be involved in infection (García and Heredia, 2006) and a number of practices including good storage conditions (Saleemullah et al., 2006) and planting genetically modified crops (Marasas and Vismer, 2003) are now employed to control aflatoxin levels. Contamination has also been reported commonly in other cereals and cereal products: wheat (Lugauskas et al., 2006; Giray et al., 2007); wheat flour (Kumar et al., 2002; Ogundare and Adetuyi, 2003) and flour products including bread (Lugauskas et al., 2006), pasta (Halt et al., 2004) and bran; barley (Lugauskas et al., 2006; Medina et al., 2006; see also Pitt and Hocking, 1997); paddy, milled and parboiled rice (see Pitt and Hocking, 1997); rice and rice bran (Sales and Yoshizawa, 2005a); sorghum (da Silva et al., 2004; Lugauskas et al., 2006); and pearl millet (Wilson et al., 2006). However, unlike the situation with crops high in oil, spoilage by *A. flavus* or aflatoxin production in small grain cereals is almost always the result of poor handling. Aflatoxin levels in properly handled small grains are usually negligible (Stoloff, 1977; Pohland and Wood, 1987).

Spices of many kinds frequently contain *Aspergillus flavus* (Elshafie et al., 2002; Gatti et al., 2003; Mandeel, 2005 and also see Pitt and Hocking, 1997). Ionising radiation (see Pitt and Hocking, 1997) and treatment of fresh plant materials before grinding or immediately after processing can reduce fungal and bacterial loads and aflatoxin levels in spices (Schweiggert et al., 2005).

Aspergillus flavus is common in green coffee beans (Martins et al., 2003; Magnani et al., 2005) and herbal drugs (Rizzo et al., 2004). Other sources of *A. flavus* include chickpeas (Singh et al., 2005), pigeon peas (Reddy et al., 2006), soybeans (Ahammed et al., 2006), olives, rapeseed, amaranth seeds (see Pitt and Hocking, 1997), mustard seeds (Ahmad and Sinha, 2006), sesame seeds (Singh et al., 2003; Pillai et al., 2003); freshly harvested and stored blackgram (Goyal and Jain, 1998), sunflower seeds (Narasimhan and Muthumary, 2005) and betel nuts (see Pitt and Hocking, 1997). Processed and smoked meats, bacon (see Pitt and Hocking, 1997), milk (Garrido et al., 2003; Ruggia and Galiero, 2005) and cheese (Hayaloglu and Kirbag, 2007) often contain *A. flavus* and sometimes aflatoxins. Some processed fish products from Nigeria and Sierra Leone supported growth of *A. flavus* but it was relatively uncommon in salted dried fish from Southeast Asia or dry cured hams in Spain (see Pitt and Hocking, 1997).

Aspergillus flavus is capable of causing spoilage of some kinds of fresh fruit and vegetables, including citrus, tomatoes, peppers, litchis, pineapples and pomegranates, but it is not usually of great importance (Snowdon, 1990, 1991). It has recently been reported to cause rot of peaches in Greece (Michailides and Thomidis, 2007).

Pathogenicity. *Aspergillus flavus* is one of the main agents of human allergic bronchial aspergillosis and of pulmonary infections in immunocompromised patients. It also causes ear infections (De Hoog et al., 2000).

Additional species. *Aspergillus oryzae* (Ahlburg) Cohn is closely related to *A. flavus* and produces colonies of similar or slightly smaller size on the standard media. It is usually distinguishable from *A. flavus* on the standard media after 7 days only by a more floccose and lightly sporing appearance and sometimes a tendency towards pale brown conidial colours. Colonies of *A. oryzae* change in conidial colour from green towards olive brown with continued incubation at 25°C for 7–14 days. Colonies of *A. flavus* and *A. parasiticus* remain yellow green or become greyish green under these conditions. Conidial heads of *A. oryzae* usually bear metulae and phialides, and conidia are usually larger than those of *A. flavus*, with thin, smooth to finely roughened walls. *A. oryzae* can be distinguished from *A. flavus* by amplified fragment length polymorphism (AFLP) (Monteil et al., 2003; Lee et al., 2004).

Aspergillus oryzae is of great economic importance, as it forms the basis of much of the fermented

food industry in Japan and other parts of Asia (see Pitt and Hocking, 1997). Tane koji, prepared by growing *A. oryzae* on cooked rice, provides a source of enzymes used in the production of shoyu (soy sauce), miso, hamanatto and other important Oriental products, which are mostly used as food flavourings (Tanaka et al., 2006).

Aspergillus oryzae is rarely isolated from sources other than fermented foods and it is reasonable to believe that it is a domesticated form of *A. flavus*, adapted by centuries of use in fermented food manufacture (Wicklow, 1983). Unlike *A. flavus, A. oryzae* is not known to produce aflatoxins. However, fermented foods may not be as free from the hazards of mycotoxins as is popularly believed as strains of *A. oryzae* were found to produce cyclopiazonic acid and kojic acid (Tanaka et al., 2006).

Additional species. *Aspergillus nomius* Kurtzman et al. (Kurtzman et al., 1986a) is also closely related to *A. flavus*. In the absence of sclerotia, the two species are morphologically indistinguishable. The major differences are that *A. nomius* produces both B and G aflatoxins and that sclerotia of *A. nomius* are bullet shaped, not spherical like those of *A. flavus*. Physiologically, *A. nomius* resembles *A. flavus*, but does not grow at quite such low water activities, with 0.83 a_w the minimum at 25 and 30°C and 0.81 at 37°C (Pitt and Miscamble, 1995).

Aspergillus nomius is a comparatively rare species, originally isolated from alkali bees in the United States (Kurtzman et al., 1986a). However, it has been reported from agricultural and native forest soils in Thailand where it was found to produce greater amounts of aflatoxins than *A. flavus* (Ehrlich et al., 2007), and we isolated it from a range of Thai commodities: maize, peanuts, soybeans, cassava and black beans, but always at low levels (Pitt et al., 1993, 1994).

References. Raper and Fennell (1965); Domsch et al. (1980); Kurtzman et al. (1986a); Klich and Pitt (1988); Klich (2002).

Aspergillus fumigatus Fresen. Fig. 8.14

Colonies on CYA 40–60 mm diam, plane or lightly wrinkled, low, dense and velutinous or with a sparse, floccose overgrowth; mycelium inconspicuous, white; conidial heads borne in a continuous, densely packed layer, greyish turquoise to dark turquoise (blue green); clear exudate sometimes produced in small amounts; reverse pale or greenish. Colonies on MEA 40–60 mm diam, similar to those on CYA but less dense and with conidia in duller colours; reverse uncoloured or greyish. Colonies on G25N less than 10 mm diam, sometimes only germination, of white mycelium. No growth at 5°C. At 37°C, colonies covering the available area, i.e. a whole Petri dish in 2 days from a single point inoculum, of similar appearance to those on CYA at 25°C, but with conidial columns longer and conidia darker, greenish grey to pure grey.

Conidiophores borne from surface hyphae, stipes 200–400 μm long, sometimes sinuous, with colourless, thin, smooth walls, enlarging gradually into pyriform vesicles; vesicles 20–30 μm diam, fertile over half or more of the enlarged area, bearing phialides only, the lateral ones characteristically bent so that the tips are approximately parallel to the stipe axis; phialides crowded, 6–8 μm long; conidia spherical to subspheroidal, 2.5–3.0 μm diam, with finely roughened or spinose walls, forming radiate heads at first, then well defined columns of conidia.

Distinctive features. This distinctive species can be recognised in the unopened Petri dish by its broad, velutinous, bluish colonies bearing characteristic, well defined columns of conidia. Growth at 37°C is exceptionally rapid. Conidial heads are also diagnostic: pyriform vesicles bear crowded phialides which bend to be roughly parallel to the stipe axis. Care should be exercised in handling cultures of this species (see Chapter 4).

Physiology. Undoubtedly the most important physiological character of *Aspergillus fumigatus* is its thermophilic nature: its minimum growth temperature is near 12°C, the optimum 40–42°C and maximum near 55°C (Panasenko, 1967; Ayerst, 1969: Evans, 1971; Domsch et al., 1980). *A. fumigatus* is a marginal xerophile, Ayerst (1969) recording 0.82 a_w as the minimum for growth, near 40°C. It required more than 15% CO_2 to reduce growth rates by half at 0.98 a_w, but only 2.5% at 0.90 a_w (Magan and Lacey, 1984b).

Mycotoxins. *Aspergillus fumigatus* produces fumitremorgens, verruculogen and gliotoxin and a role in animal disease seems likely (Cole, 1981a,b; Dorner et al., 1984; Moreau, 1990; Frisvad et al., 2006a). Evidence suggests gliotoxin is important in

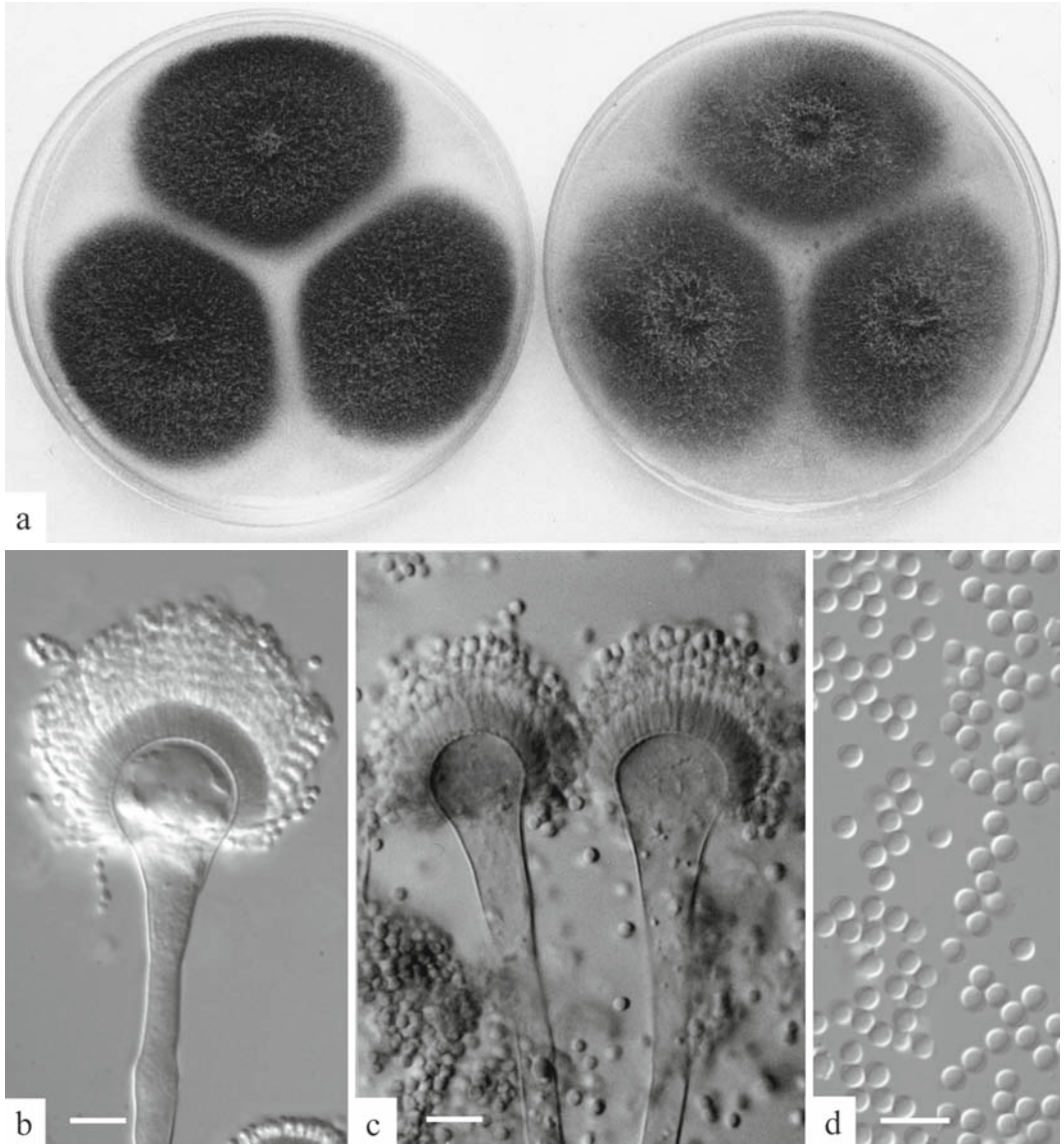


Fig. 8.14 *Aspergillus fumigatus* **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b, c)** heads, bars = 10 μm; **(d)** conidia, bar = 5 μm

the invasion of animal and bird lungs by *A. fumigatus* (Richard et al., 1994). The presence of *A. fumigatus* in pepper used in foods for hospital patients is undesirable (De Bock et al., 1989).

The fumigaclavines, produced by *Aspergillus fumigatus*, are related to ergot alkaloids (Panaccione and Coyle, 2005). *A. fumigatus* also produces fumagillin, a compound with some antibacterial and antifungal activity, which is also an angiogenesis inhibitor (Yang et al., 2003).

Ecology. The prime habitat for *Aspergillus fumigatus* is decaying vegetation, particularly grass clippings, in which it causes spontaneous heating (Cooney and Emerson, 1964). Major habitats are therefore in cocoa beans during and after fermentation and in spices (see Pitt and Hocking, 1997). It has been recorded frequently from stored commodities in the tropics, where its ability to grow simultaneously at low a_w and at high temperatures provides an ecological advantage, e.g. stored oilseeds, stored eggs,

copra, soybeans and vegetables (see Pitt and Hocking, 1997). It has been reported as one cause of "Rio" off-flavours in coffee (Liardon et al., 1992), but it is not usually an important spoilage fungus. Cereals are a common source, e.g. wheat (Soldevilla et al., 2005; Lugauskas et al., 2006), rice, parboiled rice and barley (see Pitt and Hocking, 1997). It is quite common on cured and processed meats, especially in the tropics (Sabater-Vilar et al., 2003 and see also Pitt and Hocking, 1997). *A. fumigatus* also occurs on nuts, e.g. hazelnuts, walnuts, peanuts (see Pitt and Hocking, 1997) and cashews (Adebajo and Diyaolu, 2003). Other sources include dried fish, baladi bread, corn snacks, rootstock snacks, melon seeds, mango pickles, dried onion, low fat buffalo milk cottage cheese, processed cheese (see Pitt and Hocking, 1997) and kuflu cheese (Hayaloglu and Kirbag, 2007). *A. fumigatus* isolated from marine sediments produced gliotoxin under hypersaline conditions. After 6 days in gliotoxin contaminated seawater, fresh mussels were able to accumulate gliotoxin at levels up to 2.9 μm/mg in meat (Grovel et al., 2003).

Pathogenicity. *Aspergillus fumigatus* is the main agent of pulmonary aspergillosis in man and birds. However, disseminated infections in patients without apparent immune disorders are extremely rare (De Hoog et al., 2000).

References. Raper and Fennell (1965); Domsch et al. (1980); Klich (2002).

Aspergillus niger Tiegh. *nom. cons.* Fig. 8.15

Colonies on CYA 60 mm or more diam, usually covering the whole Petri dish, plane, velutinous, of low, usually subsurface, white mycelium, surmounted by a layer of closely packed, dark brown to black conidial heads, ca. 2–3 mm high; reverse usually pale, sometimes pale to bright yellow. Colonies on MEA varying from 30 to 60 mm diam, usually smaller than those on CYA and often quite sparse by comparison, otherwise similar. Colonies on G25N 18–30 mm diam, plane, velutinous, with white or pale yellow mycelium visible at the margins, otherwise similar to those on CYA; reverse pale or occasionally with areas of deep brown. No growth at 5°C. At 37°C, colonies 60 mm or more diam, covering the available space, sometimes sulcate, otherwise similar to those on CYA at 25°C.

Conidiophores borne from surface hyphae, 1.0–3.0 mm long, with heavy, hyaline, smooth walls; vesicles spherical, usually 50–75 μm diam, bearing closely packed metulae and phialides over the whole surface; metulae 10–15 μm long, or sometimes more; phialides 7–10 μm long; conidia spherical, 4–5 μm diam, brown, with walls conspicuously roughened or sometimes striate, borne in large, radiate heads.

Distinctive features. One of the best known of all fungal species, *Aspergillus niger* is distinguished by its spherical black conidia, derived from colonies which show little or no other colouring. *A. carbonarius* (Bainier) Thom, which produces conidia 7–10 μm in diameter and *A. awamori* Nakaz., which produces finely roughened conidia, are closely related. *A. awamori* is used in food fermentations and is perhaps a domesticated form of *A. niger*.

Taxonomy. The name *Aspergillus niger*, in use for most of this century, is predated by other valid names which have priority under the Botanical Code. *A. niger* was conserved by the International Botanical Congress in 1993 (Greuter et al., 1994). The formal wording "*nom. cons.*" after the name indicates this protected status. Molecular studies have shown that *A. niger* consists of two species, *A. niger* and *A. tubingensis*, separated at first by RFLP analysis (Kusters-van Sommeren et al., 1991; Varga et al., 1994) and later by sequence analysis (Peterson, 2000). The species are morphologically indistinguishable, and for practical purposes, the name *A. niger* can be used for both.

Physiology. Growth temperatures for *Aspergillus niger* are minimum 6–8°C, maximum 45–47°C, and optimum 35–37°C (Panasenko, 1967; Leong et al., 2006b). However, Palacios-Cabrera et al. (2005) reported no growth at 8°C on three different media. *A. niger* is a xerophile: Ayerst (1966) reported germination at 0.77 a_w at 35°C. Only slight differences in growth rates were observed on media based on NaCl or glycerol, or of pH 4.0 and 6.5, at various water activities (Avari and Allsopp, 1983). *A. niger* is able to grow down to pH 2.0 at high a_w (Pitt, 1981). Microwaves were found to have no effect on conidia of *A. niger*, apart from that from substrate heat (Ishitani et al., 1981). *A. niger* was relatively resistant to UV-C radiation but more sensitive than *A. carbonarius* or *A. alternata* (Valero et al., 2007b).

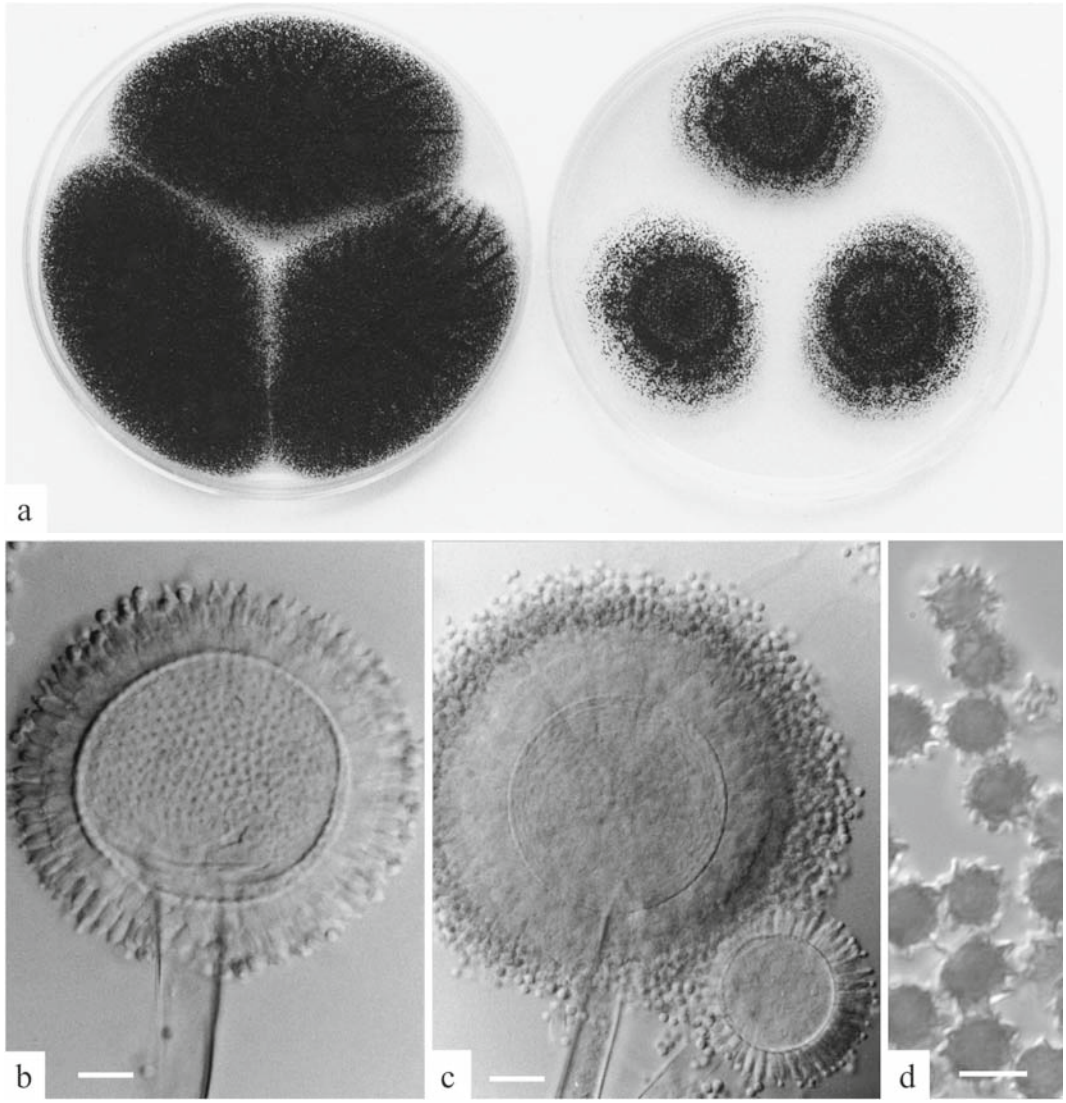


Fig. 8.15 *Aspergillus niger* **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b)** head, bar = 15 μm; **(c)** heads, bar = 10 μm; **(d)** conidia, bar = 5 μm

Mycotoxins. *Aspergillus niger* has usually been regarded as a benign fungus and has been widely used in enzyme production and ingredients for food processing. It holds GRAS (generally regarded as safe) status from the US Food and Drug Administration. However, 2 of 19 *A. niger* isolates were reported to produce ochratoxin A by Abarca et al. (1994) and further research has shown than many more isolates of *A. niger* aggregate can produce ochratoxin A under various conditions (Bellí et al., 2004; Esteban et al. 2004, 2006 a,b; Leong et al., 2006b). Usually only a low percentage of *A. niger* isolates are able to produce ochratoxin A. Frisvad and Samson (2004) reported that *A. tubingensis* does not produce ochratoxin A. However, recent studies have disagreed. Medina et al. (2005) and Perrone et al. (2006) have both reported the production of ochratoxin A by some isolates of *A. tubingensis* after identification by molecular methods, 3 of 21 (14%) and 5 of 20 (25%), respectively.

Fumonisin B_2 has recently been detected in cultures of three full genome sequenced strains of *Aspergillus niger*, including the ex type culture (Frisvad et al., 2007).

Ecology. Among the fungi most commonly reported from foods, *Aspergillus niger* is more prevalent in warmer climates, both in field situations and stored foods. The black spores provide protection from sunlight and UV light, providing a competitive advantage in such habitats (Valero et al., 2007b). *A. niger* is very frequently isolated from sun dried products, such as vine fruits (Abarca et al., 2003; Leong et al., 2004; Magnoli et al., 2004; Iamanaka et al., 2005), dried, smoked and cured fish, biltong, cocoa beans (see Pitt and Hocking, 1997) and spices (Elshafie et al., 2002; Mandeel, 2005). A similar physiology to *A. flavus* means that the two species compete in many situations. Various suggestions have been made for using *A. niger* as a competitor to *A. flavus* in the field (e.g. Wicklow et al., 1987; Shantha et al., 1990; Paster et al., 1992), but in our opinion *A. niger* is too destructive for this to be a practical proposition.

Aspergillus niger is by far the most common *Aspergillus* species responsible for postharvest decay of fresh fruit, including apples, pears, peaches, citrus, grapes, figs, strawberries, mangoes and melons (Snowdon, 1990 and also see Pitt and Hocking, 1997). Most of these diseases are sporadic and of minor significance. *A niger* has also been reported to cause serious losses in tomatoes (Muhammad et al., 2004) and breadfruit (Omobuwajo and Wilcox, 1989) and in some vegetables, especially onions and garlic (Rath and Mohanty, 1985; Snowdon, 1991; Sinha et al., 1994), stored cabbages (Lugauskas et al., 2005) and yams (Adeniji, 1970a; Ogundana, 1972). It is frequently isolated from fresh grapes where it may be involved in Aspergillus bunch rot (Valero et al., 2005; Lasram et al., 2007; Fredj et al., 2007; Ponsone et al., 2007; Iamanaka et al., 2005). It is one species causing thread mould spoilage of cheese (Hocking and Faedo, 1992).

Aspergillus niger is among the most common fungi isolated from nuts, especially peanuts, and also pecans (see Pitt and Hocking, 1997), pistachios, almonds and walnuts (Bayman et al., 2002a). It has been isolated from cashews (Adebajo and Diyaolu, 2003), kola nuts (Adebajo, 1994), coconut (Zohri and Saber, 1993) and copra (Srinivasulu et al., 2003). Cereals and oilseeds are also frequent sources, especially maize (see Pitt and Hocking, 1997; also Magnoli et al., 2007a for a recent review), corn snacks (Caldas et al., 2002) and also barley (see Pitt and Hocking, 1997; Soldevilla et al., 2005; Lugauskas et al., 2006; Viswanath et al., 2006), soybeans (Aziz et al., 2006), rapeseed (Magan et al., 1993; Ahamad et al., 2003) and rape oil (Mondal and Nandi, 1984), sorghum (Navi et al., 2002 and see Pitt and Hocking, 1997), stored and parboiled rice (Aziz et al., 2006; Taligoola et al., 2004 and see Pitt and Hocking, 1997), blackgram (Ahmad, 1993), sunflower seeds (Begum et al., 2003), chickpeas (Ahmad and Singh, 1991) and pigeon peas (Maximay et al., 1992).

Meat products are another common source (Farghaly et al., 2004; Hammad et al., 2006 and see Pitt and Hocking, 1997). Other records include cheese (Sinigaglia et al., 2004; Hayaloglu and Kirbag, 2007 and see Pitt and Hocking, 1997), olives (Roussos et al., 2006), fresh vegetables (Lugauskas et al., 2005) and strawberries, rootstock snacks and a variety of tropical products (see Pitt and Hocking, 1997).

Aspergillus niger was commonly isolated during our study of Southeast Asian commodities. The highest contamination occurred in peanuts, maize and kemiri nuts. From Thailand, sorghum (58% of samples), cashews (53%) and copra (43%) contained *A. niger*, with overall infection rates of 7, 5 and 18%, respectively. Infections in pepper and spice from Indonesia and the Philippines were also quite high. Seventy eight per cent of coriander samples from Indonesia were infected with *A. niger*, with 18% infection overall.

Infection rates with *Aspergillus niger* were lower in commodities other than the nuts and oilseeds mentioned above. Philippine soybeans were exceptional, with an infection in 47% of samples. Otherwise, soybeans from the other countries, mung beans, black beans, cowpeas, paddy rice and milled rice all were infected at no more than 2% of all particles examined (Pitt et al., 1993, 1994, 1998a and our unpublished data).

References. Raper and Fennell (1965); Domsch et al. (1980); Al-Musallam (1980); Klich (2002).

Aspergillus niveus Blochwitz **Fig. 8.16**

Teleomorph: *Fennellia nivea* (B.J. Wiley & E.G. Simmons) Samson

Colonies on CYA 18–30 mm diam, plane or lightly sulcate, dense and velutinous; mycelium white,

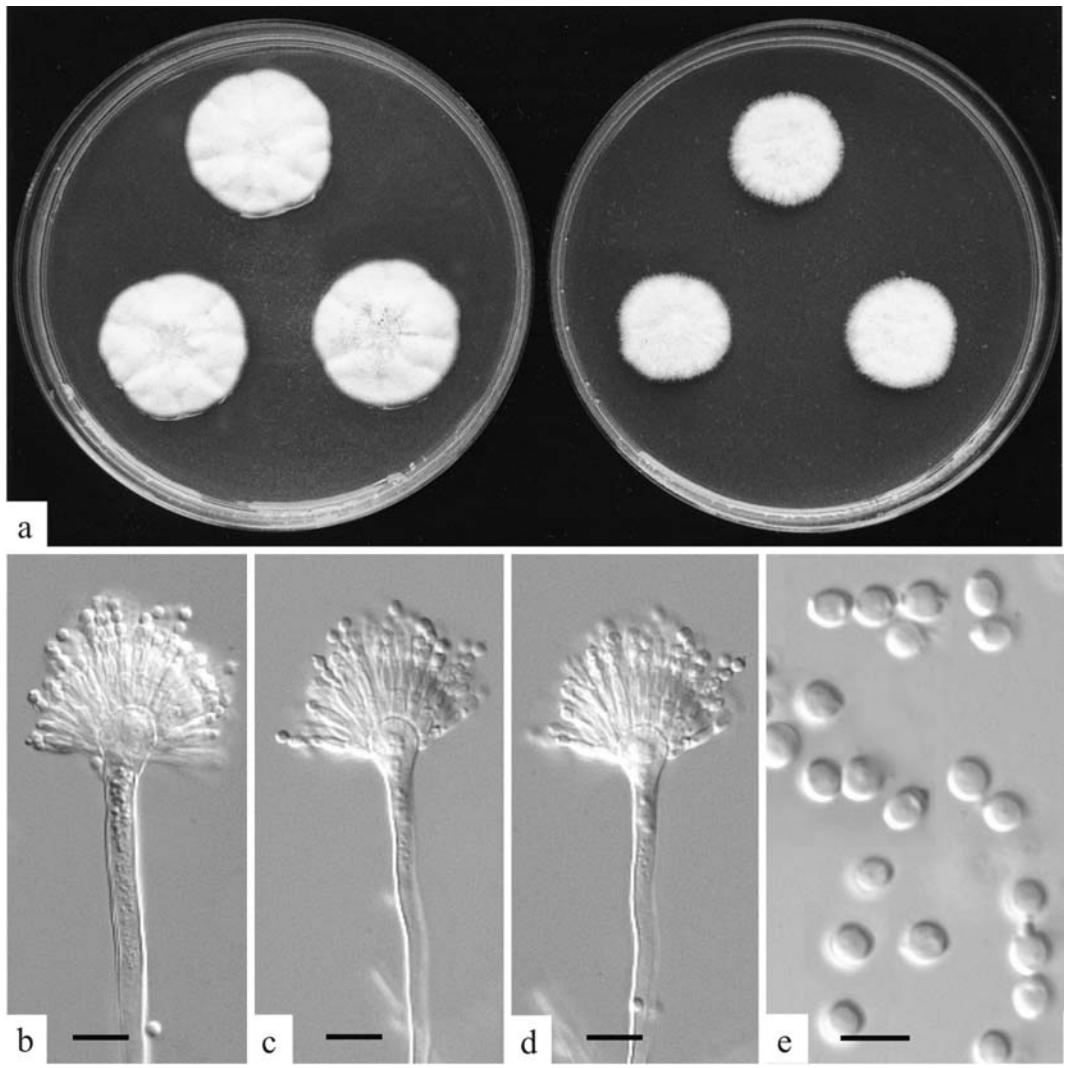


Fig. 8.16 *Aspergillus niveus*: **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b, c, d)** heads, bars = 10 μm; **(e)** conidia, bar = 5 μm

occasionally with bright yellow sectors; conidial heads closely packed, white to pale yellow; exudate clear, yellow brown or sometimes dull red; yellow soluble pigment sometimes present; reverse yellow brown to dark green. Colonies on MEA 18–30 mm diam, plane, similar to those on CYA but texture granular to floccose, exudate clear; reverse pale to dull yellow. Colonies on G25N 10–16 mm diam, dense to floccose, white; reverse pale to dull yellow. No growth at 5°C. At 37°C, colonies usually 30–45 mm diam, similar to those on CYA at 25°C, occasionally much smalle.

Cleistothecia *fide* Wiley and Fennell (1972) 80–130 μm diam, yellow, ascospores hyaline to yellow, 4.0–5.6 μm long, with an inconspicuous furrow, two low, longitudinal crests and spinose walls. Hülle cells sometimes present on CYA, spherical to ellipsoidal, 10–18 μm long, yellow. Conidiophores borne from surface hyphae, stipes usually 100–500 μm long, with uncoloured, smooth walls; vesicles hemispherical to spathulate, 8–15 μm diam, bearing crowded metulae and phialides over the upper half to two thirds; metulae and phialides 5–9 μm long; conidia spherical to

subspheroidal, 2.5–3.5 μm diam, with smooth to finely roughened walls, borne in radiate to loosely columnar heads.

Distinctive features. *Aspergillus niveus* is one of only two *Aspergillus* species with white or off-white conidia. It is distinguished from *A. candidus* by yellow conidial colours in age and more readily by vesicles which are fertile only over the upper half to two thirds, while those of *A. candidus* are fertile over the entire area.

Taxonomy. This species occasionally produces a teleomorph, which was described as *Aspergillus niveus* by Wiley and Fennell (1973), was provided with the teleomorph name *Emericella nivea* by Wiley and Simmons (1973) and transferred to *Fennellia* by Samson (1979).

Physiology. *Aspergillus niveus* grows strongly at higher temperatures, with a reported minimum of 11–13°C, an optimum near 36–42°C and a maximum at 47–48°C (Domsch et al., 1980). Water relations have not been studied.

Mycotoxins. A culture of *Aspergillus niveus* was toxic to ducklings (Scott, 1965). The toxicity of this species does not seem to have been further investigated, though Frisvad and Samson (1991) indicated that it may produce citrinin.

Ecology. The main habitat of *Aspergillus niveus* is soil in the tropics (Domsch et al., 1980) and reports from foods are rare. However, we isolated this species from peanuts in Thailand, the Philippines and Indonesia; from soybeans, sorghum and black rice in Thailand; and milled rice in Indonesia (Pitt et al., 1993, 1994, and 1998a). Pectinase enzymes isolated from *A. niveus* were reported to improve the fermentation of tea leaves when compared with a commercial enzyme preparation (Angayarkanni et al., 2002).

References. Raper and Fennell (1965); Wiley and Fennell (1973); Domsch et al. (1980), as *Emericella nivea*; Klich (2002).

Aspergillus ochraceus K. Wilh. Fig. 8.17

Colonies on CYA 40–55 mm diam, plane or sulcate, low and velutinous or lightly floccose; mycelium white; conidial heads closely packed, light yellow to golden yellow; sclerotia sometimes produced, white when young, later pink to purple; clear exudate sometimes present, some exuded from stipe walls; reverse greyish orange to brown. Colonies on MEA 40–55 mm diam, plane, similar to those on CYA but quite sparse; reverse pale yellow brown or slightly darker. Colonies on G25N 20–30 mm diam, plane, low and dense to deep and floccose, conidial production light to moderate, coloured as on CYA; reverse pale yellow or brown. No growth at 5°C. At 37°C, colonies up to 30 mm diam produced (Fig. 8.17).

Conidiophores borne from surface hyphae, stipes 1.0–1.5 mm long, with yellowish to pale brown walls, finely to conspicuously roughened; vesicles spherical, 25–50 μm diam, bearing tightly packed metulae and phialides over the entire surface; metulae 15–20 μm long; phialides 9–12 μm long; conidia spherical to subspheroidal, 2.5–3.5 μm diam, with smooth to finely roughened walls, borne in radiate heads when young, splitting into two or more broad columns with maturity.

Distinctive features. *Aspergillus ochraceus* produces yellow brown (ochre) conidia, borne on long stipes; vesicles bear metulae and phialides over the entire surface. *A. ochraceus* grows at 37°C, unlike the closely related species *A. westerdijkiae* and *A. steynii* (see below). Of the other species described here, *A. ochraceus* most resembles *A. wentii*: *A. ochraceus* grows more rapidly on CYA and MEA than does *A. wentii*, but less rapidly on G25N. Conidia of *A. wentii* are golden yellow, not ochre.

Taxonomy. Domsch et al. (1980) and Samson and Gams (1985) took up the name *Aspergillus alutaceus* Berk. and Curtis for this species as it predates *A. ochraceus*. However, this change did not meet with broad acceptance, both because of reluctance to change such a well known and apt epithet and because doubt existed as to whether these two species were synonymous. *A. ochraceus* was accepted in the list of Names in Current Use (Pitt and Samson, 1993). *A. ochraceus* is therefore the appropriate name for this species. In searches, the name *A. alutaceus* should also be checked in literature from about 1990 to 2000. Two closely related species, *A. westerdijkiae* Frisvad and Samson and *A. steynii* Frisvad and Samson (Frisvad et al., 2004), have recently been described (see Additional species at the end of this section).

Physiology. Because *Aspergillus ochraceus* was confused with *A. westerdijkiae* and *A. steynii* until recently, the physiological data reported for *A. ochraceus* sometimes refer to these other two

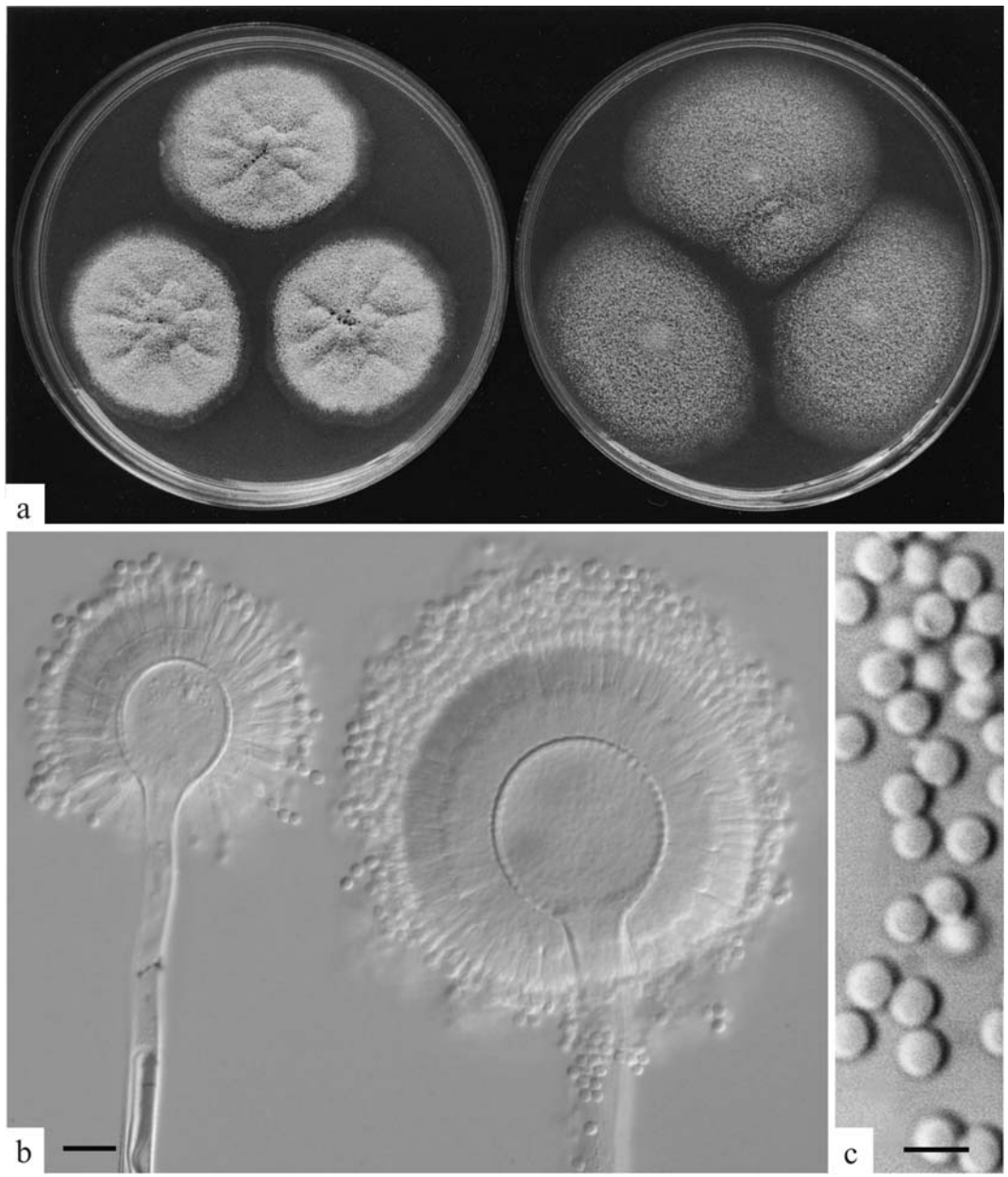


Fig. 8.17 *Aspergillus ochraceus* **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b)** heads, bar = 20 μm; **(c)** conidia, bar = 5 μm

species. In particular, *A. ochraceus* isolates grow quite strongly at 37°C, indicating a maximum for growth of at least 40°C. The other two species do not grow at 37°C. It is not clear which species were studied when Palacios-Cabrera et al. (2005) reported no growth by *A. ochraceus* at either 8 or 41°C on a selection of media.

It seems likely that all three species will have similar water relations, though it is unknown which species was studied in the reports that follow. Growth was reported down to 0.79 a_w on glucose/fructose media and down to 0.81 a_w on media based on NaCl (Pitt and Hocking, 1977). Pardo et al. (2006a) indicated germination occurred down to 0.80 a_w at 20 or

30°C and 0.85 a_w at 10°C. No germination occurred at 0.75 a_w on barley meal extract medium (Pardo et al., 2004a) or on green coffee extract agar (Pardo et al., 2005a). The optimum a_w for growth is 0.95–0.99 a_w (ICMSF, 1996a; Pardo et al., 2006a). *A. ochraceus* grew well between pH 3 and 10 and slowly at pH 2.2 (Wheeler et al., 1991).

The decimal reduction time (D_{10}) for *Aspergillus ochraceus* subjected to low dose gamma irradiation was given as 0.44 kGy on grain (O'Neill et al., 1991). Up to 4 kGy of gamma irradiation was necessary to prevent growth of *A. ochraceus* on barley (Szekely et al., 1991). Irradiation had no intrinsic effect on ochratoxin A production (Chelack et al., 1991).

In the presence of 20% O_2, *Aspergillus ochraceus* was able to grow in 60% but not 80% CO_2 (Paster et al., 1983). Germ tube elongation was significantly inhibited by 50% CO_2 at 0.90–0.95 a_w and both growth and ochratoxin A production were affected by 25 and 50% CO_2, regardless of the water activity (Cairns-Fuller, 2004).

Like *Aspergillus flavus* and *A. parasiticus*, *A. ochraceus* and related species produce a bright orange reverse on AFPA, though not the same pigment (Assante et al., 1981). Growth is much slower: reading results from AFPA plates before 5 days will avoid false positives from *A. ochraceus* and related species (Pitt et al., 1983). *A. niger* has been reported to inhibit growth of *A. ochraceus* (Paster et al., 1992), but this does not appear to have practical applications.

Mycotoxins. The toxigenic nature of *Aspergillus ochraceus* was discovered not as the result of a natural toxicosis but in laboratory studies on toxigenic fungi (van der Merwe et al., 1965). Three toxins were found: the major one was named ochratoxin A, while minor components of lower toxicity were named ochratoxins B and C. Subsequent work has shown that only a minority of *A. ochraceus* isolates are toxigenic (see Miyaki et al., 1970; Ciegler, 1972; Cvetnic, 1994; Chourasia, 1995) and that other species closely related to *A. ochraceus, A. westerdijkiae* and *A. steynii* are the main producers of ochratoxin A (Frisvad et al., 2004).

It is now believed that the well-documented occurrence of ochratoxin A in temperate climate foods results from growth of *Penicillium verrucosum*. The properties of ochratoxin A are described under that species. *Aspergillus ochraceus* and related species produce most of the ochratoxin A found in coffee, but it is likely that most of the ochratoxin A produced in tropical commodities is due to the growth of *A. carbonarius*. Possible involvement of *A. ochraceus* in the production of ochratoxin under tropical conditions should not be overlooked, however.

Pardo et al. (2004b) found optimum ochratoxin A production by *Aspergillus ochraceus* on barley grains at 0.98–0.99 a_w and 25–30°C. Similar results were observed for maximum ochratoxin A production on irradiated green coffee beans and the highest yields occurred at 20°C (Pardo et al., 2005b). The minimum water activity allowing ochratoxin A formation was reported to be 0.85 a_w on sterilised coffee beans (Taniwaki et al., 1999; Pardo et al., 2005b); however, no ochratoxin A was produced at this water activity on irradiated barley grains or on grapes (Pardo et al., 2004b, c and see Pardo et al., 2006a for a recent review).

The effect of pH and trace metals on the production of ochratoxin A by *Aspergillus ochraceus* was examined in liquid media (Mühlencoert et al., 2004). At 25°C, *A. ochraceus* synthesised ochratoxin A (up to 2 mg/l) at pH values ranging from 5.5 to 8.5, and no significant difference was observed in the amount of ochratoxin A produced at pH values within this range. Outside this pH range, however, no ochratoxin A was detected. At pH 6.5, variations in trace metal concentrations such as Zn and Fe affected ochratoxin A production (Mühlencoert et al., 2004).

From a study of the growth of *Aspergillus ochraceus* on cereals (maize and wheat) and oilseeds (peanuts, rapeseed and soybeans), it was concluded that ochratoxin production was favoured more by peanuts and soybeans than the other substrates studied (Madhyastha et al., 1990). Pardo et al. (2006b) also reported that the nature of the substrate influenced toxin production by *A. ochraceus*. The highest ochratoxin A yields were produced on green coffee beans (>2 mg/g), barley grains (ca. 1 mg/g), yeast extract sucrose medium (13.9 μg/g) and grape berries (ca. 3 ng/g). The toxigenic potential of the strains studied was independent of their origin (Pardo et al., 2006b).

Ochratoxin A production by *Aspergillus ochraceus* was similar in atmospheres with 5 or 1% O_2 as in air. In the presence of 20% O_2, low levels of CO_2 had no influence on ochratoxin A production, but it was completely inhibited by 30% CO_2 in the

presence of 20% O_2 (Paster et al., 1983). Sorbic acid had little effect on ochratoxin A production at 25°C and pH 5.0 (Bullerman, 1985).

The effect of heat on ochratoxin A produced in wheat by a strain of *Aspergillus ochraceus* was reported by Boudra et al. (1995). For dry wheat, 50% decomposition occurred in 700, 200, 12 and 6 min at 100, 150, 200 and 250°C, respectively. The addition of 50% water increased decomposition rates at 100 and 150°C, but not at higher temperatures (Boudra et al., 1995).

Aspergillus ochraceus also produces penicillic acid (Frisvad et al., 2004), a mycotoxin of lesser importance described under *Penicillium commune*. It is produced by *A. ochraceus* between about 10 and 35°C and down to 0.81 a_w (ICMSF, 1996a). Sorbic acid (500 mg/kg) had little effect on penicillic acid production at 15–35°C, but 1,000 mg/kg markedly reduced production at 15 and 25°C (Gourama and Bullerman, 1988).

Emodin is also produced by *Aspergillus ochraceus* or a sibling species and was found at low levels in chestnuts on the Canadian market (Overy et al., 2003). See *A. wentii* for details of emodin.

Ecology. An important source of *Aspergillus ochraceus* and the closely related *A. westerdijkiae* and *A. steynii* is green coffee beans (Mislivec et al., 1983; Tsubouchi et al., 1985; Téren et al., 1997; Urbano et al., 2001; Bucheli and Taniwaki, 2002; Martins et al., 2003; Taniwaki et al., 2003; Suárez-Quiroz et al., 2005) which may result in ochratoxin A contamination of roasted and instant coffee (Jørgensen, 2005; Clark and Snedeker, 2006).

Aspergillus ochraceus and related species have also been isolated from a wide range of other foods, but are more common in dried and stored foods than elsewhere. Stored foods from which they have been isolated include soybeans (Pacin et al., 2002; Aziz et al., 2006), pepper (Gatti et al., 2003) and dried fruit (Iamanaka et al., 2005). Other foods include smoked dried fish, salted dried fish, dried beans, biltong, chickpeas, rapeseed, sesame seeds before and after fermentation, blackgram seeds and rootstock snacks (see Pitt and Hocking, 1997). Nuts are also a major source, including peanuts (Aziz et al., 2006), pecans, pistachios, hazelnuts, walnuts and betel nuts (see Pitt and Hocking, 1997).

Aspergillus ochraceus and related species have been reported from cereals and cereal products, but rather infrequently. Recent records include rice (Pacin et al., 2002) and barley (Medina et al., 2006), though levels in Europe are lower than for some other fungal species (Sepitkova and Jesenska, 1986), maize (Sepulveda and Piontelli, 2005; Magnoli et al., 2006b, 2007a for a recent review) and maize meal (Souza et al., 2003), corn snacks (Adebajo et al., 1994), wheat (Aziz et al., 2006; Hajjaji et al., 2006), flour (Halt et al., 2004) and bran (Dragoni et al., 1979).

Aspergillus ochraceus and related species were the major contaminant in Bhutanese cheese (Sinha and Ranjan, 1991). They have also been reported from cheese in the temperate zone (see Pitt and Hocking, 1997). Electron beam irradiation of 0.42 kGy enabled a greatly increased shelf life for refrigerated Cheddar cheese surface inoculated with *A. ochraceus* (Blank et al., 1992). Other less common sources include spices (Almela et al., 2007), cassava (Souza et al., 2003), black olives and processed meats (see Pitt and Hocking, 1997).

We isolated *Aspergillus ochraceus* and related species from a variety of Southeast Asian commodities, including maize, peanuts, soybeans and other beans, cashews and sorghum. Its presence or absence in any sample probably related to length of storage rather than geographical location or other factors (Pitt et al., 1993, 1994, and 1998a).

Rots in garlic have been reported to be caused by *Aspergillus ochraceus*, but the species responsible is *A. alliaceus* Thom and Church (Snowdon, 1991).

Additional species. *Aspergillus westerdijkiae* Frisvad and Samson (Frisvad et al., 2004) is closely related to *A. ochraceus* with perhaps slightly faster growth rates on CYA at 25°C. No growth occurs at 37°C and sclerotia, which are sparsely produced, remain white to cream with age. Conidia of *A. westerdijkiae* are consistently finely roughened, compared with smooth conidia in *A. ochraceus*. *A. westerdijkiae* produces large amounts of ochratoxin A. It also produces penicillic acid, xanthomegnin, viomellein and vioxanthin. Some physiological studies conducted on isolates identified as *A. ochraceus* undoubtedly relate to *A. westerdijkiae* (Frisvad et al., 2004). Because of its ability to synthesise high levels of ochratoxin A, *A. westerdijkiae* is of importance in commodities such as coffee, cereals and beverages.

Additional species. *Aspergillus steynii* Frisvad and Samson (Frisvad et al., 2004) is also closely related to *A. ochraceus*. The absence of growth at 37°C, ellipsoidal conidia and pale yellow conidia distinguish this species from *A. ochraceus*. Like *A.*

westerdijkiae, *A. steynii* produces copious amounts of ochratoxin A, along with penicillic acid and xanthomegnins. Its broad distribution in commodities such as coffee, soybeans and rice makes *A. steynii* an important species (Frisvad et al., 2004).

References. Raper and Fennell (1965); Domsch et al. (1980), under *Aspergillus alutaceus*; Christensen (1982); Klich (2002) (all under *A. ochraceus*); Frisvad et al. (2004) (for *A. ochraceus, A. westerdijkiae* and *A. steynii*).

Aspergillus parasiticus Speare **Fig. 8.18**

Colonies on CYA 50–70 mm diam, plane, low, dense and velutinous; mycelium inconspicuous, white; conidial heads in a uniform, dense layer, dark yellowish green (29-30D-F6-8); sclerotia occasionally produced; reverse uncoloured or brown. Colonies on MEA 50–65 mm diam, generally similar to those on CYA but usually less dense and with reverse uncoloured. Colonies on G25N

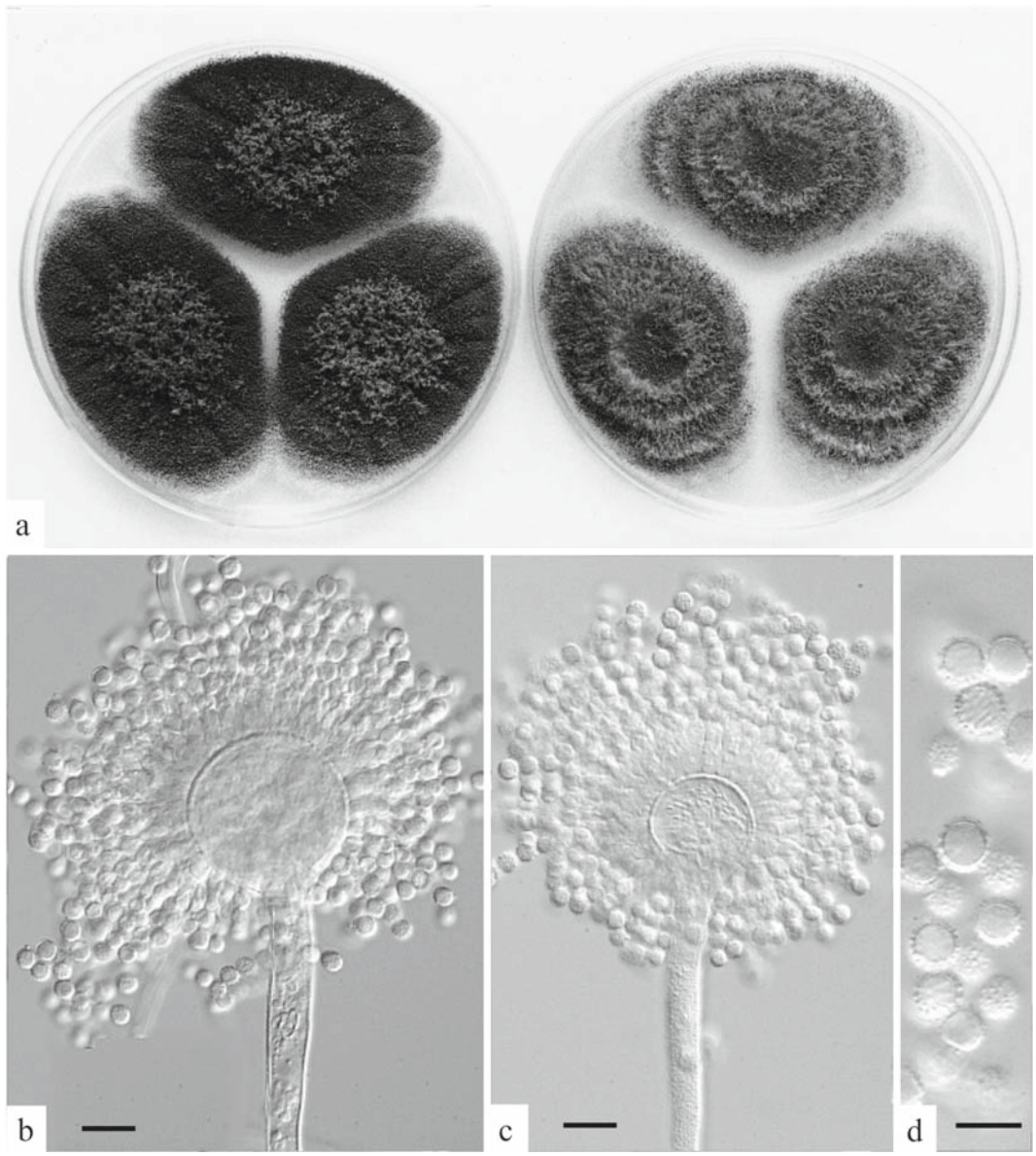


Fig. 8.18 *Aspergillus parasiticus* **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b, c)** heads, bars = 10 µm; **(d)** conidia, bar = 5 µm

20–40 mm diam, plane, low and velutinous, generally similar to those on CYA; reverse uncoloured, yellow or brown. No growth at 5°C. At 37°C, colonies covering the available area, similar to those on CYA at 25°C, or with conidia deeper green or brownish; reverse pale.

Sclerotia occasionally produced, white at first, becoming black, spherical, 400–800 μm diam. Teleomorph not known. Conidiophores borne from subsurface or surface hyphae, stipes 250–500 μm long, with colourless or pale brown, smooth walls; vesicles spherical, 20–35 μm diam, fertile over three quarters of the surface, mostly bearing phialides only, but in some isolates up to 20% of heads bearing metulae as well; phialides 7–11 μm long; conidia spherical, mostly 4.0–6.0 μm diam, with distinctly roughened walls, usually borne in radiate heads.

Distinctive features. *Aspergillus parasiticus* shares with *A. flavus* fast growth at both 25 and 37°C and distinctive bright yellow green conidial colours. *A. parasiticus* differs from *A. flavus* by the production of spherical conidia with heavy, rough walls, while *A. flavus* conidia are more variable in shape and have relatively thin, usually finely roughened walls.

Taxonomy. Production of phialides only was the principal character used by Raper and Fennell (1965) to distinguish *Aspergillus parasiticus* from *A. flavus*. Klich and Pitt (1985, 1988) showed that this criterion was unsatisfactory, as some *A. flavus* isolates produce very few metulae. Careful study showed several differences between the two species, the most useful of which are mentioned above. The criteria used here provide a 100% correlation with mycotoxin production (see below).

Kurtzman et al. (1986b) reduced *Aspergillus parasiticus* to the status of subspecies, as *A. flavus* subspecies *parasiticus* (Speare) Kurtzman et al., but as discussed under *Aspergillus flavus,* compelling reasons exist for maintaining distinct species names for these taxa, as has been done here. *A. sojae*, reduced to the status of variety by Kurtzman et al. (1986b), appears to be a domesticated form of *A. parasiticus*. As with *A. oryzae, A. sojae* is widely used in food fermentations, and recognition of it as a separate species is a practical necessity. Random amplification of polymeric DNA (RAPD) has been used to distinguish between *A. parasiticus* and *A. sojae* (Yuan et al., 1995).

Physiology. A number of earlier studies reported to be on the physiology of *Aspergillus flavus* have used misidentified strains and have really dealt with *A. parasiticus* (Pitt, 1993b). These two species are physiologically very similar, so that the studies reported under *A. flavus* are probably equally applicable to *A. parasiticus*. Growth of *A. parasiticus* has been reported over the range 12–42°C, with an optimum at 32°C (ICMSF, 1996a). A minimum a_w for growth of 0.82 at 25°C, 0.81 at 30°C and 0.80 at 37°C, similar to *A. flavus*, has been reported (Pitt and Miscamble, 1995). Data from that paper were used to provide a predictive model for *A. parasiticus* growth in relation to a_w and temperature (Gibson et al., 1994). Growth of *A. parasiticus* occurred over the pH range 2.4–10.5 at 25, 30 and 37°C, but growth did not occur at pH 2.2 at 25°C. Optimal growth occurred over a broad range from pH 3.5–8, with growth at pH 10.5 still more than half that under optimal conditions (Wheeler et al., 1991). High levels of sorbic acid (1,000 mg/kg) were needed to inhibit growth of *A. parasiticus* on synthetic media at pH 5 (Bullerman, 1983).

Conidia of *Aspergillus parasiticus* have a low heat resistance: a D_{55} up to 9 min at pH 7 in phosphate buffer (Doyle and Marth, 1975a). However, values were much higher if a_w was reduced: a D_{55} of 230 min in 16% NaCl (0.90 a_w), 210 min in 60% glucose (0.85 a_w) and 200 min in 60% sucrose (0.90 a_w) (Doyle and Marth, 1975b; ICMSF, 1996a). D values for the effect of gamma irradiation on conidia of *A. parasiticus* ranged from 0.3 to 0.6 kGy (ICMSF, 1996a).

Like *Aspergillus flavus*, *A. parasiticus* colonies exhibit a brilliant orange yellow reverse colouration after 42–48 h incubation on AFPA (Pitt et al., 1983).

Mycotoxins. The important differences in mycotoxin production between *Aspergillus parasiticus* and *A. flavus* are that *A. parasiticus* produces G as well as B aflatoxins, that *A. parasiticus* isolates often produce aflatoxins in much higher concentrations (Pitt, 1993b) and that nontoxigenic *A. parasiticus* strains are rare (Horn et al., 1994; Tran-Dinh et al., 1999). *A. parasiticus* does not produce cyclopiazonic acid (Vaamonde et al., 2003; Horn, 2003; Frisvad et al., 2005a) but does produce kojic acid, a metabolite of low toxicity now finding wide use in medicine and cosmetics (El-Aasar, 2006).

The conditions under which *Aspergillus parasiticus* produces aflatoxins are similar to those reported for *A. flavus*. The best available figures indicate production over the temperature range 12–40°C, down to 0.86 a_w, and over the pH range 3–8 or higher (ICMSF, 1996a). The ratio of aflatoxin G_1 production relative to B_1 on a rice substrate was higher at lower temperatures (at 15–18°C, G_1:B_1 was 1.3–1.5) and much reduced at high temperatures (28°C, 0.24 and 32°C, 0.06) (Sorenson et al., 1966; ICMSF, 1996a). The G_1:B_1 ratio was up to 2.5 at 15°C on peanuts (Diener and Davis, 1967).

In the presence of 20% O_2, at least 60% CO_2 was needed to prevent production of aflatoxins on a moist peanut substrate at 30°C, though the production in 60% CO_2 was only 10% of that in air (Landers et al., 1967). In reduced levels of O_2 in N_2, 5% O_2 caused aflatoxin production to be reduced to 30% of that in air, while in 1% O_2 production was only 1% of that in air (Landers et al., 1967).

Other aspects of aflatoxins, including toxicity and control, are discussed under *A. flavus*.

Ecology. Records of *Aspergillus parasiticus* from foods are relatively rare. Although *A. parasiticus* is certainly widely distributed in soils and foodstuffs in the United States, Latin America, South Africa, India and Australia, it is essentially unknown in Southeast Asia (Pitt et al., 1993, 1994, and 1998a).

Like *Aspergillus flavus*, *A. parasiticus* is a tropical and subtropical species, less prevalent in warm temperate zones and rare in the cool temperate regions of the world. Its absence from Southeast Asia indicates a limited distribution. In soils, populations of *A. parasiticus* are generally associated with peanut cultivation in the United States and Australia (Carter et al., 1998; Horn, 2003). This species has only been reported in low densities in corn fields (Horn, 2003). The most important food source is peanuts, in which we believe *A. parasiticus* is endemic, but about which reliable information is surprisingly limited. Klich and Pitt (1988) reported studying isolates from peanuts from the United States, Australia and Uganda. This species has been reported in peanuts in Argentina (Vaamonde et al., 2003) and Botswana (Mphande et al., 2004). Isolations of *A. parasiticus* from Australian peanuts are now more numerous than those of *A. flavus* (our data). Other types of nuts may also be infected: hazelnuts and walnuts, pistachios and pecans (see Pitt and Hocking, 1997).

Maize is perhaps a source (Aja-Nwachukwu and Emejuaiwe, 1994), but we believe that *Aspergillus parasiticus* is much less common on this commodity than *A. flavus*. A recent survey of Italian maize revealed that most contamination was due to *A. flavus* and *A. parasiticus* occurred in low frequency (Giorni et al., 2007). *A. parasiticus* has been reported at low frequency in rice and other rice derivatives (Sales and Yoshizawa, 2005b). Other reported sources include soybeans (Vaamonde et al., 2003), processed meats (El-Tabiy, 2006), black pepper (Gatti et al., 2003; Mandeel, 2005) as well as rootstock snacks, herbal drugs, amaranth seeds and pearl millet (see Pitt and Hocking, 1997).

References. Raper and Fennell (1965); Domsch et al. (1980); Christensen (1981); Klich and Pitt (1988); Klich (2002).

Aspergillus penicillioides Spegazzini Fig. 8.19

Colonies on CYA up to 5 mm diam, sometimes only microcolonies, of white mycelium only. Growth on MEA usually limited to microcolonies, occasionally colonies up to 5 mm diam, similar to those on CYA. Colonies on G25N 8–15 mm diam, plane or centrally raised, sometimes sulcate or irregularly wrinkled, texture velutinous or lightly floccose; mycelium usually inconspicuous, white; conidial production moderate, coloured dull green to dark green; reverse pale to dark green. No growth at 5 or 37°C on CYA.

Colonies on CY20S varying from microcolonies up to 10 mm diam, similar to those on CYA, occasionally some dull green conidial production but conidiophores poorly formed; reverse pale. Colonies on MY50G 10–16 mm diam, plane or umbonate, relatively sparse, velutinous to floccose; conidial production moderate, greyish green to dull green; reverse pale.

Conidiophores borne from surface or aerial hyphae, showing optimal development on G25N, stipes (150–)300–500 μm long, sometimes sinuous, with colourless, thin, smooth walls, enlarging gradually from the base, then rather abruptly to pyriform or spathulate vesicles; vesicles mostly 10–20 μm diam, usually fertile over two thirds of the area, bearing phialides only; phialides 8–11 μm long; conidia borne as ellipsoids, at maturity ellipsoidal to subspheroidal, 4.0–5.0 μm diam, with spinose walls, typically borne in radiate heads, uncommonly in loose columns also.

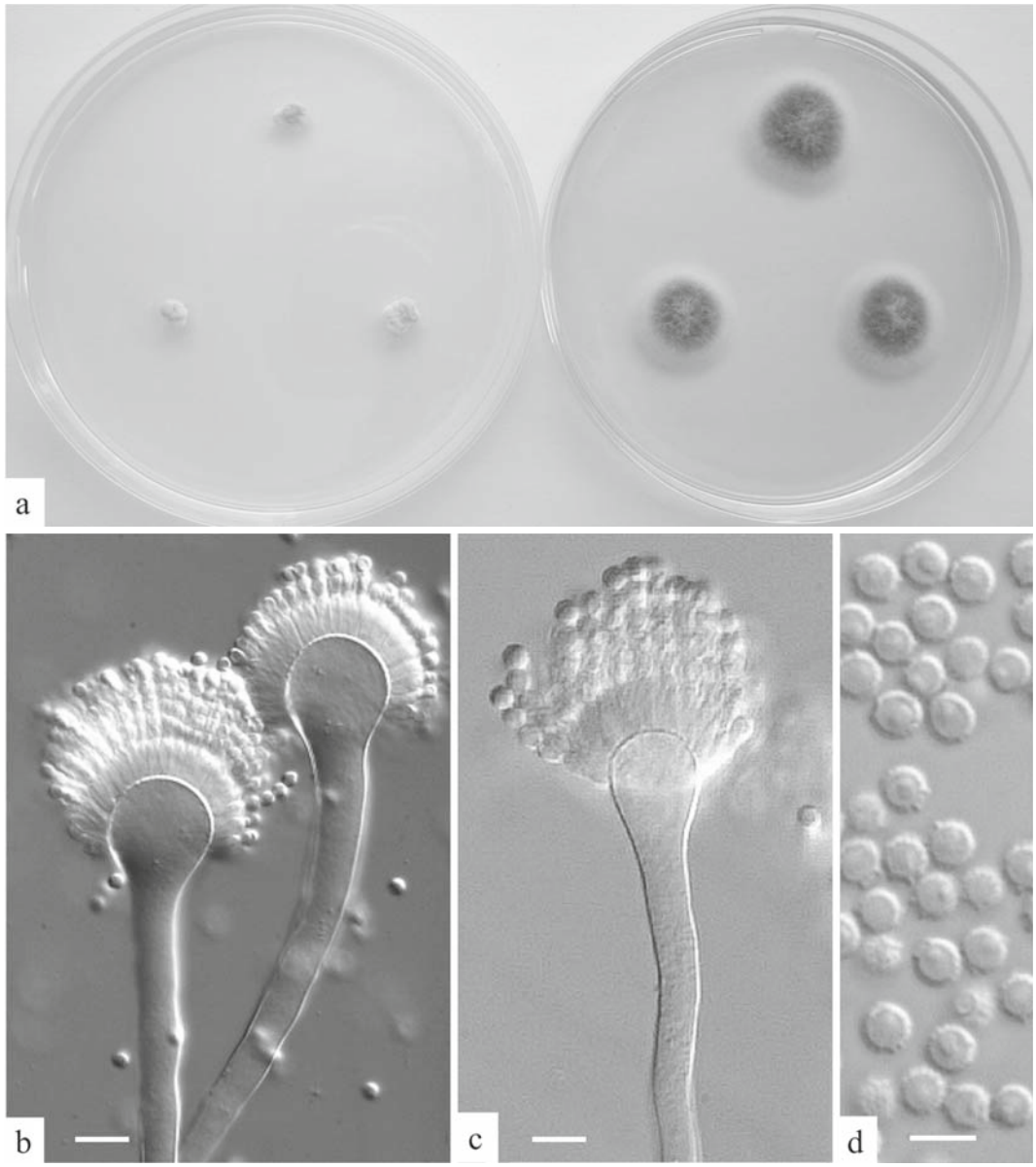


Fig. 8.19 *Aspergillus penicillioides* **(a)** colonies on CYA and MY50G, 7 days, 25°C; **(b, c)** heads, bars = 10 μm; **(d)** conidia, bar = 5 μm

Distinctive features. In common with *Aspergillus restrictus, A. penicillioides* grows very slowly under all standard conditions and produces green conidia. It differs from *A. restrictus* by very weak growth on CYA and MEA, by forming radiate conidial heads from spathulate vesicles, fertile over more than the upper half; and by bearing conidia as ellipsoids, which usually separate in liquid mounts.

Physiology. As is clear from the description above, this species grows very poorly at high a_w. The optimal a_w for growth is 0.89 at 30°C in glucose/fructose media of pH 5.5 (Gock et al., 2003) or 0.91–0.93 at 25°C on media containing either glucose/fructose or NaCl, at pH 6.5 (Andrews and Pitt, 1987). At 25°C, *Aspergillus penicillioides* is capable of germination but not growth at 0.70 a_w and pH

4.5–5.5 in media containing glucose/fructose (Gock et al., 2003). No germination is observed at this a_w at pH values above 5.5 at 25, 30 or 37°C. On NaCl-based media, the limit is 0.75 a_w (Andrews and Pitt, 1987). The minimum at 20, 30 and 34°C is higher, 0.76–0.79 a_w on glucose/fructose and 0.80–0.84 on NaCl. At 37°C, minimum figures are 0.79 and 0.88 a_w, respectively (Wheeler et al., 1988b). In natural substrates, *A. penicillioides* is capable of growth at much lower water activities: we have reproducible evidence of its ability to multiply at 0.68 a_w in wheat and pet food within 6 months (our unpublished observations).

Growth temperatures have not been accurately reported: from the above, growth does not occur at 37°C on CYA, but occurs readily at this temperature at lower a_w (Wheeler et al., 1988b; Gock et al., 2003). Growth occurs down to 15°C or lower, at 0.95–0.90 a_w at least (Wheeler et al., 1988b).

Mycotoxins. This species has not been reported to produce mycotoxins.

Ecology. Reports of *Aspergillus penicillioides* in foods are quite rare, primarily because it does not grow on the media commonly used for fungal isolation and enumeration. At best, development on DRBC or other high water activity isolation media is poor. Greatly improved results can be obtained if a low a_w medium such as DG18 is used (Hocking, 1981). It is also true that this species has frequently been misidentified as *A. restrictus*. For example, *A. restrictus* was reported to be a major cause of loss of germinability in wheat stored just above the safe moisture content (Tuite and Christensen, 1957; Christensen, 1963). In fact the species responsible is *A. penicillioides*.

In this laboratory, we have isolated *Aspergillus penicillioides*, often in very high numbers, from a wide variety of foods, including flour, dried fruit and dried fish, and from spices, including pepper and dried chillies. Other reported isolations have come from milled rice (Taligoola et al., 2004), fermented and cured meats, cocoa and peanuts (see Pitt and Hocking, 1997) and salted dried fish (Nketsia-Tabiri et al., 2003; Gräu et al., 2007). This species was quite competitive at 30°C on glucose media, but was less so on NaCl media or salt fish, or at other temperatures (Wheeler and Hocking, 1993).

From Southeast Asian commodities, we isolated *Aspergillus penicillioides* quite frequently. Although the proportion of samples infected was low, individual samples often showed high infection rates, no doubt reflecting prolonged storage at water activities just above safe limits. *A. penicillioides* was commonly isolated from soybeans in Thailand and Indonesia, where 6 and 17% of samples were contaminated, respectively. Black pepper from the Philippines showed up to 60% infection in contaminated samples and 2% infection in peppercorns overall. Lower levels of infection were found in paddy and milled rice, various other kinds of beans including mung beans and peanuts (Pitt et al., 1993, 1994, 1998a and our unpublished data).

References. Raper and Fennell (1965); Pitt and Samson (1990c); Klich (2002).

Aspergillus restrictus G. Sm. Fig. 8.20

Colonies on CYA 6–12 mm diam, sulcate or wrinkled, low, dense and velutinous; mycelium inconspicuous, white; conidial heads often poorly formed, sparse to numerous, in the latter case dull green; reverse pale to very dark green. Colonies on MEA 6–12 mm diam, occasionally smaller, similar to those on CYA or centrally raised, conidial production heavy but heads poorly formed, coloured dull green to dark green; reverse usually pale. Colonies on G25N 10–15 mm diam, plane or umbonate, usually similar to those on MEA, but heads well formed; reverse sometimes dark green. No growth at 5 or 37°C on CYA .

Colonies on CY20S 16–20 mm diam, generally similar to those on G25N apart from slightly more rapid growth. Colonies on MY50G 12–16 mm diam, plane or umbonate, with aerial growth and conidial production usually sparse, coloured greyish green to dull green; reverse pale.

Conidiophores borne from surface hyphae, developing optimally on CY20S, stipes 75–200 μm long, sometimes sinuous, with colourless, thin, smooth walls, enlarging from the base gradually then abruptly to pyriform vesicles; vesicles 10–18 μm diam, fertile over the upper hemisphere or less, bearing phialides only; phialides crowded, 8–10 μm long; conidia borne as cylinders, in long, appressed columns, adhering in liquid mounts, when mature nearly cylindrical to doliiform (barrel shaped), 4.0–6.0 μm long, with rough walls, borne in long columns under favourable growth conditions.

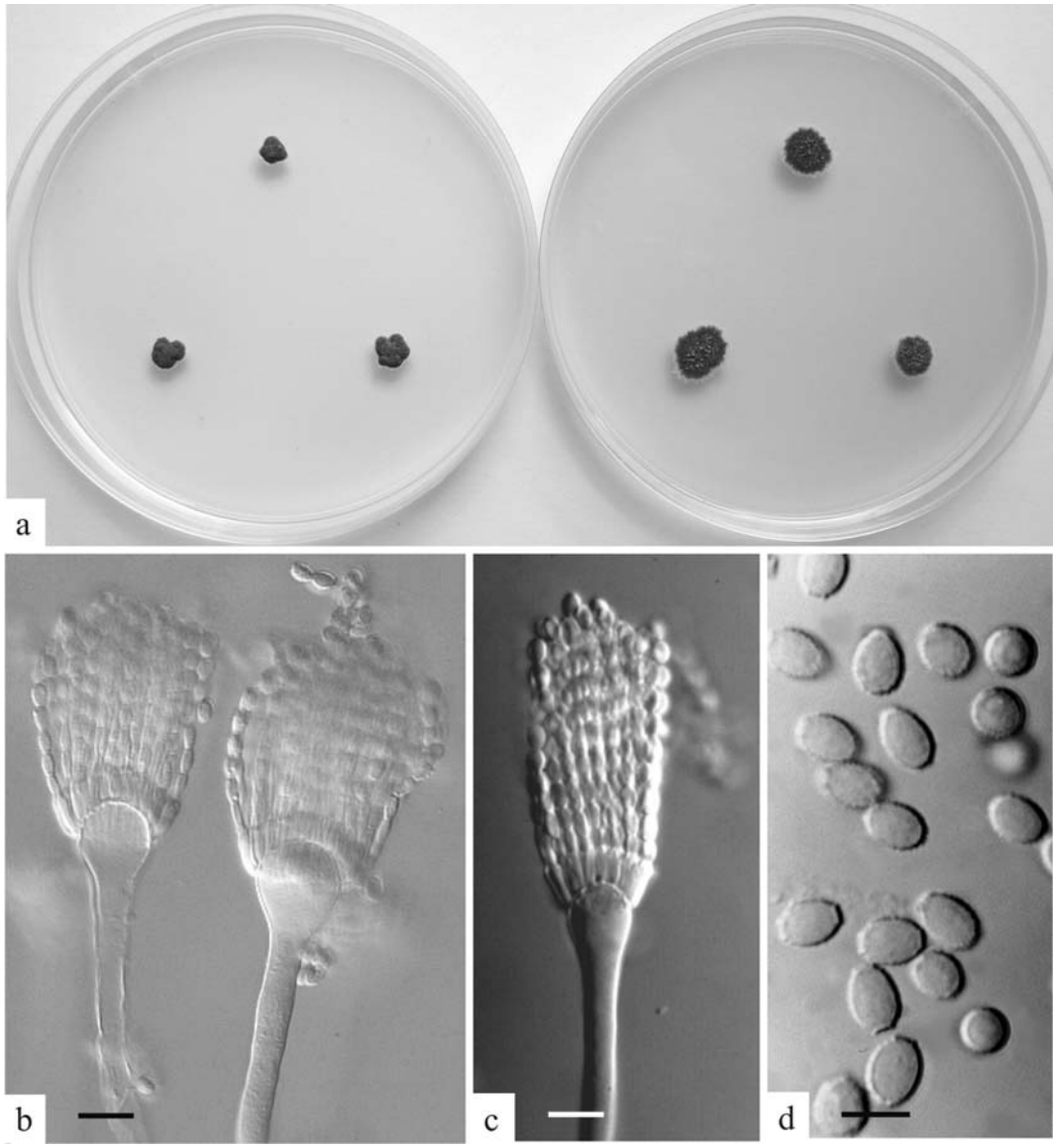


Fig. 8.20 *Aspergillus restrictus* **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b, c)** heads, bars = 10 μm; **(d)** conidia, bar = 5 μm

Distinctive features. In common with *Aspergillus penicillioides, A. restrictus* grows very slowly under all conditions and produces green conidia. It differs from *A. penicillioides* by vesicles which are fertile over the upper half or less and by conidia borne as cylinders and adhering in long columns, usually persisting in liquid mounts. *A. restrictus* grows more rapidly than *A. penicillioides* on CY20S.

Physiology. Smith and Hill (1982) reported that the temperature range for growth of *Aspergillus restrictus* was minimum 9°C, optimum 30°C and maximum 40°C. Growth of this species has been observed down to 0.75 a_w (Snow, 1949; Pelhate, 1968). This species grows strongly in 1% O_2 but not at all in 0.1% O_2 (Hocking, 1990).

Mycotoxins. This species has not been reported to produce mycotoxins.

Ecology. Considering the slow growth rate of this species, and its inconspicuous habit, *Aspergillus restrictus* has been isolated from foods quite frequently. Most records have come from dried foods: wheat, rice, maize, dried beans, pecans,

peppercorns, salted dried fish, cured meats, Japanese traditional noodles, spices, pepper, carriers for dough during breadmaking, dried prunes and health foods (see Pitt and Hocking, 1997). *A. restrictus* has been reported recently in cashew nuts (Adebajo and Diyaolu, 2003), coffee beans (Ahmad and Magan, 2003), cocoa beans (Wojcik-Stopczynska, 2006) and cured fish (Nketsia-Tabiri et al., 2003).

We isolated *Aspergillus restrictus* quite frequently from Southeast Asian commodities. As with *A. penicillioides,* the proportion of samples infected was often low, but with individual samples showing high infection rates, reflecting prolonged storage. The highest levels of *A. restrictus* found were in samples of red rice from Indonesia. Soybeans from the Philippines, Indonesia and Thailand showed low levels of infection (see Pitt and Hocking, 1997). Mung beans, red beans and cowpeas showed up to 24% infection in individual samples. Black pepper from the Philippines showed up to 100% infection in contaminated samples. Only 1 sample of kemiri nuts (of 20) from Indonesia was infected, but this species infected 30% of nuts in that sample. Low levels of infection occurred in paddy and milled rice, maize and peanuts (Pitt et al., 1993, 1994, 1998a and our unpublished data).

References. Raper and Fennell (1965); Domsch et al. (1980); Pitt and Samson (1990c); Klich (2002).

Aspergillus sydowii (Bainier & Sartory) Thom & Church Fig. 8.21

Colonies on CYA 18–30 mm diam, plane or lightly sulcate, low to moderately deep, dense and velutinous to somewhat floccose; mycelium white; conidial heads sparse to quite dense, dark turquoise to dark green, especially in marginal areas, centrally sometimes buff to orange brown; dark brown exudate and/or soluble pigment sometimes produced; reverse pale to orange brown. Colonies on MEA 16–25 mm diam, plane, dense, velutinous to lightly floccose; mycelium inconspicuous, white; conidial heads numerous, coloured like those on CYA; reverse pale. Colonies on G25N 15–20 mm diam, plane, dense; mycelium white; often heavily sporing, dull green, blue or brown; reverse pale or yellowish. Usually no growth at 5°C, occasionally germination. At 37°C, no growth or colonies up to 10 mm diam formed .

Conidiophores borne from surface or aerial hyphae, stipes 250–500 μm long, often sinuous, with heavy, pale brown, smooth walls; vesicles only slightly swollen, club shaped, 10–20 μm diam, bearing metulae and phialides over the upper two thirds to three quarters; smaller conidiophores also produced from aerial hyphae, ranging down to tiny monoverticillate penicilli; metulae 4–7 μm long; phialides 7–10 μm long; conidia spherical, 2.5–3.5 μm diam, with spinose walls, from the larger conidiophores borne in radiate heads.

Distinctive features. *Aspergillus sydowii* grows slowly at 25°C and often not at all at 37°C, produces heads with both metulae and phialides and blue conidia. Vesicles on the larger stipes are small and club shaped, and diminutive penicilli are also formed.

Taxonomy. The taxonomy of *Aspergillus versicolor* and related species, including *A. sydowii*, was reviewed and revised by Klich (1993).

Physiology. Closely related to *Aspergillus versicolor*, *A. sydowii* can be expected to have similar physiological properties. Snow (1949) reported 0.78 a_w to be the minimum for growth on a natural substrate; Wheeler and Hocking (1988) gave the same figure for growth on a glucose/fructose medium and 0.81 for growth on NaCl-based media. Only 0.7% of *A. sydowii* conidia survived heating at 50°C for 10 min (Pitt and Christian, 1970).

Mycotoxins. No significant mycotoxins have been reported from this species.

Ecology. Widely distributed, *Aspergillus sydowii* is a storage fungus. Most isolations have come from dried foods, including various nuts: peanuts, pistachios, coconut, hazelnuts, walnuts and pecans (see Pitt and Hocking, 1997); fermented milk (Ahmed and Adbel-Sater, 2003), coffee beans (Silva et al., 2003), dried fish, paddy rice, stored rice, soybeans, cured meats and biltong, dried beans, health foods, red pepper powder (see Pitt and Hocking, 1997) and spices (Ramaswamy et al., 2004). It appears to be relatively uncommon in cereals, but has been isolated from barley, wheat and flour (see Pitt and Hocking, 1997), maize and soy beans (Sepulveda and Piontelli, 2005). It has been reported as one cause of spoilage in fresh breadfruit (Omobuwajo and Wilcox, 1989).

Aspergillus sydowii was quite common in Southeast Asian commodities. We isolated it from 80% of pepper samples from Indonesia and 31% of samples from the Philippines. Levels in Indonesian

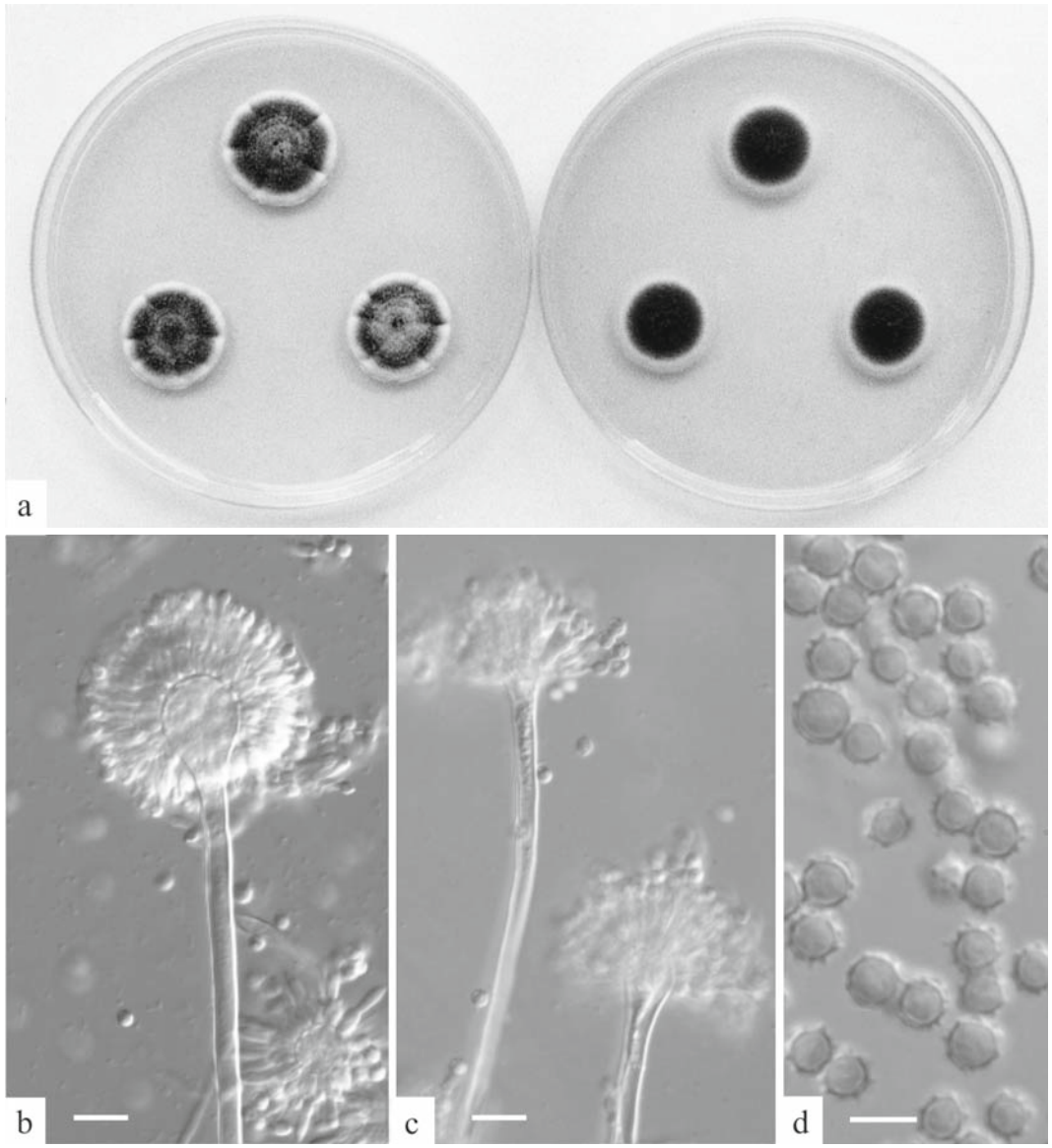


Fig. 8.21 *Aspergillus sydowii* **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b, c)** heads, bars = 10 μm; **(d)** conidia, bar = 5 μm

coriander were also high. It was significant (1–2% overall) in cashews and copra from Thailand; soybeans from the Philippines; and kemiri nuts, sorghum and black soybeans from Indonesia. It was also present at low levels in maize, peanuts, mung beans, paddy rice and milled rice from one country or more (Pitt et al., 1993, 1994, 1998a and our unpublished data).

References. Raper and Fennell (1965); Domsch et al. (1980); Klich (1993, 2002).

Aspergillus tamarii Kita — Fig. 8.22

Colonies on CYA 50–65 mm diam, plane, velutinous to lightly floccose; mycelium inconspicuous, white; conidial heads abundant, olive brown; reverse uncoloured. Colonies on MEA 55–65 mm diam, similar to those on CYA but relatively sparse and with olive conidia; reverse uncoloured. Colonies on G25N 35–40 mm diam, similar to those on CYA but coloured deep olive brown; reverse

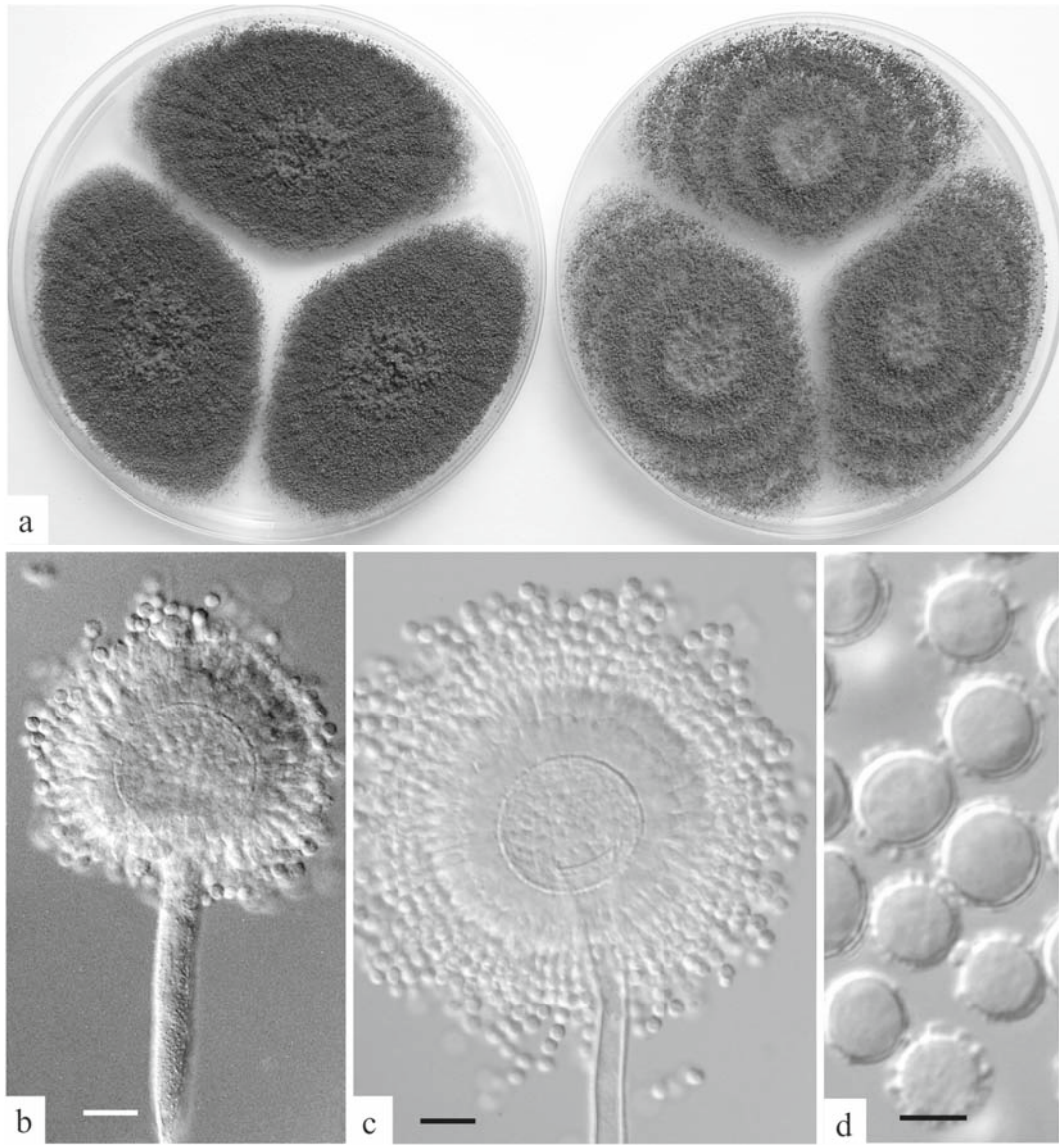


Fig. 8.22 *Aspergillus tamarii* **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b, c)** heads, bars = 20 μm; **(d)** conidia, bar = 5 μm

uncoloured. No growth at 5°C. At 37°C, colonies 50–65 mm diam, much more dense than on CYA at 25°C, low and velutinous, with conidia coloured coffee brown; reverse pale.

Conidiophores borne from subsurface or surface hyphae, stipes 300–1,500 μm long, colourless, usually with rough, thin walls; vesicles spherical to subspheroidal, 25–50 μm diam, fertile virtually all over, bearing both metulae and phialides or less commonly phialides alone; metulae 12–20 μm long; phialides 9–12 μm long; conidia spherical to subspheroidal, 5–8 μm diam, brown, with characteristic thick, rough to spiny walls, borne in radiate heads.

Distinctive features. *Aspergillus tamarii* shows an unmistakable resemblance to *A. flavus* and *A. parasiticus*. The main distinguishing features are that conidia of *A. tamarii* are coloured olive to brown on CYA and MEA and are larger, with thick, conspicuously roughened walls. On AFPA, *A. tamarii* produces a deep brown reverse colouration, in contrast to the orange yellow of *A. flavus* and *A. parasiticus*. This is a useful diagnostic aid.

Taxonomy. Domsch et al. (1980) revived the name *Aspergillus erythrocephalus* Berk. and Curtis for this species, but other authorities, e.g. Christensen (1981), maintained the use of *A. tamarii*. Examination of the type specimen of *A. erythrocephalus* (K.A. Seifert and R.A. Samson, unpublished) showed that it is distinct from *A. tamarii*. In the List of Names in Current Use (Pitt and Samson, 1993), *A. tamarii* is maintained as the correct name for this species.

Physiology. Less has been published on the physiology of *Aspergillus tamarii* than on *A. flavus*, but it appears from growth data that gross physiological behaviour of the two species, such as temperature relations, will be similar. Ayerst (1969) reported that *A. tamarii* was capable of growth down to 0.78 a_w at 33°C, a figure identical with that which he obtained for *A. flavus*. *A. tamarii* can degrade caffeine in coffee residues (Gutiérrez-Sánchez et al., 2003).

Mycotoxins. *Aspergillus tamarii* does not produce aflatoxins, but does produce cyclopiazonic acid (Dorner, 1983). This toxin is discussed in more detail under *P. camemberti*. *A. tamarii* may have been the cause of "kodua poisoning" from kodo millet seed (*Paspalum scrobiculatum*) in India (Rao and Husain, 1985). The alkaloid fumiclavine A was also found in kodo millet seed from that outbreak, and it may have contributed to the toxicity (Janardhanan et al., 1984). *A. tamarii* was identified as the source of both toxins. Antony et al. (2003) demonstrated that exposure to cyclopiazonic acid through ingestion of contaminated kodo millet may cause acute hepatotoxicity in men and animals. *A. tamarii* also produces kojic acid, a compound of low toxicity (Manabe et al., 1984).

Ecology. While not as universally encountered in foods as *Aspergillus flavus*, *A. tamarii* is nevertheless of widespread occurrence in tropical and subtropical regions. Like *A. flavus*, it occurs commonly in nuts and oilseeds (see Pitt and Hocking, 1997). Isolations from cereals have been infrequent. *A. tamarii* has been recorded in wheat, barley and sorghum (see Pitt and Hocking, 1997). Other sources include soybeans and maize (Amusa et al., 2005, Sepulveda and Piontelli, 2005), green coffee beans (Silva et al., 2003; Tharappan et al., 2006), meat products (Mohamed and Hussein, 2004), spices, peppercorns, salted, smoked and dried fish and a variety of tropical products including cocoa, palm kernels, maize, yams and melon ball snacks (see Pitt and Hocking, 1997).

Adeniji (1970a) reported isolation of *Aspergillus tamarii* from rotting yams, and it was reported as one cause of kernel rot in cashews (Esuruoso, 1974). Amusa et al. (2003) isolated this species from African star apple.

Southeast Asian food commodities we examined were commonly infected with *Aspergillus tamarii*. Indonesian pepper was severely contaminated, although peppercorns harboured slightly lower rates of infection. Levels in peanuts were high, but lower in maize. *A. tamarii* was also found on kemiri nuts from Indonesia, peppercorns and soybeans from the Philippines, copra and black beans from Thailand and paddy rice from Indonesia. Other commodities, including cashews, mung beans and soybeans from Thailand and Indonesia, mung beans and paddy and milled rice from the Philippines and cowpeas from Indonesia showed low levels of infection (Pitt et al., 1993, 1994, 1998a and our unpublished data).

References. Raper and Fennell (1965); Domsch et al. (1980), under *Aspergillus erythrocephalus*; Christensen (1981); Klich (2002).

Aspergillus terreus Thom — Fig. 8.23

Colonies on CYA 40–50 mm diam, plane, low and velutinous, usually quite dense; mycelium white; conidial production heavy, pale pinkish brown to blonde; reverse pale to dull brown or yellow brown. Colonies on MEA 40–60 mm diam, similar to those on CYA or less dense. Colonies on G25N 18–22 mm diam, plane or irregularly wrinkled, low and sparse; conidial production light, pale brown; brown soluble pigment sometimes produced; reverse brown. No growth at 5°C. Colonies at 37°C growing very rapidly, 50 mm or more diam, of similar appearance to those on CYA at 25°C.

Conidiophores borne from surface hyphae, stipes 100–250 μm long, smooth walled; vesicles 15–20 μm diam, fertile over the upper hemisphere, with densely packed, short, narrow metulae and phialides, both 5–8 μm long; conidia spherical, very small, 1.8–2.5 μm diam, smooth walled, at maturity borne in long, well defined columns.

Distinctive features. Velutinous colonies formed at both 25 and 37°C, uniformly brown, with no other colouration, and minute conidia borne in long columns make *Aspergillus terreus* a distinctive species.

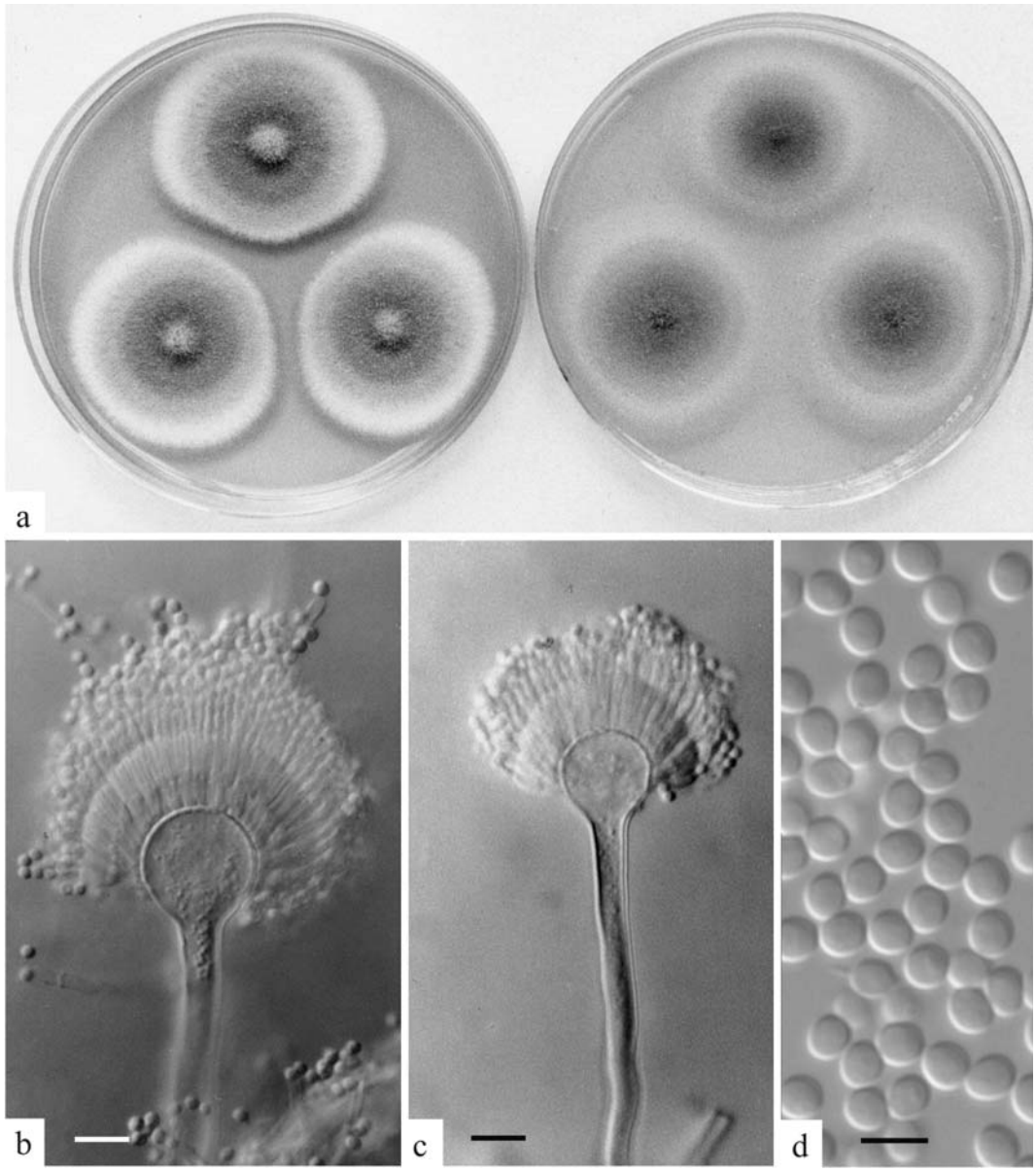


Fig. 8.23 *Aspergillus terreus* **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b, c)** heads, bars = 10 µm; **(d)** conidia, bar = 5 µm

Physiology. Growth data reported here indicate that *Aspergillus terreus* is thermophilic, but there appear to be no published data on this aspect of its physiology. A minimum a_w for growth of 0.78 at 37°C has been reported (Ayerst, 1969).

Mycotoxins. *Aspergillus terreus* produces a wide range of metabolites (Frisvad and Samson, 1991; Samson and Frisvad, 2004), but of these only territrems appear to have significant toxicity. Territrems are tremorgenic toxins which, unlike all other tremorgens, lack a moiety containing nitrogen. Maximum toxin production occurred at 28°C following 12 days' incubation in potato dextrose liquid medium (Fang and Peng, 2003). Territrem B synthesis increased at alkaline pH. Immunoelectron microscopy revealed that the toxin was localised in the cytoplasm of conidia after a minimum of 4 days growth on potato dextrose medium. No toxin was

detected in the fungal hyphae (Peng et al., 2004). These toxins have not been implicated in disease (Cole and Dorner, 1986; Ling, 1994) and their importance is a matter for conjecture.

Ecology. Although not usually a cause of spoilage in foods, *Aspergillus terreus* is of common occurrence. It is well adapted to growth in stored foods in tropical and subtropical climates. It commonly occurs in nuts, e.g. pistachios (Heidarian et al., 2006), peanuts, hazelnuts, walnuts and pecans (see Pitt and Hocking, 1997). Reports from cereals include maize (Bhattacharya and Raha, 2002; Sepulveda and Piontelli, 2005) and maize meal (Souza et al., 2003), wheat (Hajjaji et al., 2006), barley, paddy rice and parboiled rice (see Pitt and Hocking, 1997). *A. terreus* has also been reported from animal feeds containing a mixture of cereals (Magnoli et al., 2005) and silage (El-Shanawany et al., 2005). This species has been found in cassava flour (Souza et al., 2003), flour and refrigerated dough products, pasta, miso and soy sauce (see Pitt and Hocking, 1997). Other records include soybeans (Sepulveda and Piontelli, 2005), chickpeas and mungbeans (Javaid et al., 2005), rapeseed, blackgram seeds, dried beans and peas, meat and meat products, biltong (see Pitt and Hocking, 1997), spices (Mandeel, 2005), stored coffee beans (Ahmad and Magan, 2003), dry salted fish (Gräu et al., 2007), cheese and curds (Kumaresan et al., 2003).

Pathogenicity. *Aspergillus terreus* is responsible for about 10% of cases of human pulmonary aspergillosis, and it is regularly implicated in a wide range of other conditions including cutaneous and ophthalmic infections. German Shepherd dogs appear to be particularly susceptible to *A. terreus* infections (De Hoog et al., 2000).

References. Raper and Fennell (1965); Domsch et al., (1980); Klich (2002).

Aspergillus ustus (Bainier) Thom & Church Fig. 8.24

Colonies on CYA 30–40 mm diam, plane or lightly sulcate, dense, sometimes with a floccose overlay; mycelium white to greyish; conidial production sparse to quite heavy, pure grey to brownish grey; bright yellow soluble pigment usually produced; reverse greyish brown and often dull to bright yellow from soluble pigments as well. Colonies on MEA 40–50 mm diam, low, plane, dense and velutinous, or lightly floccose; mycelium white; conidial production moderate, olive brown or greyer; reverse pale green or dull brown. Colonies on G25N 10–14 mm diam, low and dense; reverse greenish or brown. At 5°C, sometimes germination by a proportion of conidia. At 37°C, colonies usually 30–50 mm diam, similar to those on CYA at 25°C, or with brown conidia; reverse brown.

Conidiophores borne from surface or aerial hyphae, stipes 100–300 μm long, sometimes curved or sinuous, with brown walls; vesicles spherical to pyriform, 10–16 μm diam, fertile over the upper two-thirds, bearing metulae and phialides, both 5–7 μm long; conidia spherical, 3.5–4.5 μm diam, brown, with rough to very rough walls, borne in small, densely packed radiate heads.

Distinctive features. *Aspergillus ustus* is distinguished by grey conidia, small heads and vesicles bearing metulae and phialides. Growth rates at 25 and 37°C on CYA are similar.

Physiology. From the growth data above, *Aspergillus ustus* grows well at high temperatures and, interestingly, is one of the very few *Aspergillus* species we have encountered which can grow at low temperatures. *A. ustus* was isolated from deep sea (5,000 m) sediments in the Central Indian Basin. Although this species grew at 5 and 30°C it was only able to synthesise an alkaline, cold tolerant protease at 30°C and 1 bar pressure (Damare et al., 2006). It is not xerophilic.

Mycotoxins. This species produces a number of secondary metabolites (Frisvad and Samson, 1991), but has not been reliably reported to produce significant mycotoxins.

Ecology. Although *Aspergillus ustus* has been reported from a wide range of foods, occurrence is rather infrequent. Recent records include soybeans (Tariq et al., 2005), cashews (Freire and Kozakiewicz, 2005), almonds (Giridhar and Reddy, 2001) and grapes (Bau et al., 2006). *A. ustus* has also been reported in wheat, barley, flour, peanuts, walnuts, pecans, betel nuts, sago, frozen meat, biltong and cheese (see Pitt and Hocking, 1997). It has not been reported to cause food spoilage.

References. Raper and Fennell (1965); Domsch et al. (1980); Klich (2002).

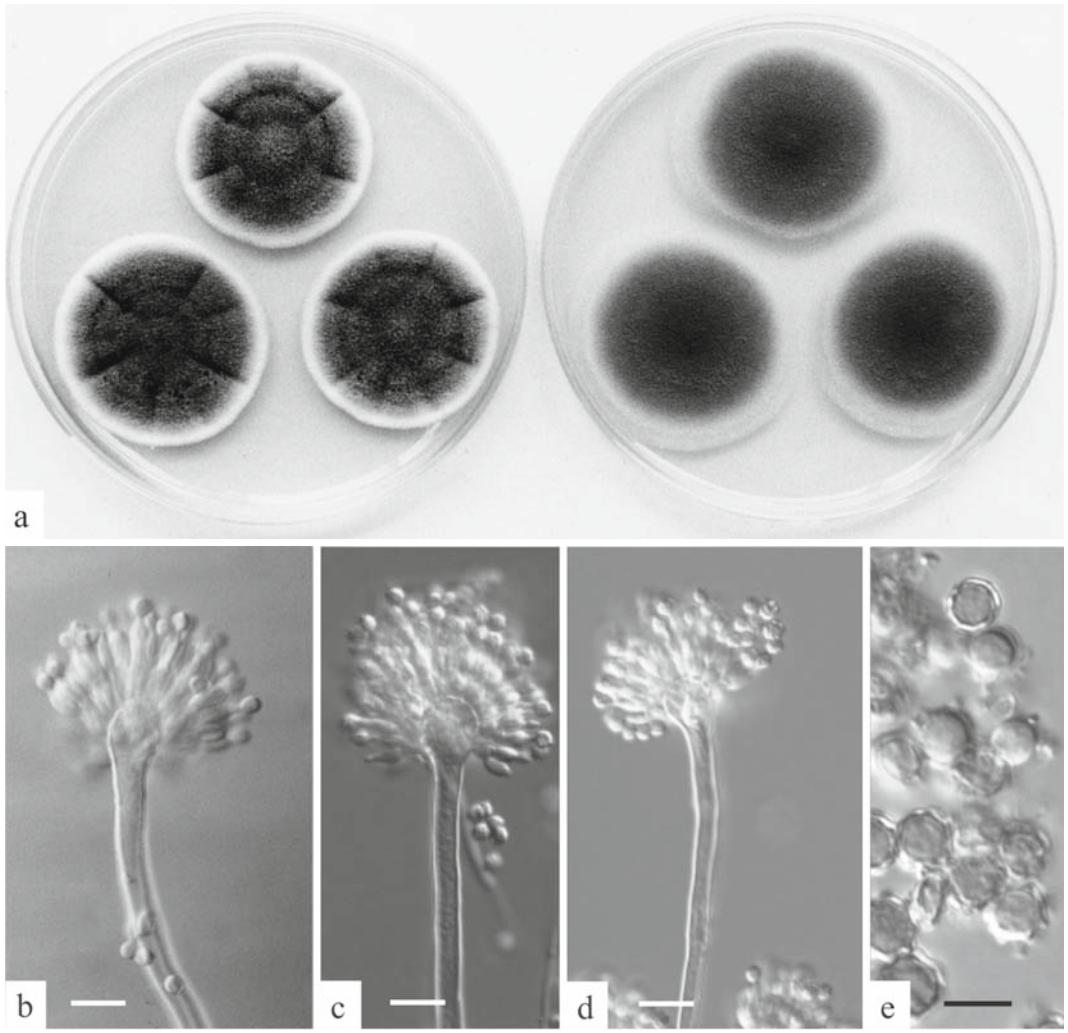


Fig. 8.24 *Aspergillus ustus* **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b, c, d)** heads, bars = 10 μm; **(e)** conidia, bar = 5 μm

Aspergillus versicolor (Vuill.) Tirab. Fig. 8.25

Colonies on CYA 16–25 mm diam, plane or lightly sulcate, low to moderately deep, dense; mycelium white to buff or orange; conidial heads sparse to quite densely packed, greyish green; pink to wine red exudate sometimes produced; reverse brownish orange or reddish brown. Colonies on MEA 12–25 mm diam, low, plane, and dense, usually velutinous; mycelium white to buff; conidial heads numerous, radiate, dull green; reverse yellow brown to orange brown. Colonies on G25N 10–18 mm diam, plane or umbonate, dense, of white, buff or yellow mycelium; reverse pale, yellow brown or orange brown. No growth at 5°C. Usually no growth at 37°C, occasionally colonies up to 10 mm diam formed.

Conidiophores borne from surface or aerial hyphae, stipes 300–600 μm long, with heavy yellow walls; vesicles variable, the largest nearly spherical, 12–16 μm diam, fertile over the upper half to two-thirds, the smallest scarcely swollen at all and fertile only at the tips, bearing closely packed metulae and phialides, both 5–8 μm long; conidia mostly spherical, very small, 2.0–2.5 μm diam, with walls finely to distinctly roughened or spinose, borne in radiate heads.

Distinctive features. *Aspergillus versicolor* grows slowly, produces both metulae and phialides from

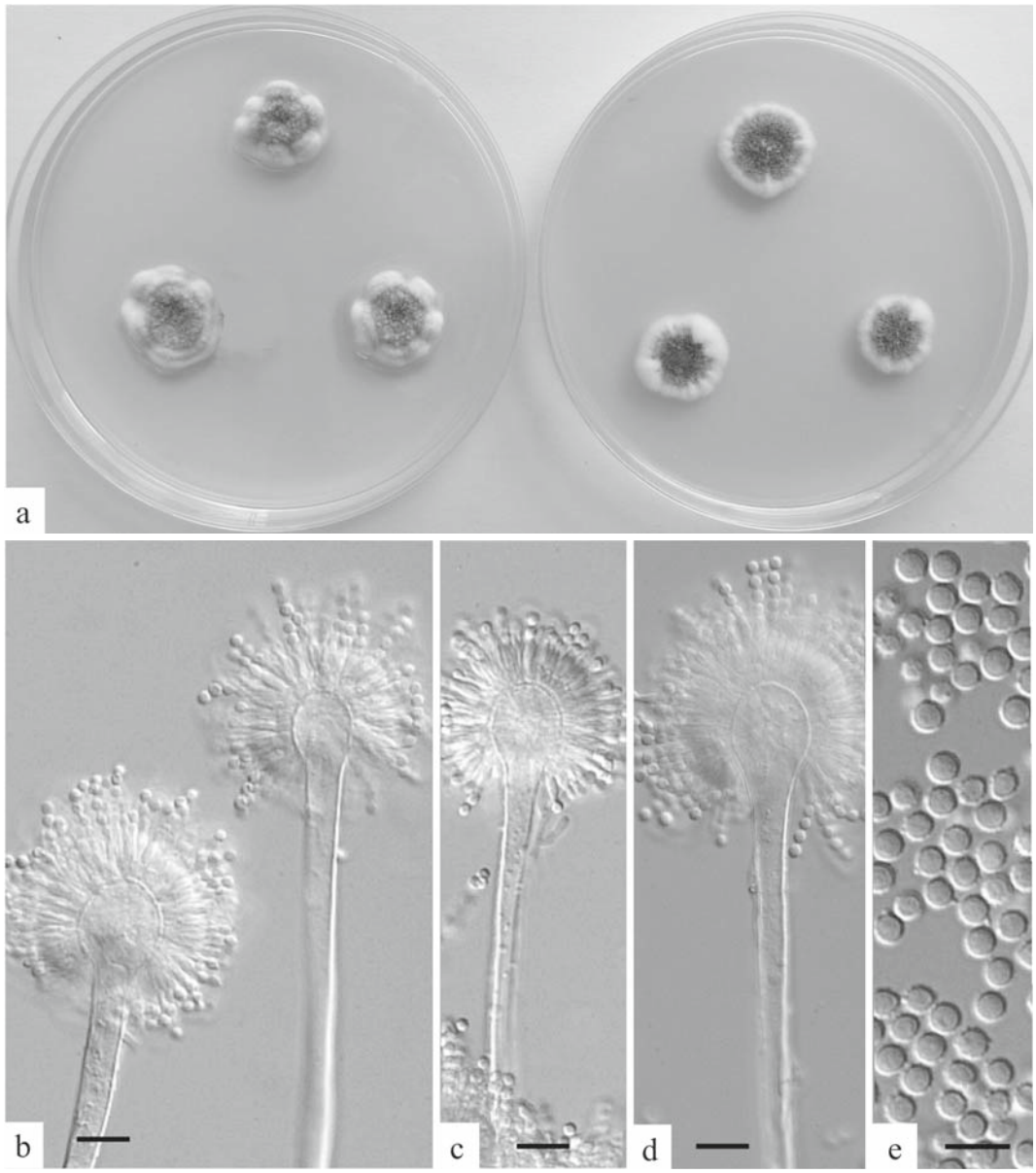


Fig. 8.25 *Aspergillus versicolor* **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b, c, d)** heads, bars = 10 μm; **(e)** conidia, bar = 5 μm

small vesicles and green conidia. Growth at 37°C is weak or absent. This species is remarkable for the wide range of mycelial and reverse pigmentation it may produce, especially if cultures are incubated for 14 days or so.

Taxonomy. Klich (1993) reviewed and revised the taxonomy of *Aspergillus versicolor* and related species.

Physiology. *Aspergillus versicolor* is a mesophile. Its minimum temperature for growth was reported to be 9°C at 0.97 a_w, its maximum 39°C at 0.87 a_w and optimum 27°C at 0.98 a_w (Smith and Hill, 1982). Fomicheva et al. (2006) reported minimum growth on CYA at 5°C, maximum growth between 35 and 37°C and optimal growth between 25 and 27°C. The minimum a_w for growth was reported to be 0.78 (Snow, 1949) or 0.80 (Pitt and Christian, 1968), both at 25°C. Growth rates at various water activities were little affected by the use of NaCl or

glycerol as controlling solute, or adjusting pH to 4.0 or 6.5 (Avari and Allsopp, 1983). Addition of NaCl to CYA to a concentration of 3.5% (0.98 a_w) favoured the growth of *A. versicolor* (Fomicheva et al., 2006). Low pH (below 3.1) prevented growth, while at 25 and 30°C growth at pH 10.2 was little different from that at pH 7.4 (Wheeler et al., 1991).

Growth of *Aspergillus versicolor* is greatly affected by modified atmospheres, growth being halved by reduction of O_2 concentration to 5%, or by addition of 12–15% CO_2, over the range 0.98–0.90 a_w (Magan and Lacey, 1984b). It is a major source of volatile compounds produced by mouldy grain (see Pitt and Hocking, 1997).

The decimal reduction time (D_{10}) for *Aspergillus versicolor* subjected to low dose gamma irradiation was given as 0.45 kGy on grain (O'Neill et al., 1991).

Mycotoxins. *Aspergillus versicolor* is the major producer of sterigmatocystin, a precursor of aflatoxins. Acute oral toxicity is low due to very low solubility in water or gastric juices, so sterigmatocystin is unlikely to be responsible for acute poisoning outbreaks in man or animals (Terao, 1983). Due to this insolubility, experimental doses given to animals are only adsorbed to a small extent. As absorption is dependent on the method of administration, LD_{50} figures lack accuracy: literature values vary from 60 to 800 mg/kg body weight. However, even low doses can cause tumours in mice (Fujii et al., 1976) and pathological changes to the livers of rats (Terao, 1983). Rats orally administrated sterigmatocystin at the rate of 8.3–16.6 mg per kg body weight per day for 10 days showed severe fatty acid changes and necrosis of hepatocytes (Aal et al., 1997).

Aspergillus versicolor is commonly reported in indoor air, and its presence in damp buildings has been linked to complaints of mouldy odour and eye, nose and throat irritation (Samson et al., 1994). A single dose of 10^8 *A. versicolor* spores inhaled by mice induced acute inflammation of the lungs (Jussila et al., 2002). Aerosols of *A. versicolor* extract administered to mice also caused upper respiratory tract irritation (Korpi et al., 2003). Continuous exposure of baby rats to *A. versicolor* spores caused pituitary tumors (Sumi et al., 1990, 1994).

As a liver carcinogen, sterigmatocystin appears to be only about 1/150th as potent as aflatoxin B_1, but this is still much more potent than most other liver carcinogens. Levels as low as 15 mg/day fed continuously, or a single 10 mg dose, caused liver cancer in 30% or more of male Wistar rats (Terao, 1983). Sterigmatocystin also increased the development of intestinal cancer in Mongolian gerbils infected with a known carcinogenic *Helicobacter pylori* toxin (Ma et al., 2003). Sterigmatocystin has the potential to cause human liver cancer and its occurrence in foods should not be taken lightly. However, unlike aflatoxins and ochratoxin, which occur in the field or during crop drying, sterigmatocystin is only produced in stored grains or cheese, a manufactured product over which control of mould contamination can be achieved (Engel and Teuber, 1980; Veringa et al., 1989). If sterigmatocystin is formed by growth of *A. versicolor* in rice, most of the toxin is removed at the milling stage (Takahashi et al., 1984). In Canada, the risk of sterigmatocystin formation in stored grains is very low (Mills, 1990).

A high proportion of *Aspergillus versicolor* isolates produce sterigmatocystin, e.g. 18 of 58 tested (Miyaki et al., 1970) or 30 of 32 (Mills and Abramson, 1986).

Ecology. A very widely distributed fungus, *Aspergillus versicolor*, has been reported from most kinds of foods. Although it occurs at harvest in crops such as wheat and barley (see Pitt and Hocking, 1997), it is of much more common occurrence in stored products. Major sources include cereals and oilseeds: wheat, cereal flakes, stored barley, milled rice, oats, rapeseed, sunflower seeds, amaranth seed and vegetable oil (see Pitt and Hocking, 1997). Recent reports include maize (Sepulveda and Piontelli, 2005), flour (Halt et al., 2004), rice (Sakai et al., 2005), soybeans (Sepulveda and Piontelli, 2005) and dried medicinal herbs, especially lemon balm (Janda and Ulfig, 2005). Other sources include peanuts, hazelnuts, walnuts, pistachios, pecans, green coffee beans, frozen meat, biltong peppercorns, spices and health foods (see Pitt and Hocking, 1997). *A. versicolor* has also been reported in sugar used in the manufacture of soft drinks (Ancasi et al., 2006), fermented and cured meats (Cantoni et al., 2007) and smoked sardines (Nketsia-Tabiri et al., 2003).

Aspergillus versicolor has been reported from cheese quite frequently (Minervini et al., 2002 and see also Pitt and Hocking, 1997) and occasionally causes spoilage (Northolt et al., 1980). It is one fungus responsible for decay in fresh breadfruit

(Omobuwajo and Wilcox, 1989) and is also one cause of the "Rio" off-flavour in coffee due to the formation of trichloroanisoles (Liardon et al., 1992).

Occurrence of *Aspergillus versicolor* in Southeast Asian commodities we examined was variable, being quite high in foodstuffs from Indonesia, but low in those from Thailand and the Philippines. This presumably reflected differences in storage times and practice in these countries. Levels in Indonesian pepper (in 12% of all peppercorns examined), kemiri nuts (11%), red beans (10%), cowpeas (5%), coriander and mung beans (2%), peanuts, paddy and milled rice (1%) are probably indicative of growth in storage rather than association with a particular kind of food substrate. In commodities from Thailand, low levels of *A. versicolor* were found in peanuts, copra, soybeans, paddy and milled rice, wheat and sorghum, while from the Philippines, incidence was limited to low levels in maize, soybeans, mung beans, paddy and milled rice, pepper and sesame seeds (Pitt et al., 1993, 1994, and our unpublished data).

References. Raper and Fennell (1965); Domsch et al., (1980); Klich (1993, 2002).

Aspergillus wentii Wehmer Fig. 8.26

Colonies on CYA 25–35 mm diam, plane or lightly wrinkled, moderately deep to deep, floccose, mycelium white to pale yellow; conidial production moderate, coloured greyish yellow; clear exudate sometimes produced; reverse pale. Colonies on MEA 22–30 mm diam, plane, relatively dense, velutinous; mycelium white; conidial production abundant, orange yellow; reverse pale. Colonies on G25N 30–45 mm diam, plane, deep, with areas of floccose white mycelium, heavily sporing on long stipes; conidia brownish yellow; reverse pale brown. No growth at 5 or 37°C.

Conidiophores borne from aerial hyphae, on CYA 500–1,200 μm long, on G25N up to 5 mm long, with thin, smooth walls; vesicles nearly spherical, on CYA 25–35 μm diam, on G25N 70–100 μm diam, with metulae and phialides densely packed over the entire surface; metulae 10–18 μm long; phialides ampulliform, 7–12 μm long; conidia spherical to broadly ellipsoidal, 3.5–5.0 μm diam, smooth walled, borne in loose, radiate heads.

Distinctive features. Golden brown colony colour, faster growth as a rule on G25N than CYA and absence of growth at 37°C are the principal characters which set *Aspergillus wentii* apart. Long stipes on G25N, production of metulae and phialides and large smooth-walled conidia are also distinctive.

Physiology. *Aspergillus wentii* is a xerophile which exhibits strong growth in both sugar and salt environments. The optimum a_w for growth is near 0.94 in both glucose/fructose and NaCl based media (Andrews and Pitt, 1987). The minimum a_w for germination at 25°C was reported to be 0.73 a_w in glucose/fructose, 0.75 in glycerol and 0.79 a_w in NaCl based media (Andrews and Pitt, 1987). A slightly higher limit (0.76 a_w in glucose/fructose) was reported by Wheeler et al. (1988b). The minimum a_w for germination in glucose/fructose was little affected by temperature: 0.77 at 20 and 30°C, 0.79 at 34°C and 0.83 at 37°C (Wheeler et al., 1988b).

Mycotoxins. Emodin is the only mycotoxin of significance produced by *Aspergillus wentii* (Wells et al., 1975). Administered to day-old cockerels, the oral LD_{50} was only 3.7 mg/kg, indicating high toxicity (Wells et al., 1975). It has also been reported to be a mutagen (Masuda and Ueno, 1984; Müller et al., 1996). There are no reports of toxicoses associated with *A. wentii*, perhaps reflecting the lack of reports of substantial growth, i.e. spoilage, in foods.

Ecology. This species has only rarely been reported as a spoilage fungus and is considered to be among the less common major *Aspergillus* species in foods. Nevertheless, in our experience it is widely distributed. We isolated it in high percentages of many of the Southeast Asian commodities we examined. Twenty three per cent of nearly 500 peanut samples examined contained *A. wentii*, at up to 100% infection in infected samples and in 6% of all kernels examined. Thirty seven per cent of kemiri nut samples from Indonesia were infected, but the maximum infection level was only 35%, with 6% of nuts infected overall. A wide range of other commodities, maize from all three countries, cashews from Thailand, pepper and soybeans from the Philippines, and mung beans, pepper, coriander and cowpeas from Indonesia, all showed 1–3% overall infection in the particles examined. Low levels of infection were seen in mung beans, soybeans, paddy rice, sorghum, red beans, cassava

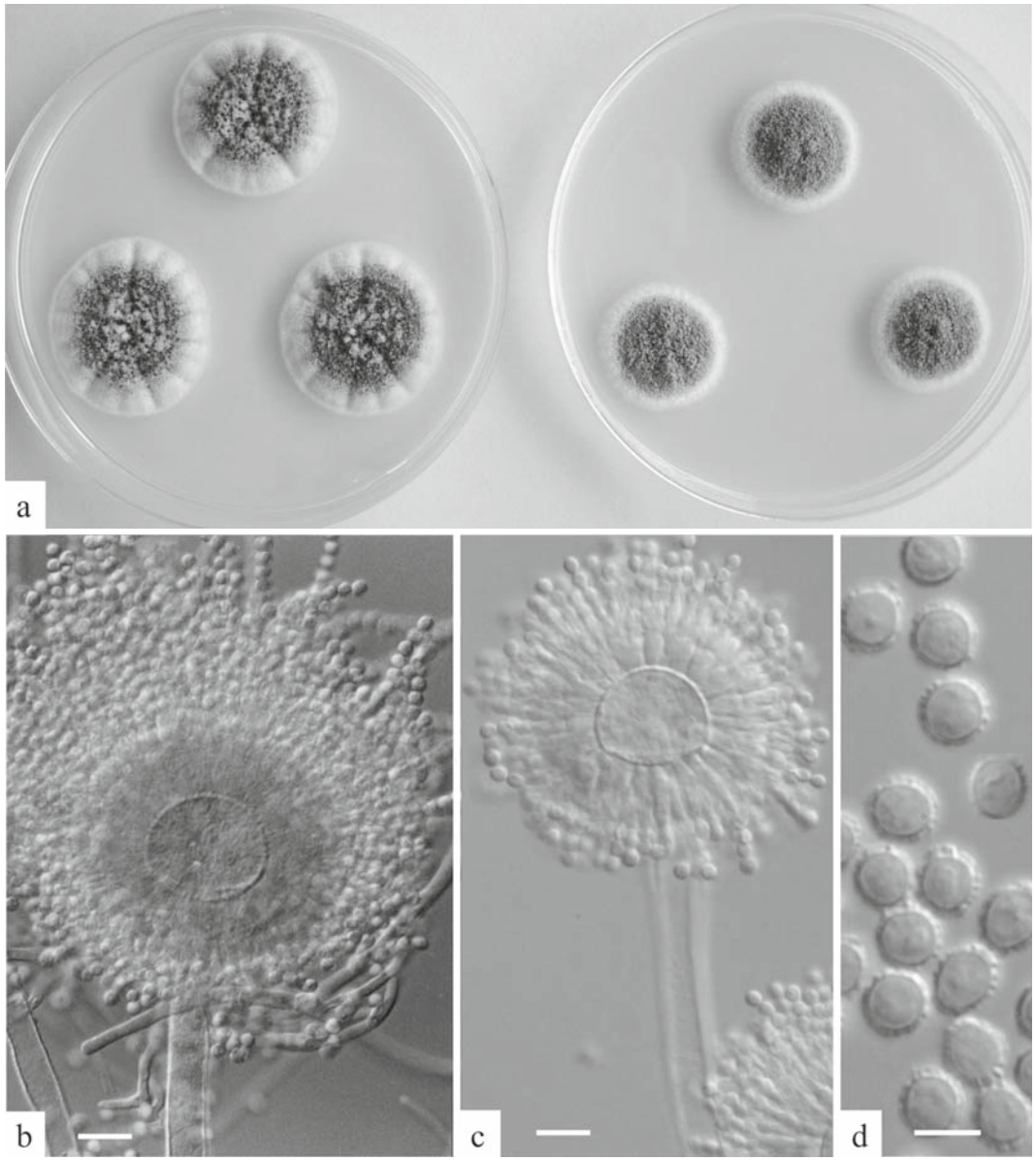


Fig. 8.26 *Aspergillus wentii* **(a)** colonies on CYA and MEA, 7 days, 25°C; **(b, c)** heads, bars = 20 μm; **(d)** conidia, bar = 5 μm

and copra from Thailand and cashews, soybeans, paddy and milled rice from Indonesia (Pitt et al., 1993, 1994, and 1998a). It was also quite common in dried fish (Wheeler et al., 1986).

Other records of *Aspergillus wentii* from foods include peanuts, pistachios, pecans, walnuts, various cereal products, wheat, barley, bread, green coffee beans, dried beans, ripened raw hams and biltong (see Pitt and Hocking, 1997) and also rice (Taligoola et al., 2004), maize and soybeans (Sepulveda and Piontelli, 2005) and grapes (Pollatro et al., 2005).

References. Raper and Fennell (1965); Domsch et al., (1980); Klich (2002).

Chapter 9
Xerophiles

Xerophilic fungi are distinguished by their ability to grow under conditions of reduced water activity, i.e. to complete their life cycles on substrates that have been dried or concentrated, in the presence of high levels of soluble solids such as salts or sugars. Early usage (Scott, 1957) confined the word "xerophile" to filamentous fungi and used the term "osmophile" for yeasts; the term "halophile" was used rather indiscriminately for moulds, yeasts and bacteria with the ability to grow on concentrated salt solutions.

Pitt (1975) discussed the terminology used for fungi which grew at low a_w. He pointed out that osmophile was an inappropriate term, because high osmotic pressures were not involved in the growth of these fungi, as they balance the outside environment with internal solutes, maintaining just sufficient osmotic pressure to enable growth. The crucial point was that they preferred to grow at reduced water activities. In the absence of a suitable term for "lovers of low water activity", xerophile seemed the most suitable appellation, for both moulds and yeasts.

Some other authors have used the term "xerotolerant" in place of xerophile, arguing that these fungi exhibit "tolerance of" not "love for" reduced a_w. However, xerotolerant is an inappropriate term for extremely xerophilic fungi such as *Xeromyces bisporus, Chrysosporium fastidium* and *Basipetospora halophila*, which have an absolute requirement for, not tolerance of, reduced a_w. Second, although many xerophiles grow optimally at quite high a_w, most are basically slowly growing fungi and cannot compete in mixed cultures at high a_w levels such as prevail in soils or fresh foods. Survival of xerophiles in nature depends on access to environments of reduced a_w where competition is restricted or eliminated. In this vital sense they are lovers of lowered water activity, i.e. xerophiles.

Xerophilic fungi may be defined in a variety of ways, usually relating to minimal or optimal a_w for growth. The definition of Pitt (1975) is used here: a xerophile is a fungus capable of growth, under at least one set of conditions, at a water activity below 0.85. This has proved to be a practical working definition.

In discussing the use of the word halophile for fungi, Pitt (1975) wrote that it was inappropriate because there were no known fungi with a preference, let alone an obligate requirement, for salt environments. More recently, however, such fungi have been shown unequivocally to exist, exhibiting quite superior growth on media with NaCl as controlling solute. *Scopulariopsis halophilica* Tubaki was the first; Pitt and Hocking (1985) added *Polypaecilum pisce* and transferred *S. halophilica* to *Basipetospora* (as *B. halophila*). Perhaps there are others. Such fungi are still classed as xerophiles: if there is a need to differentiate them from other xerophiles, the term "halophilic xerophile" is appropriate (Andrews and Pitt, 1987).

In this book, for practical reasons, "xerophile" has a different and much narrower circumscription than the definition given above. Here the definition is derived solely from responses to the standard media. A species has been included, or at least keyed, in this chapter if, after 7 days at 25°C, colony diameters on G25N exceed those on CYA and MEA. Many marginally xerophilic fungi which meet the definition of Pitt (1975) do not meet this criterion and have simply been keyed in other appropriate chapters.

J.I. Pitt, A.D. Hocking, *Fungi and Food Spoilage*, DOI 10.1007/978-0-387-92207-2_9,
© Springer Science+Business Media, LLC 2009

In this chapter, *Eurotium* species and a few Aspergilli are keyed out with a miscellaneous group of other fungi. To maintain an orderly presentation, the *Eurotium* and *Aspergillus* species have been described in Chapter 8. The miscellaneous fungi are described below, in alphabetical order.

Note that in the key which follows, couplets 1–3 are based on growth on G25N, and sort out species which are placed elsewhere, plus one of those described below. It is not possible satisfactorily to differentiate the remaining xerophiles on this medium. The subsequent couplets, 4–8, are based on colonies grown on MY50G agar (see Chapter 4). So before entering the key at couplet 4 it will be necessary to inoculate the unknown isolate onto MY50G agar and incubate at 25°C for 14 days, and perhaps longer, until fruiting structures differentiate and mature.

Key to xerophilic fungi

1	Bright yellow, barely macroscopic spherical bodies (cleistothecia) visible in the aerial mycelium of colonies on G25N. (Grow on CY20S agar and use key in Chapter 8)	*Eurotium*
	Cleistothecia not visible in colonies on G25N	2
2 (1)	Colonies on G25N showing yellow or green conidial colours (Use key in Chapter 8)	*Aspergillus*
	Colonies on G25N white, brown or black	3
3 (2)	Colonies on G25N chocolate brown	*Wallemia*
	Colonies on G25N white, pale brown or black (Grow on MY50G)	4
4 (3)	Colonies on MY50G white or pale brown	5
	Colonies on MY50G black or with black areas	*Bettsia* (see *Chrysosporium*)
5 (4)	Colonies on CYA and MEA 10 mm diam or more in 7 days; conidia on MY50G lemon-shaped	*Polypaecilum*
	Colonies on CYA and MEA not exceeding 10 mm diam	6
6 (5)	On MY50G, solitary asci produced, containing or releasing mature ascospores in 14 days	*Eremascus*
	Mature asci and ascospores not evident in cultures on MY50G in 14 days	7
7 (6)	Colonies on MY50G producing spherical to cylindroidal aleurioconidia or similar conidia in 14 days	8
	Colonies on MY50G not producing aleurioconidia in 14 days; 3 celled cleistothecial initials or developing cleistothecia may or may not be evident	*Xeromyces*
8 (7)	Intercalary chlamydoconidia and arthroconidia present; aleurioconidia on tiny pedicels or solitary	*Chrysosporium*
	Intercalary chlamydoconidia and arthroconidia absent; aleurioconidia on short conidiophores, sometimes in short chains	*Basipetospora*

9.1 Genus *Basipetospora* G.T. Cole and W.B. Kendr.

Basipetospora was described by Cole and Kendrick (1968) as the anamorph of the Ascomycete genus *Monascus* (see Chapter 5). The two genera have until recently only been found occurring together, as holomorphic species. Following a light and scanning electron microscopic study, however, Pitt and Hocking (1985) transferred the salt-tolerant xerophile *Scopulariopsis halophilica* Tubaki, a species with no known teleomorph, to *Basipetospora*. Conidium formation in this fungus appeared to occur by the process characteristic of the *Monascus* anamorphs.

In *Basipetospora*, conidia (aleurioconidia) are borne in short chains from simple conidiophores,

cut off successively from the tip of the conidiophore so that it shortens as the conidia form. Conidia sometimes separate at maturity. The distinction from *Chrysosporium* is that in the latter genus aleurioconidia are formed solitarily. Moreover conidia borne along the lengths of the vegetative hyphae, i.e. chlamydoconidia and arthroconidia, are not produced by *Basipetospora* species but are usually present in *Chrysosporium* species.

Basipetospora halophila (J.F.H. Beyma) Pitt & A.D. Hocking Fig. 9.1

Oospora halophila J.F.H. Beyma
Scopulariopsis halophilica Tubaki

No growth on CYA at 5, 25 or 37°C or on MEA. Colonies on G25N at 14 days 4–8 mm diam, occasionally 12 mm, of dense and tough mycelium, centrally raised, sulcate or irregularly wrinkled; mycelium persistently white, with little or no sporulation; reverse pale. Colonies on MY50G at 14 days 4–8 mm diam, similar to those on G25N. Colonies on MY5-12 at 14 days 10–16 mm diam, low or umbonate, plane or irregularly wrinkled, of dense mycelium overlaid by floccose to funiculose aerial hyphae; mycelium persistently white, sporulation light to moderate in lower layers of the mycelium; reverse pale to yellow brown. Colonies on MY10-12 at 14 days 18–22 mm diam, similar to those on MY5-12, but growth more rapid and vigorous; sporulation in surface mycelial layers moderate to heavy; mycelium and conidia persistently white; reverse pale to yellow brown.

Reproductive structures short, solitary conidiophores borne at irregular intervals along vegetative hyphae, sometimes bearing a short chain of conidia, but more commonly a single developing conidium, shed at maturity and succeeded by another blown out terminally from the conidiophore, the conidiophore shortening a little with each successive conidium; conidiophores often curved, usually cylindrical, 2.0–3.0 μm diam, but sometimes narrowing towards the apex; when young 8–20 μm long, in age down to 3–4 μm long, smooth walled; mature conidia spherical to broadly ellipsoidal or pyriform with a truncate base, 3.5–6.0 μm diam, with heavy walls, smooth to finely roughened, in wet mounts usually solitary, but sometimes in chains of 3 or 4. No teleomorph known.

Distinctive features. Although it superficially resembles a *Chrysosporium* species, *Basipetospora halophila* is readily distinguished by (1) very slow growth on MY50G; (2) aleurioconidia sometimes in short chains; and (3) the absence of intercalary conidia in the vegetative hyphae.

Taxonomy. This distinctive species was described as *Oospora halophila* by van Beyma (1933). The name was not used by later authors, however. Tubaki (1973) described *Scopulariopsis halophilica* in terms which did not lead to association with van

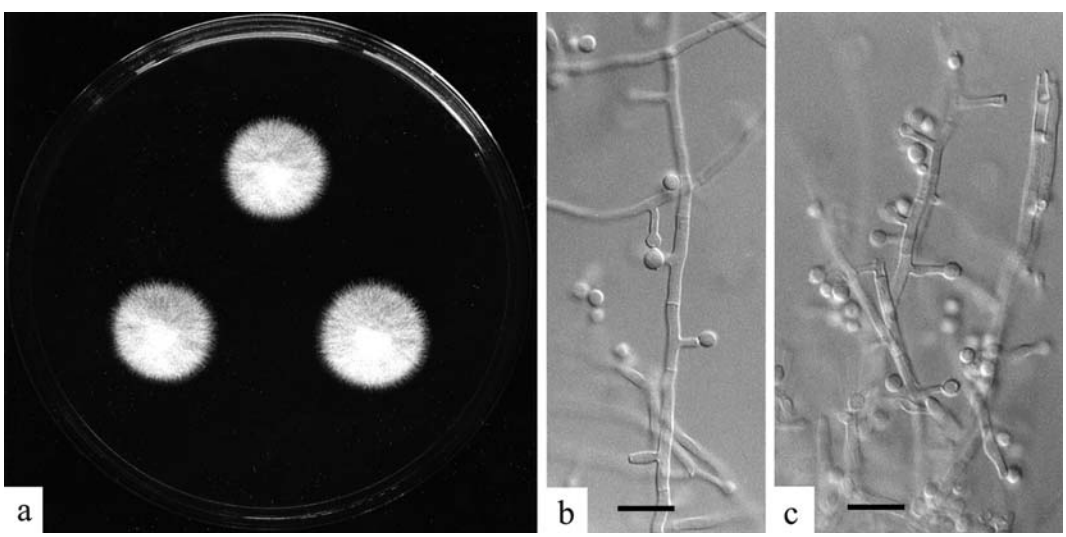


Fig. 9.1 *Basipetospora halophila* **(a)** colonies on MY5-12, 14 d, 25°C; **(b,c)** conidiophores and aleurioconidia, bars = 20 μm

Beyma's species. Pitt and Hocking (1985) established that *O. halophila* and *S. halophilica* were a single species. Studies of *S. halophilica* by scanning electron microscopy showed that it was not a *Scopulariopsis*, but belonged in *Basipetospora*.

Physiology. The differences in rates of growth of this fungus on MY50G and MY5-12, media of similar water activity and nutritional status, show clearly that *Basipetospora halophila* is a true halophilic xerophile. Detailed studies on the water relations of this species confirmed this observation (Andrews and Pitt, 1987; Wheeler et al., 1988c). Three isolates of *B. halophila* germinated in 6–11 days on a saturated NaCl medium (0.747 a_w). Germination was slower on media containing glucose/fructose or glycerol as controlling solute; however, one isolate germinated at 0.73 a_w in 40 days on glucose/fructose. The lowest a_w for germination on glycerol media was 0.78. Growth rates of three isolates were similar, but showed marked differences on the different media: the radial growth rate on NaCl at 0.75 a_w (10 μm/h) was comparable with the maximum rate observed on glucose/fructose at 0.90 a_w. Maximum growth rates on NaCl, ca. 30 μm/h, occurred at 0.87–0.88 a_w. Strong growth of *Basipetospora* on NaCl based media was also demonstrated by Diyaolu and Adebajo (1994).

The optimum temperature for growth of *Basipetospora halophila* is near 30°C, although it was more tolerant of high a_w conditions at lower temperatures, growing at 0.99 a_w on NaCl based media at 25°C. At 37°C, *B. halophila* demonstrated slow growth on NaCl based media, and over only a limited a_w range, 0.86–0.77 a_w (Wheeler et al., 1988c). The maximum temperature for growth is probably not much higher than 37°C. The minimum temperature for growth is not known.

Basipetospora halophila is well adapted to its ecological niche – dried, salted seafoods and similar substrates. Studies on competition between common xerophiles such as *Eurotium* species and halophilic xerophiles growing together on salted fish have clearly shown that *B. halophila* and the other halophilic xerophile, *Polypaecilum pisce,* can outgrow the Eurotia below about 0.87 a_w (Wheeler and Hocking, 1993).

Mycotoxins. No mycotoxins are known from this species.

Ecology. *Basipetospora halophila* is a halophilic xerophile which is restricted to salty environments. Most isolates of *B. halophila* have come from salted, dried fish: it has been reported from dried or cured fish from Japan, Indonesia, the Philippines, Sri Lanka and Nigeria (see Pitt and Hocking, 1997). Other sources include dried food grade seaweed in Japan, a gelatine hydrolysate (Tubaki, 1973) and sea salt (as *Scopulariopsis halophilica*; CBS, 2007).

Reference. Pitt and Hocking (1985).

9.2 Genus *Chrysosporium* Corda

Chrysosporium is a genus of Hyphomycetes characterised by the formation of solitary, hyaline, smooth walled aleurioconidia. These are produced on the sides of vegetative hyphae either directly (sessile) or on small pedicels. Some species also produce similar conidia terminally on hyphae (terminal chlamydoconidia). In a few species, the vegetative hyphae themselves also differentiate either partially or almost totally into conidia: some are nearly spherical (intercalary chlamydoconidia); others are produced from unswollen hyphal segments (arthroconidia). These two spore types may intergrade. Speciation in this genus largely depends on the size, shape and proportion of these various types of conidia.

Most *Chrysosporium* species are found in soil, or on the hair or skin of animals, and are dependent on high water activities for growth. Some are pathogenic. Species which occur in foods, however, are not pathogenic and are xerophiles. However, molecular work in our laboratory has indicated that they are genetically related to some of the non-xerophilic *Chrysosporium* species (N. Tran-Dinh, unpublished).

Most *Chrysosporium* species are strictly anamorphic. Some of the dermatophyte species form teleomorphs in *Gymnoascus, Arthroderma* and other related genera, outside the scope of this work. One of the foodborne species, *C. farinicola,* sometimes produces a teleomorph classified in *Bettsia* Skou, an Ascomycete which forms unique black cleistothecia. Molecular studies have shown that it belongs to the Eurotiales (N. Tran-Dinh, unpublished). Skou (1992)

described seven new species and three new varieties of *Chrysosporium* from the pollen and nectar of mason bees. Although not all of Skou's species are molecularly distinct (N. Tran-Dinh, unpublished), his work indicated that the natural habitat for xerophilic *Chrysosporium* species is associated with bees, beehives and honey. Kinderlerer (1995) developed a medium, Czapek casein 50% glucose agar (CZC50G) to assist in the identification of xerophilic foodborne *Chrysosporium* species.

The key to xerophilic *Chrysosporium* species deals only with the four species most commonly encountered in foods and is based on growth on MY50G agar for 7 days at 25°C. Confirmation of identity may depend on colony maturation which can take 2–3 weeks.

Key to xerophilic *Chrysosporium* species

1	Colonies on MY50G exceeding 15 mm diam in 7 days; predominant conidial type solitary aleurioconidia	2
	Colonies on MY50G not exceeding 15 mm diam in 7 days, and not usually exceeding 30 mm in 14 days; predominant conidial type intercalary chlamydoconidia and arthroconidia	*C. inops* *C. xerophilum*
2 (1)	Colonies on MY50G pale yellow or brown with a yellow brown reverse	*C. fastidium*
	Colonies on MY50G persistently white with a pale reverse, or showing dark grey to black areas	*C. farinicola*

Chrysosporium farinicola (Burnside) Skou

Fig. 9.2

Ovularia farinicola Burnside

Teleomorph: *Bettsia alvei* (Betts) Skou
Pericystis alvei Betts

No growth on CYA at 5, 25 or 37°C or on MEA. Colonies on G25N 10–20 mm diam, of low, white mycelium, or if the teleomorph is present, centrally grey from the production of immature cleistothecia; margins fimbriate; reverse pale, but grey centrally if the *Bettsia* state is being produced. Colonies on MY50G growing relatively rapidly, at 7 days 20–30 mm diam, low, plane, persistently white, or showing sectors becoming translucent or greyish if the *Bettsia* state is present, reverse pale beneath white areas, but darker beneath grey sectors; at 14 days, 40–65 mm diam, colonies remaining white if only the anamorph is present, but with dark grey to black areas in the presence of the teleomorph; reverse pale, or dark under the teleomorph (Fig. 9.2).

Reproduction on MY50G predominantly solitary aleurioconidia, broadly ellipsoidal to pyriform, thick walled, highly refractile, 7–11 μm long. Fertile hyphae mostly undifferentiated, dissolving in age. In isolates producing the teleomorph, areas or sectors of translucent growth developing. At maturity such areas becoming grey and then black as small cleistothecia form. Cleistothecia formed on MY50G at 25°C, dark brown to black, usually maturing only after several weeks at 15–25°C, 25–60 μm diam, with walls thin and smooth, and without internal structure; initials a row of three short cells, 12–18 × 6–8 μm overall, adhering to the cleistothecial wall as a distinctive appendage; ascospores not liberated readily, spherical, 5–6 μm diam, with dark walls, smooth to minutely roughened.

Distinctive features. *Chrysosporium farinicola* is distinguished from *C. fastidium* by the following features: (1) colonies on MY50G grow more rapidly; (2) colonies on G25N and MY50G remain pure white, with reverses virtually uncoloured; and (3) terminal chlamydoconidia are often larger, up to 13–18 μm in diameter. In addition, some isolates of *C. farinicola* produce the *Bettsia* state. No teleomorph is known for *C. fastidium*.

Mycotoxins. No mycotoxins are known from this species.

Physiology. No studies on the water relations of this species are known to us, but *Chrysosporium farinicola* would be expected to resemble *C. fastidium* in its response to a_w. Studies on recovery of heat stressed *C. farinicola* aleurioconidia showed that older cultures were less heat sensitive than 14 day cultures (Beuchat and Pitt, 1990b). There was less than one log reduction in 20 day old aleurioconidia heated at 56°C for 10 min. Aleurioconidia survived

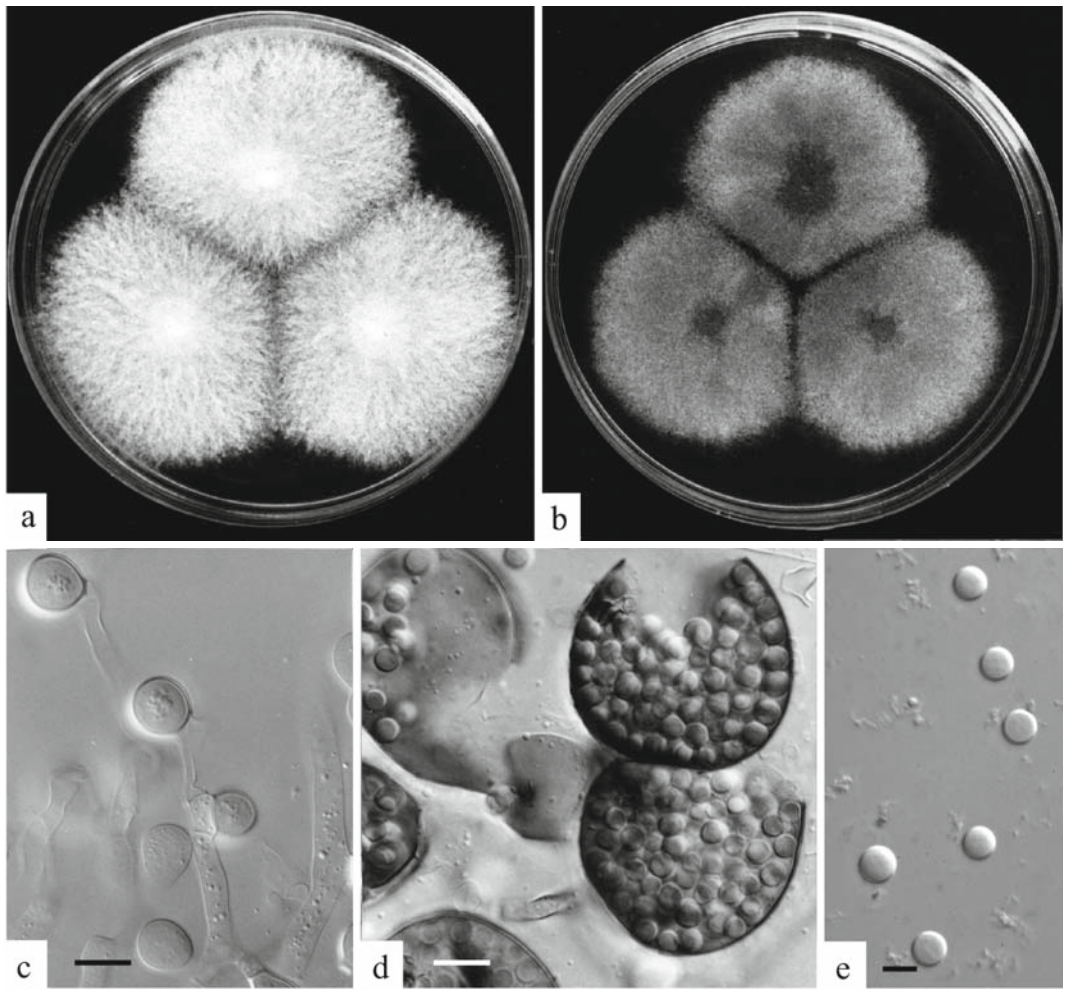


Fig. 9.2 *Chrysosporium farinicola* and *Bettsia alvei* **(a)** colonies of the *Chrysosporium* state alone and **(b)** with the *Bettsia* state also present, both on MY50G, 14 d, 25°C; **(c)** aleurioconidia, bar = 10 μm; **(d)** mature cleistothecia-containing ascospores, bar = 25 μm; **(e)** ascospores, bar = 5 μm

well in maize starch at 0.43 and 0.71 a_w at 1 and 25°C, showing no reduction in numbers after 45 days storage. Heat stressed *C. farinicola* aleurioconidia grew better on glucose based media than on media containing sorbitol, glycerol or NaCl (Beuchat and Pitt, 1990b). Isolates of *C. farinicola* were able to grow in the presence of 1.05–1.76 % (w/v) ethanol and at temperatures ranging from 4 to 25°C (Kinderlerer, 1997). Growth in controlled atmosphere was recorded in a mixture of 10% CO_2 and 20% O_2 or 6% O_2 (Kinderlerer, 1997). Weak protease activity and moderate lipolytic activity were reported for *C. farinicola* from Kenyan coconut (Ismail, 2001).

Ecology. *Chrysosporium farinicola* has been isolated from a range of low a_w substrates, including honeycomb, prunes and prune processing equipment, sultanas, mixed dried fruit, chocolate, jelly crystals and coconut from Australia, the United Kingdom, Sri Lanka, Czechoslovakia, Denmark and Kenya (Kinderlerer, 1997; Ismail, 2001; Food Science Australia, 2006; CBS, 2007). In coconut, *C. farinicola* can cause cheesy butyric spoilage (Kinderlerer, 1984a).

References. Skou (1975); van Oorschot (1980).

Chrysosporium fastidium Pitt — Fig. 9.3

No growth on CYA at 5, 25 or 37°C, or on MEA. Colonies on G25N 1–5 mm diam, of low, dense,

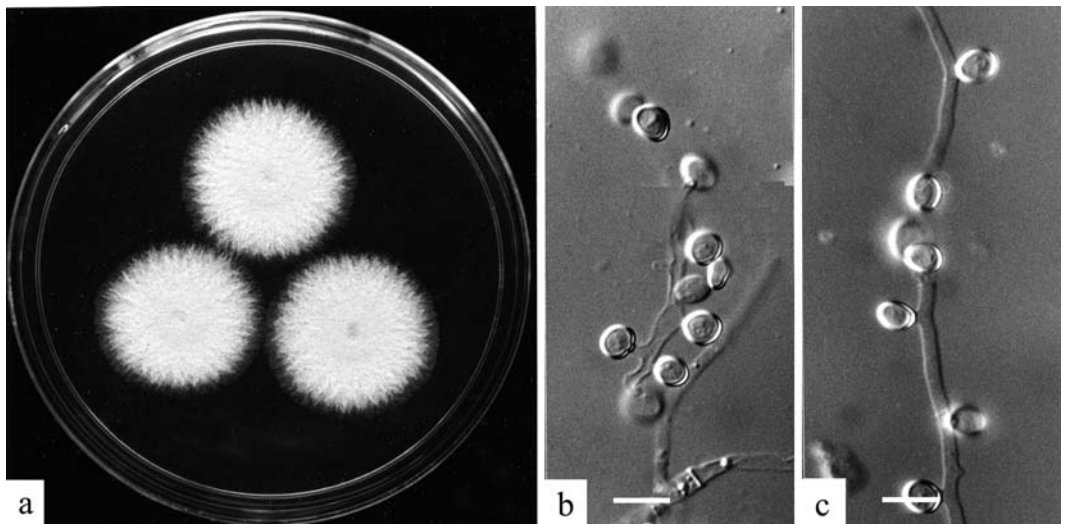


Fig. 9.3 *Chrysosporium fastidium* **(a)** colonies on MY50G, 14 d, 25°C; **(b,c)** aleurioconidia, borne on pedicels, bar = 10 µm

white mycelium. Colonies on MY50G at 7 days 15–22 mm diam, low, plane and sparse, pale yellow or brown, reverse yellow brown; at 14 days, 35–42 mm diam, low and plane, margins sparse and fimbriate, white, centres more dense, dull yellow; reverse yellow to pale brown.

Reproduction on MY50G predominantly by smooth walled aleurioconidia borne singly on short pedicels or less commonly sessile, spheroidal (oblate or prolate) to broadly ellipsoidal, 6–9 × 5–8 µm, in age released by dissolution of the pedicels; terminal chlamydoconidia, spherical to pyriform or pedunculate, 8–12 × 6–10 µm, also produced, but intercalary chlamydoconidia and arthroconidia rare. No teleomorph known.

Distinctive features. *Chrysosporium fastidium* forms dull yellow to yellow brown colonies; conidia are predominantly aleurioconidia with few intercalary chlamydoconidia or arthroconidia produced on G25N or MY50G. It most closely resembles *C. farinicola* from which is can be distinguished by colony colour (*C. farinicola* is persistently white or has dark grey areas where the *Bettsia* teleomorph is formed), and slower growth on MY50G and Czapek casein 50% glucose agar (Kinderlerer, 1995).

Taxonomy. Van Oorschot (1980) placed *Chrysosporium fastidium* in synonymy with *C. farinicola*, but this is not accepted here. The two species are readily distinguished and only *C. farinicola* forms a teleomorph.

Physiology. A mesophilic obligate xerophile, *Chrysosporium fastidium* has a maximum a_w for growth of 0.98 and a minimum of 0.69 (Pitt and Christian, 1968). It does not utilise nitrate and appears to require accessory factors for growth. Conidia have an unexceptional heat resistance (Pitt and Christian, 1970).

Mycotoxins. No mycotoxins are known from this species.

Ecology. This species has been repeatedly isolated from prunes (dried and high moisture) and prune-processing machinery in N.S.W., Australia. There are no records of isolation from other substrates or other locations, indicating that *Chrysosporium fastidium* is a rare species with a restricted habitat.

Reference. Pitt (1966).

Chrysosporium inops J.W. Carmich. Fig. 9.4

Colonies on CYA and MEA microscopic or up to 3 mm diam. Colonies on G25N 2–9 mm diam, varying from low and translucent to deep and floccose, white; reverse pale to amber or duller yellow brown. No growth on CYA at 5 or 37°C. Colonies on MY50G at 7 days 6–10 mm diam, at 14 days 12–20 mm diam, varying from low, sparse and translucent to moderately deep, dense and with a floccose surface, white or if translucent, uncoloured; reverse uncoloured to pale yellow brown.

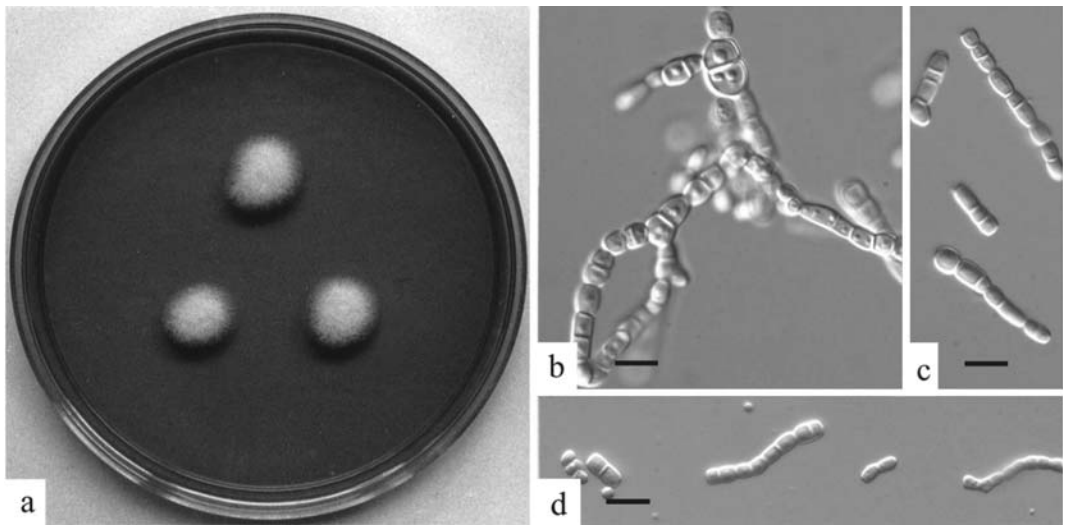


Fig. 9.4 *Chrysosporium inops* **(a)** colonies on MY50G, 14 d, 25°C; **(b,c,d)** chains of arthroconidia, borne by hyphal transformation, bars = 10 μm

Reproductive structures on G25N or MY50G at 7 days primarily short chains of chlamydoconidia and arthroconidia, borne by retrogressive differentiation from hyphal tips and as intercalary chains; some terminal chlamydoconidia also present; at maturity on MY50G (2–4 weeks), clusters of chlamydoconidia and arthroconidia also present, formed by retrogressive differentiation of groups of short branching hyphae with a lateral stipe as their common origin. Chlamydoconidia spherical, 4–7(–10) μm diam; arthroconidia cylindroidal or doliiform (barrel shaped), 3–8 × 3–6 μm, those of greater width intergrading with chlamydoconidia; aleurioconidia, the conidial type characteristic of *Chrysosporium*, uncommon, ellipsoidal to pyriform, 5–8 μm diam or longer. Large chlamydoconidia, up to 25 μm diam, with walls up to 2 μm thick, produced by some isolates. Teleomorph unknown.

Distinctive features. In culture, *Chrysosporium inops* (and *C. xerophilum*; see below) differ from *C. fastidium* and *C. farinicola* by slower growth rates, especially on MY50G. The two species under consideration here also produce predominantly chlamydoconidia and arthroconidia. On CZC50G medium, some isolates of *C. inops* produce a red reverse, but most are yellow (Kinderlerer, 1995). *C. inops* grows more slowly than *C. xerophilum* on this medium. In young colonies (7 days), terminal chlamydoconidia are often the dominant conidium type in *C. inops*. Aleurioconidia are rare.

Physiology. Van Oorschot (1980) reported that *Chrysosporium inops* had a minimum growth temperature of 20°C, an optimum of 25°C and a maximum of 30°C. However, her data were obtained on media of very high a_w, and limits are undoubtedly wider under optimal conditions. The water relations of this species have not been studied in detail, but isolations in our laboratory have come from growth under controlled a_w levels as low as 0.72. It is clear, contrary to previous reports (Pitt and Christian, 1968; van Oorschot, 1980), that *C. inops* is an extreme xerophile. However, it is also able to grow (very slowly) at the very high a_w of CYA and MEA.

Arthroconidia of *Chrysosporium inops* are moderately heat resistant, and their heat resistance increases with maturity. The decimal reduction time at 66°C (D_{66}) was 1.67 min for 3 week old spores, 1.95 min for 4 week old spores and 5.49 min for 6 week old spores (Kinderlerer, 1996). A small population of heat resistant arthroconidia survived heating at 66°C for 1 h, representing approximately 0.001% of the population (Kinderlerer, 1996). The heating menstruum in these experiments was 0.1% peptone. A higher heat resistance could be expected in high sugar environments.

Chrysosporium inops can produce ethanol from glucose (Kinderlerer, 1987). Its ability to utilise the

Embden–Meyerhof–Parnas pathway may explain why this species and the closely related *C. xerophilum* may become dominant in low a_w foods stored at low oxygen tension (Kinderlerer, 1987).

Mycotoxins. No mycotoxins are known from this species.

Ecology. This appears to be an uncommon species, but as with other xerophilic Chrysosporia, this may only reflect the need for well-chosen isolation techniques and identification procedures. We have isolated *Chrysosporium inops* from a variety of spoiled products: nutmeg powder, Chinese five spice powder, mixed spice powder, chopped Chinese dates and a gelatine confection made in a starch mould. In the latter case, 30 tonnes of starch were heavily contaminated with this fungus, causing a serious loss of confectionery products. There was no evidence that the starch had ever been more than marginally above a safe a_w level. *C. inops* has also been isolated from table jelly (Kinderlerer, 1987) and spoiled chocolate and hazelnuts in the United Kingdom (J.L. Kinderlerer, unpublished). Christensen (1978b) reported *C. inops* from samples of maize stored for 12 months or more at low moisture in tightly closed containers. His identification was in error (see below); however, we have recovered *C. inops* from a sample of safflower seeds stored in a similar way in our laboratory.

Additional species. *Chrysosporium xerophilum* Pitt is closely related to *C. inops*. It differs by (1) faster growth, especially on MY50G, where colonies at 7 days are 10–15 mm diam and at 14 days 25–32 mm diam; (2) higher numbers of aleurioconidia, which measure 7–8 × 5–7 μm; (3) larger terminal chlamydoconidia, 10–12(–15) μm diam; and (4) at maturity, almost complete differentiation of vegetative hyphae into intercalary chlamydoconidia and arthroconidia, even aleurioconidium pedicels often becoming thick walled spores. CZC50G medium may be useful in differentiating these two species (Kinderlerer, 1995).

A mesophilic xerophile, *Chrysosporium xerophilum* has a minimum a_w for growth of 0.71 (Pitt and Christian, 1968) and, like *C. inops*, a high maximum limit. Although most conidia of this species have a low heat resistance, surviving less than 10 min at 60°C, a small proportion of conidia appear to be quite resistant, surviving at least 70°C for 10 min (Pitt and Christian, 1970). A similar effect was observed for conidia of *C. inops* (Kinderlerer, 1996).

For a long time this species was known only from the type isolate, from moist Australian prunes. Christensen (1978b) isolated it (reported as *Chrysosporium inops*) from US maize and other oilseed samples which had been stored at moisture contents of 15–16% in sealed containers for periods of 1–10 years. Other isolates have come from coconut and chocolate (Kinderlerer, 1997) in the United Kingdom and Australia (our laboratory). In spoiled coconut, *C. xerophilum* formed a wide range of volatile compounds including aliphatic methyl ketones, esters and secondary alcohols as well as free medium chain length fatty acids (Kinderlerer et al., 1988).

References. Pitt (1966); van Oorschot (1980).

9.3 Genus *Eremascus* Eidam

Eremascus is distinguished by the formation of asci which are borne singly from undifferentiated hyphae without any surrounding wall or hyphal network. In this characteristic, it resembles *Byssochlamys*. However, no anamorph is produced as a rule. Molecular analysis of the ITS region places *Eremascus* in the Eurotiomycetidae along with *Aspergillus, Penicillium* and other genera described here (Berbee and Taylor, 1992). *Eremascus* species are strict xerophiles, growing only at reduced a_w. Colonies are floccose and remain persistently white, and apparently sterile. Under the compound microscope, abundant asci can be seen. There are two closely related species, *E. albus* and *E. fertilis*, distinguished by differences in the cells supporting the asci. Both have been reported extremely rarely, but are of sufficient interest to be included here, partly in the hope that this will lead to further isolations of these unusual xerophilic fungi.

Eremascus albus Eidam **Fig. 9.5**

No growth on CYA at 5, 25 or 37°C, or on MEA. Colonies on G25N at 7 days 2–3 mm diam, convex, of white mycelium; at 14 days, 11–12 mm diam, convex, centrally 3–4 mm deep, of floccose white

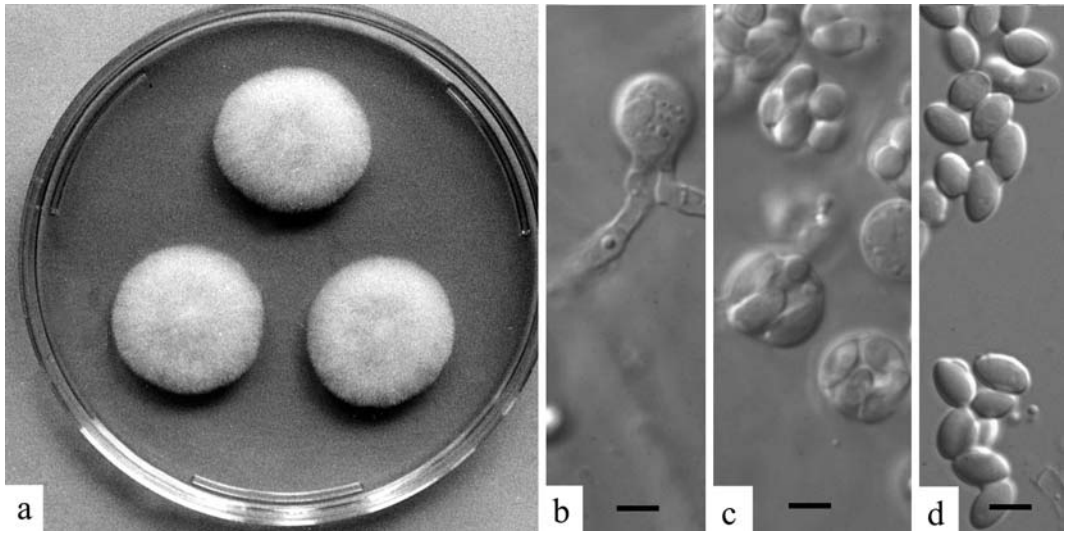


Fig. 9.5 *Eremascus* species **(a)** colonies of *E. albus* on MY50G, 7 d, 25°C; **(b,c,d)** *E. fertilis* **(b)** conjugating hyphal elements forming an ascus, bar = 5 μm; **(c)** asci containing ascospores, bar = 5 μm; **(d)** ascospores, bar = 5 μm

mycelium; reverse uncoloured; occasionally no growth at all. Colonies on MY50G at 7 days, 4–5 mm diam, of low, sparse white mycelium; at 14 days, 14–17 mm diam, deep and floccose, similar to those on G25N.

Reproductive structures solitary asci borne laterally from vegetative hyphae on a pair of spiral suspensors (ascus initials) coiled 2–3 turns and originating from adjacent cells; asci maturing within 14 days on G25N and MY50G, 12–14 μm diam; ascospores broadly ellipsoidal, 6–8 × 5.0–6.5 μm, hyaline and smooth walled. Terminal chlamydoconidia, measuring 7–15 μm diam, occasionally produced.

Distinctive features. See genus preamble.

Physiology. *Eremascus albus* is a mesophilic obligate xerophile, with a maximum a_w for growth between 0.98 and 0.997 and a minimum of 0.70 a_w (Pitt, 1975). Heat resistance of the ascospores is not known, but may be expected to be quite high.

Mycotoxins. No mycotoxins are known from this species.

Ecology. This is a rare fungus: described in 1881 by Eidam, it was not reported again until nearly 70 years later, following its discovery in several samples of English mustard stored for long periods (Harrold, 1950). In this laboratory, it has been isolated once from high moisture prunes and once from spoiled mustard. Occasional further isolations from foods have been recorded in culture collection catalogues.

Additional species. *Eremascus fertilis* Stoppel is a closely related and equally rare species. It is distinguished from *E. albus* primarily by forming asci in which the suspensors are not coiled around each other. There are other differences: *E. fertilis* will sometimes grow slightly on CYA or MEA, and asci and ascospores are smaller, 7–10 μm diam and 5–7 × 2.5–3.5 μm, respectively.

Nothing is known of this species' physiology and little of its ecology. It has been reported only rarely, one occasion being from high moisture prunes in this laboratory (Pitt and Christian, 1968), other isolations being from preserved fruit, honeycomb and cake (CBS, 2007).

Reference. Harrold (1950).

9.4 Genus *Polypaecilum* G. Sm.

This genus is a Hyphomycete characterised by the production of cells which resemble phialides but from which conidia are produced at more than one aperture. These structures are known as **polyphialides**. It is not clear whether the types of polyphialides grouped together in this genus are really the same type of structure, so that *Polypaecilum* may well be heterogeneous. The only species in *Polypaecilum* of interest here is *P. pisce*, which is a halophilic xerophile found on salt fish in tropical

regions. In this species, the polyphialides are very large, up to 60 µm long, sometimes with a distinct resemblance to a human forearm, hand and fingers.

Polypaecilum pisce A.D. Hocking & Pitt — Fig. 9.6

On CYA and MEA at 14 days, colonies 15–20 mm diam, usually low and sparse, sometimes centrally umbonate or irregularly wrinkled, with a dense, velutinous or weakly funiculose texture; sporulation light, mycelium and conidia persistently white; on CYA, sclerotia formed by a few isolates, white to buff, 250–400 µm diam, with walls of pseudoparenchymatous cells, becoming firm at maturity; reverse pale to yellow brown. Colonies on G25N at 14 days 22–26 mm diam, low and sparse, often deeply and irregularly wrinkled centrally, texture usually velutinous, sometimes floccose or funiculose centrally; mycelium persistently white; conidia sparsely produced, uncoloured; exudate and soluble pigment not produced; reverse pale. Colonies on MY50G at 14 days 10–18 mm diam, plane and sparse, margins entire, velutinous to floccose, heavily sporing; mycelium and conidia persistently white; exudate and soluble pigment absent; reverse pale. Colonies on MY5-12 at 14 days 35–45 mm diam, radially sulcate or irregularly wrinkled and often centrally raised; some isolates with growth low, dense and velutinous, others with

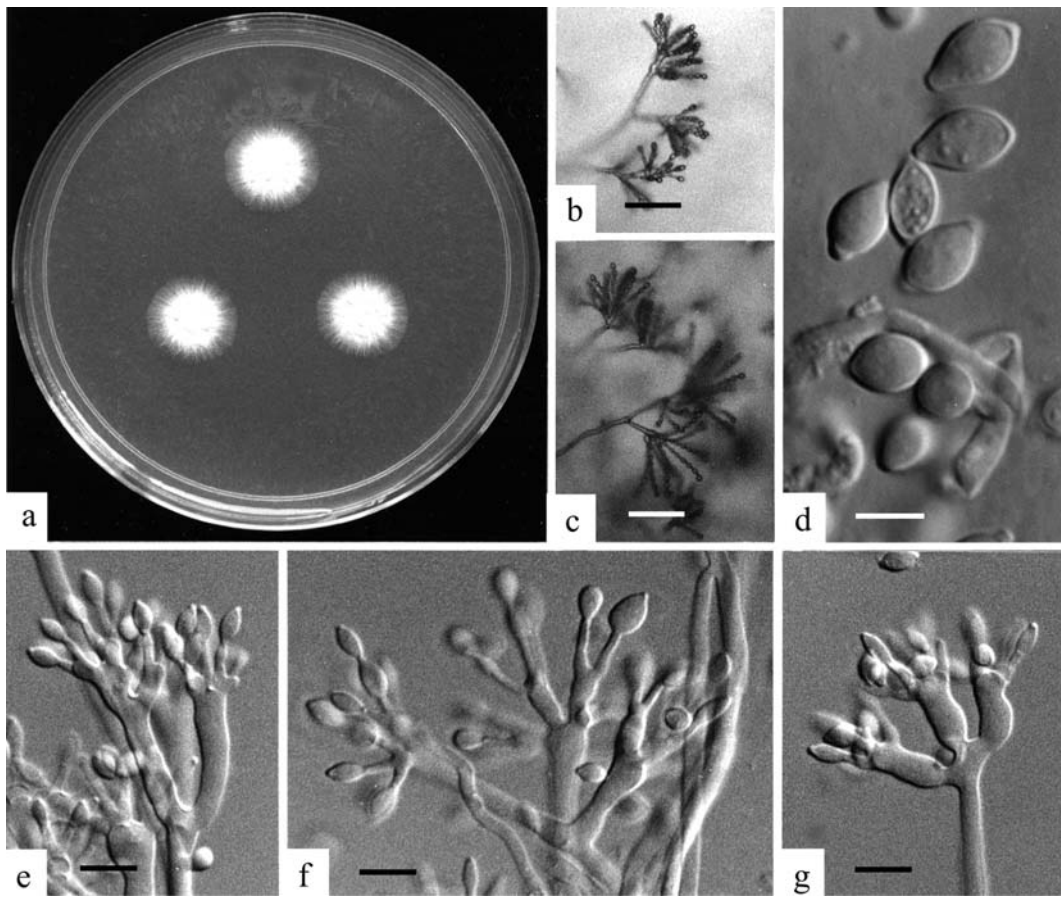


Fig. 9.6 *Polypaecilum pisce* **(a)** colonies on MY5-12, 7 d, 25°C; **(b,c)** conidiophores with polyphialides and conidia in situ, bars = 50 µm; **(d)** conidia, bar = 5 µm; **(e,f,g)** polyphialides and developing conidia, bars = 10 µm

rudimentary fascicles bearing conidial structures terminally, or sometimes with quite well developed funicles, up to 1 mm high, with scattered conidial structures; conidial structures borne in profusion, mostly terminal on ascending or trailing hyphae, each bearing several short chains of conidia clearly visible under the low power microscope; mycelium and conidia persistently white; exudate and soluble pigment not produced; reverse pale to buff. No growth on CYA at 5 or 37°C.

Reproductive structures polyphialides, borne solitarily on short conidiophores from vegetative hyphae; polyphialides large and complex, with a body 15–60 μm long and of varying width, usually 3–5 μm, with thin, smooth walls, unbranched or more commonly dichotomously or irregularly branched near the apex, each branch terminating in 2–5 necks, 3.0–5.0 μm long, each bearing conidia; conidia ellipsoidal to lemon shaped, 5–8 μm long, hyaline, smooth walled, borne in long chains, breaking up in wet mounts.

Distinctive features. See the genus preamble.

Taxonomy. Other species in this genus, *Polypaecilum insolitum* G. Sm. and *P. capsici* (J.F.H. Beyma) G. Sm., have not been reported to grow on media of reduced water activity. A close relationship to *P. pisce* is therefore doubtful.

Physiology. *Polypaecilum pisce* is a tropical halophilic xerophile with an optimum a_w range for growth between 0.96 and 0.87 a_w. Growth is faster and the growth optimum occurs over a broader a_w range on NaCl based media than on glucose/fructose or glycerol based media (Andrews and Pitt, 1987; Wheeler et al., 1988c). The optimum temperature for growth is near 30°C although *P. pisce* also grows strongly at 37°C over a narrower a_w range (Wheeler et al., 1988c). At 30°C, the minimum a_w for germination in NaCl media was 0.75 (saturated NaCl) after 17 days, and in glucose/fructose 0.71 a_w after 7 days (Wheeler et al., 1988c). At 25°C, *P. pisce* germinated at 0.83 a_w in 7.5 days in NaCl media, in glucose/fructose 0.77 a_w after 4 days and in glycerol 0.75 a_w after 7 days (Andrews and Pitt, 1987). The maximum radial growth rate of 85–90 μm/h was achieved over the temperature range 30–37°C and a_w range of 0.95–0.88 a_w. This rate of growth was also achieved in glucose/fructose at 37°C but only at 0.92 a_w (Wheeler et al., 1988c). *P. pisce* accumulates glycerol as an internal solute when growing under conditions of reduced a_w on NaCl-based media (Hocking, 1986).

Polypaecilum pisce is very well adapted to its habitat of dried salted fish. Studies on the interactions of five xerophilic fungi isolated from salt fish (*P. pisce*, *Basipetospora halophila, Eurotium rubrum, Aspergillus wentii* and *A. penicillioides*) on NaCl based media and on salt fish at 30°C have shown that below about 0.88 a_w *P. pisce* outgrows the other fungi, despite relatively slow growth rates at higher a_w values (Wheeler and Hocking, 1993).

Mycotoxins. No mycotoxins have been reported from this species.

Ecology. This species was initially isolated in our laboratory from imported Asian dried fish in 1979. It appeared to be a curiosity. During a much more extensive study on dried fish, however, it became apparent that *Polypaecilum pisce* is a major cause of spoilage of dried fish in Indonesia. This species was isolated from 42% of 74 samples of Indonesian fish; 20% showed profuse growth of this fungus (Wheeler et al., 1986). *P. pisce* appears to be of restricted distribution: all cultures in the FRR collection have come from Indonesian dried seafood with the exception of the original isolate, FRR 2185 (now lost), which came from the Philippines. The CBS database records one isolate from yeast extract in the Netherlands in 1998 (CBS, 2007).

Reference. Pitt and Hocking (1985).

9.5 Genus *Wallemia* Johan-Olsen

Recent studies have shown that the tiny brown fungus *Wallemia sebi* comprises three species, *W. sebi, W. muriae* and *W. ichthyofaga*, all of which are xerophilic (Zalar et al., 2005). These species are distinguishable by differences in conidial size, water relations and molecular sequence data. It is impossible to know which of the numerous isolations described as *W. sebi* in the pre-2005 literature relates to which of these species. However, we believe that the most common foodborne species is *W. sebi* which grows over an exceptionally wide range of a_w and is indifferent to solute type as well.

Wallemia sebi (Fr.) Arx — Fig. 9.7

Sporendonema sebi Fr.
Sporendonema epizoum (Corda) Cif. & Redaelli

Colonies on CYA and MEA 1–6 mm diam, plane or crateriform, velutinous, margins narrow, coloured uniformly brown; reverse deep brown. Colonies on G25N at 7 days 2–6 mm diam, as on CYA. No growth on CYA at 5 or 37°C. Colonies on MY50G at 7 days 2–5 mm diam, paler and less dense than on CYA.

Reproductive structures short fertile hyphae, septating into segments during elongation, then segments subdividing into four cylindrical cells, subsequently rounding up into conidia; conidia 1.5–3.0 (–4.0) µm diam, with walls finely roughened or spinose, adhering in short chains.

Distinctive features. *Wallemia* species are unique foodborne fungi, readily distinguished by the formation of small, brown colonies on the standard media at 25°C. The reproductive structures are unique.

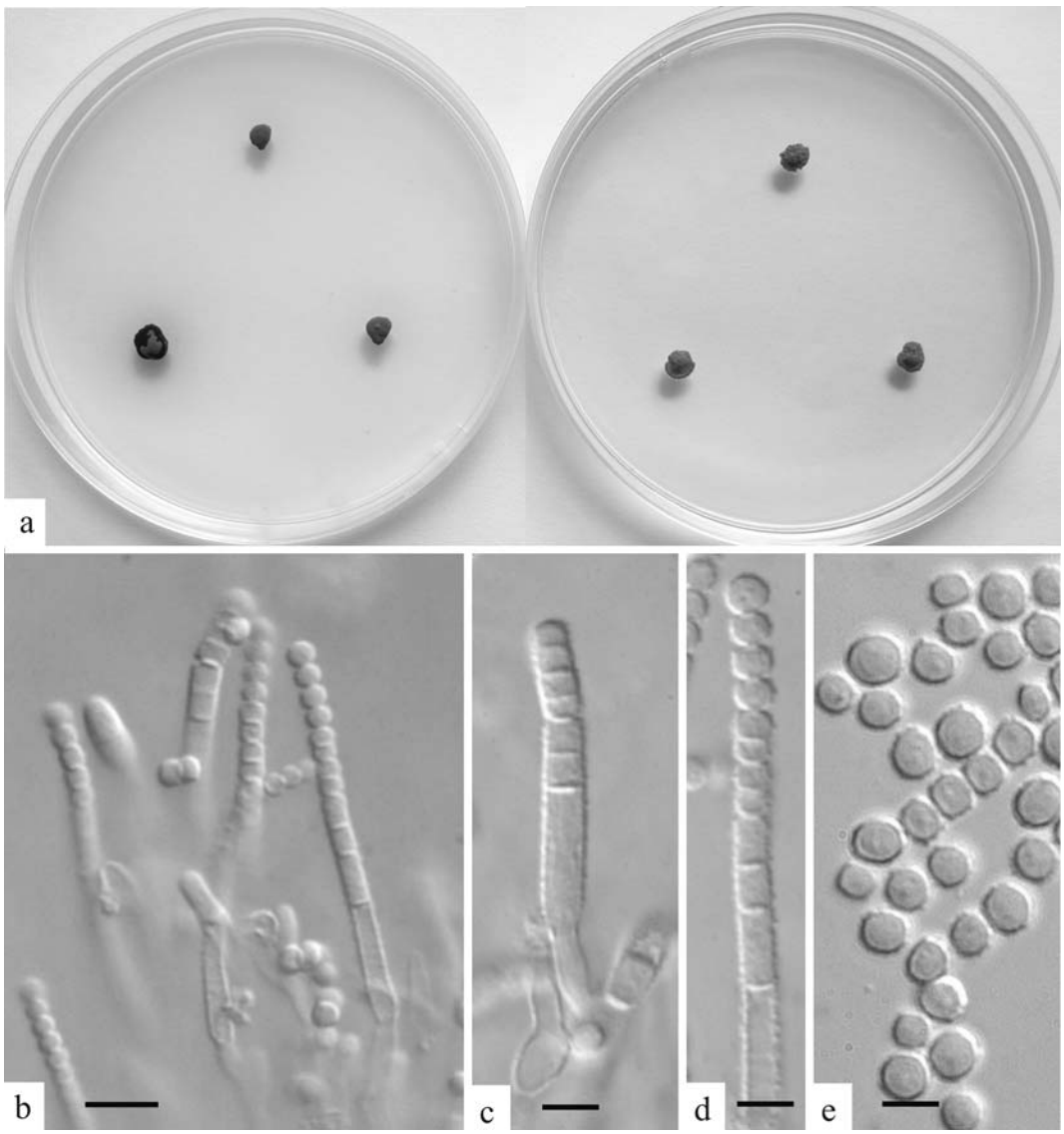


Fig. 9.7 *Wallemia sebi* **(a)** colonies on CYA and MY50G, 7 d, 25°C; **(b,c,d)** conidiophores with developing conidia, **(b)** bar = 10 µm; **(c,d)** bars = 5 µm; **(e)** conidia, bar = 5 µm

Although the exact manner of conidium formation is still in dispute, the end result is the rapid and virtually complete differentiation of aerial hyphae into small spherical brown conidia. If mature colonies are jarred, the aerial growth, consisting almost entirely of conidia, is released as a powdery mass. *W. sebi* is distinguished from the other two species by having smaller conidia (1.5–2.5 μm diam) and ability to grow on high a_w media. *W. muriate* conidia are 2.5–3.0 μm diam, and those of *W. ichthyophaga* are 3.5–5.0 μm diam. Neither *W. muriae* nor *W. ichthyophaga* is able to grow on CYA or MEA.

Taxonomy. For the first half of this century, this fungus was generally known as *Sporendonema sebi* or *S. epizoum*, but the type species of *Sporendonema* is unrelated. Barron (1968) revived the little known name *Wallemia*, and von Arx (1970) made the combination which gave priority to the earliest epithet. Moore (1986) suggested that *Wallemia* could be a Basidiomycete. This was confirmed by Zalar et al. (2005) and Matheny et al. (2006), who used molecular analysis of nuclear small subunit ribosomal DNA and sequence data of the ITS rDNA and several genes. Zalar et al. (2005) proposed a new basidiomycetous class Wallemiomycetes, covering an order Wallemiales. The only genus within this order is *Wallemia*, in which Zalar et al. (2005) recognised and neotypified three species: *W. ichthyophaga, W. sebi* and *W. muriae*.

Physiology. Conidia of *Wallemia sebi* are produced in vast numbers, and extremely rapidly. *W. sebi* can sporulate within 1–2 days of germination on media between 0.997 and 0.92 a_w and within 5 days at 0.85 a_w (Hocking, 1986). However, conidia are short lived on both media and natural substrates. They are relatively sensitive to low pH (Ormerod, 1967) and are of low heat resistance (Pitt and Christian, 1970; Beuchat and Pitt, 1990a). A $D_{57.5}$ value of 1 min, and a z value of 8.1C° for conidia heated in phosphate buffer, pH 5.5, with 400 g/l sucrose has been reported (Baggerman and Samson, 1988).

Wallemia sebi is capable of growth over a very wide a_w range, about 0.997–0.69 at 25°C in glucose/fructose media at pH 4.0 (Pitt and Hocking, 1977) and 0.92–0.74 at pH values 4.5–7.5 (Gock et al., 2003). In media with NaCl as the major solute, the lower limit for growth at 25°C has been reported as 0.80 a_w at pH 4 and 0.75 a_w at pH 6.5 (Pitt and Hocking, 1977). At 20°C, *W. sebi* germinated at 0.75 a_w (saturated NaCl) in 15 days and in glucose/fructose at 0.69 a_w in 46 days, but its minimum a_w for growth at this temperature was 0.78 a_w in NaCl and 0.80 in glucose/fructose based media (Wheeler et al., 1988a).

In glucose/fructose based media at 20°C, optimum growth occurred between 0.98 and 0.93 a_w, whereas at 30°C, optimum growth occurred over a lower a_w range, 0.93–0.85 a_w. This temperature effect was not observed on NaCl based media. The fastest growth rate for *Wallemia sebi* (18–20 μm/h) was observed on glucose/fructose media at 20 and 30°C over the a_w ranges discussed above. The lowest a_w at which growth occurred at 20°C (0.78 a_w) was on NaCl based media (Wheeler et al., 1988a). Vindelov and Arneborg (2002) reported growth at 10°C in crystalline sucrose at 0.82 a_w but not at 0.76 a_w.

Wallemia sebi accumulates glycerol as its main compatible solute when growing under conditions of reduced a_w. In media containing glucose and fructose, these two solutes were accumulated along with glycerol and traces of mannitol and arabitol (Hocking and Norton, 1983). On NaCl based media, glycerol was the major compatible solute (Hocking, 1986).

Mycotoxins. Two related, but distinct toxic compounds have been reported from *Wallemia sebi*, walleminol A and B (Wood et al., 1990). Walleminol A appears to be a tricyclic dihydroxysesquiterpene with an LD_{50} of 40 μg/ml for brine shrimp and an MIC of 50 μg/ml for rat liver cells. There is no indication of toxicity *in vivo* in commonly used animal systems.

Ecology. Its ability to grow at almost any a_w supporting microbial growth, its rapid sporulation and small, easily dispersed conidia ensure that *Wallemia sebi* is ubiquitous. It has long been considered to be the principal fungus spoiling dried and salt fish (Frank and Hess, 1941), on which it is known as "dun" mould. It is rare on tropical fish in our experience, however (Wheeler et al., 1986).

It has been isolated in our laboratory from a very wide range of foods, especially dried commodities, including dried prunes, dried peas, maple syrup, sultanas, jams and rice (see Pitt and Hocking, 1997). A straw hat imported from the Philippines became completely covered in *Wallemia sebi* during

a particularly humid Sydney summer. Counts in dried chillies and pepper have exceeded 10^8 and 10^9 conidia per gram, respectively (Hocking and Pitt, 1980; Hocking, 1981). Almost any sample of Australian rice, wheat or bread, suitably moistened and incubated, will yield this fungus. During storage trials, we have observed populations of *W. sebi* develop in wheat stored at 20°C at a_w values between 0.71 and 0.75 over a period of 1 year. Data sheets of the International Mycological Institute, Egham, Surrey, record isolations from bread, milk, condensed milk, jams, jellies, dates, marzipan cakes, suet, gingerbread, etc.

Wallemia spp. are commonly isolated from hypersaline waters of salterns (Zalar et al., 2005); however, records of *W. sebi* in the literature are relatively rare. This must be due to oversight or inadequate isolation techniques. *W. sebi* is ubiquitous: it has been reported from Australia, Japan, Southeast Asia, the United Kingdom, Europe, Scandinavia, Canada, the United States and more recently from the Shaanxi Province, China (Sun et al., 2006). Isolations have been reported from rice, jam, pecans, meat products including Italian salami (Cantoni et al., 2007), rapeseed and Japanese noodles (see Pitt and Hocking, 1997). We have found *W. sebi* in maize, peanuts, cashews and soybeans from Thailand (Pitt et al., 1993, 1994); peanuts, maize, paddy and milled rice, soybeans and mung beans from Indonesia; and peanuts, maize, soybeans, mung beans and black peppercorns from the Philippines (Pitt et al., 1998a and our unpublished data). It is interesting that C.M. Christensen who with his coworkers examined the fungi on North American grains for many years (see for a summary Christensen and Kaufmann, 1965) first mentioned *W. sebi* in grains (Christensen, 1978a) after Pitt (1975) drew attention to this omission.

Wallemia sebi is significant in indoor environments (Samson and Hoekstra, 1994), but is often not reported because high a_w media are used in air sampling equipment. The use of DG18 in air sampling equipment is recommended for detection of xerophilic fungi in indoor air (Samson and Hoekstra, 1994). Since that recommendation, *W. sebi* is now commonly reported from indoor environments worldwide and has been implicated as one cause of farmer's lung disease (Reboux et al., 2001). Using DG18, we have isolated *W. sebi* in high numbers from mould affected buildings.

Reference. Barron (1968); Zalar et al. (2005).

9.6 Genus *Xeromyces* L.R. Fraser

The genus *Xeromyces* has a single species, *X. bisporus*, which is distinctive. It has the lowest requirement for available water of any known organism. Growth will not occur on media of high a_w, usually not even on G25N. On more favourable media, and carbohydrate rich substrates at lower a_w, it grows quite rapidly and produces colourless cleistothecia, with evanescent asci containing two "D"-shaped ascospores.

Xeromyces bisporus L.R. Fraser **Fig. 9.8**

Monascus bisporus (L.R. Fraser) Arx

No growth on CYA at 5, 25 or 37°C, or on MEA. On G25N, at 7 days, no germination to microcolony formation; at 14 days no germination to dense colonies up to 4 mm diam. Colonies on MY50G at 7 days, 3–6 mm diam, low and sparse; at 14 days, 15–20 mm diam, low and dense, translucent with a glistening surface, colourless or very pale red brown; reverse uncoloured; at 4 weeks, 50–70+ mm diam, low, translucent and sometimes glistening, colourless or faintly red brown, with contiguous layers of colourless cleistothecia visible under the low-power microscope; reverse uncoloured.

Cleistothecial initials evident on MY50G at 2 weeks, commencing as three short cells, then developing distinctive finger-like processes from the bottom cell, enveloping the second, the latter then enlarging to form the cleistothecium; cleistothecia maturing in 4–6 weeks, 40–120 μm diam, with walls thin and structureless; asci inconspicuous and evanescent, containing two ascospores only; ascospores ellipsoidal, flattened on one side ("D"-shaped), 10–12 × 4.0–5.0 μm, smooth walled. Aleurioconidia developing below 0.90 a_w, solitary, usually measuring 15–20 × 12–15μm.

Distinctive features. In culture, *Xeromyces bisporus* is distinguished by its inability to grow on

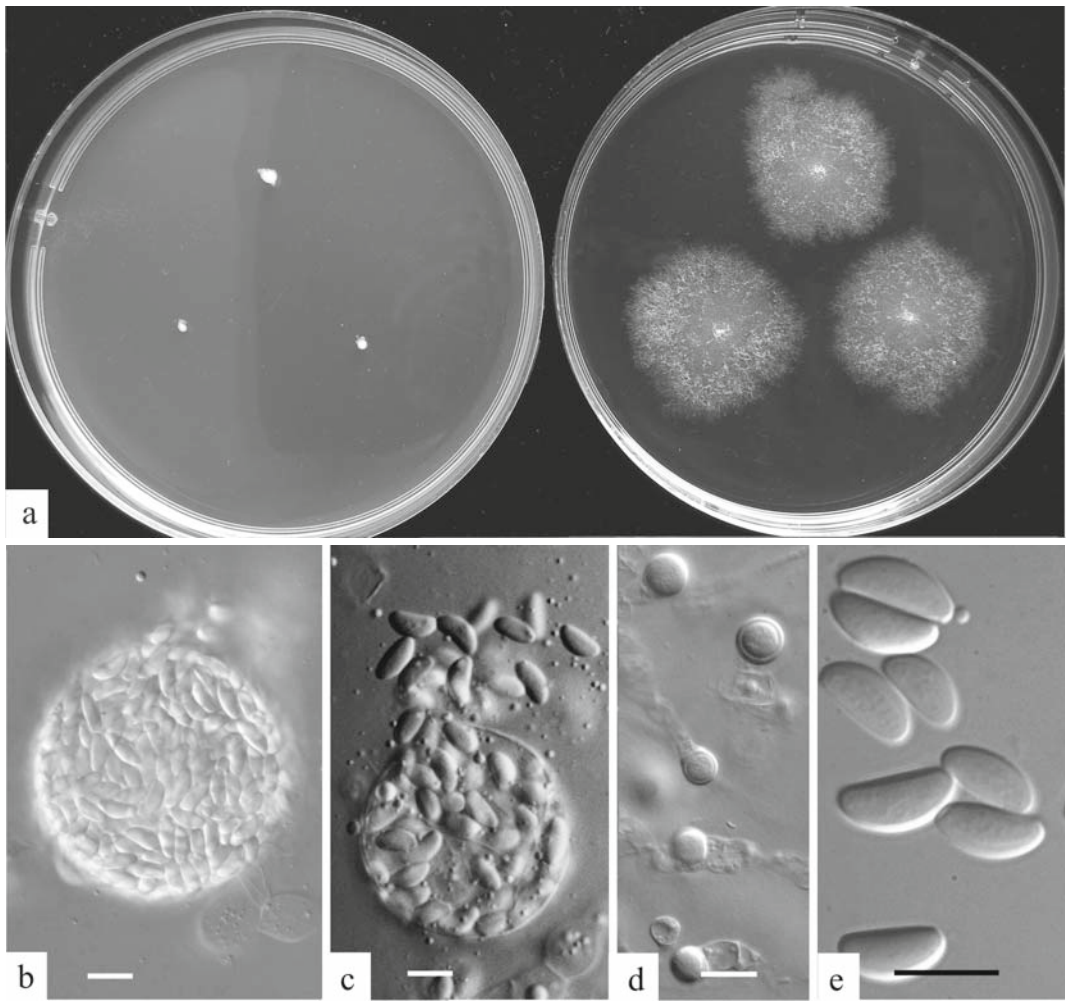


Fig. 9.8 *Xeromyces bisporus* **(a)** colonies on G25N and MY50G, 14 d, 25°C; **(b)** developing cleistothecium, bar = 25 μm; **(c)** mature cleistothecium releasing ascospores, bar = 25 μm; **(d)** aleurioconidia, bar = 10 μm; **(e)** ascospores, bar = 10 μm

CYA, MEA or (usually) G25N; by relatively fast but, in young cultures, strictly mycelial growth on MY50G, with cleistothecial formation occurring only after about 2 weeks incubation at 25°C; and, later, by its distinctive D-shaped ascospores.

On carbohydrate rich foods of 0.75 a_w or less, the presence of *Xeromyces* may be inferred from luxuriant but low white or translucent growth. The observation of D-shaped ascospores under the microscope is diagnostic for *X. bisporus*. Xerophilic *Chrysosporium* species may produce similar growth, but it is powdery and under the microscope, mostly consists of aleurioconidia or chlamydoconidia.

Taxonomy. Von Arx (1970) transferred *Xeromyces bisporus* to *Monascus*. However, other taxonomists have maintained *Xeromyces* as a distinct genus related to *Monascus*. Analysis of the D1/D2 region of the large subunit rDNA suggests that *Xeromyces* and *Monascus* are monophyletic, justifying placement of the two genera in the same family within the *Eurotiales* (Park and Jong, 2003), but the issue of a separate genus for *Xeromyces* requires further investigation.

Physiology. *Xeromyces bisporus* is unable to grow above 0.96 a_w, and it has the lowest requirement for available water (0.61 a_w) of any known organism (Pitt and Christian, 1968). Germination at this a_w

required 120 days. Its optimum a_w for growth is 0.85 (Pitt and Hocking, 1977). Germination and growth at low a_w are extraordinarily rapid in comparison with other xerophiles, its radial growth rate on glucose/fructose media at 0.75 a_w (ca. 25 μm/h) still being nearly half that of its optimum. Germination was recorded at 0.70 a_w at 25, 30 and 37°C and the maximum growth rate occurred at 37°C (Gock et al., 2003).

Colonies on glucose/fructose media produced aleurioconidia in 80 days at 0.66 a_w; ascospores were observed in cultures at 0.67 a_w in a similar time period. The ability to complete a sexual life cycle at water activities lower than almost any other life form can grow is remarkable. Only *Zygosaccharomyces rouxii* has a comparable ability.

Growth of *Xeromyces bisporus* is much more rapid on media containing glucose/fructose as controlling solute than on other media. Maximum growth rates on glycerol based media are less than one third of those on glucose/fructose at the optimal a_w. On these media, pH 4.0 or 6.5 had little effect on growth rates. Growth on NaCl media occurred only at pH 4.0 and only over the range 0.96–0.87 a_w (Pitt and Hocking, 1977).

Like the xerophilic *Chrysosporium* species, *Xeromyces bisporus* appears to have a high tolerance of CO_2. Dallyn and Everton (1969) reported growth in an atmosphere of 95% CO_2, in the presence of 1% O_2. However, in work carried out in our laboratory, *X. bisporus* failed to grow on MY50G agar in atmospheres of 80% CO_2 with 20% O_2 or 20% CO_2 with <0.5% O_2 (Taniwaki, 1995).

Ascospores of *Xeromyces bisporus* are quite heat resistant. Pitt and Christian (1970) reported that a small proportion (0.1%) survived 10 min heating at 80°C, while Dallyn and Everton (1969) observed that to kill 2,000 ascospores in a medium of 0.9 a_w and pH 5.4 required more than 2 min at 90°C, 4 min at 85°C and 9 min at 80°C. Using these data, Pitt and Hocking (1982) constructed a thermal death–time curve, which was defined by a *z* value of 16.0C° and a $D_{82.2}$ of 2.3 min.

Mycotoxins. No mycotoxins are known from this species.

Ecology. *Xeromyces bisporus* is probably a much less rare fungus than the literature would indicate. The original isolation (Fraser, 1953) was from liquorice, and it has been seen in our laboratory causing spoilage of this product twice since. Dallyn and Everton (1969) reported it from British table jelly, dried prunes, tobacco, currants (of 0.67 a_w) and chocolate sauce. It was the most common spoilage mould on Australian prunes in the study reported by Pitt and Christian (1968), but its dominance on that substrate has been less marked in recent years. We have isolated *Xeromyces* from spice powders, nutmegs, imported Chinese dates of 0.72 a_w, fruit cakes of 0.75–0.76 a_w, gelatine confectionery, mixed dried fruit and cookies containing a high proportion of dried fruit pieces, liquorice allsorts, fudge and strawberry fruit snacks. Growth on the dates and fruit cakes was luxuriant and commercial losses high.

Xeromyces bisporus can become established in plants producing low a_w products such as fruit cakes and cookies, providing a continuous low level inoculum. Ascospores appear capable of surviving the baking process, leading to sporadic spoilage which is often not manifested until many months after production. Tracing the source of the contamination can be difficult, if not impossible, particularly in view of the elapsed time between production and visual spoilage. Careful attention to cleaning of all product residues from production lines and equipment before sanitation is essential for eliminating this type of spoilage.

Xeromyces bisporus has been reported from the Australian and UK sources discussed above, and in date honey from Israel, tobacco in the Netherlands, and chocolate in the United Kingdom (CBS, 2007).

References. Dallyn and Everton (1969); Pitt and Hocking (1982).

Chapter 10
Yeasts

Yeasts are fungi which reproduce vegetatively by means of single cells which bud, or less commonly, divide by fission. This property enables yeasts to increase rapidly in numbers in liquid environments, which favour the dispersal of unicellular microorganisms. Many yeasts grow readily under strictly anaerobic conditions, again favouring their growth in liquids. On the other hand, reproduction as single cells restricts spreading on, or penetration into, solid surfaces, where filamentous fungi are at an advantage. Being eukaryotic organisms, yeasts reproduce more slowly than do most bacteria, and hence do not compete in environments which favour bacteria, i.e. at pH values near neutral or at very high temperatures. In common with filamentous fungi, many yeasts are tolerant of acid conditions. In broad terms, then, yeasts are more likely to be active in acidic, liquid environments than elsewhere. However, many yeasts also appear to be highly resistant to sunlight and desiccation and occur widely in nature on the surfaces of leaves, fruits and vegetables.

When defined as above, i.e. as budding, nonphotosynthetic eukaryotes, yeasts are a heterogeneous assembly of often quite unrelated fungi. Barnett et al. (2000) recognised almost 680 species of yeasts in all, divided into over 90 genera: 57 were classified as Ascomycetes, 36 as Basidiomycetes with 2 unclassified. It is not surprising, then, that yeasts possess diverse properties.

As with other fungi, yeasts are classified into genera primarily on the type and appearance of spores, in this case ascospores or basidiospores. Because yeasts possess limited morphological variability, classification at species level has traditionally relied on biochemical tests, principally the utilisation of various carbon or nitrogen sources, and vitamin requirements. Some physiological properties, i.e. growth at high temperature and reduced water activity, are used in a secondary role. The 2000 edition of "Yeasts: characteristics and identification" listed 90 separate tests, including utilisation of 47 carbon sources, 10 nitrogen sources, vitamin requirements, fermentation patterns, growth at various temperatures and in the presence of elevated glucose and NaCl levels and morphological characteristics. Clearly, taxonomy of that kind is beyond the scope of the present work.

In recent years, DNA sequencing has become a major classification tool and is now commonly used for yeast identification. Molecular methods have clarified some of the bases for taxonomy of yeasts, but at the same time have led to increasing complexity, with a seemingly random reassortment of species into genera with each new monograph.

Teleomorph–anamorph connections. As with other fungi of industrial significance, ascomycetous and basidiomycetous yeasts frequently reproduce in nature or in the laboratory as their anamorphic states, and hence possess anamorphic names. Diligent study has in recent years enabled yeast specialists to link many anamorphs with teleomorphs, and in many cases the anamorph name has fallen into disuse. As with some filamentous fungi, however, a case for dual nomenclature exists because establishing the anamorph–teleomorph connection is often difficult. Sometimes too the link is tenuous, with only a few isolates carrying the life cycle to completion. Dual names have been retained here for most species discussed.

J.I. Pitt, A.D. Hocking, *Fungi and Food Spoilage*, DOI 10.1007/978-0-387-92207-2_10,
© Springer Science+Business Media, LLC 2009

Yeasts in foods. By comparison with many strictly filamentous fungi, yeasts possess limited biochemical pathways and quite fastidious nutritional requirements. Foodstuffs, generally substrates rich in the hexose sugars, minerals and vitamins, which many yeasts require for growth, are an ideal substrate for yeasts. Their association with the phyllosphere of many crops ensures their presence on fruit and vegetables, and their entry into food processing plants.

Relatively few yeast species cause significant spoilage in processed foods, most being adventitious contaminants from natural sources. Deák and Beuchat (1996) list 99 yeast species which occur in foods including fruit, beverages, wine, beer, meat, dairy products, low a_w products and low pH products. Many of these yeasts grow poorly if at all in properly formulated processed foods, as they are intolerant of reduced water activity, heat processing or preservatives. Even if limited metabolism by such yeasts does occur, it is usually of little consequence unless there is significant gas production. It is doubtful whether any yeasts produce mycotoxins, and few produce even marginally unacceptable off-odours. There are, of course, exceptions. Certain species must be classified as spoilage yeasts because they possess one or more undesirable properties. These commonly occurring spoilage species form the basis for most of the subject matter of this chapter.

Spoilage yeasts. In our experience, only about ten species of yeasts are responsible for spoilage of foods which have been processed and packaged according to normal standards of good manufacturing practice. These species are responsible for major losses of processed foods around the world every year. We believe that relatively simple tests can be used to identify them when taken in conjunction with their spoilage habitats. These species are listed in Table 10.1, with their principal undesirable properties. Two species which do not cause spoilage, but are of widespread occurrence in foods, are also included in Table 10.1, and in subsequent descriptions in this chapter.

It is important to note that if good manufacturing practice is neglected, i.e. if factory hygiene is poor, if preservatives are omitted, either deliberately or unintentionally, if pasteurising temperatures are inadequate, or filling machinery or factory premises are unsanitary, raw materials are of poor quality, brining or syruping procedures are poorly controlled, etc., many other adventitious yeast contaminants can develop in a product. In such cases the following text will be of little value and neither will identification of the yeasts concerned. The correct approach, and often the only recourse in such

Table 10.1 Spoilage yeasts

Yeast	Important properties
Brettanomyces bruxellensis	Production of off-odours in beer, cider and soft drinks
Candida krusei	Preservative resistant; film formation on olives, pickles and sauces
C. parapsilosis	Lipolysis, fermentative; causes spoilage in a wide range of foods including cheese, margarines, dairy and fruit products
Debaryomyces hansenii	Growth at low water activities in foods preserved with NaCl, especially salt meat
Kazachstania exigua	Moderately preservative resistant; of common occurrence in olive brines, relevantly rarely the cause of spoilage of sauerkraut or of juices, dairy products or soft drinks
Kloeckera apiculata	Spoilage of fresh and processed fruit
Pichia anomala	Spoilage of processed fruit and yoghurts containing fruit
P. membranaefaciens	Preservative resistant; film formation on olives, pickles and sauces
Rhodotorula mucilaginosa *R. glutinis*	Common food contaminants; spoilage of dairy products; occasional spoilage of fresh fruits
Saccharomyces cerevisiae	Common food contaminant; sometimes fermentative spoilage of soft drinks; some strains preservative resistant
Schizosaccharomyces pombe	Preservative resistant; relatively rare spoilage yeast
Zygosaccharomyces bailii	Preservative resistant; fermentative spoilage of acid, liquid preserved products such as juices, sauces, ciders and wines
Z. bisporus	Preservative resistant; properties intermediate between *Z. bailii* and *Z. rouxii*
Z. rouxii	Growth at extremely low water activities; fermentative spoilage of juice concentrates, honey, jams, confectionery, packaged dried fruits, etc.

cases, is to pay attention to manufacturing guidelines, so that this kind of problem is positively eliminated.

Identification of spoilage yeasts. Considerable work has been carried out in recent years to develop simplified systems for identification of foodborne yeasts, as yeast identification procedures based on biochemical reactions are too complex and take too long to be of value in the food or industrial microbiology laboratory. Several automated systems are now commonly used, as well as simpler laboratory-based systems, and these are discussed in some detail in Chapter 4. Identification using DNA sequencing is increasingly becoming the method of choice, as extensive databases such as GenBank are freely available for identification purposes. The 600–650 nucleotide D1/D2 region of the large subunit (26S) ribosomal DNA is the most widely targeted section of the genome, and sometimes the ITS region may also be used (Kurtzman et al., 2003). However, a word of caution. Results presented by a computer identification system or DNA sequence database still need to be interpreted with care: computers are not infallible, and sequences in DNA databases do not always have the correct species name! Does the result make sense? Keep in mind the source of the yeast isolate and the spoilage problem being investigated. Is the yeast reported a relatively common foodborne yeast, or a rare yeast only ever isolated once or twice from some obscure source? If the computer key gives a list of yeasts differing by one result (one discrepant test), check this list along with the discrepant test to see if your yeast is really more likely to be one of those listed as a second option. A weak reaction may have been incorrectly interpreted as negative. Perhaps carrying out one or two other tests can give better results.

A simplified approach has been used here which differentiates the species of yeasts most commonly responsible for actual spoilage of processed foods and beverages. Table 10.2 lists a series of media and conditions which will enable differentiation of the species listed in Table 10.1. To simplify and expedite identification as far as possible most media and conditions specified here have been used in the identification of filamentous fungi elsewhere in this book. The exceptions are the use of malt acetic agar, Czapek agar and growth on MEA at 37°C. The first of these media distinguishes preservative-resistant yeasts from others; on the second only yeasts which utilise nitrate as a sole carbon source can grow. MEA is used at 37°C rather than the customary CYA because some yeasts grow poorly on CYA. Czapek agar is made with the same ingredients and procedures as CYA but yeast extract is omitted.

It is emphasised that the procedures used here will work only for yeasts causing actual spoilage: the system is not designed to cope with the myriad species of yeasts than can occur adventitiously in foods. Even so, the procedures are not rigorous and may on occasion misidentify an excluded yeast as being one of those discussed here. Provided all tests are carried out as specified and morphological observations made, such occasions will be infrequent. In particular preservative-resistant yeasts, the most important yeasts in food spoilage will usually be readily recognised by the techniques described below.

Table 10.2 Media and conditions for identification of spoilage yeasts[a]

Medium	Purpose
Czapek agar	Assessing ability to utilise nitrate as a sole nitrogen source
Malt Extract Agar (MEA)	Colony and cell morphology
Malt Acetic Agar (MAA)	Assessing preservative resistance
MEA at 37°C	Assessing growth at elevated temperatures
Malt Yeast 50% Glucose Agar (MY50G)	Assessing growth at reduced water activities in the presence of high carbohydrate levels
Malt Yeast 10% Salt 12% Glucose Agar (MY10-12)	Assessing growth at reduced water activities in the presence of high sodium chloride

[a] Incubation is at 25°C unless specified. Inspection should be at 3 and 7 days after inoculation.

Procedures for yeast identification. Pour Petri dishes with the media listed in Table 10.2. From a 3 to 7 day old slant culture of the yeast, preferably growing on MEA, disperse a small loopful of cells in 3–5 ml sterile water or 0.1% peptone. Streak each plate with a loopful of cells from this inoculum. A suitable streaking technique is described in Chapter 4. Incubate plates for 3 days, then examine each for presence or absence of growth. Note also colony colour and the size and shape (regular or irregular) of well separated colonies. Make a wet microscopic mount in water, lactic acid or lactofuchsin (see

Chapter 4) from the MEA plate grown at 25°C and from malt acetic agar. Record approximate cell size and shape, position of budding and the presence or absence, type and number of ascospores (see the following figures for a guide). Reincubate plates and repeat observations at 7 days.

Salient properties on these media of yeasts included here are listed in Table 10.3. The key which follows is based on growth for 7 days on the media in Table 10.2. Cell sizes are from colonies on MEA at 25°C, aged between 3 and 7 days. The species are described and discussed below in alphabetical order.

Brettanomyces bruxellensis Kuff. & van Laer Fig. 10.1

Brettanomyces intermedius (Krumbholz & Tauschan.) van der Walt & Keuken
Dekkera intermedia van der Walt

Teleomorph: *Dekkera bruxellensis* van der Walt

Colonies on MEA at 3 days minute, white; at 7 days 1.5–2.0 mm diam, white, convex, margins circular, surface glistening. Cells on MEA at 3 days mostly ellipsoidal to ogival (pointed at one end, rounded at the other), less commonly spherical or cylindrical, 4.5–7 × 3.0–4.0 µm, reproducing by budding, terminally or subterminally, but not laterally; occurring singly, in pairs, short chains or clusters. Teleomorph not produced under conditions used here. An acetic acid or other sharp or fruity off-odour usually produced.

No growth on Czapek agar; growth at 37°C more rapid than at 25°C; no growth on malt acetic agar; slow growth on MY50G; no growth on MY10-12.

Distinctive features. Species of *Brettanomyces* are distinguished by the formation of ogival cells, exclusively terminal budding and the production of acetic acid from glucose under aerobic conditions. Cultures are usually short lived unless 2% calcium carbonate is incorporated in the growth medium to neutralise the acid produced.

Most *Brettanomyces* species have similar properties; identification of an isolate to genus is usually sufficient. *B. bruxellensis,* in our experience and that of others, is the species most commonly isolated from foods.

Taxonomy. Kurzman and Fell (1998) and Barnett et al. (2000) agree that *Brettanomyces intermedius* is a synonym of *B. bruxellensis*.

Physiology. As noted above, the most important characteristic of *Brettanomyces bruxellensis* and other *Brettanomyces* species is the ability to produce acetic acid from glucose. Pitt (1974a) reported growth of this species down to pH 1.8 in a medium acidified with HCl and to pH 2.3 in citric acid. *Brettanomyces* species are resistant to acetic acid and SO_2 (Stratford and James, 2003), although Loureiro and Malfeito-Ferreira (2006) report 70–75 mg/l to be the maximum

Table 10.3 Salient properties of yeasts at 7 days

Species	Cell length MEA (µm)	Colony diam MEA (mm)	Colony colour MEA	Growth on Czapek	MEA 37°C	MAA	MY50G	MY10-12
B. bruxellensis	4.5–7	1.5–2	White	0	+	0	w	0
C. krusei	3–25	5–8	White	w	+	+	0	0
C. parapsilosis	3–20	3–4	White	w	+	v	+	+
D. hansenii	2.5–4	2.5–4	White	w	0	0	w	+
Kaz. exigua	4–5	1–2	White	0	0	0	0	0
K. apiculata	3.5–6	2–4	White	±	0	0	0	0
P. anomala	3–7	3–4	White	+	0–vw	0	vw	+
P. membranaefaciens	4–6	3–4	White	W	vw	+	0	0
R. mucilaginosa, R. glutinis	4.5–5	5–10	Red	±	0	0	0	0
S. cerevisiae	5–12	2.5–4	White	W	+	w	0	0
Sch. pombe	5–7	1–2	White	W	+	+	w	0
Z. bailii	5–8	2–3	White	0	0	+	+	0
Z. bisporus	3.5–7	2–3	White	0	0	0	+	0
Z. rouxii	5–7	2–3	White	0	0	0	+	+

0, no growth; w, weak; vw, very weak; + 1 mm diam or more in 7 days.

Key to spoilage yeasts

1	Colonies on MEA white, off-white or brownish	2
	Colonies on MEA pink or red	*Rhodotorula mucilaginosa* *Rhodotorula glutinis*
2 (1)	Cells dividing by transverse fission	*Schizosaccharomyces pombe*
	Cells dividing by budding	3
3 (2)	Growth on MY10-12	4
	No growth on MY10-12	6
4 (3)	Cells nearly spherical	*Debaryomyces hansenii*
	Cells ellipsoidal to elongate	5
5 (4)	Growth on Czapek agar, ascospores hat shaped	*Pichia anomala*
	No growth no Czapek agar, ascospores smooth walled	*Zygosaccharomyces rouxii*
6 (3)	Growth on malt acetic agar	7
	No growth on malt acetic agar	11
7 (6)	Growth on MY50G	8
	No growth on MY50G	9
8 (7)	Strong growth at 37°C	*Candida parapsilosis*
	No growth at 37°C	*Zygosaccharomyces bailii*
9 (7)	Cells mostly 4–6 μm long; growth at 37°C weak at most	*Pichia membranaefaciens*
	Cells often exceeding 6 μm long; growth at 37°C vigorous	10
10 (9)	Larger cells cylinders, up to 25 μm long; isolated colonie on MEA at 25°C often exceeding 5 mm diams	*Candida krusei*
	Larger cells ellipsoids, rarely exceeding 12 μm long; isolated colonies on MEA at 25°C not exceeding 5 mm diam	*Saccharomyces cerevisiae*
11 (6)	Growth at 37°C	12
	No growth at 37°C	14
12 (11)	Colonies white, cells narrow ellipsoids 4–7 μm long; isolated colonies on MEA at 25°C not exceeding 2.5 mm diam	*Brettanomyces bruxellensis*
	Colonies off white to cream, cells narrow to broadly ellipsoidal, often exceeding 7 μm long; colonies on MEA at 25°C exceeding 2.5 mm diam	15
13 (12)	Cells narrow ellipsoids, long (>10 μm) cells often present	*Candida parapsilosis*
	Cells broad ellipsoids, 5–12 μm long	*Saccharomyces cerevisiae*
14 (11)	No growth on MY50G	15
	Growth on MY50G	*Zygosaccharomyces bisporus* (See *Z. bailii*)
15 (14)	Larger cells 7–9 μm long, budding terminally only	*Kloeckera apiculata*
	Larger cells 4–5 μm long, budding irregularly	*Kazachstania exigua*

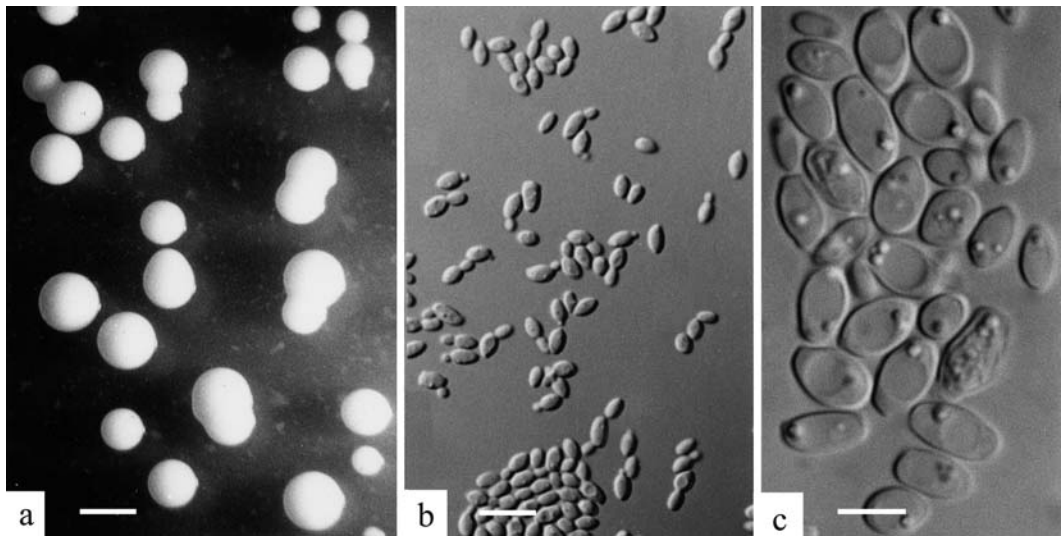


Fig. 10.1 *Brettanomyces bruxellensis* **(a)** colonies on MEA, 7 d, 25°C, bar = 2 mm; **(b,c)** vegetative cells **(b)** bar = 10 μm; **(c)** bar = 5 μm

SO_2 level tolerated at pH 3.5. *B. bruxellensis* can grow in 15.5% ethanol at pH 3.5 (Loureiro and Malfeito-Ferreira, 2006). The biochemical activities of *Brettanomyces* species contribute to off flavours in beverages spoiled by these yeasts: extracellular enzymes such as pectinesterases and proteases are formed (Rosi, 1993; Panon et al., 1995) and various volatile compounds detrimental to wine aroma may be produced (Ciolfi, 1991). Production of phenolic compounds in fermenting grape juice is a characteristic of sporulating cultures (Heresztyn, 1986; see Suárez-Quiroz et al., 2005 for a review). However, growth of *Brettanomyces* species also makes a positive contribution to the acetic flavour of lambic beers (Deák and Beuchat, 1996; Stratford and James, 2003). *Brettanomyces* species sometimes survived heating for 10 min, but not 20 min, at 60°C, but did not survive 10 min at 62.5°C (Put et al., 1976). Couto et al. (2005) demonstrated that heat inactivation of *B. bruxellensis* begins at 50°C in tartrate buffer at pH 4.0. However, in wine, significant inactivation was apparent at 35°C due to the presence of ethanol, phenolic compounds, low pH and other wine parameters (Couto et al., 2005).

Ecology. *Brettanomyces bruxellensis* and other *Brettanomyces* species have been isolated almost exclusively from beer and similar beverages (Fleet, 1992; and see Pitt and Hocking, 1997), wines (Kalathenos et al., 1995; Deák and Beuchat, 1996; Renouf et al., 2007) and soft drinks (see Pitt and Hocking, 1997; Stratford and James, 2003; and in our laboratory). Spoilage is often due to undesirable odours. *Brettanomyces* species have also been reported from spoiled fruit yoghurt (Comi et al., 1982) and from sour dough (Haznedari, 1976).

References. Kurtzman and Fell (1998); Barnett et al. (2000) both under *Dekkera bruxellensis*; Loureiro and Malfeito-Ferreira (2006).

Candida krusei (Castell.) Berkhout Fig. 10.2

Teleomorph: *Issatchenkia orientalis* Kudrjanzev

Colonies on MEA at 3 days 2–4 mm diam, white, convex, with margins irregularly lobate or fimbriate and surface matt; at 7 days colonies large, 5–8 mm diam, white, often centrally umbonate, margins characteristically filamentous. Cells on MEA at 3 days varying from short ellipsoids (3.0–4.0 × 2.0–10 μm) to long cylinders (10–25 × 3.0–4.0 μm), occasionally with larger ellipsoids (10–15 × 5–7 μm) also; reproducing by irregular budding, occurring singly and in chains. Ascospores rarely observed, one per ascus, smooth walled. Weak growth on Czapek agar; strong growth at 37°C (3–4 mm diam in 7 days); growth on malt acetic agar; no growth on MY50G or on MY10-12.

Distinctive features. *Candida krusei* grows strongly at 37°C and grows on malt acetic agar, although slowly. Large cylindrical cells are produced in cultures on MEA.

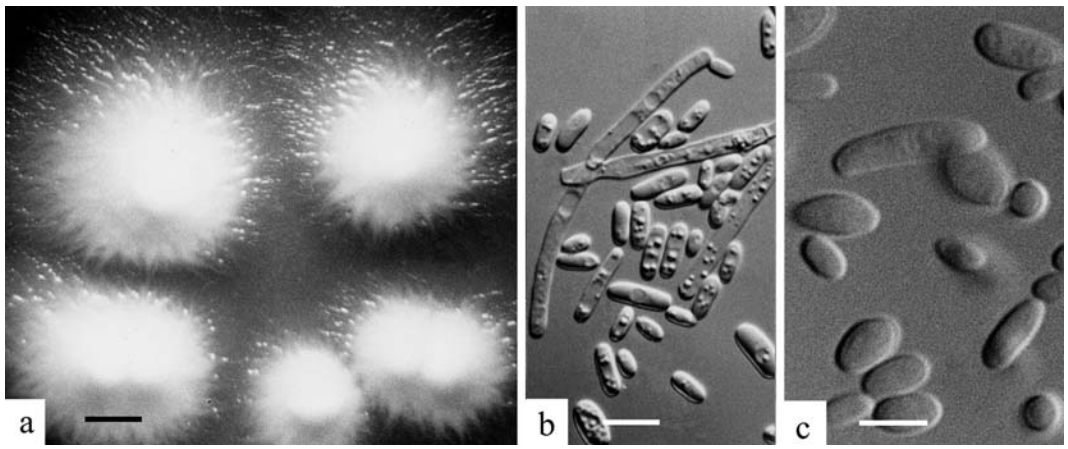


Fig. 10.2 *Candida krusei* **(a)** colonies on MEA, 7 d, 25°C, bar = 2 mm; **(b,c)** vegetative cells **(b)** bar = 10 µm; **(c)** bar = 5 µm

Physiology. The most important physiological characteristic of *Candida krusei* in the present context is its ability to grow in the presence of preservatives (Pitt and Richardson, 1973). *C. krusei* was able to grow anaerobically at pH 3.5 in the presence of 3 mM (335 ppm) sorbic acid, 3 mM (360 ppm) benzoic acid or 0.48 mM (30 ppm) free SO_2 (Warth, 1985). For *C. krusei* cells adapted to benzoic acid the minimum inhibitory concentrations (MICs) of various preservatives were acetic acid, 13.5 g/l; propanoic acid, 8.0 g/l; benzoic acid, 440 mg/l and methyl paraben, 1.0 g/l (Warth, 1989c). The ability of this yeast to grow in the presence of acetic acid has been linked to the citrate synthase gene *cs1* (Casey and Dobson, 2003). *C. krusei* also grows at exceptionally low pH: in a medium acidified with HCl, at pH 1.3, equivalent to 0.05 N HCl; and at pH 1.7–1.9 in media acidified with H_3PO_4 and organic acids, respectively (Pitt, 1974a). *C. krusei* has a minimum growth temperature near 8°C and a maximum near 47°C (Miller and Mrak, 1953). It has been reported as relatively heat resistant, surviving heating at 56°C for 80 min, though inactivation took less than 2 min at 65°C (Engel et al., 1994).

Ecology. Although it ferments glucose, *Candida krusei* is usually a surface growing, film forming yeast on foods. It has caused spoilage of African cocoa beans (Maravalhas, 1966), United States figs and Australian tomato sauce (see Pitt and Hocking, 1997). It has also been isolated from citrus and other fruit products, fermenting mango, olives, soft drinks (Deák and Beuchat, 1996; see also Pitt and Hocking, 1997; Stratford and James, 2003), grapes with sour rot (Fleet, 2003a), low salt bacon (Samelis and Sofos, 2003), cassava flour (Okagbue, 1990), spoiled wine (Deák and Beuchat, 1996), fresh and matured cheeses and fermented milk products (see Pitt and Hocking, 1997; Frölich-Wyder, 2003). *C. krusei* is associated with a number of fermentations: cocoa beans (Schwan and Wheals, 2003; Jespersen et al., 2005; Nielsen et al., 2005); sour dough (Spicher et al., 1979; Spicher, 1986a; Gobbetti et al., 1994); idli batter in India (Venkatasubbaiah et al., 1985); maize dough in West Africa (Hounhouigan et al., 1994; Jespersen et al., 1994; Obiri-Danso, 1994); kamu, a fermented millet cake from Nigeria (Oyeyiola, 1991); fermented cassava flour (Nigerian fufu; Oyewole, 2001); and various other fermented foods such as cheese (Bockelmann et al., 2005; Shuangquan et al., 2006) and beverages (Batra and Millner, 1976; Deák and Beuchat, 1996).

References. Kurtzman and Fell (1998); Barnett et al. (2000) as *Issatchenkia orientalis*.

Candida parapsilosis (Ashford) Langeron & Talice Fig. 10.3

Monilia parapsilosis Ashford

Colonies on MEA at 3 days 1.5–2.5 mm diam, off white; convex, margins circular, surface matt; at 7 days 3–4 mm diam, off white to cream coloured, often centrally umbonate, margins appearing entire, but showing thin filamentous growth when viewed with the stereomicroscope. Cells on MEA varying widely in size and shape, from short ellipsoids 3.5–4.0 × 2.5–3.5 µm to long, irregularly

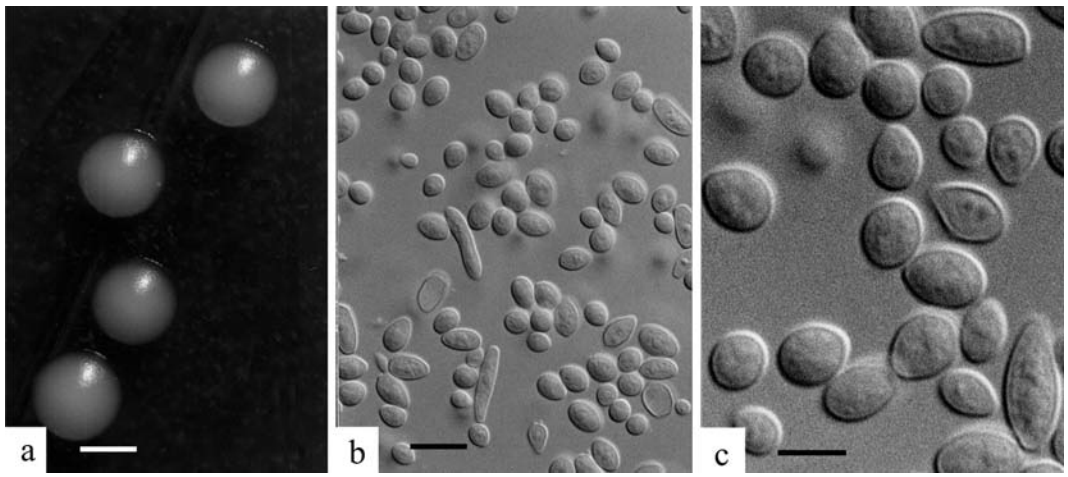


Fig. 10.3 *Candida parapsilosis***(a)** colonies on MEA, 7 d, 25°C, bar = 2 mm; **(b,c)** vegetative cells **(b)** bar = 10 µm; **(c)** bar = 5 µm

cylindroidal cells, straight or curved, 10–15(–20) × 1.5–3.0 µm; reproducing by irregular budding, occurring singly or in short chains. Ascospores not produced. Strong growth at 37°C; weak growth on Czapek agar; sometimes growth on malt acetic agar; growth on MY50G and MY10-12.

Distinctive features. The production of elongated cells and pseudohyphae is a feature of *Candida parapsilosis*. Smaller colonies on MEA and the ability to grow on MY50G distinguish it from *C. krusei*.

Taxonomy. Studies on small subunit rRNA gene sequences indicate that *Candida parapsilosis* may be the anamorph of the ascomycete yeast *Lodderomyces elongisporus* (James et al., 1994a).

Physiology. *Candida parapsilosis* grows between 8 and 42°C, with an optimum temperature near 35°C (Suzzi et al., 2003) and is potentially pathogenic to man. It is lipolytic (Bours and Mossel, 1973) and is strongly proteolytic at 37–42°C (Kobatake et al., 1992; Suzzi et al., 2003). It has average heat resistance, with a D_{62} of 2.2 min (Hur et al., 1993), is tolerant of NaCl (5–10%) and actidione (100 and 1,000 mg/l) (Suzzi et al., 2003).

Ecology. *Candida parapsilosis is* often associated with high fat foods such as butter, cheese, margarine, salad dressing and yoghurt (Deák and Beuchat, 1996; see also Pitt and Hocking, 1997; Suzzi et al., 2003) and has also been isolated from human breast milk (Rosa et al., 1990). *C. parapsilosis* has been reported from raw meat and meat products (Deák and Beuchat, 1996; see also Pitt and Hocking, 1997; Hammad, et al., 2006), raw seafood and shellfish, a fermented fish product in the Philippines, vegetables, fruit products, fruit juice concentrates and soft drinks, chalky bread (Deák and Beuchat, 1996; see also Pitt and Hocking, 1997), pickles and olive brines (Deák and Beuchat, 1996; Mourad and Nour-Eddine, 2006).

Pathogenicity. *Candida parapsilosis* is an opportunistic pathogen that can cause infections in burns patients and those with impaired immunity. It can also form biofilms, colonising tubing and prostheses (De Hoog et al., 2000).

References. Kurtzman and Fell (1998); Barnett et al. (2000).

Debaryomyces hansenii (Zopf) Lodder & Kreger Fig. 10.4

Debaryomyces membranaefaciens H. Nagan.

Anamorph: *Candida famata* (F.C. Harrison) S.A. Mey. & Yarrow

Torulopsis candida Saito
Torulopsis famata (F.C. Harrison) Lodder & Kreger

Colonies on MEA at 3 days 1–2 mm diam, off-white, becoming brown when ascospores produced, convex to hemispherical with circular margins, surface glistening; at 7 days colonies 2–4 mm diam, similar to at 3 days, but relatively less deep and with surface sometimes matt. Cells on MEA at 3 days spherical to subspheroidal, 2.5–4.0 µm diam, with some larger ellipsoidal cells, up to 8 µm long; reproduction by irregular budding, sometimes simultaneously at more than one site on the mother cell; occurring

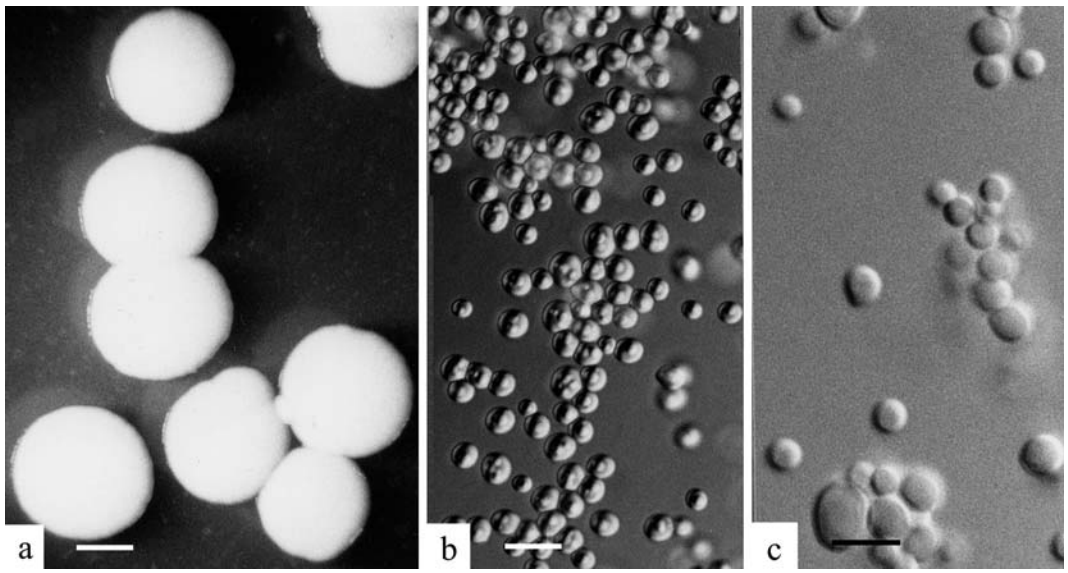


Fig. 10.4 *Debaryomyces hansenii* **(a)** colonies on MEA, 7 d, 25°C, bar = 2 mm; **(b,c)** vegetative cells **(b)** bar = 10 μm; **(c)** bar = 5 μm

singly, in pairs or in small clusters. Ascospores sometimes observed in older cultures; ascus formation occurring in the mother cell after conjugation between mother and daughter cells; ascospores 1, rarely 2, per ascus, spherical, with finely roughened walls. Weak growth on Czapek agar; rarely growth at 37°C; no growth on malt acetic agar; slow growth on MY50G (up to 1 mm diam in 7 days); rapid growth on MY10-12 (1–4 mm in 7 days).

Distinctive features. Spherical cells and rapid growth on MY10-12 agar distinguish *Debaryomyces hansenii* from other species considered here, and, indeed, from almost all other yeasts.

Physiology. The most important physiological feature of *Debaryomyces hansenii* is its ability to grow in salt concentrations as high as 24% (w/v; 0.84 a_w; Mrak and Bonar, 1939; Prista et al., 2005; Corte et al., 2006; review by Breuer and Harms, 2006). Tilbury (1980) reported growth of one isolate of *Torulopsis famata* (= *Candida famata*) at 0.65 a_w in sucrose/glycerol syrups. This species is capable of utilising a wider range of carbon sources than most other spoilage yeasts (Barnett et al., 2000; Breuer and Harms, 2006) and is lipolytic (Marquina et al., 1992; Baruzzi et al., 2006). Pectinase activity has been reported (Deák and Beuchat, 1996).

Debaryomyces hansenii grows between 2 and 33°C in YM broth (Kobatake et al., 1992) but its T_{max} increases to around 38°C in the presence of 60% (w/w) glucose (Jermini and Schmidt-Lorenz, 1987b). Its optimum temperature range for growth is 24–25°C in 10% (w/w) glucose increasing to 27–30°C in the presence of 60% (w/w) glucose (Jermini and Schmidt-Lorenz, 1987b). The pH range for growth at 25°C is 2.0–2.5 up to at least pH 8.0 (the highest pH tested), with one strain able to grow in 50% glucose at pH 2.0 (Praphailong and Fleet, 1997). Sørensen and Jakobsen (1997) used flow cytometry to study the combined effects of temperature (10–30°C), pH (4.7–6.0) and NaCl concentration (1–12% w/v) on growth of *D. hansenii* and used the data to construct a predictive model. *D. hansenii* was unable to grow in 250 mg/l sorbic or benzoic acid at pH 3.0, but some strains grew in 500 mg/l benzoic acid at pH 5.0 (Praphailong and Fleet, 1997). Approximately 10^5 vegetative cells/ml were found to survive 20 min at 55° and 10 min at 60°C; they did not survive 20 min at 60° or 10 min at 62.5°C (Put et al., 1976).

Ecology. The high salt tolerance of *Debaryomyces hansenii* accounts for its frequent occurrence in salt brines including those used for olives (Deák and Beuchat, 1996; Quintana et al., 2005), cheeses (see Pitt and Hocking, 1997), fermented milk (Kebede et al., 2007) and in soy sauce koji (Lee and Lee, 1970). *D. hansenii* has been isolated from fermented and cured meats on many occasions (see Pitt and Hocking, 1997; Samelis and Sofos, 2003;

Simoncini et al., 2007) and has been shown to cause dark spots on the casing of ripening salamis (Papa et al., 1995). *D. hansenii* has also been isolated from fresh meat (Dalton et al., 1984; Deák and Beuchat, 1996; Samelis and Sofos, 2003).

Debaryomyces hansenii is a common component of the microflora of cheeses (see Pitt and Hocking, 1997) where it may have a role in ripening (Roostita and Fleet, 1996). *D. hansenii* is sometimes added as part of a combined lactic acid bacteria-yeast starter culture mixture in certain types of cheeses including Pecorino Romano (Deiana et al., 1984), soft cheeses (Kang et al., 1980), Cheddar cheese (Ferreira and Viljoen, 2003) and other varieties (Fleet, 1990).

Debaryomyces hansenii has caused spoilage of fruit juice, marzipan, and canned figs (Deák and Beuchat, 1996; Pitt and Hocking, 1997). It has been isolated from a variety of fruit (Deák and Beuchat, 1996), candied pumpkin (Martorell et al., 2005a), soft drinks (Ancasi et al., 2006), raw tomatoes (Wade et al., 2003), sugar cane (Azeredo et al., 1998) and been reported in high numbers in yoghurt (Suriyarachchi and Fleet, 1981; Yamani and Abu-Jaber, 1994). It has also been isolated from malting barley (Petters et al., 1988). Barnett et al. (2000) list milk, rancid butter, miso, mushrooms, fruit and berries, wine, beer and salt beans as other sources. In our laboratory *D. hansenii* has been isolated from chilli sauce, tomato paste, fruit purée, ham, blue-vein cheese, pickling brine, boiled peanuts, chalky bread and kangaroo biltong. *D. hansenii* has been evaluated as a biological control agent against fungal pathogens on fruits and vegetables including *Penicillium digitatum* postharvest rots in citrus, *Botrytis cinerea* on apples (Droby et al., 1989; Wilson and Chalutz, 1989; Santos et al., 2004) and *Rhizopus macrosporus* on peaches (Singh, 2004).

References. Kurtzman and Fell (1998); Barnett et al. (2000).

Kazachstania exigua (Reess) Kurtzman

Fig. 10.5

Saccharomyces exiguus Reess

Anamorph: *Candida holmii* (A. Jörg.) S.A. Mey. & Yarrow

Torulopsis holmii (A. Jörg.) Lodder

Colonies on MEA at 3 days 4–5 mm diam, white, circular with a smooth margin, and a low, convex, glistening surface; at 7 days 6–8 mm diam, appearance as at 3 days. Cells on MEA small, ellipsoidal, 4.0–5.0 × 2.5–3.5 μm, reproducing by irregular budding, occurring singly or in pairs. Ascospores not usually formed under the conditions used here; asci formed directly from vegetative cells; ascospores 1–4 per ascus, spherical to ellipsoidal and smooth walled. No growth on Czapek agar or at 37°C; slow growth on malt acetic agar; no growth on MY50G or MY10-12.

Distinctive features. *Kazachstania exigua* and its anamorph *Candida holmii* are of similar appearance to *Saccharomyces cerevisiae,* but do not grow on Czapek agar or at 37°C.

Taxonomy. After extensive studies involving multigene sequencing and phylogenetic analysis (Kurtzman and Robnett, 2003), *Saccharomyces exiguus* was transferred to *Kazachstania* as *K. exigua* by Kurtzman (2003). The two genera (*Saccharomyces* and *Kazachstania*) are difficult to distinguish using morphological criteria, however, and the name *Saccharomyces exiguus* is still in common use for this taxon. The anamorph of *S. exiguus* is generally accepted to be *Candida holmii* (Kurtzman and Fell, 1998; Barnett et al., 2000). However, McGinnis (1980) considered *Torulopsis holmii* to be the correct name for this species, regarding *Torulopsis* as a valid genus which has priority over *Candida*.

Physiology. *Kazachstanina exigua* is preservative resistant. Pitt (1974a) reported growth in 400 mg/kg benzoic or sorbic acid at pH 4.0, and Frölich-Wyder (2003) reported growth in 500 and 800 mg/kg sorbic and benzoic acid, respectively, at the same pH, but with considerable strain to strain variation. *K. exigua* is also capable of growth under very acid conditions, pH 1.5 in HCl, pH 1.7 in H_3PO_4, and pH 1.9–2.1 in organic acids (Pitt, 1974a). Suihko and Mäkinen (1984) demonstrated that *K. exigua* was relatively resistant to acetate, propionate and sorbate at pH 4.5 and had better dough raising power than *S. cerevisiae* when these compounds were present. Growth at 4–7°C within 1–3 weeks has been reported (Fleet, 2006). This species vigorously ferments a wide range of sugars.

Ecology. *Kazachstania exigua* is of common occurrence in brines during the early stages of pickle fermentation (Etchells et al., 1952, 1953). Steinbuch (1965, 1966) reported that *K. exigua* and *Candida holmii,* regarded at that time as distinct species,

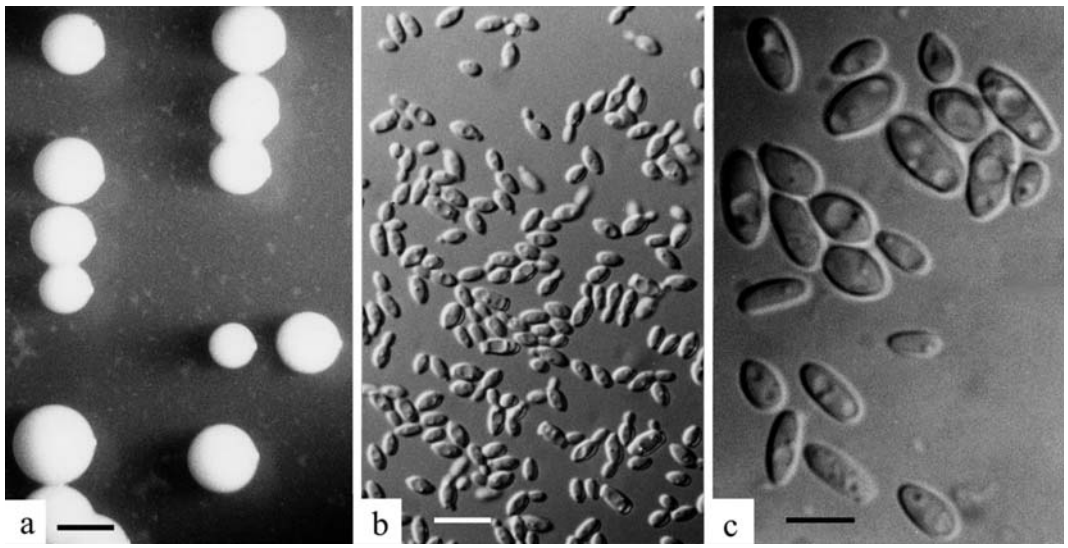


Fig. 10.5 *Kazachstania exigua* **(a)** colonies on MEA, 7 d, 25°C, bar = 2 mm; **(b,c)** vegetative cells **(b)** bar = 10 μm; **(c)** bar = 5 μm

were the cause of pink or grey discolouration in sauerkraut. *K. exigua* has been identified as an important component of the microflora of sour dough in France (Infantes and Schmidt, 1992), Italy (Galli et al., 1988; Gobbetti et al., 1994; Pulvirenti et al., 2004), Finland (Salovaara and Savolainen, 1984), Germany (Spicher et al., 1979; Spicher, 1986a), in San Francisco sour dough (Sugihara et al., 1971) and in Italian panettone dough (Galli and Ottogalli, 1973). It has also been identified as an important component of the microflora of silage made from maize (Middelhoven, 1998) and grass (O'Brien et al., 2007).

Kazachstania exigua has been isolated from spoiled soft drinks in Australia (Pitt and Richardson, 1973) and elsewhere (Frölich-Wyder, 2003), and it is a common spoilage yeast in delicatessen salads (Baumgart et al., 1983; Fleet, 1992; Bonestroo et al., 1993; Hunter et al., 1994). Other recorded sources include green olives (Mrak et al., 1956), citrus products (Recca and Mrak, 1952), kefir (Iwasawa et al., 1982; Frölich-Wyder, 2003; Latorre-Garcia et al., 2007), fresh cheese (quarg) (Engel, 1988), yoghurt (Viljoen, et al., 2003b) and occasionally from other dairy and meat products (Fleet, 2006).

References. Kurtzman and Fell (1998); Barnett et al. (2000) and Fleet (2006) as *Saccharomyces exiguus*; Kurtzman (2003) as *Kazachstania exigua*.

Kloeckera apiculata (Reess) Janke Fig. 10.6

Teleomorph: *Hanseniaspora uvarum* (Niehaus) Shehata et al.

Colonies on MEA at 3 days 1–2 mm diam, off-white, almost hemispherical, margins circular, surface glistening; at 7 days 2–4 mm diam, pale brown, low to convex, margins circular, surface glistening. Cells on MEA at 3 days ranging from small narrow ellipsoids, 3.0 × 1.5 μm, and larger, broader ellipsoids, 5–6 × 3.0–4.0 μm, to characteristic apiculate cells, 7–9 × 3.0–4.0 μm, budding terminally only, occurring singly or in pairs. Ascospores sometimes produced in old cultures on MEA, under a coverslip; asci formed from single cells; ascospores 1–2 per ascus, not released in age, spherical, finely roughened or with a minute equatorial ledge. No growth on Czapek agar; sometimes growth at 37°C; no growth on malt acetic agar, on MY50G or on MY10-12.

Distinctive features. The genus *Kloeckera* and its teleomorph *Hanseniaspora* are distinguished by the formation of cells which bud only terminally. *K. apiculata* (= *H. uvarum*) is the most common species in foods. It is distinguished from other species by minor differences in the utilisation of carbon sources and in ascospore ornamentation.

Physiology. Miller and Mrak (1953) reported a minimum growth temperature for *Kloeckera*

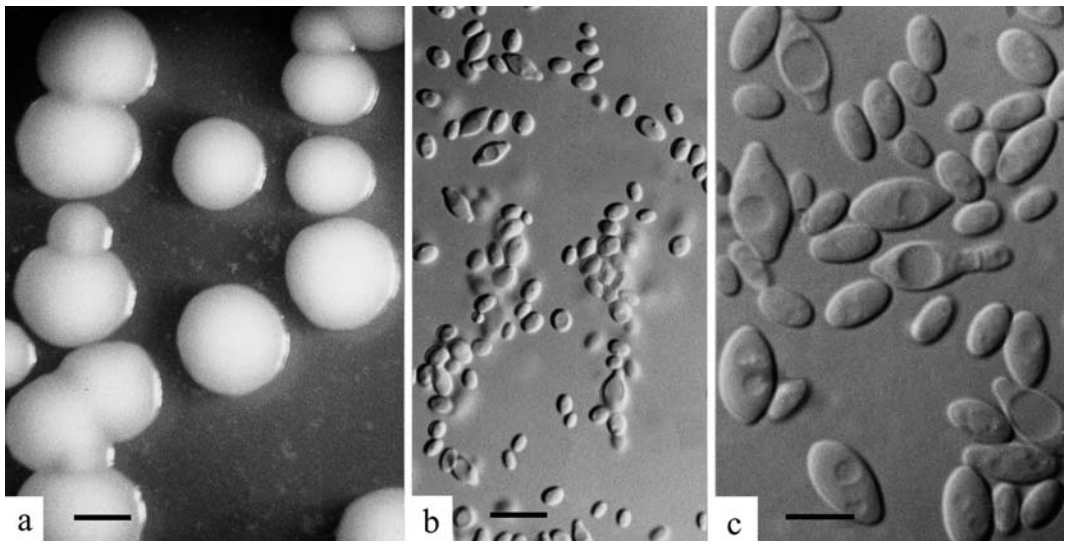


Fig. 10.6 *Kloeckera apiculata* **(a)** colonies on MEA, 7 d, 25°C, bar = 2 mm; **(b,c)** vegetative cells **(b)** bar = 10 μm; **(c)** bar = 5 μm

apiculata near 8°C and a maximum near 40°C. It is capable of growth in 9% ethanol and tolerates 12.5% ethanol at 15°C, but becomes more sensitive at 10 and 30°C (Gao and Fleet, 1988). Pina et al. (2004) reported tolerance to 22.5% v/v ethanol when grown aerobically before exposure to ethanol. *K. apiculata* is extremely acid tolerant, growing at pH 1.5 in inorganic buffer, and pH 2.0 in citrate-phosphate buffer at 25°C in yeast nitrogen base with 0.5 or 5.0% glucose. However, it was unable to grow at pH 8.0 under these conditions (Praphailong and Fleet, 1997). *K. apiculata* is quite sensitive to preservatives: the MIC of benzoic acid was 188 mg/l at pH 3.5 and 25°C (Warth, 1989c). However, it was reported to tolerate levels of SO_2 up to 100 mg/l in red wine (Heard and Fleet, 1988b). Praphailong and Fleet (1997) reported no growth in 250 mg/l benzoate or sorbate at pH 3.0, but at pH 5.0, *K. apiculata* grew in 750 mg/l benzoate or 250 mg/l sorbate. *K. apiculata* grew in 12.5% (w/v) NaCl and 50% (w/v) sucrose from pH 2.0 to 7.0 (Praphailong and Fleet, 1997). Heat resistance is average, with cells (10^5/ml) of this species surviving for 20 min at 55°C but not 10 min at 60°C (Put et al., 1976). Later work reported that 10^4–10^5 cells when heated at 56°C in either milk or Ringers solution did not survive 30 s (Engel et al., 1994).

Kloeckera apiculata is a strongly fermentative yeast which exhibits a wide range of biochemical activities, producing extracellular proteases (Rosi, 1993), polygalacturonase (Masoud and Jespersen, 2006), α-glucosidase (Rosi et al., 1994), and also acetoin (Romano et al., 1993), erythritol and sorbitol (Sponholz et al., 1986) and ethanol (Heard and Fleet, 1988a; Duenas et al., 1994). It can be isolated from mixed cultures of yeasts in natural fermentations using lysine agar (Heard and Fleet, 1986b).

Ecology. The natural habitat of *Kloeckera apiculata* (and *Hanseniaspora uvarum*) is fruit, particularly grapes, and it may play an active role during the early stages of fermentation of grape juice (Fleet et al., 1984; Heard and Fleet, 1986a,b; Suarez-Lepe, 1991; Deák and Beuchat, 1996). It is also associated with cocoa fermentation (Ravelomanana et al., 1984; Mazigh, 1994; Masoud and Jespersen, 2006), malting barley (Petters et al., 1988) and cider fermentation (Morrissey et al., 2004). *K. apiculata* has been reported to spoil figs, tomatoes, canned black cherries and in our laboratory, strawberry topping and fruit flavoured yoghurt (see Pitt and Hocking, 1997). *K. apiculata* has been isolated from fresh strawberries, blackcurrants and wine grapes and is associated with sour rot of grapes (see Pitt and Hocking, 1997) and fermenting mango (Suresh et al., 1982). Other sources include citrus, orange concentrate, fruit juices and fruit syrups (see Pitt and Hocking, 1997; Las Heras-Vazquez et al., 2003).

References. Miller and Phaff (1958) under both *Kloeckera* and *Hanseniaspora*; Kurtzman and Fell (1998); Barnett et al. (2000), under *Hanseniaspora*.

Pichia anomala (E.C. Hansen) Kurtzman

Fig. 10.7

Hansenula anomala (E.C. Hansen) Syd. & P. Syd.

Anamorph: *Candida pelliculosa* Redaelli

Colonies on MEA at 3 days 1.5–2.0 mm diam, off-white, convex, margins circular, surface matt; at 7 days 3–4 mm diam, off-white, convex, smooth, margins entire. Cells on MEA at 3 days very variable in size and shape, from small and subspheroidal to large and ellipsoidal, 3.0–7 × 2.5–5.0 µm, with smaller numbers elongate to cylindroidal, up to 12 × 3.0 µm, reproducing by multilateral budding. Ascospores produced without conjugation in some of the larger ellipsoidal cells, bowler hat shaped, 2–4 per ascus. Moderate growth on Czapek agar, colonies 1.0–2.0 mm diam at 7 days, white and convex, margins entire; growth on MY10-12 only a little slower than on MEA, 2–3 mm diam at 7 days, colonies of similar appearance to those on MEA. Very slow growth on MY50G (pinpoint colonies at 7 days). Usually no growth at 37°C, no growth on MAA.

Distinctive features. *Pichia anomala* utilises nitrate as a sole nitrogen source, and most isolates of this species readily produce characteristic bowler hat-shaped ascospores.

Taxonomy. The genera *Hansenula* and *Pichia* were originally separated on the basis of the ability of the former to utilize nitrate as a sole nitrogen source. Later taxonomists did not consider this difference sufficient to justify two genera, and species of *Hansenula* with hat-shaped ascospores were transferred to *Pichia* (Kurtzman, 1984). However, a more recent phylogenetic study (Kurtzman and Robnett, 1998) has found that most members of the former genus *Hansenula* form a separate clade from *Pichia*. A phylogenetic argument for maintaining *Hansenula* may exist, but further work is required.

Physiology. *Pichia anomala* grows between 3 and 37°C, and from pH 2.0 to 12.4 at 25°C, but not at pH 1.9 (Fredlund et al., 2002). *P. anomala* was able to grow at 0.85 a_w with glycerol as the controlling solute, but when NaCl was used, the lowest a_w at which growth was observed was 0.92 a_w (Fredlund et al., 2002). Praphailong and Fleet (1997) reported growth of *P. anomala* in 15% (w/v) NaCl (*ca* 0.91 a_w) at pH 3–5 or 70% (w/v) sucrose (*ca* 0.89 a_w) at pH 2–7. Quintana et al. (2003) recorded growth of *P. anomala* at 7°C in 8% NaCl during fermentation of table olives.

Pichia anomala is moderately resistant to preservatives. At pH 5, *P. anomala* grew in 500 mg/l benzoic acid or 250 mg/l sorbic acid, but at pH 3, was unable to grow in 250 mg/l of either preservative (Praphailong and Fleet, 1997). Fredlund et al.

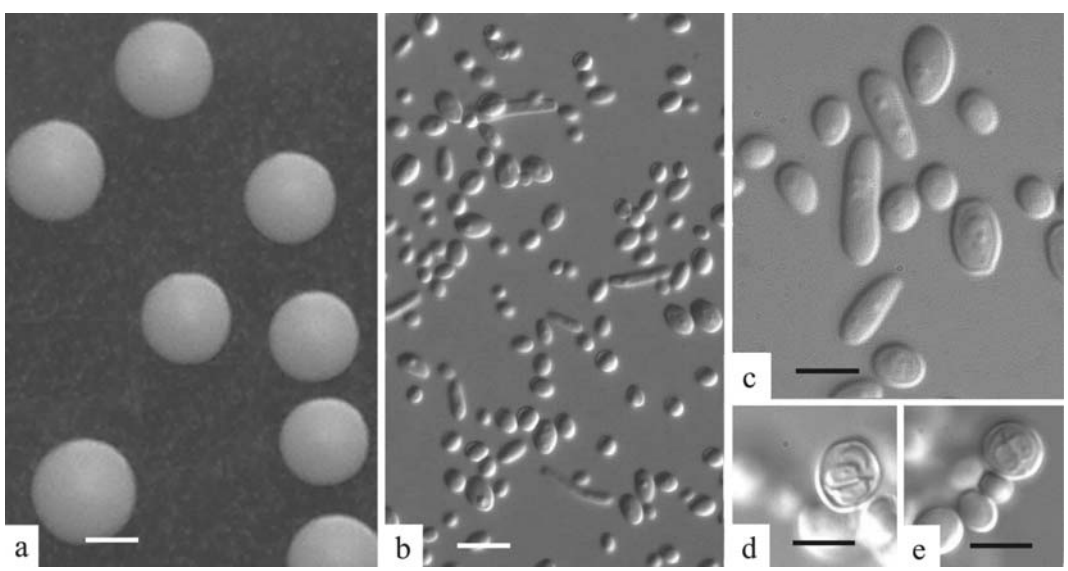


Fig. 10.7 *Pichia anomala* **(a)** colonies on MEA, 7 d, 25°C, bar = 2 mm; **(b,c)** vegetative cells **(b)** bar = 10 µm; **(c)** bar = 5 µm; **(d,e)** asci and ascospores, bar = 5 µm

(2002) reported MICs for *P. anomala*: for propionic acid, 1,000–5,000 mg/l at pH 3.6; for potassium sorbate, 100–500 mg/l at pH 5.3; and for sodium benzoate, 100–500 mg/l at pH 5.2. Stratford et al. (2007) reported that *P. anomala* has the gene (PAD1) that enables decarboxylation of sorbic acid to 1,3-pentadiene. *P. anomala* is able to produce ethyl acetate from glucose (Passoth et al., 2006), and this compound may be formed during growth on bread, resulting in spoilage taints. *P. anomala* produces a killer toxin that is effective against other yeasts and possibly some moulds (Passoth et al., 2006).

Ecology. Deák and Beuchat (1996) list *Pichia anomala* as the third most frequently reported foodborne yeast, after *Saccharomyces cerevisiae* and *Debaryomyces hansenii*. It is most commonly associated with low pH products, but also fruits, beverages, wine and beer, meat and dairy products and low a_w products. However, it is not always associated with spoilage. *P. anomala* appears to play an important role in olive fermentation (Marquina et al., 1992; Quintana et al., 2003; Coton et al., 2006; Hernandez et al., 2007), but may also be implicated in deterioration of olives at the end of fermentation (Faid et al., 1994; Asehraou et al., 2000). *Pichia anomala* has been reported from coffee (Schwann and Wheals, 2003; Masoud et al., 2004; Masoud and Kaltoft, 2006) and cocoa fermentations (Schwann and Wheals, 2003). It is involved in a number of indigenous fermentations including brem, a Balinese rice wine (Sujaya et al., 2004), gowe (fermented sorghum) in Benin (Vieira-Dalode et al., 2007), murcha cakes in India (Tamang and Sarkar, 1995) and idli (fermented lentils) in the Indian subcontinent (Nout, 2003).

The antifungal activity of *Pichia anomala* makes it a potentially useful biocontrol organism. It has been shown to be active against *Botrytis cinerea* on apples (Jijakli and Lepoivre, 1998; Santos et al., 2004) and grapes (Masih et al., 2000) and against the growth of *Penicillium roqueforti* and other moulds in grain silage (e.g. Björnberg and Schnürer, 1993; Druvefors et al., 2005; Passoth et al., 2006).

Pichia anomala has been reported to cause spoilage in a wide range of foods. It can produce ethyl acetate in "chalky" bread (Legan and Voysey, 1991; Bonjean and Guillaume, 2003, and in our laboratory). It has been reported from butter (Mushtaq et al., 2007), wine (Deák and Beuchat, 1996; Menke et al., 2007), fruit juices and soft drinks (Deák and Beuchat, 1996), fruit juice concentrates (Deák and Beuchat, 1993), yoghurt (Kosse et al., 1997; Caggia et al., 2001) and cream-filled cakes (Lanciotti et al., 1998). In our laboratory we have isolated it consistently from fruit purees and flavourings used as ingredients for the dairy industry. We have also isolated it from spoiled yoghurt, sugar syrup, chocolate syrup, tomato dip and bakery products.

Pathogenicity. *Pichia anomala* can cause infections in immunocomprised and paediatric patients (De Hoog et al., 2000; Paula et al., 2006; Bhardwaj et al., 2007). An identification and strain typing method has been developed based on the ribosomal intergenic spacer region IGS1 (Bhardwaj et al., 2007).

References. Kurtzman and Fell (1998); Barnett et al. (2000).

Pichia membranaefaciens E.C. Hansen

Fig. 10.8

Anamorph: *Candida valida* (Leberle) Uden & H.R. Buckley

Colonies on MEA at 3 days 1–3 mm diam, off-white, convex but not hemispherical, margins irregular, surface usually matt; at 7 days 3–4 mm diam, white, margins circular, centrally heaped up or wrinkled, surface dull and granular. Cells on MEA at 3 days small and ellipsoidal to cylindrical, 4.0–6 × 2.0–4.0 μm, reproducing by irregular budding. Ascospores regularly formed on malt acetic agar after 7 days at 25°C; asci formed from single cells; ascospores usually 4 per ascus, tiny, shaped like a bowler hat, quickly liberated from the ascus but adherent to each other in clumps. Very weak growth on Czapek agar; usually no growth at 37°C; growth on malt acetic agar (1 mm diam in 3 days); no growth on MY50G or on MY10-12.

Distinctive features. A diagnostic feature for *Pichia membranaefaciens* is the formation of tiny bowler hat shaped ascospores on malt acetic agar within 7 days. Spores usually number 4 per ascus but are quickly liberated.

Physiology. Miller and Mrak (1953) reported a minimum growth temperature near 5°C for *Pichia membranaefaciens* and a maximum near 37°C. Mrak et al. (1956) reported poor but positive growth of this species in the presence of 15.2% NaCl: assuming the percentage is weight in volume,

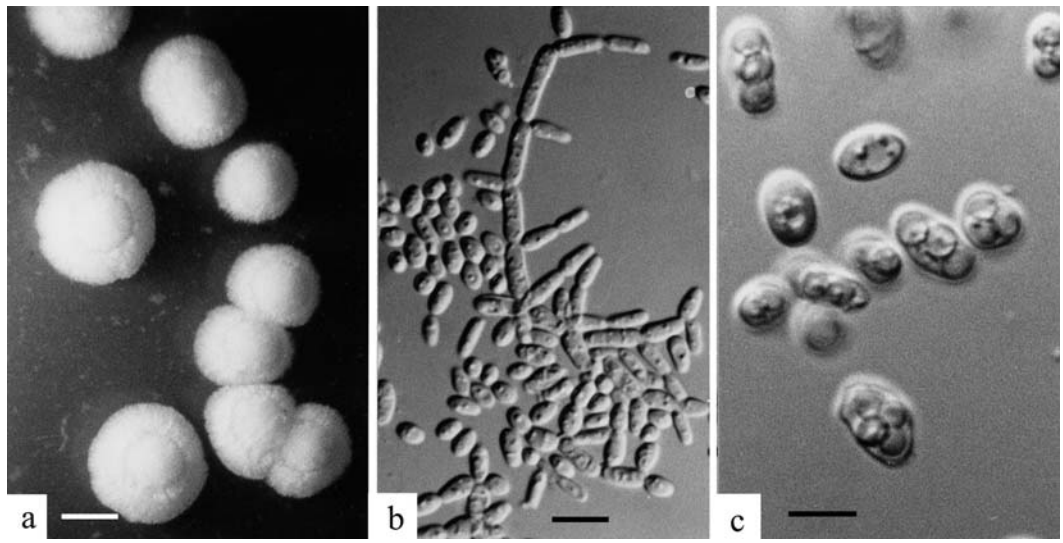


Fig. 10.8 *Pichia membranaefaciens* **(a)** colonies on MEA, 7 d, 25°C, bar = 2 mm; **(b)** vegetative cells, bar = 10 μm; **(c)** asci and ascospores, bar = 5 μm

this is equivalent to 0.90 a_w. Praphailong and Fleet (1997) reported growth of *P. membranaefaciens* in 12.5% (w/v) NaCl at pH 3, but at pH 2.0 only 2.5% (w/v) NaCl was tolerated, and at pH 5.0, 5.0% NaCl. *P. membranaefaciens* was able to grow in 50% (w/v) sucrose from pH 2.0 to 7.0 (Praphailong and Fleet, 1997). Pitt and Richardson (1973) reported growth in the presence of 1% acetic acid; Pitt (1974a) observed growth in the presence of 400 mg/kg benzoic acid at pH 4.0; Ethiraj and Suresh (1988) isolated a strain from spoiled mango pulp that grew in the presence of 1,500 mg/kg sodium benzoate at pH 4.0, and in 3,000 mg/kg benzoate at pH 4.5. Praphailong and Fleet (1997) reported growth in 750 mg/l benzoate or 250 mg/l sorbate at pH 5.0, but no growth was observed with 250 mg/l of either preservative at pH 3.0. Growth occurred down to pH 1.9 in media acidified with HCl and pH 2.1–2.2 in media acidified with organic acids (Pitt, 1974a). Praphailong and Fleet (1997) reported similar results, with growth between pH 2.0 and 7.0 in inorganic buffer and between 2.5 and 7.5 in citrate–phosphate buffer. No growth occurred at pH 8.0.

Pichia membranaefaciens is sensitive to heat: Put et al. (1976) reported survival of vegetative cells (10^5/ml) for 10 min at 55°C, but not 20 min at 55°C or 10 min at 60°C. Engel et al. (1994) reported survival of *P. membranaefaciens* (10^4–10^5 cells) heated in Ringers solution or milk for 30 min at 56°C.

Enzymic activity of *Pichia membranaefaciens* includes production of pectin methylesterase (Gauthier et al., 1977; Panon et al., 1995) and extracellular proteases (Rosi, 1993). *P. membranaefaciens* produced 2-propanol and 2-hexanone when inoculated into grape must (Mamede and Pastore, 2006).

Ecology. The most common food associated source of *Pichia membranaefaciens* has been olive brines (Mrak and Bonar, 1939; Mrak et al., 1956; Marquina et al., 1992; Duran et al., 1994; Oliveira et al., 2004), but it also occurs in cheese brines (Kaminarides and Laskos, 1993) and appears to be an important component of cocoa fermentations (Gauthier et al., 1977; Nielsen et al., 2005, 2007). Vaughn et al. (1943) associated this and other yeasts with "stuck" olive fermentations, in which carbohydrate is depleted without the desired build up in lactic acid concentration. Dakin and Day (1958) reported the isolation of *P. membranaefaciens* from a variety of acetic acid preserves including onions, gherkins, pickles, beetroot and sauerkraut. Muys et al. (1966a) and Pitt and Richardson (1973) reported spoilage of tomato sauce due to film formation by this yeast. It is also relatively common in delicatessen salads (Bonestroo et al., 1993; Hunter et al., 1994) and salad dressings and mayonnaise (our observations).

Pichia membranaefaciens has been isolated from "chalk mould" spoilage of bread (Spicher, 1986b), fermented milk products (Rohm et al., 1992), fresh and

ripened cheeses (see Pitt and Hocking, 1997; Westall and Filtenborg, 1998; Lioliou et al., 2001), fresh sausages (Dalton et al., 1984), Brazilian guava fruit (Abranches et al., 2000) and in our laboratory from cherries in brine. Other recorded sources of *Pichia membranaefaciens* include cereal grains and flours (Spicher and Mellenthin, 1983), citrus and citrus products including orange concentrate, fermenting mango, grape must and soft drink processing lines (see Pitt and Hocking, 1997).

Pichia membranaefaciens shows promise as a biocontrol agent against *Monilia fructicola* on cherries (Qin et al., 2006), *Botrytis cinerea* on grapes (Masih and Paul, 2002; Santos et al., 2004; Santos and Marquina, 2004), *Rhizopus* rot of nectarines (Fan and Tian, 2000) and post harvest pathogens of apples (Chan and Tian, 2005). Activity appears to be due to production of a killer toxin (Santos and Marquina, 2004) and extracellular enzymes such as ß–1, 3-glucanase and chitinase (Masih and Paul, 2002).

References. Kurtzman and Fell (1998); Barnett et al. (2000).

Rhodotorula mucilaginosa (A. Jörg.) F.C. Harrison Fig. 10.9

Rhodotorula rubra (Demme) Lodder

Colonies on MEA at 3 days 1.5–3 mm diam, coloured dull to bright pink to red, margins circular or spreading, convex, surface glistening or appearing mucoid; at 7 days 5–10 mm diam, with appearance as at 3 days. Cells on MEA at 3 days mostly ellipsoidal, 4.0–5.5 × 3.0–3.5 µm; reproduction by irregular budding; occurring singly or in pairs. Ascospores not produced. Growth on Czapek agar weak to quite strong; sometimes weak growth at 37°C; no growth on malt acetic agar, MY50G, or MY10-12.

Distinctive features. *Rhodotorula mucilaginosa* and the closely related *R. glutinis* (see below) are distinguished by pink to red, often mucoid colonies and their inability to grow on malt acetic agar or MY50G.

Taxonomy. The name *Rhodotorula rubra* (Demme) Lodder was used for this species in Kreger-van Rij (1984), but because that name is a later homonym of *Rhodotorula rubra* (Saito) F.C. Harrison it is illegitimate. The situation is further confused by the fact that *Rhodotorula rubra* (Saito) F.C. Harrison is a synonym of *R. glutinis* (Barnett et al., 2000). *R. mucilaginosa,* which is the second name in order of priority, is the correct one for this species (Barnett et al., 2000).

Physiology. *Rhodotorula mucilaginosa* has a minimum growth temperature between 0.5 and 5°C and a maximum near 35°C, according to Miller and Mrak (1953). Other reports suggest that some *Rhodotorula* species can grow well below 0°C (Walker, 1977; Table 10.1), but these reports are often unsubstantiated. Collins and Buick (1989)

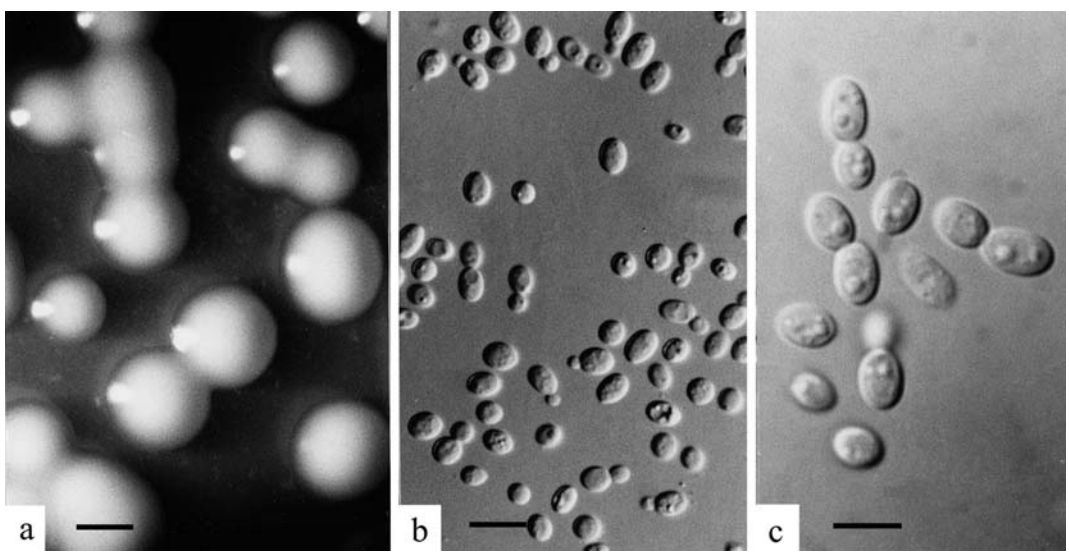


Fig. 10.9 *Rhodotorula mucilaginosa* **(a)** colonies on MEA, 7 d, 25°C, bar = 2 mm; **(b,c)** vegetative cells **(b)** bar = 10 µm; **(c)** bar = 5 µm

reported good growth of *R. glutinis* on blanched frozen peas stored at 0°C for 8 weeks, and a significant increase in yeast numbers after 24 weeks at –18°C. A minimum a_w for growth near 0.92 was reported by Bem and Leistner (1970). pH 2.2 was the minimum for growth in the presence of HCl or organic acids; growth was inhibited by 100 mg/kg or less of benzoic or sorbic acid at pH 4 or below (Pitt, 1974a). Cultures of *R. mucilaginosa* and *R. glutinis* (10^5 cells/ml) sometimes survived heating at 62.5°C for 10 min (Put et al., 1976). This is a high heat resistance for an asporogenous yeast. However, heat resistance is lower in orange juice. *R. mucilaginosa* had a D_{50} value of 2.6 min and a *z* value of 9.8C° (Barreiro et al., 1981) and Shearer et al. (2002) reported D_{60} values between 0.12 min at pH 3 and 0.16 min at pH 4.0 with *z* values of 4.5–4.7C° in 0.1 M citrate buffer.

Rhodotorula species produce extracellular lipases and proteases (Rapp and Backhaus, 1992; Rosi, 1993).

Ecology. Both *Rhodotorula mucilaginosa* and *R. glutinis* are of widespread occurrence on fresh fruits and vegetables (e.g. Recca and Mrak, 1952; Buhagiar and Barnett, 1971). Reports of spoilage are rare. Leaves and plant stems are major habitats, and as a result, these species sometimes occur in cereals and flours, dough, malting barley, olives, soaking soybeans, citrus products and fruit juice concentrates (see Deák and Buechat, 1996; Pitt and Hocking, 1997).

The ability to grow rapidly at refrigeration temperatures means that *Rhodotorula* species are commonly associated with and may cause spoilage in dairy products such as yoghurts, cream, butter and cheeses (Fleet and Mian, 1987; see also Deák and Beuchat, 1996; Pitt and Hocking, 1997; Frölich-Wyder, 2003; Viljoen et al., 2003a). In our laboratory, we have frequently encountered *Rhodotorula* species causing spoilage of cream, sour cream and dairy desserts. They are also common in seafoods, including fish, shellfish and crustaceans and in marine waters (Hood, 1983; Comi et al., 1984; Kobatake et al., 1992). *Rhodotorula* species are also associated with fresh and processed meats (Dillon and Board, 1991; see also Deák and Beuchat, 1996; Pitt and Hocking, 1997; Samelis and Sofos, 2003).

Spoilage by *Rhodotorula* species has been reported in heat treated apple sauce and strawberries (Put et al., 1976) and cut potato chips packaged in 97–99.8% carbon dioxide (Cerny and Granzer, 1984). *Rhodotorula* species have been isolated from ready to eat airline meals (Saudi and Mansour, 1990). We have isolated *R. mucilaginosa* from apple pie filling and *R. glutinis* from soft cheeses.

Rhodotorula species produce ß-carotene and other carotenoids (Martin et al., 1993; Frengova et al., 1994) and the yeast can be cultivated as a source of these compounds and protein feed for fish in aquaculture (Hari et al., 1992).

Additional species. *Rhodotorula glutinis* (Fresen.) F.C. Harrison [synonym *Rhodotorula rubra* (Saito) F.C. Harrison] differs from *R. mucilaginosa* by the ability to use nitrate as a nitrogen source. It occurs in a similar range of habitats (see above).

References. Kurtzman and Fell (1998); Barnett et al. (2000).

Saccharomyces cerevisiae Meyen Fig. 10.10

Colonies on MEA at 3 days 1–2 mm diam, off-white, convex, margins circular, surface glistening; at 7 days 2–3 mm diam, as at 3 days except margins sometimes becoming fimbriate. Cells usually spherical to subspheroidal, 5–12 × 5–10 μm, occasionally also ellipsoidal to cylindrical, 5–20(–30) × 3–9 μm, reproducing by irregular budding, occurring singly, in pairs or in chains. Ascospores sometimes formed on MEA after prolonged incubation; asci formed directly from vegetative cells without conjugation; ascospores 1–4 per ascus, spherical to subspheroidal and smooth walled. Weak growth on Czapek agar; growth at 37°C usually as fast as, or faster than, that at 25°C; growth on malt acetic agar very weak or absent; no growth on MY50G or MY10-12.

Distinctive features, *Saccharomyces cerevisiae* is included here as an example of a strongly fermentative yeast which commonly occurs on foods but only infrequently causes spoilage. Colony and cell characteristics together with sporulation of the kind described above are reasonably diagnostic. In the absence of sporulation, identification remains a matter of conjecture unless the full identification systems of Barnett et al. (2000) are used or molecular methods are applied.

Taxonomy. Kurtzman and Robnett (2003) used multigene sequence analysis to examine *Saccharomyces* and related genera. After extensive phylogenetic analysis they concluded that the genus should

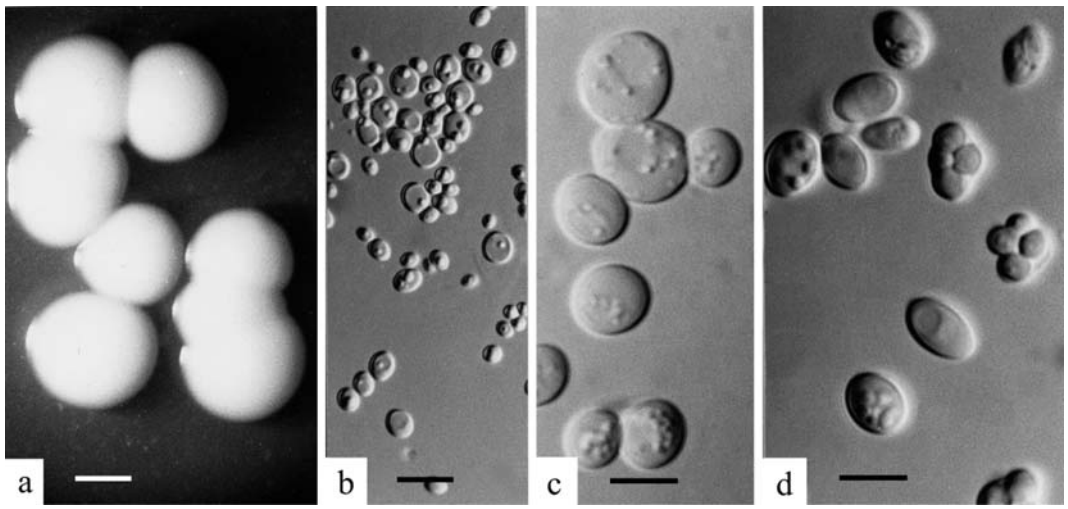


Fig. 10.10 *Saccharomyces cerevisiae* **(a)** colonies on MEA, 7 d, 25°C, bar = 2 mm; **(b,c)** vegetative cells **(b)** bar = 10 μm; **(c)** bar = 5 μm; **(d)** asci and ascospores, bar = 5 μm

be split into a number of genera, with *Saccharomyces* reserved for *S. cerevisiae* and closely related species, whilst the remaining *Saccharomyces* species were assigned to several other genera, some of which were newly described (Kurtzman, 2003). *Saccharomyces exiguus* was included in the genus *Kazachstania*. Fleet (2006) provided a useful summary of the changes in taxonomic treatment of these yeasts.

Barnett et al. (2000) list almost 120 synonyms for *Saccharomyces cerevisiae,* including many subspecies and varieties encompassing bread making, brewing, wine and cider yeasts, as well as yeasts from the natural environment. Consequently, the biochemical reactions given for *S. cerevisiae* are highly variable.

Physiology. *Saccharomyces cerevisiae* grows optimally between 33 and 35°C in 10–30% glucose. Its minimum growth temperature is reported as 4°C in 10% glucose and 13°C in 50% glucose, with a maximum growth temperature of 38–39°C (Jermini and Schmidt-Lorenz, 1987b). Juven et al. (1978) demonstrated growth of *Saccharomyces cerevisiae* down to 0.89 a_w in glucose media at neutral pH. Praphailong and Fleet (1997) reported growth in 7.5% (w/v) NaCl (0.95 a_w) at pH 5 and pH 7, but at pH 3, only 5% (w/v) NaCl was tolerated. The lowest pH at which Praphailong and Fleet (1997) recorded growth was pH 2.5 in yeast nitrogen base with 5% glucose in citrate–phosphate buffer. Over the pH range 3.0–7.0, *S. cerevisiae* grew in 50% but not 60% (w/v/) sucrose. *S. cerevisiae* was capable of growth down to pH 1.6 in HCl, 1.7 in H_3PO_4 and 1.8–2.0 in organic acids with a maximum tolerance to benzoic acid of 100 mg/kg at pH 2.5–4.0 and to sorbic acid of 200 mg/kg at pH 4.0 (Pitt, 1974a). *S. cereviaise* can degrade sorbic acid to 1,3-pentadiene (Stratford et al., 2007).

Using about 20 isolates, Put and de Jong (1982) determined that vegetative cells of *Saccharomyces cerevisiae* had a D_{60} of 0.1–0.3 min, while ascospores were more resistant, with a D_{60} of 5.1–17.5 min. Splittstoesser et al. (1986) reported that S. *cerevisiae* ascospores were over 100-fold more heat resistant than vegetative cells of the same strain, with a D_{60} of 6.1 min for ascospores in apple juice, and a *z* value of 3.8C°. Juven et al. (1978) found that heating cells of *S. cerevisiae* in media of reduced a_w greatly enhanced their heat resistance. In a medium based on fruit juice (pH 3.1, 0.99 a_w, 12° Brix), D_{60} was 0.3–2 min, but at 0.93 a_w D_{60} was 5 min or more. Using a 0.5% glucose heating medium, Török and King (1991) obtained a value for D_{60} of 1.05–2.67 min and a *z* value of 4.0–6.2C°, while Hur et al. (1993) reported a D_{62} of 3.5 min and a *z* value of 7.2C° for ascospores in 0.05 M phosphate citrate buffer. Shearer et al. (2002) assessed the heat resistance of *S. cerevisiae* in a range of buffers and juices. The highest D_{60} they recorded was 6.9 min in calcium-fortified apple juice at pH 3.9. Dry heat resistance

was much greater. When 10^8 *S. cerevisiae* cells were dried in wheat flour or skim milk powder to a_w values between 0.10 and 0.70, then heated at 150 or 200°C for 5–30 s, survival was greatest in skim milk powder at 0.40 a_w, with less than 1 $\log_{10}$ reduction at 200°C for 5 s, and 5 log reduction after 30 s at 200°C, indicating the difficulty of sterilising dry powders (Laroche et al., 2005).

High pressure inactivation of 4×10^5 *Saccharomyces cerevisiae* cells in apricot nectar required 700 MPa for 5 min (Gola et al., 1994), although Hocking et al. (2006b) reported a 4–5 $\log_{10}$ reduction in *S. cerevisiae* cells suspended in 20°Brix sucrose at pH 4.2 after treatment at 400 MPa for 2 min. Concentrated solutions provide protection against high pressure processing: a treatment of 600 MPa for 30 s resulted in less than 1 log reduction in viability of *S. cerevisiae* cells in glycerol, sucrose or NaCl solutions at 0.86 a_w (Goh et al., 2007). High intensity pulsed electric field treatment has also been used to inactivate *S. cerevisiae* in orange juice (Elez-Martinez et al., 2004).

Saccharomyces cerevisiae produces polygalacturonase (Panon et al., 1995; Djokoto et al., 2006) and extracellular proteases (Rosi, 1993).

Ecology. Best known for its domesticated role in the manufacture of breads and alcoholic beverages, *Saccharomyces cerevisiae* is also of widespread natural occurrence, in nectars and exudates, and on leaves and fruits. Not surprisingly, then, it occurs widely in foods and can be a source of spoilage. Soft drinks commonly contain S. *cerevisiae* (see Deák and Beuchat, 1996; Pitt and Hocking, 1997). Sand and van Grinsven (1976) and Put et al. (1976) reported spoilage of some cold sterilised products. The other major source of *S. cerevisiae* is fruit juices and concentrates (see Deák and Beuchat, 1996; Pitt and Hocking 1997; Boekhout and Robert, 2003; Fleet, 2006). Back and Anthes (1979) reported spoilage of fruit juice drinks and Put et al. (1976) of heat processed cherries. We have also isolated *S. cerevisiae* from spoiled fruit juices, tomato paste, fruit purees and bottling machinery and preservative resistant strains from spoiled sports drinks, mineral water with added *Aloe vera* and orange fruit juice cordial.

Saccharomyces cerevisiae has been isolated from a variety of dairy products. It was the dominant yeast in Egyptian Kareish cheese (Zein et al., 1983) and was relatively common in labaneh, a strained, concentrated yoghurt (Yamani and Abu-Jaber, 1994) and in some other yoghurts (Green and Ibe, 1987; Hur et al., 1992). Rohm et al. (1992) found *S. cerevisiae* in fermented milk products, kefir and cheeses. It has also been isolated from cocoa fermentation (Mazigh, 1994; Schwan and Wheals, 2003).

Rapid methods for identification of *Saccharomyces cerevisiae* have been reported based on genetic techniques such as multiplex PCR of plasmid DNA (Pearson and McKee, 1992), electrophoretic karyotyping, DNA–DNA hybridisation (Török et al.,1993) and real time PCR (Martorell et al., 2005b).

References. Fleet (2006) for an overview of *Saccharomyces* in foods; Boekhout and Robert (2003) for industrial and food uses, and application of molecular methods; Kurtzman and Fell (1998) and Barnett et al. (2000) for taxonomy.

Schizosaccharomyces pombe Lindner Fig. 10.11

Colonies on MEA at 3 days very small, up to 0.5 mm diam, white, circular with a smooth margin, surface convex, glistening; at 7 days 1–2 mm diam, of similar appearance. Cells on MEA dividing by lateral fission, at 3 days short cylinders with rounded ends, 5–7 × 3.0–5.0 μm, longer before division, at maturity becoming ellipsoidal or becoming wider at one end than the other, and sometimes showing fission scars. Ascospores often formed on MEA at 25 or 37°C within 1 week, asci of irregular shape, formed by fusion of two cells, usually more or less end to end, dehiscing at maturity, usually containing four ascospores; ascospores ellipsoidal, 3.0–4.0 μm long, with rough walls. Growth on Czapek agar very weak or absent; growth at 37°C often more rapid than at 25°C; growth on malt acetic agar equivalent to on MEA at 25°C; weak growth on MY50G; no growth on MY10-12.

Distinctive features. The genus *Schizosaccharomyces* is characterised by reproducing vegetatively by lateral fission. *S. pombe* forms asci with four ascospores and grows well at 37°C and on malt acetic agar.

Physiology. *Schizosaccharomyces pombe* grows at least as vigorously at 37°C as at 25°C. It is xerophilic, growing at 0.81 a_w in glucose based media

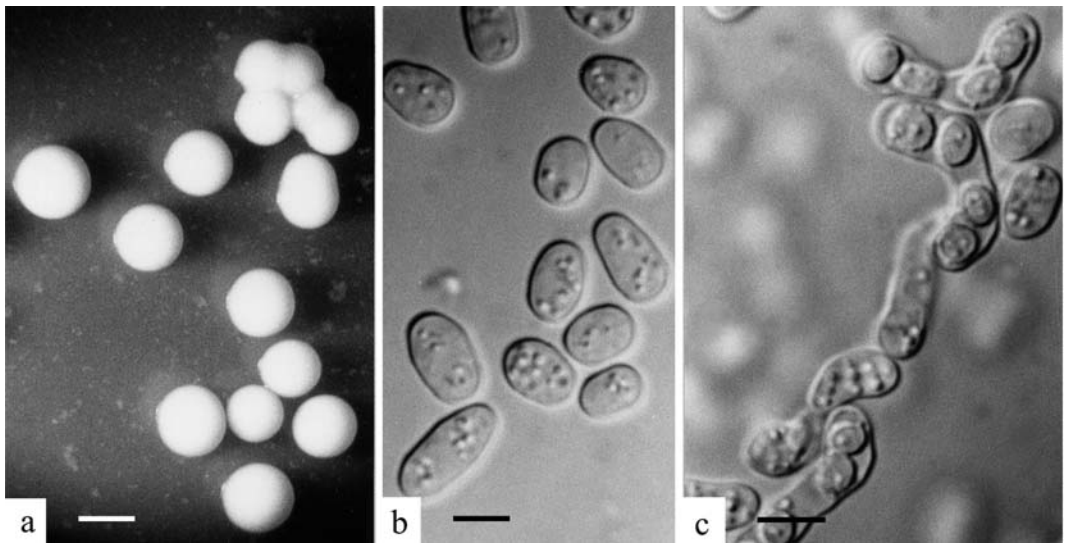


Fig. 10.11 *Schizosaccharomyces pombe* **(a)** colonies on MEA, 7 d, 25°C, bar = 2 mm; **(b)** vegetative cells, bar = 10 μm; **(c)** asci and ascospores, bar = 5 μm

(van Eck et al., 1993). However, in NaCl based media, the minimum a_w is only 0.97–0.95 (van Eck et al., 1993; Deák and Beuchat, 1996). Papouskova and Sychrova (2007) reported that the presence of 600 mM NaCl or KCl or 15% sorbitol enhanced the ability of *S. pombe* to grow at 34 °C and 37°C, but at 25°C and 30°C, the opposite was the case. Corry (1976) studied the heat resistance of vegetative cells of *S. pombe* at 0.95 a_w in various solutes. Sucrose was the most protective and gave a D_{65} value of 1.48 min. Glucose, fructose and glycerol were much less protective, with D_{65} values of 0.41, 0.27 and 0.21, respectively. *S. pombe* is resistant to the common food preservatives, as shown by its ability to grow on malt acetic agar. It is resistant to free SO_2 at levels up to 120 mg/kg (Warth, 1985) and up to 600 mg/l benzoic acid (Warth 1988, 1989c). *S. pombe* may produce high levels of H_2S off-flavours if sulphite is present (Rankine, 1964).

Ecology. *Schizosaccharomyces pombe* is a relatively uncommon spoilage yeast. However, its ability to grow at reduced water activities, in the presence of preservatives, particularly SO_2, and at 37°C gives it great potential to cause spoilage in warmer regions. It has been isolated on several occasions in our laboratory from sugar syrups undergoing fermentative spoilage with the production of H_2S. The syrups, used in the manufacture of glace fruits, had been preserved with substantial levels of SO_2. We have also isolated *S. pombe* from raspberry cordial concentrate, of 45° Brix and pH 3.0, containing 250 mg/kg SO_2.

In the wine industry, *Schizosaccharomyces pombe* may be used to deacidify wine because of its ability to metabolise L-malic acid (Sousa et al., 1993, 1995; Gao and Fleet, 1995), but it may also be responsible for off flavours under some circumstances (Unterholzner et al., 1988). *S. pombe* may also be used in fermentation of sugar cane juice in rum production (Pech et al., 1984; Fahrasmane et al., 1988) and in the production of palm wine (Chrystopher and Theivendirarajah, 1988; Sanni and Loenner, 1993). It is associated with cocoa fermentation (Ravelomanana et al., 1984; Mazigh, 1994; Schwan and Wheals, 2003) and has also been reported from coffee fermentation (Silva et al., 2000).

References. Kurtzman and Fell (1998); Barnett et al. (2000).

Zygosaccharomyces bailii (Lindner) Guillierm. Fig. 10.12

Saccharomyces bailii Lindner
Zygosaccharomyces acidifaciens W.J. Nick.
Saccharomyces acidifaciens (W.J. Nick.) Lodder & Kreger

Colonies on MEA at 3 days small, less than 2 mm diam, white, almost hemispherical, margins circular, surface glistening; at 7 days colonies up to

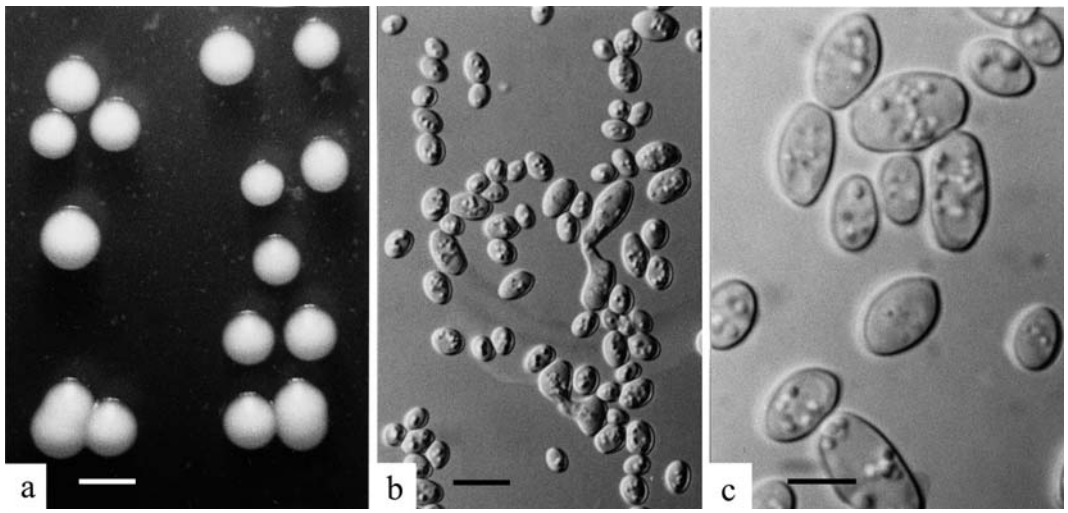


Fig. 10.12 *Zygosaccharomyces bailii* **(a)** colonies on MEA, 7 d, 25°C, bar = 2 mm; **(b)** vegetative cells, asci and ascospores, bar = 10 μm; **(c)** vegetative cells, bar = 5 μm

3 mm diam, of similar appearance as at 3 days. Cells large, ellipsoidal, usually 5–8 × 3.0–5.0 μm, reproducing by budding, characteristically subapically, or at an acute angle to the cell longitudinal axis, leaving a flat subapical "shoulder" on one side only of both mother and daughter cells; occurring singly or in pairs, rarely in short chains. Ascospores formed by most isolates in 7 days on MEA or malt acetic agar or both; asci formed by union of two cells to give characteristic "dumbbell" or less regular shapes; ascospores 1–4 per ascus, spheroidal to ellipsoidal, smooth walled and refractile, not readily liberated from the ascus. No growth on Czapek agar; rarely growth at 37°C; growth on malt acetic agar, only slightly slower than on MEA; slow growth on MY50G; no growth on MY10-12.

Distinctive features. Using the methods and media described here, *Zygosaccharomyces bailii* is a readily recognised species. Growth on MEA is slow and colonies are hemispherical; growth on malt acetic agar is only slightly slower. Asci are produced on one of these media or the other within 7 days by conjugation of two cells and contain 1–4 smooth walled ascospores.

Taxonomy. For a long time, there was controversy over whether highly fermentative yeasts related to *Saccharomyces* but undergoing cell conjugation before sporulation should be classified in *Saccharomyces* or as a separate genus, *Zygosaccharomyces*. *Zygosaccharomyces* is now accepted as the appropriate name for those taxa where cell conjugation precedes ascospore formation (von Arx et al., 1977; Kurtzman and Fell, 1998; Barnett et al., 2000).

Genetic techniques have been used to examine the relationships between species in the genus *Zygosaccharomyces* (James et al., 1994b) and now offer the most reliable means of species identification (James and Stratford, 2003). PCR targeting a short region of the 18S rRNA gene was able to discriminate to species level (Stubbs et al., 1994). Plasmid DNA was used as the target for multiplex PCR to distinguish between *Z. bailii, Z. rouxii* and *Saccharomyces cerevisiae* as the basis for a rapid identification system (Pearson and McKee, 1992), microsatellite PCR fingerprinting analysis was able to discriminate between the closely related species *Z. bailii* and *Z. bisporus* (Baleiras Couto et al., 1996) and RFLP of the ITS-5. 8S rRNA gene was able to distinguish between 10 species within the genus (Esteve-Zarzoso et al., 2003). The gene encoding the D1/D2 region of the 26S ribosomal RNA has been used recently as a target for sequencing and primer design to enable real-time PCR detection of *Z. bailii* in beverages (Rawsthorne and Phister, 2006). Isoenzyme patterns have also been used to differentiate *Z. bailii* from other closely related *Zygosaccharomyces* species (Duarte et al., 2004).

Physiology. Physiologically, the most outstanding feature of *Zygosaccharomyces bailii* is its resistance

to weak acid preservatives such as sorbic, benzoic, acetic and propionic acids and SO_2. The mechanisms of this resistance and effects of other parameters such as solute type, pH and a_w, have been extensively studied by Warth (1977, 1985, 1986, 1989a,b,c, 1991), Lenovich et al. (1988, as *Z. rouxii*) and Jermini and Schmidt-Lorenz (1987d). *Z. bailii* is remarkable in another characteristic: exposure to low levels of preservative, such as may occur in imperfectly cleaned filling lines, causes adaptation to preservative, and the ability to survive and grow in much higher concentrations than before adaptation (J.I. Pitt, unpublished; Warth, 1977, 1986, 1988). Warth (1986) reported an MIC for benzoic acid of 1,000–1,400 mg/l at pH 3.5 in adapted cells of *Z. bailii*. Growth in commercially packed fruit-based cordials – of pH 2.8–3.0, 40–45° Brix, preserved with 800 mg/l benzoic acid – has been observed by us on several occasions. A similar level of resistance to sorbic acid was reported by Neves et al. (1994) in strains of *Z. bailii* from spoiled foods and alcoholic beverages. *Z. bailii* is also resistant to combinations of alcohol and acids present in wine (Kalathenos et al., 1995). Sousa et al. (1996) reported that ethanol inhibits uptake of acetic acid by *Z. bailii* cells, which could explain this protective effect.

Like *Saccharomyces cerevisiae, Zygosaccharomyces bailii* vigorously ferments glucose solutions to CO_2, and the reaction is not inhibited until at least 80 psig (= 560 kPa) overpressure is reached; unlike S. *cerevisiae, Z. bailii* can carry out this fermentation in the presence of 400 mg/kg or more of benzoic or sorbic acid. In the absence of high CO_2 pressure, *Z. bailii* can tolerate relatively low pH and low a_w as well. Tolerance of low pH is not as great as for some other yeasts: according to Pitt (1974a), pH 2.2–2.5 is the minimum for growth.

Jermini and Schmidt-Lorenz (1987b) reported that the optimum temperature for growth of *Zygosaccharomyces bailii* ranged from 30–32°C in 10% (w/w) glucose to 34–36°C in 60% (w/w) glucose. The minimum temperature for growth of *Z. bailii* was 6.5°C in 10 and 30% (w/w) glucose, rising to 13°C in 60% glucose. *Z. bailii* grew at 37°C in the lower glucose concentrations, while its maximum growth temperature in 60% (w/w) glucose was 40°C. López-Malo and Palou (2000) reported growth of *Z. bailii* at 5°C in mango puree (0.97 a_w, pH 3.5) containing 1,000 mg/l benzoate after 29 days, but no growth under equivalent conditions with sorbate instead of benzoate. *Z. bailii* is a xerophile, capable of growth down to at least 0.80 a_w at 25°C (Pitt, 1975), although at 30°C, Jermini and Schmidt-Lorenz (1987a) reported a minimum a_w for growth in fructose of 0.86.

Mathematical modelling has been used to predict growth/no growth boundaries and time to visible growth of *Z. bailii* under various combinations of conditions. López-Malo and Palou (2000) based their model on mango puree, containing either 1,000 mg/l potassium sorbate or sodium benzoate, pH 3.5, at 0.97–0.99 a_w over the temperature range 5–25°C. Jenkins et al. (2000) based their model on yeast nitrogen broth, incubated at 30°C with combinations of acetic acid (1.0–2.8% v/v), fructose (7.0–32.0% w/v) and NaCl (2.6–4.2% w/v).

Put and de Jong (1982) reported that vegetative cells of *Zygosaccharomyces bailii* showed a low heat resistance, with a D_{50} value of 0.1–0.3 min. Ascospores were more resistant; for three isolates the D_{60} value for ascospores ranged from 8 to 14 min. Data of Jermini and Schmidt-Lorenz (1987c) confirmed this, reporting a D_{60} of 14.9 min for *Z. bailii* ascospores heated in yeast extract glucose broth with 60% (w/w) glucose, 0.86 a_w, at pH 4.5. *Z. bailii* can also be inactivated by high hydrostatic pressure (Palou et al., 1997a), although ascospores are more resistant than vegetative cells (Raso et al., 1998) and high concentrations of sugars can provide some protection (Palou et al., 1997b).

Zygosaccharomyces bailii is sensitive to ozone, with 10^4 cells killed instantaneously in ozonated water in a recirculating concurrent reactor (Restaino et al., 1995). *Z. bailii* cells can also be inactivated by hydrostatic pressure: a reduction of 10^7 cells was achieved with 3,000 atm for 10 min at 25°C in spaghetti sauce with meat at pH 3.0, 4.0 or 5.0, and at 2,500 atm for 20 min at pH 5.0 (Pandya et al., 1995).

Ecology. Outside the food and beverage industries, *Zygosaccharomyces bailii* is a virtually unknown yeast, although reports of spoilage caused by it date back to the beginning of the century (Thomas and Davenport, 1985). Only within the past 20 years has *Z. bailii* been isolated from "natural" sources, from fermenting fruit in vineyards and orchards (see Pitt and Hocking, 1997; Nisiotou et al., 2007).

Within the food and beverage industries, in contrast, *Zygosaccharomyces bailii* has become notorious. The preservation of acid, liquid products

against fermentative yeasts has traditionally relied on chemical preservatives; i.e. sorbic and benzoic acids, and sulphur dioxide, and the "natural" preservatives acetic acid and ethanol. The resistance of *Z. bailii* to all of these compounds at permitted (and against other yeasts, effective) levels means that such products must really be packed sterile or be pasteurised in the final sealed container. Products at risk and in which fermentation or explosive spoilage has been observed are listed by Thomas and Davenport (1985) and include tomato sauce, mayonnaise, salad dressing, soft drinks including sports drinks, fruit juices and concentrates, ciders and wines (see Pitt and Hocking, 1997; James and Stratford, 2003; Kurtzman and James, 2006). We and others (Put et al., 1976; R.R. Davenport, unpublished) have also seen spoilage in cordial concentrates, fruit syrups intended for cake and confectionery manufacture and a variety of other products. *Z. bailii* has recently been reported from cough mixture (Charnock et al., 2005), mustard (Buchta et al., 1996), balsamic vinegar (Solieri et al., 2006), Haipao and Kombucha tea fermentation (Liu et al., 1996; Teoh et al., 2004), fermenting cassava (fufu; Oyewole, 2001) and black olives (Kotzekidou, 1997b). Losses to *Z. bailii* around the world run to many millions of dollars per annum.

Our experiments with adapted and unadapted *Zygosaccharomyces bailii* inoculated into canned carbonated soft drinks (J.1. Pitt, unpublished) have shown that only five cells per can of adapted *Z. bailii* are sufficient to cause spoilage of a high percentage of containers. Noncarbonated products will certainly fare no better. It seems probable that a single healthy, adapted cell of *Z. bailii* per container of any size will ultimately lead to spoilage in a high percentage of cases. Prevention of spoilage, then, must usually rely on the total exclusion of living *Z. bailii* cells from the final product. Pasteurisation in the final, sealed container is the method of choice, though not always practical. Centre temperatures of 65–68°C for an appreciable number of seconds are an adequate pasteurisation treatment. Heat treatments of this kind are insufficient to kill ascospores (Put and de Jong, 1982), so it appears that ascospores of *Z. bailii* are not a common problem. If pasteurisation precedes filling, as is often the case with a wide range of products, rigorous daily cleaning of filling machinery is essential if spoilage by *Z. bailii* is to be avoided. Common sites for yeast proliferation include filler heads, diaphragm valves, pressure gauges and dead ends in pipes. Yeasts can also survive in lubricating oils and can infect product from aerosols generated by moving machinery (Thomas and Davenport, 1985). Membrane filtration immediately before filling is an effective treatment, but of course is only practicable for clear products such as ciders and white wines. Membrane filtration is widely practiced by the Australian wine and cider industries.

Where possible, synthetic products such as soft drinks and water ices should be manufactured without a utilisable nitrogen or carbon source. *Zygosaccharomyces bailii* is unable to ferment sucrose, and the use of sucrose not glucose as a sweetener in such products is highly recommended. Manufacturers should be aware that the commercially desirable practice of adding fruit juices to soft drinks or mineral waters is microbiologically hazardous. Far more stringent cleaning procedures are essential with such products than with purely synthetic or mineral products.

Additional species. *Zygosaccharomyces bisporus* (H. Nagan.) Lodder and Kreger shares many characters with *Z. bailii* including the formation of similar colonies on MEA and malt acetic agar. *Z. bisporus* is distinguished by smaller cells, 3.5–7 × 2.5–4.5 μm, which often adhere in short chains, and do not produce ascospores on either of the above media in 7 days. Relatively little has been published about this species, which appears to be less common than *Z. bailii*, but it has a similar capability to cause food spoilage. It can be safely assumed to have similar physiological properties to *Z. bailii*. It is more xerophilic, however. Tilbury (1980) reported growth down to 0.70 a_w in sucrose/glycerol syrups. Put et al. (1976) reported survival of ascospores (5×10^4/ml) after 10 min at 60°C, but not 20 min.

References. Kurtzman and Fell (1998); Barnett et al. (2000); James and Stratford (2003); Kurtzman and James (2006).

Zygosaccharomyces rouxii (Boutroux) Yarrow Fig. 10.13

Saccharomyces rouxii Boutroux

Anamorph: *Candida mogii* Vidal-Leir.

Colonies on MEA at 3 days 0.2–0.5 mm diam, white, margins circular, almost hemispherical, surface

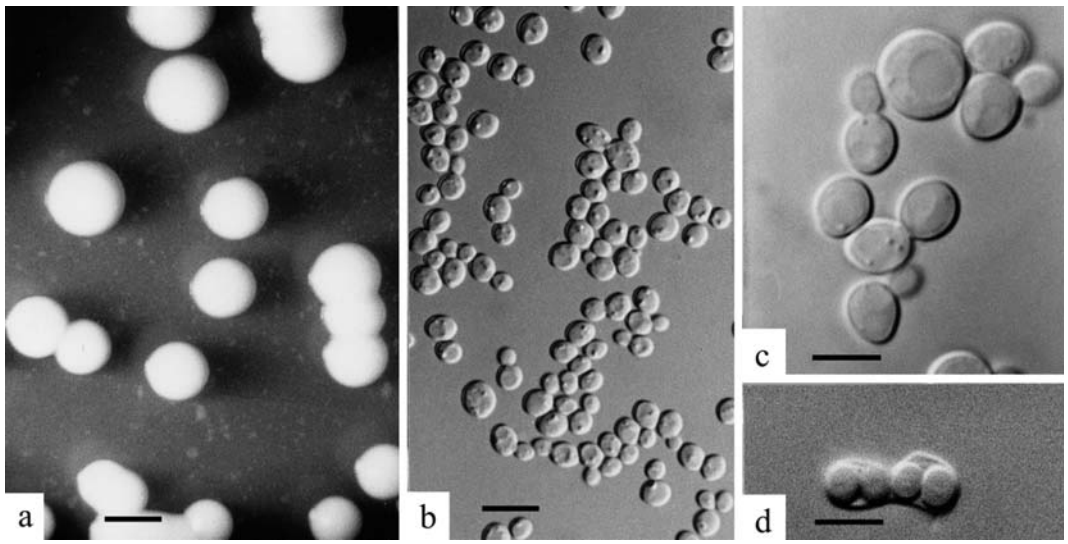


Fig. 10.13 *Zygosaccharomyces rouxii* **(a)** colonies on MEA, 7 days, 25°C, bar = 2 mm; **(b)** vegetative cells, bar = 10 μm; **(c)** vegetative cells, bar = 5 μm; **(d)** ascospores, bar = 5 μm.

glistening; at 7 days 2–3 mm diam, appearance as at 3 days. Cells on MEA at 3 days subspheroidal to ellipsoidal, 4.0–9 × 2.5–7 μm, mostly 5–7 × 4.0–5 μm, budding irregularly, occurring singly, in pairs or in small groups. Ascospores rarely seen on artificial media, but in our experience frequently observed on such low a_w, high-sugar substrates as prunes. Asci of irregular shape, usually formed by conjugation of two cells; ascospores 1–4 per ascus, spherical to subspheroidal, with walls smooth or finely roughened. No growth on Czapek agar; sometimes weak growth at 37°C; no growth on malt acetic agar; growth on MY50G and on MY10-12, with macroscopic colonies in 3 days (Fig. 10.13).

Distinctive features. *Zygosaccharomyces rouxii* shares with *Debaryomyces hansenii* the ability to grow on both MY50G and MY10-12. Unlike *D. hansenii, Z. rouxii* does not grow on Czapek agar; also *Z. rouxii* does not produce the small (2.5–4.0 μm) spherical cells characteristic of young cultures of *D. hansenii*.

Taxonomy. *Zygosaccharomyces* is differentiated from *Saccharomyces* by undergoing cell conjugation before ascospore formation. However, Yarrow (1984) noted that *Z. rouxii* sometimes does not conjugate before ascospore formation. As with *Z. bailii* (see above), genetic techniques are now being used to investigate relationships within the genus *Zygosaccharomyces* and applied to detection and identification systems for *Z. rouxii* (see Kurtzman and James, 2006).

Physiology. *Zygosaccharomyces rouxii* has the distinction of being the second most xerophilic organism known (Pitt, 1975), being able to grow down to 0.62 a_w in fructose solutions (von Schelhorn, 1950) and to 0.65 a_w in sucrose/glycerol (Tilbury, 1980). Comi et al. (1992) reported growth down to 0.63 a_w at 30°C but only down to 0.76 a_w at 4°C in ice cream mix. In our laboratory, formation of ascospores has been observed down to 0.70 a_w on a favourable substrate (prunes). The optimum temperature for growth varies with a_w, from 24°C in 10% (w/w) glucose (0.99 a_w) to about 33°C in 60% (w/w) glucose (0.87 a_w) (Jermini and Schmidt-Lorenz, 1987b). In 10% glucose, Z. *rouxii* grew at 4°C, but its minimum temperature for growth in 60% glucose was 7°C. The maximum temperature for growth was 37°C in 10% glucose, but 42°C in 60% glucose (Jermini and Schmidt-Lorenz, 1987b). Martorell et al. (2007) reported that *Z. rouxii* was unable to grow at 4 or 37°C at pH 4.0 in a medium containing only 2% glucose. English (1954) reported growth of Z. *rouxii* over the range pH 1.8–8.0 in a medium containing 46% glucose. Onishi (1963) showed that, while the pH range for growth in 1 M NaCl solutions was very

broad, in 2–3 M NaCl it was greatly restricted, in the latter case to within the range pH 3.0–6.0. *Z. rouxii* was able to grow in 15% (w/v) NaCl at pH 3 and pH 5, but at pH 7, only 7.5% (w/v/) was tolerated. No growth occurred at pH 2.0 (Praphailong and Fleet, 1997). The pH range for *Z. rouxii* in 5% glucose medium reported by Praphailong and Fleet (1997) was pH 2.5–7.5 in citrate-phosphate buffer, but only pH 3.0–7.0 in inorganic buffer.

Put et al. (1976) reported that 10^5 cells/ml of *Candida mogii,* the anamorph of *Zygosaccharomyces rouxii,* barely survived heating at 62.5°C for 10 min. The calculated D_{60} value for *Z. rouxii* in 0.1 M citrate buffer was reported by Shearer et al. (2002) to be 0.039 min at pH 3.5 and 0.008 min at pH 4.0, with *z* values of 3.3 and 2.1C°, respectively. Marked differences were observed in the influence of reduced water activities as generated by glucose and sucrose solutions on the heat resistance of *Z. rouxii* (probably actually *C. mogii*) (Gibson, 1973). At 55°C, D values ranged from less than 0.1 min at 0.995 and 0.98 a_w through 0.6 min at 0.94 a_w and 7 min at 0.90 a_w to 55 min at 0.85 a_w. At 60°C, the D value at 0.94 a_w or above was less than 0.1 min, but at 0.85 a_w was 10 min. At 65.5°C, D values were all less than 0.1 min except at 0.85 a_w, where it was 0.4 min. These very large effects may not occur with less xerophilic yeasts. At 0.90 and 0.85 a_w, *z* values were about 8C°. Török and King (1991) obtained a similar D_{55} value to that reported by Gibson (1973): 0.16 min in 0.5% glucose, with a *z* value of 5.5C°. Jermini and Schmidt-Lorenz (1987c) reported a D_{60} value for *Z. rouxii* asci of 3.5 min at 0.86 a_w, with D values for asci being 20–50 fold higher than for vegetative cells at 0.96 a_w. Corry (1976) compared the effect of different solutes on the heat resistance of *Z. rouxii* (again, probably only vegetative cells) at a single a_w, 0.95. Sucrose was the most protective solute, D_{65} being 1.9 min in that substrate. Glucose, fructose and glycerol produced rather variable results, with D_{65} values ranging from 0.2 to 0.6 min.

Zygosaccharomyces rouxii is moderately resistant to preservatives. Martorell et al. (2007) reported MIC values for *Z. rouxii* of a range of antimicrobial compounds in a basal medium containing 2% glucose, pH 4.0: sorbic acid 2.8–2.85 mM (approx, 310–320 ppm), benzoic acid 3.05 to >3.6 mM (*ca* 370 to >440 ppm) and acetic acid 106–110 mM (6,360–6,600 ppm). Praphailong and Fleet (1997) reported growth in YNB + 5% glucose at pH 5 with 750 ppm benzoic acid or 500 ppm sorbic acid. At pH 3.0, *Z. rouxii* was unable to grow in the presence of 250 ppm of either preservative. In yeast extract glucose broth at pH 4.0, *Z. rouxii* was able to grow in 400 ppm potassium sorbate or 600 ppm sodium benzoate (El-Halouat and Debevre, 1996). However, these authors reported that preservative tolerance was reduced when the yeast was grown in 80% CO_2 compared with growth in air. CO_2 concentration (20–80%, balance N_2) compared with air had little effect on growth rate of *Z. rouxii* at 30°C at 0.94 or 0.90 a_w. However, at 0.83 a_w, elevated CO_2 levels reduced initial (4 days) growth rates, but by 12 days at 30°C there was no significant difference in final yeast cell numbers (El-Halouat and Debevre, 1996). *Z. rouxii* is able to metabolise sorbate to 1,3-pentadiene (Casas et al., 1999, 2004) and has also be reported to degrade the fungicide iprodione (Zadra et al., 2006).

Ecology. The combined ability to grow at exceptionally low water activities and to vigorously ferment hexose sugars makes *Zygosaccharomyces rouxii* second only to *Z. bailii* as a cause of fermentative food spoilage. Tilbury (1980) reported isolations by himself and others from raw cane sugar, malt extract, fruit juice concentrates, ginger and glace cherries. *Z. rouxii* has also been reported from wine musts (Delfini et al., 1990), traditional balsamic vinegar (Giudici, 1990; Solieri, 2006), honey (Poncini and Wimmer, 1986; Schneider et al., 2003; Beckh et al., 2005), hydrated prunes (El-Halouat and Debevere, 1997) and soy sauce koji (Lee and Lee, 1970; O'Toole, 1997). Jermini et al. (1987) isolated *Z. rouxii* from honey, apple, orange and other fruit juice concentrates, marzipan, sugar syrup and candied pineapple. The unpublished list of sources in our laboratory includes jams, ginger, glucose syrup, liquid malt extract, fruit juice concentrates, cake icings, chocolate sauce, dried and high moisture prunes, sultanas, candied citrus peel and flavouring syrups. The list of sources in Barnett et al. (2000) is equally wide.

Concentrated liquid foods and ingredients, which rely on their low a_w for microbial stability, simply cannot be made concentrated enough to inhibit growth and fermentation by *Zygosaccharomyces rouxii*. As is the case with *Z. bailii* in preserved foods, concentrated foods must be free of *Z. rouxii* to be stable. Raw materials such as orange and apple

concentrates are usually stored in large (200 l) drums. Even very low initial contamination rates with *Z. rouxii* cells can eventually cause huge losses. The short term cure for 200 l drums with swelling ends is immediate refrigeration and, if possible, rapid utilisation. The only satisfactory long term solution, other than continued refrigeration near 0°C, appears to be dilution, pasteurisation and reconcentration. Refrigeration temperatures must eventually kill the yeast, but no figures appear to exist on the length of time necessary. Preservatives are effective against *Z. rouxii,* but are rarely permitted in concentrated or dried foods. In any case, dispersal of a preservative through large masses of a viscous concentrate would be difficult to achieve.

Confectionery fillings and marzipan are also highly susceptible to infection by *Zygosaccharomyces rouxii* and leakage in soft centred chocolates almost always is indicative of *Z. rouxii* spoilage (see Pitt and Hocking, 1997). Losses are often high because the inoculum per chocolate block need be only a few cells. *Z. rouxii* may grow continuously in lines and feed into the manufactured product for a long period before spoiled product is detected.

Generally speaking, the growth of *Zygosaccharomyces rouxii* on dried, loosely packaged foods is not a problem, although *Z. rouxii* may be isolated from dried foods such as dried condiments (Ekundayo, 1987). "Sugaring" on dried prunes or dates often contains cells of *Z. rouxii* which act as crystallisation nuclei for glucose, but most Pure Food Acts do not regard this as spoilage.

Once such commodities are packaged, however, usually at somewhat higher a_w levels, CO_2 production by *Zygosaccharomyces rouxii* can rapidly lead to spoilage (Pitt, 1963; Bolin et al., 1972).

Jams do not usually spoil due to this yeast because the hot fill process is sufficient for inactivation. Entry of a *Zygosaccharomyces rouxii* cell after a jam is opened will often lead to fermentative spoilage, but such occurrences are rare and outside the manufacturer's control. We have seen marmalade spoil due to *Z. rouxii;* Zaake (1979) has reported a similar occurrence.

References. Onishi (1963); Kurtzman and Fell (1998); Barnett et al. (2000); James and Stratford (2003); Kurtzman and James (2006).

Chapter 11
Fresh and Perishable Foods

In relation to spoilage by fungi, foods can be divided onto two broad classes: fresh or perishable foods and stored or processed foods. Spoilage of these two classes of foods occurs in quite different ways and is caused by separate types of fungi, so is treated in two separate chapters. Many kinds of foods, however, can undergo spoilage both when fresh and after processing and so have been included in both chapters.

In considering spoilage, fresh foods can again be divided into two types: foods composed of living cells, including fruit, vegetables, nuts and cereals, and non-living foods, such as meat, milk or fruit juice. Again the kind of spoilage which may occur and the types of fungi which cause spoilage are quite different.

11.1 Spoilage of Living, Fresh Foods

Living foods are biologically active to a greater or less degree, and plants have developed extremely effective defence mechanisms against invasion by their natural predators, the fungi. The fungi in turn have sought evolutionary pathways to enable them to invade living plant tissue. Plant tissues are invariably of high a_w, of neutral or acid pH, and grow at mesophilic temperatures so that invasion is much less related to physical factors than in other types of food spoilage. It is a contest between plant defence mechanisms and the ability of the fungi to overcome them. Here we are dealing with plant–parasite relationships, not with the physical parameters which govern spoilage of other kinds of foods.

The interaction between plant and parasite is very complex and often poorly understood; hence here as in other similar publications we can do little more than catalogue the common forms of spoilage and provide general references to methods for reducing losses. Just as food mycology is a relatively neglected part of food microbiology, so postharvest spoilage of fruits and vegetables is a relatively unexplored area both of food mycology and plant pathology.

Spoilage of living fresh foods can again be usefully subdivided into two categories: foods such as fruit and vegetables which are perishable unless rapidly processed and foods such as nuts and cereals which tend to dry naturally and become stable in the field.

The basic difference in the spoilage of fresh fruits and vegetables lies in the pH of the living tissue. Fruit are usually quite acidic, in the pH range 1.8–2.2 (passionfruit, lemons) to 4.5–5.0 (tomatoes, figs; for a list see Splittstoesser, 1987), and are quite resistant to invasion by bacteria. Microbial spoilage of fruit and fruit products is almost always caused by fungi. Vegetables on the other hand are of near-neutral pH and are susceptible to bacterial invasion as well. Bacterial and fungal spoilage of vegetables are of roughly equal importance, with *Erwinia, Pseudomonas* and *Xanthomonas* the most commonly implicated genera (Brackett, 1987; Snowdon, 1991).

11.2 Fruits

Defence mechanisms in fruit appear to be highly effective against nearly all fungi, as only a relatively few genera and species are able to invade and cause serious losses. Some of these are highly specialised

J.I. Pitt, A.D. Hocking, *Fungi and Food Spoilage*, DOI 10.1007/978-0-387-92207-2_11,
© Springer Science+Business Media, LLC 2009

pathogens, attacking only one of two kinds of fruit, others have a more general ability to invade fruit tissue.

Fruits become increasingly susceptible to fungal invasion during ripening, as the pH of the tissue increases, skin layers soften, soluble carbohydrates build up and defence barriers weaken. The storage of fresh fruit postharvest is a branch of science in its own right, with the need to balance desirable maturity against storage life and transportability, ripening against overripening, balanced maturation against breakdown of desirable qualities, all with the ever present problems of controlling fungal invasion and spoilage as well. The postharvest diseases and disorders of fruit have been comprehensively documented by Snowdon (1990), and the reader is referred to this text for a complete guide. The most important fungal diseases of fruits are briefly described below.

11.2.1 Citrus Fruits

By far the most common causes of citrus fruit decay throughout the world are the *Penicillium* rots due to *P. italicum* and *P. digitatum,* termed blue rot and green rot, respectively. Fruit can be attacked by these species at any stage after harvest. Invasion initially requires damage to skin tissue, which readily occurs in modern bulk handling systems. Decay spreads by contact from fruit to fruit. As would be expected from their physiology, growth of these species in citrus is rapid at 20–25°C but very slow below 5°C or above 30°C (Hall and Scott, 1977).

Control relies primarily on careful handling of the fruit. Postharvest treatments are based on washes heated to 40–50°C and containing detergents, weak alkali and/or fungicides such as benomyl, thiabendazole, imazalil, guazatine or sodium *o*-phenylphenate (SOPP). Pyrimethanil has recently been approved for postharvest use on citrus fruit to control *Penicillium digitatum* and other species, which have developed a resistance to common fungicides (Smilanick et al., 2006). In Australia, fungicides containing guazatine are currently restricted to domestic markets. Therefore, the use of generally regarded as safe (GRAS) compounds such as sodium bicarbonate or sodium carbonate and gibberellic acid in combination with heat treatments has been investigated to control *P. digitatum* and *Geotrichum candidum* in citrus fruit destined for export markets (Cunningham and Taverner, 2007). In a search for alternatives to chemical treatment, biological control of postharvest rots is an active research area. Bacteria such as *Bacillus subtilis, Pseudomonas cepacia* and *P. syringae* and the yeasts *Debaryomyces hansenii* and *Candida guilliermondii* have been shown to be active against the main postharvest pathogen *P. digitatum* (Droby et al., 1989; Wilson and Chalutz, 1989; Arras, 1993; Huang et al., 1993; McGuire, 1994). *Candida oleophila* and *Pichia anomala* at inoculum levels of 10^6 to 10^8 cfu/ml were effective in protecting Clementine and Valencia oranges against *Penicillium italicum* and *P. digitatum* (Lahali et al., 2004).

Penicillium ulaiense is a recently emerged pathogen of citrus (Holmes et al., 1993, 1994). This fungus is resistant to the commonly used preservatives and is posing problems in citrus packing plants in many countries. Rots are distinguished from those of *P. italicum* by the formation of coremia on the fruit (Holmes et al., 1994). Originally described from decaying oranges in Taiwan (Hsieh et al., 1987), *P. ulaiense* has been isolated from citrus fruit in the United States (Holmes et al., 1993, 1994), Australia (Hocking and Pitt, 1996) and Argentina (Carrillo, 1995). *P. ulaiense* is effectively controlled with pyrimethanil based fungicides (Smilanick et al., 2006).

Geotrichum candidum causes sour rot of citrus, primarily in lemons and limes, in all parts of the world (Butler et al., 1965; Snowdon, 1990). The rot is a pale, soft area of decay which later develops into a creamy, slimy surface growth. At favourable temperatures of 25–30°C, fruit will rot completely in 4 or 5 days, and the disease can spread by contact (Hall and Scott, 1977; Ryall and Pentzer, 1982; Snowdon, 1990). The characteristic cylindrical conidia of *Geotrichum* are readily seen in mounts made from advanced, slimy rots. Infection usually occurs in overmature fruit after long, high temperature storage. Control relies on storage at temperatures below 5°C.

Black centre rot of oranges, caused by an *Alternaria* species generally known as *Alternaria citri* M.B. Ellis and N. Pierce, appears as an internal blackening of the fruit. Peever et al. (2005) showed

that *Alternaria* species associated with black rot of fruits belong to a single *A. alternata* clade and that the name *A. citri* to describe species associated with citrus black rot is not justified. Culturing of blackened areas on DCMA will lead to growth of dark colonies bearing characteristic *Alternaria* conidia. Simmons (2007) lists 22 *Alternaria* species that may be found on citrus plants and fruit. *Cladosporium sphaerospermum* has been reported to produce a soft rot of oranges in India, causing postharvest losses (Gaur and Chenulu, 1981). Anthracnose of tangerines, caused by *Colletotrichum gloeosporioides* has also been reported (Timmer et al., 1998; Teixeira et al., 2001).

Less common and usually less serious spoilage of citrus can be produced by a variety of fungi not described in this book. They include *Guignardia citricarpa* Kiely, which produces black spots; *Septoria depressa* McAlpine, the cause of *Septoria* spot; *Sphaceloma fawcettii* Jenkins, causing scab on lemons; and stem end rots caused by *Diaporthe citri* F.A. Wolf (anamorph: *Phomopsis* sp.) and *Lasiodiplodia theobromae* (Hall and Scott, 1977; Splittstoesser, 1987; Ryall and Pentzer, 1982; Brown et al., 1988; Snowdon, 1990).

11.2.2 Pome Fruits

The most common and destructive fungal spoilage agent in pome fruits (apples and pears) is again a *Penicillium* causing a blue rot, in this case *P. expansum* (Fox, 1994). Decay commences as a soft, light coloured spot which rapidly spreads across the surface and also deeply into the fruit tissue. As growth spreads, blue green coremial fruiting structures appear on the surface. *P. expansum* grows at low temperatures, so cold storage retards, rather than prevents, spoilage (Hall and Scott, 1977). Damaged and overmature fruit are the most susceptible.

Diagnosis may be made by inspection in advanced cases, or by culturing on DRBC and then CYA and CSN. Infected fruit held for a few days at room temperature will develop blue green coremia of *Penicillium expansum*.

Control measures include careful handling and the use of fungicides such as benomyl or SOPP. Because the rot spreads by contact from fruit to fruit, it is also common practice to pack fruit on fibre board trays which reduce physical contact between individual fruits. Biological control using yeasts and bacterial species is being investigated in an effort to reduce the dependence on fungicides. *Aureobasidium pullulans* (Bencheqroun et al., 2007), *Pseudomonas syringae* (Nunes et al., 2007), *Metschnikowia pulcherrima* and *Cryptococcus laurentii* (Conway et al., 2007) have all been reported to reduce *Penicillium expansum* infection on apples.

Penicillium solitum is another *Penicillium* causing apple decay (Pitt et al., 1991; Sanderson and Spotts, 1995). Like *P. ulaiense, P. solitum* is resistant to the commonly used fungicides. *P. funiculosum* has also been reported from stored apples in South Africa (Vismer et al., 1996). Postharvest infection with *P. expansum* and occasionally other species of *Penicillium* may lead to significant levels of patulin contamination in stored apples (Vismer et al., 1996; Baert et al., 2007b; Morales et al., 2007b).

Another spoilage fungus in pome fruits is *Botrytis cinerea,* which causes grey mould rot in cold stored pears (Hall and Scott, 1977) and, less commonly, apples. The rot is firmer than blue mould rot and becomes covered in ash grey spore masses. The mould invades through wounds or abrasions and can spread rapidly in packed fruit. Control measures are similar to those used for *Penicillium expansum*. As well as benomyl, dichloran (Allisan, Botran) is an effective fungicide against *B. cinerea*. Storage of fruit at –0.5 to 0°C also provides good control.

Other fungi which can cause rots of pome fruits include *Gloeosporium album* Osterw. (teleomorph *Pezicula alba* E.J. Guthrie), which causes "bulls eye rot"; *Phytophthora* sp., which produces brown rots; and *Spilocaea pomi* Fr.: Fr. [teleomorph *Venturia inaequalis* (Cooke) G. Winter] and *Fusicladium pyrorum* (Lib.) Fuckel (teleomorph *V. pirina* Aderh.), which produce black spots on apples and pears, respectively (Hall and Scott, 1977; Splittstoesser, 1987; Wicks and Blumbieris, 1981; Snowdon, 1990). *Fusarium solani* and *F. equiseti* have been reported causing soft or dry rots of apples and pears in India (Tandon et al., 1975; Geeta et al., 1979; Jain et al., 1982). *Penicillium purpurogenum* and *Paecilomyces variotii* have also been found from postharvest rots of pears (Tandon et al., 1975). *Alternaria alternata* and the *Stemphylium*

state of *Pleospora herbarum* (Pers.: Fr.) Rabenh have been reported causing postharvest rots of apples in Spain and Taiwan (Viñas et al., 1992; Ko et al., 1994); however, Simmons (2007) lists *Alternaria mali* and *A. pomicola* as species associated with apples.

As with citrus fruit, the search for effective biological control agents for the two major postharvest pathogens of pome fruit is ongoing. Activity against *Penicillium expansum* has been demonstrated by cultures of the bacteria *Bacillus subtilis* (Sholberg et al., 1995), *Pseudomonas cepacia* (Smilanick and Denis-Arrue, 1992), *P. syringae* and the pink yeast *Sporobolomyces roseus* (Janisiewicz et al., 1994; Janisiewicz and Bors, 1995) and against *Botrytis cinerea* by *S. roseus* and *Candida oleophila* (Janisiewicz and Bors, 1995; Mercier and Wilson, 1995). Nunes et al. (2002) reported that a 50:50 mixture of *Candida sake* and *Pantoea agglomerans* reduced grey mould incidence in pears in cold storage by 95% and reduced blue and grey mould infection on apples at room temperature by 90%. *Pseudomonas syringae* was shown to be effective in controlling *B. cinerea*, *Penicillium expansum* and *Rhizopus stolonifer* on apples and pears (Nunes et al., 2007). *Pseudomonas fluorescens* was tested as a biological control of *Penicillium expansum* and *P. solitum* and found to reduce blue mould in apples (Etebarian et al., 2005). However, none of these treatments is yet in commercial application, and the food safety aspects of these strategies still need to be fully investigated. For a review of biological control of postharvest diseases in fruits, see Janisiewicz and Korsten (2002).

11.2.3 Stone Fruits

Stone fruits (peaches, plums, apricots, nectarines and cherries) are all susceptible to brown rot caused by *Monilia fructicola* and in some places the closely related species *M. fructigena* (Pers.) Pers. and *M. laxa* (Ehrenb.) Sacc. and Voglino (Snowdon, 1990). These species are also commonly known by their teleomorph names *Monilinia fructicola* (G. Winter) Honey [synonym *Sclerotinia fructicola* (G. Winter) Honey], *Monilinia fructigena* (Aderh. and Rühle) Honey and *Monilinia laxa* (Aderh. and Rühle) Honey, but the *Monilia* state is the only one occurring on commercial fruit or in Petri dish culture. Brown rot is the most important market disease of apricots, peaches and nectarines. Early symptoms of this rot are water-soaked spots on the fruit, which within 24 h become brown, enlarging and deepening rapidly, then producing a dusting of pale brown conidia. The whole fruit may rot in 3–4 days (Hall and Scott, 1977). Infection commences in the orchard. Rigorous preharvest spray programmes with benomyl or similar benzimidazole fungicides are necessary to achieve control, but postharvest dips with dichloran, iprodione or triforine may assist (Fourie and Holz, 1987; Sharma et al., 1989; Adaskaveg and Ogawa, 1994). Storage temperatures below 5°C are recommended.

A second major rot in all kinds of stone fruits is transit rot, so named because it usually develops in boxed fruit during transport. It is caused by *Rhizopus stolonifer* (synonym *R. nigricans*) and *R. oryzae* (Heaton, 1980; Snowdon, 1990). Soft rot commences in a single fruit, which then becomes surrounded by a coarse, loose "nest" of mycelium. Growth spreads rapidly, engulfing several fruit adjacent to the originally infected one, and sometimes all the fruit in a box, in only 2–3 days. This characteristic growth form and sporangia which are white when young but darken as they mature are diagnostic. *Mucor* species, particularly *Mucor piriformis*, but also *M. racemosus* and *M. plumbeus*, can also cause postharvest rots in stone fruits (Sholberg, 1990; Michailides, 1991).

Dichloran is an effective fungicide against *Rhizopus* (Sharma et al., 1989). A combined benomyl and dichloran preharvest spray programme for the control of both *Monilia* and *Rhizopus* is recommended for peaches, apricots and nectarines (Hall and Scott, 1977; Ryall and Pentzer, 1982). Wade and Gipps (1971) reported almost complete control of *Monilia* and greatly reduced losses from *Rhizopus* in fruit dipped in a mixture of benomyl and dichloran.

Penicillium expansum causes blue mould rot in cherries and plums, but is uncommon in the other types of stone fruits (Ryall and Pentzer, 1982). *Alternaria* spp. and *Botrytis cinerea* can cause spoilage of stone fruits (Splittstoesser, 1987; Fourie and Holz, 1985, 1995), but are generally of lesser economic importance. Infection of peaches with *Absidia corymbifera* and *Botryosphaeria ribis* H.L. Gross and Duggar has been reported from India (Singh

and Prashar, 1988). Alternative postharvest measures to control brown rot and blue mould in stone fruits include exposure to ozone (0.3 ppm, v/v; Palou et al., 2002), hot water brushing (Karabulut et al., 2002) and biological control (*Cryptococcus laurentii*) in combination with a salicylic acid spray (Yu et al., 2007).

11.2.4 Tomatoes and other Solanaceous Fruit

Tomatoes, with an internal pH of 4.2–4.5, and other solanaceous fruit (capsicums and eggplant) can be affected by both fungal and bacterial diseases. Several of those produced by fungi are important.

All solanaceous fruit are susceptible to rots caused by *Alternaria alternata*. Alternaria rots appear as dark brown to black, smooth, only slightly sunken lesions, which are of firm texture and can become several centimetres in diameter. Infection can occur at the stem end of the fruit, or through mechanical injury, cracking from excessive moisture during growth, or chilling (Snowdon, 1991). *Alternaria solani* causes early blight rot in tomatoes (Snowdon, 1991) but not in other solanaceous fruit.

Alternaria rots develop at all acceptable handling temperatures and can be avoided only by rapid marketing. As several other diseases have a similar appearance, diagnosis is best carried out by culturing on a medium such as DCMA.

Chilling injury allows the entry of other fungi also. Cladosporium rot caused by *Cladosporium herbarum* and grey mould rot due to *Botrytis cinerea* can both be potentiated by chilling injury. *B. cinerea* can also affect mechanically damaged green fruit, on which it forms "ghost spots", small whitish rings, often with darker centres. Rots can spread rapidly at higher temperatures during packing and transport (Ryall and Lipton, 1979). Diagnosis of both types of rot is best done by culturing the fungus.

Rhizopus species appear to be able to attack almost any kind of fruit or vegetable, and tomato is no exception. In severe cases of Rhizopus rot, and there seem to be no mild ones, the fruit resembles "a red, water-filled balloon" (Ryall and Lipton, 1979). When the fruit collapses, grey mycelium, a fermented odour and white to black spore masses become visible. The disease starts in cracked or injured fruit but may spread by contact thereafter. Mucor rot of tomato caused by *Mucor hiemalis* (Sonoda et al., 1981) and of tomato, capsicum and eggplant caused by *M. mucedo* (L.) Bref. (Reyes, 1990) has been reported.

Sour rot in tomatoes is caused by *Geotrichum candidum*. Lesions are a light greenish grey and may extend as a sector from end to end of the fruit. Tissue remains firm at first, but later weakens and emits a sour odour. White mycelium may become visible and in wet mounts can be seen to consist mainly of arthroconidia, with their characteristic microscopic appearance. This disease also invades only damaged or cracked fruit and is disseminated by *Drosophila* flies (Ryall and Lipton, 1979).

Tomatoes grown without stakes or trellises can develop soil rot caused by *Rhizoctonia solani* J.G. Kahn. Small brown spots of this disease develop concentric rings when they become 5 mm or more in diameter (Snowdon, 1991). Injury is not necessary for the development of this rot, but soil contact is.

Other postharvest pathogens reported from solanaceous fruits include *Fusarium* species, *F. oxysporum* (Onwuzulu et al., 1995), *F. semitectum, F. acuminatum* (Kusum, 1992), *F. avenaceum* and *F. equiseti* (Snowdon, 1991), *Trichothecium roseum* (Takahashi et al., 1995), *Sclerotinia, Phytophthora, Pythium,* and *Diaporthe* species (Ryall and Lipton, 1979; Snowdon, 1991).

11.2.5 Melons and other Cucurbits

Water melons sometimes develop anthracnose from *Colletotrichum lagenarium* (Pass.) Ellis and Halst. This disease forms circular or elongate welts which are initially dark green and later become brown, disfiguring the melon surface. Pink *Colletotrichum* conidia may be produced in acervuli if humidities remain high. Rock melons (cantaloupes) may be affected by several diseases, the most important being Alternaria rot due to *Alternaria alternata*. Mould invasion usually takes place at the stem scar, producing dark brown to black lesions and eventually invading the flesh, forming firm, adherent areas. Diagnosis can be made from microscopic examination of cultures on DCMA.

Cladosporium species can also invade melons through the stem scar, forming a rot similar to

that caused by *Alternaria*. In both cases prompt shipping and cool storage will limit the losses from these diseases.

Several *Fusarium* species, particularly *F. oxysporum, F. solani, F. semitectum* and *F. culmorum*, can invade melons, especially when storage temperatures are high or storage periods become excessive. *F. acuminatum* has been reported to cause rot in pumpkins (Elmer, 1996). *Penicillium* species may also occasionally cause problems under these conditions (Ryall and Lipton, 1979; Hawthorn, 1988; Snowdon, 1991). On muskmelons and watermelons, pink rot caused by *Trichothecium roseum* has been reported from Japan, United States, South America, India and United Kingdom (Snowdon, 1991; Takahashi et al., 1995). Silicon oxide and sodium silicate significantly reduced the severity of pink rot in rock melons (Guo et al., 2007). Infection with *Myrothecium roridum* Tode: Fr. occurs on various melons and cucumbers in United States, Mexico and India (Snowdon, 1991; Kuti and Boehm, 1994). *Mucor mucedo* and *Botrytis cinerea* can cause rots of stored cucumbers (Reyes, 1990).

Other diseases of cucurbits are caused by *Macrophomina phaseolina* (charcoal rot), *Geotrichum candidum* (sour rot), *Epicoccum nigrum* (red rot), *Pythium* spp. (cottony leak of cucumbers and melons), *Verticillium dahliae* Pleb. (cavity rot of wintermelons), *Phytophthora* spp., *Rhizopus* spp., *Sclerotium rolfsii* Sacc. and *Rhizoctonia solani* (soil rot) (Wade and Morris, 1982; Snowdon, 1991; Gubler and Bernhardt, 1992; Bruton et al., 1993; Smith et al., 1993).

11.2.6 Grapes

Botrytis cinerea is regarded as the highly desirable "noble rot" in certain wine grapes (Coley-Smith et al., 1980), but it is by far the most serious cause of spoilage in table grapes (Ryall and Pentzer, 1982; Nair et al., 1987; Snowdon, 1990). In the early stages of invasion, the fungus develops on stems and inside the berry; later growth erupts at the surface and produces grey conidia. Growth then expands rapidly through tight bunches where humidity is high, and large "nests" of rot may develop rapidly. Under the microscope the characteristic conidiophores of *Botrytis* are readily seen.

Control involves the use of preharvest sprays with benomyl and rapid transfer of fruit to cool stores after picking. Postharvest treatments with sulphur dioxide or benomyl are also effective (Hall and Scott, 1977). Esterio et al. (2007) report the first incidence of fenhexamid resistance in *Botrytis cinerea* in Chilean grapes, prompting new research into alternative control measures. Recent alternatives to control postharvest infection of table grapes include the use of natural antimicrobial compounds such as carvacrol (Martínez-Romero et al., 2007) and ozone (Tzortzaki et al., 2007; for a review on the application of ozone to postharvest fruit and vegetable processing, see Karaca and Velioglu, 2007). Spray application of calcium chloride, sodium bicarbonate or sodium carbonate (1%) 90 and 30 days before harvest significantly reduced postharvest grey mould (Nigro et al., 2006).

Yeasts are a major component of the natural flora on the surface of fresh grapes. The apiculate yeast *Kloeckera apiculata* (teleomorph *Hanseniaspora uvarum*) is usually the dominant species, comprising 50–75% of isolations. *Metschnikowia pulcherrima*, *Candida stellata* and various species of *Cryptococcus, Rhodotorula, Pichia* and *Kluyveromyces* also are common. Fermentative species such as *Saccharomyces cerevisiae* are usually present in low numbers (Fleet and Heard, 1992; Fleet et al., 2002; Fleet, 2003b).

Penicillia do not usually attack grapes before harvest, but are prevalent in stored grapes, with *Penicillium expansum* the most commonly encountered species (Hall and Scott, 1977; Ryall and Pentzer, 1982; Snowdon, 1990). *P. aurantiogriseum, P. brevicompactum, P. chrysogenum, P. citrinum, P. crustosum, P. decumbens* and *P. glabrum* have also been isolated from spoiled grapes in Israel, Morocco and Italy (Barkai-Golan, 1974; Benkhemmar et al., 1993; Pollastro et al., 2005). As with *Botrytis,* postharvest control relies on treatments with benomyl or sulphur dioxide.

Black *Aspergillus* species such as *Aspergillus niger* can cause Aspergillus bunch rot in grapes, particularly in warmer climates (Barkai-Golan, 1980; Nair, 1985; Snowdon, 1990; Pollastro et al., 2005; Leong et al., 2006a; Hocking et al., 2007). *Aspergillus carbonarius* also occurs on grapes and is the primary producer of ochratoxin A (Leong et al., 2006a). Other diseases of grapes include

Alternaria alternata rot in cold stored grapes in South Africa (Swart and Holz, 1994), black rot due to *Guignardia bidwellii* (Ellis) Viala and Ravaz in Europe and eastern United States, Cladosporium rot in some US varieties, and Rhizopus rot in market fruit stored at elevated temperatures (Barbetti, 1980; Snowdon, 1990).

11.2.7 Berries

Because of their shape and proximity to the ground during growth, berries are readily contaminated with soil and fungal spores. They are also readily damaged during picking and handling and are vulnerable to fungal invasion. Most kinds of berries have similar susceptibilities to disease fungi and can be considered as a group.

The principal fungal rots in most berry crops are caused by *Botrytis cinerea, Rhizopus stolonifer* and *Mucor piriformis* (Snowdon, 1990; Tournas and Katsoudas, 2005). *Botrytis* causes soft rots in cane berries such as raspberries and loganberries, but a firm, dry rot in strawberries. In both cases the fruit become covered with a growth of grey mould. Losses in strawberries can be high as the fungus spreads by contact and forms "nests" of rotting fruit. Preharvest spraying programmes are important for control, as is refrigerated storage. Postharvest antifungal treatments are of little benefit; however, Park et al. (2005b) reported that chitosan (2%) based coatings supplemented with potassium sorbate proved useful in reducing *Cladosporium* sp. and *Rhizopus* sp. infection on strawberries.

Rhizopus stolonifer and *Mucor piriformis* cause a large proportion of marketing losses on all berry fruits. Rotting fruit collapse completely, exuding juice, and at favourable temperatures (above 20°C) the fungi spread rapidly. Lower temperatures reduce growth markedly, so control is mainly based on low temperature storage and handling (Ryall and Pentzer, 1982; Snowdon, 1990).

Strawberries can also be invaded by *Rhizoctonia solani,* which causes a dry, spongy, black rot, and by *Phytophthora cactorum* (Lebert and Cohn) J. Schröt., which causes dry, tough "leather rot". Overripe or damaged berries can be invaded by *Penicillium* and *Cladosporium* species. Greenhouse grown strawberries are susceptible to anthracnose caused by *Colletotrichum acutatum* J.H. Simmonds (Smith and Gupton, 1993). Various other field disorders occur in berries, but they are usually not of major significance (Ryall and Pentzer, 1982; Snowdon, 1990).

Yeasts are normal colonisers of strawberries, being present at up to 10^5/g in macerates of mature berries (Buhagiar and Barnett, 1971; Fleet, 2003a). A wide variety of yeast species were isolated by these authors, and although spoilage of strawberries by yeasts is uncommon (Dennis, 1983), it can occur in tissues damaged by modified atmosphere storage (Snowdon, 1990). Lowings (1956) reported spoilage of English strawberries by *Kloeckera apiculata*.

Botrytis cinerea is the major postharvest pathogen of kiwifruit, causing stem end rots. The presence of this disease may predispose the fruit to invasion by other fungi such as *Botryosphaeria, Fusicoccum, Diaporthe, Fusarium, Phoma, Glomerella, Colletotrichum* and *Cryptosporiopsis* (Brook, 1992). *Phialophora* species have been reported causing lesions on and below the skin of stored kiwifruit in Italy (Marchi et al., 1994).

11.2.8 Figs

There are few fungal diseases of figs. However, smut caused by *Aspergillus niger* and endosepsis caused by growth and sporulation of *Fusarium verticillioides* in Calimyrna figs (Michailides and Morgan, 1998) have been investigated with a view to using *Paecilomyces lilacinus* as a biocontrol agent (Subbarao et al., 1993). Doster and Michailides (2007) reported high incidences of *A. niger*, *Alternaria alternata* and *Ulocladium atrum* in two cultivars of Californian figs. Other reported diseases of figs are due to *A. alternata, Botrytis cinerea, Cladosporium, Penicillium* and *Rhizopus* species (Snowdon, 1990); however, Simmons (2007) considers that *Alternaria* diseases in figs are due to *A. fici* and *A. ficini.*

Miller and Phaff (1962) documented the invasion of Smyrna figs by yeasts. This type of fig is pollinated by the fig wasp which introduces the yeast *Candida guilliermondii* and a bacterium, *Serratia* species. These microorganisms do not cause spoilage, but at maturity attract *Drosophila* flies which

carry spoilage yeasts. The spoilage yeasts are *Hanseniaspora* and *Kloeckera* species and *Torulopsis stellata* (Kroemer and Krombh.) Dodder, which produce "souring" of the figs by acid production.

11.2.9 Tropical Fruit

Fruit from tropical areas are susceptible to an array of diseases quite different from those grown in subtropical or temperate climates. Study of such diseases is still a developing science with many pressing problems, not the least being that tropical fruit are injured by low temperatures, so disease control cannot be assisted by refrigeration.

Bananas are the most important tropical fruits in international trade. Most postharvest diseases of bananas are due to fungal rots in the stalks and crowns, less commonly on the sides of the fruit (Eckert et al., 1975; Snowdon, 1990). A comprehensive study of bananas shipped from the Windward Islands to England (Wallbridge, 1981) showed that nearly 20 fungal species can cause crown rots. The most important were *Colletotrichum musae* (Berk. and M.A. Curtis) Arx (synonym *Gloeosporium musarum* Cooke and Massee) and *Fusarium semitectum,* with several other *Fusarium* species also significant. *Verticillium theobromae* (Turconi) E.W. Mason and S. Hughes, *Lasiodiplodia theobromae, Phomopsis musae* S. Joly*, Alternaria alternata* and *Nigrospora spherica* are less common (Wallbridge, 1981; Wade et al., 1993). Other studies have reported *Fusarium verticillioides, F. subglutinans, F. solani, F. oxysporum* and *F. proliferatum* to be important in postharvest disease of bananas (Chakrabarti et al., 1977; Dharam, 1977; Jiménez et al., 1993; Wade et al., 1993; Vesonder et al., 1995). A recent survey of Brazil bananas in Brazil identified *C. musae*, *Trichoderma harzarium*, *Fusarium equiseti, Penicillium* sp., *Aspergillus parasiticus*, *Trichothecium roseum*, *Colletotrichum acutatum*, *Alternaria* sp., *Cladosporium musae* and *Curvularia lunata* as spoilage agents (Moraes et al., 2006). Benomyl, thiabendazole and prochloraz, chlorine and hot water have all been quite successful in controlling banana rots (Eckert et al., 1975; Wade et al., 1993).

Anthracnose and stem end rots are the most destructive postharvest diseases of avocados and mangoes. Anthracnose is manifested as brown or black spots on the skin which at best reduce crop value and may eventually destroy the fruit. Anthracnose is usually caused by *Colletotrichum gloeosporioides* (often referred to as *Gloeosporium* in the literature), *C. acutatum*, *Botryosphaeria parva*, *B. dothidea* and *Phomopsis* sp. (Everett and Timudo-Torrevilla, 2007) and stem end rots by *Lasiodiplodia theobromae* and *Dothiorella* species (Snowdon, 1990; Johnson et al., 1991). *C. gloeosporioides* can also cause pepper spot of avocados (Willingham et al., 2000). The use of boscalid/pyraclostrobin and dithianon fungicides proved effective in controlling spore germination and may be an alternative to copper sprays (Everett and Timundo-Torrevilla, 2007). Mangoes are also susceptible to black spot disease, the agent of which is *Alternaria alternata* (Johnson et al., 1990; Prusky et al., 1993). Control may rely on benomyl, prochloraz or a variety of other fungicides. Hot water dips with or without fungicides for 2–5 min at 52–55°C have been quite effective in control of anthracnose and stem end rots in mangoes (Smoot and Segall, 1963; Johnson et al., 1990) and other fruit. The postharvest quality of mangoes was improved by UV C treatment (González-Aguilar et al., 2007).

Papayas (paw-paws) are affected by the same types of anthracnose and stem end rots as mangoes, by black rot caused by *Mycosphaerella caricae* Syd. and P. Syd., Phytophthora rots, Fusarium rots caused by *Fusarium solani, F. equiseti* and *F. oxysporum* and a variety of other rots (Simmonds, 1965; Quimio et al., 1975; Snowdon, 1990; Persley and Ploetz, 2003).

Pineapples are susceptible to a number of diseases of fungal origin, including core rots (black rot) caused by *Ceratocystis paradoxa,* fruitlet core rots (*Penicillium funiculosum*) and skin blemishes such as leathery spot caused by *Penicillium purpurogenum* and *Fusarium moniliforme* or *F. verticillioides* (Lim, 1983; Mourichon, 1983; Snowdon, 1990; Damayanti et al., 1992; Rohrbach and Schmitt, 2003). Postharvest control of black rot may be achieved by a hot (54°C) water dip for 3 min (Wijeratnam et al., 2005) or by biological antagonism using *Pichia guilliermondii* (Reyes et al., 2004).

In passionfruit, *Alternaria passiflorae* H.J. Simmonds causes brown spot (Inch, 1978; Fullerton, 1982), and a number of other fungi are associated

with more generalised rots (Snowdon, 1990; Manicom et al., 2003). In guava fruit, the most significant postharvest pathogens are *Rhizopus oryzae, Lasiodiplodia theobromae* and *Colletotrichum acutatum* (Snowdon, 1990; Mukta and Bora, 1993; Patel and Pathak, 1993).

11.3 Vegetables

As noted earlier, the near neutral pH of vegetables increases their susceptibility to bacterial invasion and reduces the dominant role of fungal pathogens to near equality. However, vegetables are susceptible to attack from a wide range of fungi, and these have been extensively documented and illustrated by Snowdon (1991). Bacterial rots are usually distinguishable from those of fungal origin by a watery or slimy appearance, lack of visible mycelium and disagreeable odour. Wet mounts stained with lactofuchsin can be a useful aid.

11.3.1 Peas

The most common fungal rot of peas is caused by *Botrytis cinerea*. Water soaked spots enlarge and develop grey mycelium and spores (Ryall and Lipton, 1979; Snowdon, 1991). Peas are also susceptible to a number of other postharvest diseases including anthracnose (*Colletotrichum*), Ascochyta pod spot (*Ascochyta pisi* Lib.) and Alternaria blight caused by *Alternaria alternata* (Snowdon, 1991). Control is by refrigerated storage.

11.3.2 Beans

Beans are susceptible to several pathogens, the most important being anthracnose due to *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and Cavara, "cottony leak" caused by *Pythium butleri* Subram., and "soil rot" (small rusty brown lesions) from *Rhizoctonia solani* (Snowdon, 1991). Beans are also susceptible to *Botrytis cinerea* (Reyes, 1990). Careful sorting, rapid cooling and low temperature transport provide control.

11.3.3 Onions and Garlic

As a hypogean vegetable, the onion is enveloped in fungi during growth and maturation. However, onions are highly resistant to invasion, and some diseases only develop after harvest. Few *Aspergillus* species cause plant diseases, but *A. niger* is a well known pathogen of onions, producing unsightly deposits of black conidia between the outer scales. Lesions may also be produced (Ryall and Lipton, 1979; Morris and Ward, 1986; Snowdon, 1991). *Aspergillus alliaceus* Thom and Church (teleomorph *Petromyces alliaceus* Malloch and Cain) has also been reported to be capable of causing rots in onions (Filtenborg et al., 1996). Various species of *Fusarium* including *F. solani* and *F. oxysporum* and *Botrytis* (*B. allii* and *B. cinerea*) may also invade in the field and develop in storage (Hussein et al., 1977; Dang and Singh, 1982). A number of *Penicillium* species have been reported to cause blue rot of onions around the world (Snowdon, 1991). *P. glabrum* causes spoilage of Australian onions (our unpublished observations) and has been reported elsewhere (Filtenborg et al., 1996). A recent survey of fungal pathogens of onions in Puerto Rico found that *Aspergillus niger*, *Phoma sorghina*, *Penicillium purpurogenum* and *Sclerotium rolfsii* were pathogenic to mature bulbs (Velez-Rodrigez and Rivera-Vargas, 2007). Prompt drying and curing, reduced temperatures and humidities are the most effective control for onion diseases (Ryall and Lipton, 1979; Snowdon, 1991).

Garlic bulbs are susceptible to *Fusarium, Penicillium* and *Botrytis* rots, particularly if bulbs are damaged (Böttcher and Pohle, 1993). Many postharvest pathogens of garlic may be carried in seed cloves (Dugan et al., 2007). *Fusarium* species reported include *F. oxysporum, F. verticillioides, F. solani* and *F. camptoceras* Wollenw. and Reinking (Roy et al., 1977; Rath and Mohanty, 1986; Gargi and Roy, 1988; Koch and Taanami, 1995; Mahmoody, 1998; Dugan et al., 2007), *F. proliferatum* and *F. acuminatum* in our laboratory. *F. proliferatum* was the most common pathogen of onions and garlic in Serbia (Stankovic et al., 2007). *Aspergillus niger* and *A. ochraceus* have also been isolated from garlic cloves (Dugan et al., 2007). *Penicillium allii* causes rots in garlic (Vincent and Pitt, 1989; Bertolini and Tian, 1996; Dugan et al., 2007, as *P. hirsutum*) and has recently been reported as a field pathogen also (Valdez et al., 2006).

11.3.4 Potatoes

Potatoes are mostly affected by bacterial rots. Fungal diseases are usually caused by *Fusarium* species, particularly *F. solani* var. *coeruleum* and *F. sulphureum* Schltdl. (responsible for dry rot). Potatoes are also susceptible to diseases such as silver scurf (*Helminthosporium solani* Durieu and Mont.) and skin spot [*Polyscytalum pustulans* (M.N. Owen and Wakef.) M.B. Ellis]. *Phytophthora, Pythium, Phoma* and a number of other genera can also cause diseases (Hide et al., 1994). Infection often occurs through wounds: lesions are brown and tissues shrink and become wrinkled as the decay progresses (Snowdon, 1991). Control relies on careful handling and sorting, surface drying and refrigerated storage. A hot water treatment of 60°C was effective in controlling postharvest pathogens such as *Fusarium oxysporum*, *Botryodiplodia theobromae* and *Rhizopus oryzae* (Salami and Popoola, 2007).

11.3.5 Roots and Tubers

Carrots may be invaded by *Stemphylium radicinum* (Meier et al.) Neerg., by *Rhizopus* species, by *Botrytis cinerea* and by *Sclerotinia sclerotiorum* (Lib.) de Bary (Ryall and Lipton, 1979; Snowdon, 1991). Root rot caused by *Fusarium avenaceum* and *F. solani* and sour rot (*Geotrichum candidum*) have also been described (El-Tobshy et al., 1979; Marziano et al., 1992; Arjona et al., 1996). None of these diseases causes large commercial losses as a rule.

Sweet potatoes are susceptible to several severe diseases, one of the most serious being black rot caused by *Ceratocystis fimbriata* Ellis and Halst. *Ceratocystis* is an ascomycete genus forming perithecia with long necks and long narrow ascospores. It invades sweet potatoes in the field, but causes losses only after storage. Lesions start as small, round spots which may enlarge to 20–50 mm in diameter. Perithecia may often be seen at this stage. Chemical treatments are ineffective, but heat treatments may reduce the severity of this rot with little effect on the tubers (Snowdon, 1991).

Macrophomina phaseolina (Tassi) Goid., *Diaporthe batatis* Harter and E.C. Field and *Lasiodiplodia theobromae* [synonym *Diplodia tubericola* (Ellis and Everh.) Taubenh.] all cause firm or dry, brown to black rots of sweet potatoes (Snowdon, 1991; Tournas, 2005). In contrast, *Rhizopus stolonifer* and *R. oryzae* produce a soft, watery rot with little colour change. Under moist conditions, the characteristic coarse mycelium of these fungi envelopes the decaying tubers. This serious disease affects sweet potatoes in most areas of the world (Ryall and Lipton, 1979; Snowdon, 1991; Clark, 1992). Control relies on careful handling and culling of damaged tubers. Other diseases include Java black rot (*L. theobromae*) and Fusarium rots (*F. solani, F. oxysporum, F. semitectum*) (Clark, 1980, 1992; Snowdon, 1991; Ray et al., 1994; Ray and Misra, 1995; Ray and Ravi, 2005).

Ginger is affected mainly by Fusarium rot caused by various species especially *F. oxysporum* (Teakle, 1965). *Pythium* (Snowdon, 1991) and *Sclerotium rolfsii* (Pegg et al., 1974) also cause postharvest diseases of ginger.

11.3.6 Yams

Yams are a very important crop in many parts of Africa. Decay of yams in storage has been intensively studied in Nigeria, where losses may be as high as 10% of the crop (Ogundana, 1972). The principal fungi causing decay in yams are *Lasiodiplodia theobromae, Fusarium verticillioides, Penicillium sclerotigenum* W. Yamam., and *Aspergillus niger* (Adeniji, 1970a; Ogundana et al., 1970; Obi and Moneke, 1986; Snowdon, 1991; Aboagye-Nuamah et al., 2005). A number of other fungi, including other *Penicillium* species and *Rhizopus stolonifer,* occur as secondary invaders (Snowdon, 1991). Benomyl and thiabendazole are the most effective fungicides for prevention of storage rots of yams (Ogundana, 1972; Ogundana and Dennis, 1981). Reducing insect damage in storage barns may also be an effective way of minimising postharvest fungal attacks (Nweke and Enujeke, 2006).

11.3.7 Cassava

Cassava is an important staple food in many parts of three continents: Africa, South America and Asia. The two major postharvest spoilage rots of

cassava are caused by *Lasiodiplodia theobromae* and *Fusarium solani* (Snowdon, 1991). *Rhizopus* (*R. oryzae* and *R. stolonifer*) and *Aspergillus* species are also important (Clerk and Caurie, 1968; Ray et al., 1990; Snowdon, 1991; Obadina et al., 2007).

11.3.8 Leafy and other Green Vegetables

The most generally damaging postharvest fungal diseases of leafy vegetables such as lettuces, celery and fennel are caused by *Botrytis cinerea, Rhizopus stolonifer, Rhizoctonia solani* and *Alternaria* species. Overall, *Botrytis* is the most destructive fungal pathogen on these vegetables. It is readily recognised microscopically once sporulation commences. Control is difficult, low temperature storage being recommended (Ryall and Lipton, 1979; Snowdon, 1991; Tournas, 2005). Storage under modified CO_2/O_2 atmosphere may also retard fungal growth at low temperatures (Tournas, 2005).

Cabbages and broccoli may be attacked by *Botrytis cinerea, Alternaria* species, including *A. alternata* and *A. brassicicola,* which cause dark spots, and also *Phytophthora* and *Fusarium* species during cool storage (Mercier et al., 1991; Snowdon, 1991; Leifert et al., 1993; Heimann, 1994; Tournas, 2005).

Stored asparagus spears are susceptible to rots of the bracts caused by *Fusarium verticillioides, Phytophthora* rots predominantly at the base of the spears and basal zone rots caused by *Penicillium hirsutum* (Snowdon, 1991; Montealegre and Palma, 1994). Elmer (2001) reported *Fusarium oxysporum* and *F. proliferatum* causing spear rots in asparagus. *Geotrichum candidum* and *Botrytis cinerea* can also cause damage in stored asparagus (Tournas, 2005).

11.4 Dairy Foods

Fresh milk, a liquid of neutral pH, is highly susceptible to bacterial spoilage, and hence fungi are rarely a problem. In milk processed to cream, cottage cheese or butter, the growth of lactic acid bacteria will cause the pH to fall, favouring the growth of spoilage yeasts. Yeasts may cause gas and off-flavour production in cream and cottage cheese and rancidity or other flavour defects in butter (Walker and Ayres, 1970; Frölich-Wyder, 2003). *Geotrichum candidum* can also cause spoilage of cream (Marth, 1978; Craven et al., 2001) as a result of unclean machinery on farms. We have isolated several *Penicillium* species from spoiled sour cream, including *P. glabrum, P. commune* and *P. chrysogenum*. *Penicillium* species, particularly *P. chrysogenum* and *P. glabrum,* as well as *Aspergillus sydowii*, have also been isolated in our laboratory from spoiled dairy desserts containing light sour cream and yoghurt

Fungal spoilage of UHT dairy products sometimes occurs, usually as a result of post-processing contamination, for example with *Geotrichum candidum*. We have isolated *Fusarium oxysporum* on several occasions from UHT flavoured milk drinks. *F. oxysporum* is not recognised as being heat resistant, although it does form thick walled chlamydoconidia. Its ability to grow at very low oxygen tensions enables this species to cause fermentative spoilage.

Heat resistant fungi rarely cause spoilage in UHT dairy products, but as milk can be contaminated with soil, such occurrences are not unknown. We have seen *Talaromyces avellaneus* (Thom and Turesson) C.R. Benj. causing spoilage of a UHT custard product and *Talaromyces macrosporus* in strawberry yogurt. We have isolated heat resistant fungi on three separate occasions from cream cheese. The species involved were *Byssochlamys nivea, T. avellaneus, Neosartorya fischeri* var. *spinosa* and *Eupenicillium brefeldianum*. *B. nivea, T. macrosporus* and *N. fischeri* have also been isolated from heat treated dairy beverages made from reconstituted powdered ingredients.

Yeasts are very common in yoghurts and can sometimes cause spoilage, particularly in products containing fruit. Species of *Candida,* especially *C. parapsilosis, C. famata* (anamorph of *D. hansenii*) and *C. diffluens* Ruinen, *Rhodotorula* species, particularly *R. mucilaginosa* (A. Jörg.) F.C. Harrison, *Kluyveromyces marxianus* (E.C. Hansen) van der Walt and *Yarrowia lipolytica* (Wick. et al.) van der Walt and Arx, are most common (Suriyarachchi and Fleet, 1981; Fleet and Mian, 1987; Green and Ibe, 1987; Rohm et al., 1990; Fleet, 1990; Rohm et al., 1992; Frölich-Wyder, 2003; Viljoen et al., 2003b). We have repeatedly isolated *Pichia anomala* from fermenting yoghurts containing fruit or

flavouring syrups. The characteristics which enable yeasts to cause spoilage in yoghurt are (1) growth at low temperatures (<10°C); (2) production of proteolytic and lipolytic enzymes to hydrolyse milk proteins and fats; (3) ability to ferment or utilise lactose and sucrose, the main sugars of plain and flavoured yoghurts; and (4) ability to assimilate lactic and citric acids which are the main organic acids in yoghurt (Fleet and Mian, 1987). Yoghurts containing fruit can also be spoiled by fungi introduced with the fruit preparation. We have seen fermentative spoilage of fruit flavoured yoghurt caused by *Mucor circinelloides* which grows strongly at refrigeration temperatures and can grow under extremely low oxygen tensions. *Mucor* species (*M. racemosus* and *M. hiemalis*) and *Penicillium aurantiogriseum* have also been reported from spoiled yoghurt in Italy (Foschino et al., 1993).

Solid perishable dairy foods and substitutes such as butter and margarine are susceptible to the growth of spoilage fungi. Muys et al. (1966b) studied the fungal flora of margarine and concluded that *Geotrichum candidum, Moniliella suaveolens* (synonym *Cladosporium suaveolens*), *Cladosporium herbarum* and the yeast *Yarrowia lipolytica* could cause spoilage by their lipolytic action. As few as 500 cells of *Y. lipolytica* may produce perceptible off-flavours. *Cladosporium butyri* (probably *M. suaveolens*) was a particularly undesirable contaminant in milk, cream, butter or margarine, because it caused rancidity as a result of the production of ketones, detectable in very low concentrations. Muys et al. (1966b) also outlined specific methods for the detection of undesirable fungi in butter and margarine.

Low salt margarines are more susceptible to fungal spoilage than those containing the normal amount of salt. In Australia, we have observed that the most common spoilage fungi are *Penicillium* species, particularly *P. glabrum, P. expansum* and *P. chrysogenum* and *Cladosporium* species, with *C. cladosporioides* by far the most common (Hocking, 1994). As well as being lipolytic, these fungi can cause spoilage due to off-flavours, particularly earthy taints from the production of 2-methylisoborneol and geosmin.

11.5 Meats

"There has been little study during the last 50 years of mould spoilage of meats, although it is still of importance … moulds … only spoil meat if the spoilage conditions prevent bacterial growth, but there are few firm data on the time and temperature requirements for visible mould growth to develop …". This quotation from Gill and Lowry (1982) summed up the situation then and little has changed since. Moulds only compete with bacteria on meat when storage temperatures are lowered to 0°C or below, or when the meat surface dries to an a_w that enables fungi to compete. In earlier literature, spoilage of chilled or frozen meat by fungi was usually attributed to Mucorales, especially *Thamnidium elegans* and *Mucor* species which grew as "whiskers" on cold stored meat (Brooks and Hansford, 1923; Empey and Scott, 1939). Campano et al. (1985) reported that the most commonly isolated fungi from beef aging at refrigeration temperature were *Thamnidium elegans, Mucor mucedo* and *Helicostylum pulchrum* (Preuss) Fidopl. and Milko (synonym *Chaetostylum fresenii* Tiegh. and G. Le Monn.). Hadlok and Schipper (1974) reported very infrequent isolation of Mucorales from meat and questioned their significance, but it seems more probable that techniques for meat storage have changed rather than that the prewar meat technologists were wrong.

Michener and Elliott (1964) cited several reports of bacteria and fungi growing on meats at –5°C, with yeasts and moulds predominating as temperatures were further lowered, to a limit at about –12°C. Schmidt-Lorenz and Gutschmidt (1969) reported that moulds and yeasts grew on chickens stored at –7.5 and –10 ± 0.2°C for 1 year. Spoilage of chilled meats in postwar years has principally been the result of "black spot", traditionally believed to be due to *Cladosporium herbarum* (Brooks and Hansford, 1923). Gill et al. (1981) cultured such black spots and identified *Cladosporium cladosporioides, Penicillium hirsutum* and *Aureobasidium pullulans* as well as *C. herbarum*. All were capable of producing black spots on meat at –1°C. Gill and Lowry (1982) showed that C. *herbarum* would take 4 months to produce a visible colony 1 mm in diameter at –5°C and concluded that this temperature was near the practical limit for spoilage of meat by black spot fungi.

Yeasts occur in low numbers on freshly slaughtered cuts of red and poultry meats, but can proliferate in minced or ground meats. Yeasts may reach 10^6 to 10^7 cells per gram, but effects are insignificant compared with those of bacteria. The yeasts most frequently isolated from comminuted meats are *Candida zeylanoides* (Castell.) Langeron and Guerra, *C. famata*

(*D. hansenii*), *C. sake* (Saito and M. Ota) Uden and H.R. Buckley, *Yarrowia lipolytica* and *Cryptococcus laurentii* (Kuff.) C.E. Skinner (Fleet, 1992). Dipping fresh turkey breast fillets in 0.5% fumaric acid solution or 5% sodium tripolyphosphate solution reduced yeast counts immediately by 0.4 and 0.7 log units, respectively, and extended shelf life by 4 days (Baysal and Unluturk, 2007).

11.6 Cereals, Nuts and Oilseeds

The fungi growing on crops which will subsequently be dried have been divided traditionally into "field" and "storage" fungi. Although this distinction has become blurred in recent years with the discovery that certain species, especially *Aspergillus flavus,* are equally at home in both situations, it is still a useful concept.

Field fungi are plant pathogens, which invade the growing seed or nut before harvest. Deterioration or spoilage of a particular crop usually results from invasion by a specific fungus, because climatic conditions, plant variety or agricultural practice produce circumstances where invasion by that species can occur on a large scale. Control, if it exists, is by refinements in agricultural practice. Field fungi rarely play a significant role in further deterioration of the crop postharvest. Here storage fungi become dominant, as will be discussed in Chapter 12.

All cereal, nut and oilseed crops are subject to growth of field fungi. Only the most important will be described here.

11.6.1 Wheat, Barley and Oats

From a comprehensive 2 year survey of the mycoflora of Scottish wheat, barley and oats, Flannigan (1970) concluded that field contamination of these cereal crops was similar. The most commonly occurring fungus was *Alternaria alternata,* which was present on more than 85% of kernels examined. *A. alternata* causes downgrading of cereals due to grey discolouration and the production of mycotoxins (Watson, 1984). *Cladosporium* species were also very common in barley and oats (85 and 95% of grains, respectively) but rather less so in wheat (77%). Grey discolouration can result from growth of these species also. Other commonly occurring fungi were *Epicoccum nigrum* and *Penicillium* species. *A. alternata* was also the dominant species on English barley, occurring on more than 75% of a large number of experimental plots sampled over three seasons (Hill and Lacey, 1983). These authors reported large numbers of *Penicillium* species, principally *P. verrucosum, P. aurantiogriseum, P. hordei, P. piceum* Raper and Fennell and *P. roqueforti* in barley, with *Cladosporium cladosporioides, Aureobasidium pullulans* and *E. nigrum* also very common (Hill and Lacey, 1984).

A 2 year study of malting barley grains in Slovakia confirmed that *Alternaria* species were the most prevalent fungi. Other species isolated included *Cochliobolus sativus* [anamorph *Bipolaris sorokiniana* (Saccardo) Shoemaker] and *Fusarium avenaceum* (Hudec, 2007). Similarly, *Alternaria alternata* was the most common species found in Argentinean grains (Broggi et al., 2007).

From freshly harvested wheat grains in Egypt, 77 fungal species from 26 genera were isolated, including 16 species of *Aspergillus* and 21 of *Penicillium* (Moubasher et al., 1972). Other genera of importance were *Alternaria, Cladosporium* and *Fusarium*. No indication was given that any of these species were causing spoilage or unacceptable deterioration. The dominant species were *A. niger* and *P. chrysogenum*.

In a study of freshly harvested barley in Egypt, Abdel-Kader et al. (1979) isolated 37 genera and 109 species. The dominant genera were *Aspergillus,* represented by 25 species, *Penicillium* (32 species), *Rhizopus, Alternaria, Fusarium* and *Drechslera*.

The principal invasive field fungi in US wheat and barley have been reported to be *Alternaria, Fusarium, Drechslera* and *Cladosporium*. *Alternaria* was present in nearly 100 % of grains (under the pericarp) and of barley (under the hull) (Christensen, 1965, 1978b, 1987). *Fusarium graminearum* and *F. poae* are the most common Fusaria found in North American wheat (Miller, 1994a).

In Japan, an important disease of wheat and barley is termed red mould disease, which is caused predominantly by *Fusarium graminearum* (Yoshizawa et al., 1979). Grains contained trichothecene mycotoxins. Korean barley also showed heavy infection with *Fusarium* species (Park and Lee, 1990).

In contrast, Thai wheat showed little invasion by *Fusarium* species (Pitt et al., 1994). Principal fungi found were *Alternaria alternata, Cladosporium cladosporioides* and *Penicillium aurantiogriseum*.

Alternaria alternata was found to be the most prevalent species in Australian wheat from Northern NSW and Southern Queensland affected by black point (Webley et al., 1997). In a survey of Australian wheat carried out in our laboratory, the most common species of field fungi were *Alternaria infectoria, A. alternata* and *Epicoccum nigrum*. *Nigrospora oryzae* and *Cladosporium cladosporioides* were also common (our unpublished data). *Fusarium graminearum* was not a common component of the postharvest mycoflora, usually invading less than 1% of grains (our unpublished data). Other *Fusarium* species isolated were *F. acuminatum, F. avenaceum* and *F. equiseti,* also at very low levels. The low incidence of *Fusarium* species in Australian wheat is due mainly to the much drier and hotter growing conditions in the main wheat belts of Australia compared with North America and Europe (Burgess et al., 1981). The distribution of *F. graminearum* is restricted to warm temperate to subtropical regions where rainfall is moderate to high in summer (Backhouse and Burgess, 2002). Southwell et al. (2003) reported an outbreak of Fusarium head blight in durum wheat grown in the Liverpool Plains in NSW in 1999. The disease incidence ranged from 2 to 100% of crops and *F. graminearum* was the predominant pathogen.

11.6.2 Rice

Southeast Asian rice carries a wide variety of fungi up to the point of husk removal. Most common are *Trichoconiella padwickii, Curvularia* species, *Fusarium semitectum, Bipolaris oryzae* (Breda de Haan) Shoemaker, *Nigrospora oryzae, Chaetomium* species, *Phoma* species and *Diplodia maydis* (Berk.) Sacc. (Iizuka, 1957, 1958; Majumder, 1974; Kuthubutheen, 1979; Pitt et al., 1994). Mallick and Nandi (1981) reported the same genera as the dominant flora of freshly harvested Indian rice. On Egyptian paddy rice, a number of *Aspergillus* species (*A. flavus, A. sydowii, A, terreus, A. fumigatus* and *A. ochraceus*) and *Penicillium* species (*P. chrysogenum* and *P. corylophilum*) were isolated, along with *Fusarium oxysporum, Alternaria alternata, Cladosporium cladosporioides, Trichoderma viride* and *Mucor racemosus*. In a survey of Spanish cereals the most common fungi isolated from rice were *Penicillium* and *Rhizopus* species, while *Fusarium* species were less common (Cantalejo et al., 1997). In paddy rice from Northeastern Argentina and Southern Paraguay, Tonon et al. (1997) found that the major internal contaminants were *Penicillium citrinum* and *P. islandicum*.

When cellulose agar was used, the most prevalent fungi were *Stachybotrys chartarum* (Ehrenb.) S. Hughes, *S. bisbyi* (Sriniv.) G.L. Barron, *Aspergillus niger, A. flavus, Fusarium oxysporum, Alternaria alternata, Bipolaris sorokiniana* and *Acremonium striatum* (Abdel-Hafez et al., 1987). In Australia, information is limited: weather damaged rice examined in our laboratory contained *Fusarium proliferatum, F. equiseti* and *F. anthophilum* (A. Braun) Wollenw. Mycoflora of freshly harvested rice may be similar to that found in Thailand (Pitt et al., 1994).

11.6.3 Maize

Developing ears of maize are encased in a strong, protective husk which greatly reduces invasion by fungi. *Fusarium* is the principal pathogenic fungal genus causing spoilage of maize in the ear, the most commonly occurring species being *F. graminearum, F. verticillioides* (= *F. moniliforme*) and *F. subglutinans* (Burgess et al., 1981; Marasas et al., 1984). *F. graminearum* usually causes a generalised rot, with a pronounced reddish discolouration of grains and husk and with pinkish to red mycelium also visible on the grain surface. Most *Fusarium* species invade through the sites of insect damage and perhaps also through the silks (Miller et al., 2007). However, *F. verticillioides* and related species including *F. proliferatum* appear to be commensals and are endemic in maize in most parts of the world (Desjardins, 2006; Leslie and Summerell, 2006): in the United States (Cole et al., 1973; Bullerman and Tsai, 1994), Europe (Visconti and Doko, 1994), South Africa (Marasas et al., 1979), Zambia (Marasas et al., 1978), China (Yoshizawa et al., 1994), Thailand (Pitt et al., 1993; Yoshizawa et al., 1996), other parts of Asia (Pitt et al., 1998a) and Australia (Leslie and Summerell, 2006). Control of Fusaria in maize is very difficult (Burgess et al., 1981).

The economic importance of *Fusarium* diseases in maize is exacerbated by the fact that all produce potent mycotoxins, of considerable, even devastating, significance to the health of man and domestic animals. For further information see Marasas et al. (1984); Miller and Trenholm (1994); Desjardins (2006).

Of no less importance than the *Fusarium* diseases is the fact that the mycotoxigenic fungus *Aspergillus flavus* also invades maize, although it is not considered to be a true pathogen. In the early literature, *A. flavus* was regarded only as a storage fungus, but in the mid 1970s the realisation came that freshly harvested maize in the southeastern United States was sometimes infected by *A. flavus* with the consequent production of aflatoxins (Lillehoj et al., 1976a, b; Shotwell, 1977). Maize from the cooler areas in the midwestern maize belt, however, showed little if any preharvest invasion. Insect damage to cobs is probably the major means of entry for the fungus (Lillehoj et al., 1980; Hesseltine et al., 1981), but it has also been shown that *A. flavus* can invade maize cobs down the silks without an insect vector (Williams et al., 2006; Windham and Williams, 2007). Invasion is favoured by high growing temperatures, above 30°C, and plant stress also appears to be important, at least under laboratory conditions (Lillehoj, 1983). In contrast with *A. flavus, A. parasiticus* appears to be an infrequent invader of maize. For a review, see Horn (2007).

Maize from Southeast Asia is also heavily invaded by *Aspergillus flavus*. It was present in more than 85% of 150 samples of Thai maize, at up to 100% of grains in some infected samples (Pitt et al., 1993). Similar figures were obtained from Indonesia and the Philippines (Pitt et al., 1998a). The first step towards aflatoxin control in countries such as these relies on rapid drying immediately after harvest (Siriacha et al., 1988).

Aspergillus flavus and *A. parasiticus* are not common in Europe, but were first reported from Italian maize in 2003. *A. flavus* was found on 93% of samples examined and 70% produced aflatoxins (Giorni et al., 2007).

Maize is also invaded preharvest by *Penicillium* species. Mislivec and Tuite (1970a) found 6.4% of some hundreds of samples of US midwestern maize were infected with Penicillia, the most common species being *P. oxalicum* and *P. funiculosum*. *Eupenicillium ochrosalmoneum* has been isolated from US maize and shown to produce significant levels of the mycotoxin citreoviridin in naturally infected kernels (Wicklow et al., 1988). Moubasher et al. (1972) found a much less extensive mycoflora in maize than in wheat, with numbers of both genera and species being only 50% of those in the latter crop. Again, however, *Aspergillus niger* and *Penicillium chrysogenum* were dominant. In Australia, *Penicillium funiculosum* and *P. pinophilum* have been isolated from maize (Burgess and Hocking, unpublished). The most commonly encountered *Penicillium* species in Thai maize were *P. citrinum,* found in 64% of 154 samples, and *P. funiculosum* (in 42% of samples).

Other commonly isolated fungi were *Lasiodiplodia theobromae* and *Fusarium semitectum,* with significant levels of *Rhizoctonia solani, Rhizopus oryzae* and *Trichoderma harzianum* encountered in some samples (Pitt et al., 1993). The role of Penicillia and these other species in subsequent spoilage is uncertain.

11.6.4 Soybeans and Mung Beans

In soybeans and mung beans from Thailand, the most commonly isolated field fungi were *Eupenicillium semitectum, Lasiodiplodia theobromae, Macrophomina phaseolina, Chaetomium* (*Chaetomium brasiliense, C. globosum* and *C. funicola*) and *Cladosporium* species (*C. cladosporioides* and *C. sphaerospermum*) (Pitt et al., 1994). *Aspergillus flavus* was detected in 67% of the 49 samples of soybeans examined, but was less common in mung beans (45% of samples). *Alternaria alternata* was the most commonly isolated fungus on freshly harvested soybeans in Argentina (Broggi et al., 2007). Weather damaged soybeans from the midwest of the United States were heavily infected with *A. alternata, Fusarium graminearum* and *Phomopsis* species. Soybean seedlings in Ohio were also heavily infected with *F. graminearum* (Broders et al., 2007). Zearalenone, zearalenol, deoxynivalenol and diacetoxyscirpenol were detected in damaged soybeans (Jacobsen et al., 1995). *Fusarium* species were present in only 5% of Argentinian soybeans surveyed, the most common species being *F. equiseti, F. semitectum* and *F. verticillioides* (Vaamonde et al., 1987). Freshly harvested soybeans in Ecuador were the source of *F. verticillioides*, *F. semitectum*, *A. flavus* and *A. ochraceus* (Pacin et al., 2002). *F. semitectum* was also common in weather damaged Australian soybeans (Hocking and Pitt, 1996).

11.6.5 Other Beans and Pulses

Chickpeas are an important crop in many tropical countries. In India, a survey of chickpea pods from their earliest stages of development revealed a prevalence of *Alternaria alternata, Cladosporium herbarum* and *Fusarium* spp. in the standing crop. After harvesting, *Aspergillus flavus* dominated (Singh and Ahmed, 1989). Australian weather damaged chickpeas contained *A. alternata, Botrytis cinerea, Stemphylium botryosum, Eupenicillium acuminatum* and *Chaetomium* and *Cladosporium* species, with *A. flavus, Eurotium amstelodami, E. chevalieri, Penicillium brevicompactum* and *Rhizopus oryzae* also present. Weather damaged faba beans examined at the same time contained a similar range of fungi (Hocking and Pitt, 1996).

11.6.6 Sunflower Seed

There is little information in the literature on the mycoflora of smaller oilseeds such as sunflower. One study from Pakistan reported field fungi: *Rhizoctonia solani, Macrophomina phaseolina, Fusarium verticillioides, F. solani, F. semitectum, F. equiseti, Drechslera* species, *Alternaria alternata* and *A. tenuissima,* along with a high incidence of *Aspergillus flavus* and *A. niger* infection (Shahnaz and Ghaffar, 1991). From India, *M. phaseolina, A. alternata* and *A. flavus* were reported from sunflower by Vijayalakshmi and Rao (1993). Gowdar et al. (2007) found similar fungi, but reported *Alternaria helianthi* as the most commonly occurring species. Weather damaged Australian sunflower seeds examined in our laboratory were heavily invaded by *Botrytis,* with *A. alternata, Cladosporium,* and *Stemphylium* species also isolated.

11.6.7 Sorghum

The mycoflora detected in Southeast Asian sorghum was rather different from that found in other commodities. *Aspergillus flavus* was present in very high levels, comparable to those found in maize. The presence of high numbers of *Curvulria lunata, C. pallescens,* other *Curvularia* species, *Alternaria alternata, A. longissima* Dreighton and MacGarvie, *Fusarium moniliforme, F. semitectum, Lasiodiplodia theobromae, Nigrospora oryzae* and *Phoma* species indicated that sorghum is host to a wide range of field fungi (Pitt et al., 1994). A similar range of fungi were reported from Indian sorghum (see Pitt and Hocking, 1997; Raj et al., 2007) and sorghum in Egypt (Osman et al., 1988). *A. flavus*, *Fusarium verticillioides* and *F. proliferatum* were isolated from freshly harvested and stored sorghum in Brazil (da Silva et al., 2004).

The dominant species in Australian sorghum samples examined in our laboratory was *Alternaria alternata*. Other fungi commonly isolated were *Alternaria infectoria, Phoma sorghina* (Sacc.) Boerema et al., *Bipolaris sorghicola* (Lefebvre and Sherwin) Alcorn, *Exserohilum rostratum* (Drechsler) K.I. Leonard and Suggs and *Cladosporium* species.

11.6.8 Peanuts

Freshly harvested peanuts and peanut shells may be colonised by a diversity of fungi. The intimate contact of soil with the shells of developing nuts is an ideal situation for fungal colonisation. Although the shell represents a physical barrier and protects the developing kernels from most fungal invasion, fungi may still enter via cracks in the shells, or via the pegs.

In peanut kernels, *Aspergillus* species, particularly *A. niger* and *A. flavus,* have been reported frequently (see Pitt and Hocking, 1997). In Thai peanuts, 95% of 109 samples contained *A. flavus,* with invasion of 41% of all kernels examined. Figures for peanuts from the Philippines were similar, while from Indonesia, 98% of 256 samples contained *A. flavus,* with invasion of 61% of all kernels examined (Pitt et al., 1993, 1998a). *A. parasiticus* is also very common in peanuts in the United States (Horn et al., 1994; Horn, 2007) and Australia (Pitt, 1989b; Pitt and Hocking, 2006) but rare in Southeast Asia (Pitt et al., 1993). *A. niger*, *A. glaucus* and *A. ochraceus* were found in Spanish peanuts (Jiménez and Mateo, 2001).

Other commonly occurring fungi in peanuts are *Fusarium* species (*F. solani, F, semitectum* and *F. oxysporum*), *Macrophomina phaseolina, Rhizoctonia*

solani, Rhizopus species (*R. stolonifer* and *R. oryzae*), *Chaetomium* and *Curvularia* species. *Penicillium* species are also relatively common on peanuts, with *P. funiculosum* most frequently reported, but also *P. citrinum, P. aurantiogriseum, P. glabrum, P. chrysogenum, P. pinophilum* and *P. janthinellum* (see Pitt and Hocking, 1997).

11.6.9 Cashews and Brazil Nuts

Similar fungi occur in cashews as in peanuts, but at much lower levels. The dominant species detected on cashews from Thailand were *Aspergillus* species (*A. flavus, A. niger* and *A. sydowii*), *Chaetomium* species (*C. globosum*, and *C. funicola*), *Nigrospora oryzae*, *Penicillium citrinum* and *Cladosporium cladosporioides* (Pitt et al., 1993). In Brazil, dwarf cashew nuts were contaminated with species of *Aspergillus* (*A. niger* and *A. flavus*), *Penicillium* (*P. brevicompactum* and *P. glabrum*), *Chaetomium globosum*, *Eurotium chevalieri* and *E. amstelodami*, *Nigrospora oryzae* and *Rhizopus oryzae* (Freire et al., 1999). In Nigeria, *Aspergillus flavus, A. tamarii, Rhizopus stolonifer, Fusarium* and *Gliocladium* species were associated with dry rot disease of cashew kernels (Esuruoso, 1974).

Freire et al. (2000) isolated 17 fungal species from Brazil nuts. *Aspergillus flavus* was dominant followed by *A. niger*. *Penicillium citrinum* and *P. glabrum* were the only *Penicillium* species found. Other fungi reported included *Acremonium curvulum, Cunninghamella elegans, Exophiala* sp., *Fusarium oxysporum, Rhizopus oryzae, Scopulariopsis* sp., *Thielavia terricola* and *Trichoderma citrinoviride*. Arrus et al. (2005) sampled Brazil nuts from several forest areas and found that yeasts, *Penicillium, Acremonium* and an unidentified Coelomycete species were dominant on the whole pods, whole in-shell nuts yielded a similar suite of fungi, and from aseptically shelled nuts, the unidentified Coelomyces was most common, followed by *Penicillium* spp. and a few colonies of *Aspergillus wentii*. *A. flavus* was isolated from intact pods, but *A. parasiticus* was not detected. The *Penicillium* species identified included *P. glabrum, P. funiculosum, P. citrinum, P. sclerotiorum* and *P. wortmanii*.

11.6.10 Almonds, Hazelnuts, Walnuts and Pecans

Tree nuts are protected by thick shells, which, while they remain intact, protect the nuts against fungal invasion. In almonds from California, *Aspergillus niger* and *Penicillium glabrum* were reported most frequently, with late-harvested nuts showing lower fungal counts than those harvested earlier (King and Schade, 1986). In non-surface sterilised almonds from California, Bayman et al. (2002a) found that *Penicillium* spp. and *A. niger* were most prevalent, followed by *Rhizopus, Aspergillus ochraceus* group, *A. melleus, A. flavus* and *A. fumigatus*. One survey of 149 samples of hazelnuts reported *Rhizopus stolonifer* and *Penicillium aurantiogriseum* as the most common of 33 species of fungi isolated (Senser, 1979). In hazelnuts and walnuts from Egypt, *Aspergillus* (*A. flavus, A. fumigatus, A. niger*), *Cladosporium* (*C. cladosporioides, C. herbarum*), *Penicillium* (*P. chrysogenum, P. citrinum, P. oxalicum*) and *Fusarium* species (*F. equiseti, F. moniliforme, F. oxysporum*) were reported. At 45°C, *A. fumigatus* and *Rhizomucor pusillus* were commonly isolated (Abdel-Hafez and Saber, 1993). In freshly harvested Californian walnuts, *Penicillium* spp. were most common, then *Rhizopus, A. niger, A. tamarii, A. nidulans* and *A. flavus* (Bayman et al., 2002a). Tannins in walnut skin have been shown to inhibit aflatoxin production and are the probable reason why walnuts are less susceptible to aflatoxin contamination than other tree nuts (Molyneux et al., 2007).

In pecans, most published information relates to stored nuts. However, dominant field fungi recovered from pecans include *Cladosporium, Penicillium, Alternaria* and *Epicoccum,* with *Alternaria alternata, Trichothecium roseum, Pestalotia* (*Pestalotiopsis*) spp. and *Fusarium* spp. also reported (Wells, 1980). If weather conditions are conducive (wet and warm), pecan kernels may be affected by pink mould disease caused by *T. roseum*, as occurred in California in 2002 (Sparks, 2007).

11.6.11 Pistachios

Aspergillus species appear to be the most frequently reported fungi from pistachios, but this may reflect the greater interest in this genus because of the

potential for aflatoxin production in pistachios, rather than the real frequency of occurrence. In Californian pistachios, Doster and Michailides (1994) reported 14 *Aspergillus* species, with *A. niger* common in early splitting nuts. *A. flavus, A. parasiticus, A. ochraceus* and *A. melleus* were also reported. In freshly harvested nuts, Bayman et al. (2002a) also found that *A. niger* was the most prevalent species, followed by *Penicillium* spp., *A. nidulans, A. ochraceus* group and *A. flavus*. In Turkish pistachios, *A. flavus* was present in a significant proportion of 143 samples of freshly harvested pistachios (Heperkan et al., 1994). In Iran, *Aspergillus* and *Penicillium* species were common in pistachios during various stages of development. Of the Aspergilli, *A. niger, A. flavus* and *Neosartorya fischeri* var. *spinosa* occurred most frequently (Mojtahedi et al., 1979). Early split nuts and cracked seedless shells contribute most to *A. flavus* and aflatoxin contamination of pistachio nuts in Iran (Bonjar, 2004), whereas insect damage was also reported to be a contributing factor in Californian pistachios (Campbell et al., 2003).

In freshly harvested Australian pistachios, *Fusarium* species, particularly *F. equiseti* and *F. acuminatum,* were found to be common, with *Aspergillus flavus*, *A. niger* and *A. terreus, Alternaria* species, Coelomycetes and basidiomycetous yeasts also important components of the postharvest mycoflora (Hocking and Pitt, 1996). *Rhizopus* species quickly developed on freshly harvested, unhulled nuts that were left unbrined.

11.6.12 Copra

Coconut meat is probably almost sterile before the fruit is opened, but because it is then dried on the ground, it rapidly becomes contaminated. In 21 samples of Thai copra, *Aspergillus flavus* was the dominant species encountered. Other fungi present included other Aspergilli (*A. niger, A. tamarii, A. sydowii, A. versicolor, A. clavatus*), *Rhizopus oryzae, Sordaria fimicola* (Desm.) Ces. and De Not., *Penicillium citrinum, Mucor* species, *Nigrospora oryzae* and *Eurotium* species (Pitt et al., 1993). In India and the Philippines, fungi associated with deterioration of copra included *R. oryzae, Mucor hiemalis, P. citrinum, Lasiodiplodia theobromae* and species of *Curvularia, Paecilomyces* and *Aspergillus* (*A. niger, A. flavus, A. tamarii*) (Susamma and Menon, 1981; Susamma et al., 1981; Morantte et al., 1986). *Rhizopus, A. niger* and *Penicillium italicum* were reported from Nigerian copra (Chuku et al., 2007). In Brazil, *L. theobromae* has been reported causing postharvest blackening and splitting of coconuts (Viana et al., 2002).

Chapter 12
Spoilage of Stored, Processed and Preserved Foods

It is trite to say that dried foods must be kept dry, heat processed foods must be heated enough to inactivate all relevant spores and preservative concentrations must be high enough to inhibit all fungi. In reality, the science of preserving foods, like so many other disciplines, requires compromise. Really dry foods, i.e. of a safe a_w, may be impossible to obtain for climatic or economic reasons or be unacceptable to the consumer; a sufficient heat process may destroy desirable flavours; and permitted preservative levels are set by law. Some fungi, by virtue of specific attributes, simply cannot be processed out of certain types of foods. Of particular importance are *Xeromyces bisporus* and *Zygosaccharomyces rouxii* – extreme xerophiles which grow in concentrated foods; *Byssochlamys* spp., *Talaromyces* spp. and *Neosartorya fischeri* with ascospores of very high heat resistance which can survive heat processing and grow in heat processed acid foods; and *Zygosaccharomyces bailii,* a preservative resistant yeast. Making foods safe from these fungi requires that they be absent from raw materials or destroyed by pasteurisation and then excluded from the processing and packing lines.

Some other fungi, especially *Aspergillus* and *Penicillium* species, cause less specific problems than the species mentioned above. However, these genera are ubiquitous and often rapid colonisers, and so will cause spoilage whenever processing is inadequate, formulation incorrect or moisture content too high. Still others are opportunists, capable of explosive growth if storage conditions break down as a result of water leakage, moisture migration in shipping containers, etc. This chapter briefly discusses commodities and processed foods at greatest risk from such spoilage fungi. Details of the fungi themselves, their physiology and methods for isolation have been given in earlier chapters.

12.1 Low Water Activity Foods: Dried Foods

Dried foods are categorised here as solid foods, low in moisture and soluble carbohydrate, and include cereals, nuts, dried meat (biltong and jerky), dried milk and spices. Spoilage of such foods is due to the normal range of xerophilic fungi which are capable of rapid growth above about 0.77 a_w and of slow growth at 0.75 a_w and below – down to about 0.68 a_w. Of particular importance are *Eurotium* species, which have no apparent preference for substrate; *Wallemia sebi,* which is especially common in cereals and spices; and *Aspergillus penicillioides,* which because of limited growth on high a_w media is often overlooked. Nuts are very susceptible to invasion by *Aspergillus* species, especially *A. flavus, A. niger, A. candidus, A. ochraceus* and their close relatives. Cereals always become contaminated with *Penicillium* species. Control of these fungi in foods normally relies on keeping the a_w sufficiently low to prevent growth. A good rule of thumb is that for long term storage (1 year or more) foods must be held at or below 0.68 a_w; for 6 months shelf life 0.72 a_w is adequate; and a_w levels above 0.77 are unsafe except in the short term. These a_w figures apply to normal ambient temperatures, i.e. 20–30°C. Refrigerated storage will prolong shelf life at any a_w, provided that the air is effectively dehumidified.

J.I. Pitt, A.D. Hocking, *Fungi and Food Spoilage*, DOI 10.1007/978-0-387-92207-2_12,
© Springer Science+Business Media, LLC 2009

The moisture contents corresponding to these water activities vary widely. For humidity isotherms for particular products see Iglesias and Chirife (1982).

12.1.1 Cereals

Many studies have been carried out on the mycoflora of dried cereals and flours. Dried cereals often show high levels of field fungi, especially *Alternaria* and *Fusarium* species, which are incapable of growth or toxin production in dried crops, as well as the more xerophilic fungi capable of causing spoilage. For wheat, see for example Christensen and Kaufmann (1965, 1969), Pelhate (1968), Moubasher et al. (1972), Wallace et al. (1976), Sauer et al. (1984), Mills et al. (1995) and Riba et al. (2008); for barley Flannigan (1969), the review by Apinis (1972), which has more than 60 references, Abdel-Kader et al. (1979) and Stenwig and Liven (1988); for rice, Tsuruta and Saito (1980), Mallick and Nandi (1981), Mheen et al. (1982) and Pitt et al. (1994, 1998a).

Reported results are coloured by the kinds of media used. Dilute media such as DRBC, DCPA, CZID or PDA (see Chapter 4) will often produce quite different results from media of reduced a_w, such as DG18. PDA and CZID will be biased towards the field fungi and will give a picture of the history of samples preharvest. DRBC will give a comprehensive picture of the common Aspergilli, Penicillia and other ubiquitous flora, as will DG18. DG18 will also provide information about *Aspergillus penicillioides, Eurotium* species and *Wallemia*

The most common causes of spoilage of dried cereals are *Eurotium* species, particularly *E. chevalieri, E. repens, E. rubrum* and *E. amstelodami,* and *Aspergillus penicillioides*. The latter, often misidentified as *A. restrictus,* is a major cause of loss of germinability in cereal grains (Christensen, 1978b). *A. penicillioides* is probably the pioneer species in the development of fungal populations in stored grains (Wicklow, 1995; Hocking 2003). In wheat stored at 0.68 a_w, for 12 months, we observed development of *A. penicillioides,* followed later by *Eurotium* species and *Wallemia sebi* (Hocking, unpublished). *Wallemia* and other *Aspergillus* species are commonly present in stored cereals, but, with the possible exception of *Aspergillus candidus,* relatively rarely cause spoilage.

Counts of Penicillia are often high, probably reflecting growth during the drying period. However, some species may have specific associations with particular cereals. The most common species in wheat, barley and oats are from *Penicillium* subgen. *Penicillium,* especially *P. aurantiogriseum, P. chrysogenum, P. brevicompactum* and *P. crustosum* (Scudamore et al., 1993; Mills et al., 1995; Filtenborg et al., 1996). *P. verrucosum* appears to have a specific association with wheat, barley, oats and rye in Europe (Olsen et al., 2006). *P. verrucosum* is the source of ochratoxin A in these cereal grains in Europe and other cool climates (Lund and Frisvad, 2003; Frisvad et al., 2005b). *P. verrucosum* contaminates grain postharvest, the most common sources of infection being harvesting and grain handling equipment and silos. Rapid drying is key to prevention of ochratoxin formation (Olsen et al., 2006). Of species from other *Penicillium* subgenera, *P. citrinum* and *P. glabrum* are ubiquitous.

In rice and other small grains stored in tropical conditions Penicillia from other subgenera (particularly *Biverticillium* and *Furcatum*) are more common. *Penicillium citrinum* was the most common *Penicillium* species encountered in milled rice samples from Thailand, Indonesia and the Philippines. *Penicillium islandicum* was present in 5% of the Indonesian samples, but was rarely detected in the samples from the Philippines, and not found at all in rice from Thailand (Pitt et al., 1994, 1998a). *P. citrinum* was reported to be common in rice from northern regions of South America (Tonon et al., 1997), Vietnam (Trung et al., 2001) and Korea (Park et al., 2005a). Tonon et al. (1997) also found high levels of *P. islandicum* in rice from northeastern Argentina and southern Paraguay. *Aspergillus* and *Eurotium* are far more significant components of the storage mycoflora in tropical conditions than *Penicillium* species. Important are the four common *Eurotium* species, plus *Aspergillus flavus, A. candidus, A. niger, A. versicolor, A. wentii* and *A. fumigatus* (see Pitt and Hocking, 1997). In 139 Indonesian milled rice samples, the most frequently encountered species was *A. candidus,* present in 56% of samples, sometimes infecting up to 100% of grains, followed by *A. flavus, E. chevalieri, E. rubrum, A. fumigatus* and *A. niger* (Pitt et al., 1998a). In 73 samples of milled rice from the Philippines, *A. flavus* was the most common species (present in 53% of samples),

followed by *A. candidus, E. chevalieri, A. niger* and *A. fumigatus* (Pitt et al., 1998a). *A. flavus, A. candidus, A. niger* and *A. ochraceus* were the most common *Aspergillus* species in rice from Vietnam (Trung et al., 2001), Korea (Park et al., 2005a; Oh et al., 2008), northern areas of South America (Tonon et al., 1997) and Nigeria (Makun et al., 2007a).

Yellowing of rice during storage has been attributed to fungal activity. Yellowing can occur in dry grain, but may be more closely related to mould growth before and during drying than growth during storage. Although *Penicillium islandicum* has been implicated, the exact species of fungi responsible and their role in rice yellowing remains unclear (Miyaki et al., 1970; Phillips et al., 1988). Historically, toxic yellow rice responsible for acute cardiac beri beri in Japan was probably due to the growth of *Penicillium citreonigrum* and citreoviridin production (Uraguchi, 1969; Ueno and Ueno, 1972; El-Banna et al., 1987b).

12.1.2 Flour

The kinds of fungi found in wheat are reflected in those found in flour and in goods baked from it (Graves and Hesseltine, 1966; Kurata and Ichinoe, 1967; Dragoni et al., 1980a; Eyles et al., 1989; Berghofer et al., 2003). However, it is evident that the numbers of field fungi that can be isolated from flour are much lower than those present in the wheat or rice before milling, and that the numbers of Penicillia and Aspergilli are markedly increased. Both these changes reflect the degree of sporulation which has taken place. Field fungi produce relatively few spores; Penicillia and Aspergilli relatively many. *Aspergillus candidus* was the most common species encountered in two types of flour in Germany, with *Penicillium aurantiogriseum* the next most common (Weidenbörner et al., 2000). In an extensive survey of Australian flour and mill products, *Penicillium citrinum* was the most common species, followed by *P. aurantiogriseum,* with *Aspergillus versicolor* and *A. penicillioides* the most commonly encountered Aspergilli (Hocking, unpublished data). *Wallemia sebi, Eurotium* and *Cladosporium* species may also be frequently isolated from flour (Eyles et al., 1989; Weidenbörner et al., 2000; Berghofer et al., 2003), and the uncommon xerophile *Geomyces pulvereus* A.D. Hocking and Pitt has been reported from Australian flour (Hocking and Pitt, 1988). Yeasts are common (Kurtzman et al., 1970; Spicher and Mellenthin, 1983; Eyles et al., 1989; Berghofer et al., 2003), and spores of mucoraceous fungi, such as *Rhizopus, Mucor* and *Absidia* species, have also been reported in flour (see Pitt and Hocking, 1997; Weidenbörner et al., 2000; Berghofer et al., 2003).

12.1.3 Pasta

Dry pasta products are usually safe from mould spoilage. However, improperly dried pasta can be spoiled by xerophilic fungi. We have encountered *Eurotium* species, *Aspergillus candidus, Wallemia sebi,* Penicillia and yeasts in such products. Halt et al. (2004) reported *A. candidus* as the most common species in 132 samples of dried pasta, followed by *Aspergillus flavus*. *Penicillium* and *Mucor* species were also frequently isolated. *Epicoccum nigrum* has been reported causing red spots on the surface of gnocchi (a fresh pasta dumpling containing potato). *Mucor racemosus* was isolated from the same product (Dragoni and Cantoni, 1979). *Paecilomyces variotii, Aspergillus versicolor*, *Eurotium amstelodami, Geotrichum candidum, Penicillium crustosum* and *P. solitum* were reported from fresh, modified atmosphere packaged pasta products (Colavita et al., 1999).

12.1.4 Bakery Products

Spoilage of baked goods is very much dependent on water activity. High a_w products such as bread and some pastries spoil rapidly from Penicillia (e.g. *P. roqueforti, P. brevicompactum* and *P. chrysogenum*), *Wallemia, Eurotium* species and other common moulds including the red bread mould, *Chrysonilia sitophila, Rhizopus* and *Mucor* species (see Pitt and Hocking, 1997; Abellana et al., 1997; Vytřasová et al., 2002, and our unpublished observations). *Monascus ruber,* which is resistant to propionic acid, can produce red spots on white and multi-grain breads (Spicher and Isfort, 1988). Growth of white yeast-like

fungi (*Endomyces fibuliger* and *Hyphopichia burtonii*) and yeasts (*Zygosaccharomyces bailii, Saccharomyces cerevisiae* and *Pichia anomala*) can cause "chalk mould" defects on bread (Seiler, 1980; Spicher, 1984b; Legan and Voysey, 1991). Flavour defects such as the production of ethyl acetate by *P. anomala* can also occur (Legan and Voysey, 1991) and we have seen several such spoilage incidents in white and mixed grain bread. We have also seen spoilage of Lebanese bread due to *Geosmithia putterillii* on more than one occasion and even spoilage caused by *Aspergillus flavus,* probably due to severe build-up of these particular kinds of fungi in inaccessible parts of bakeries or bakery equipment.

European rye breads are susceptible to spoilage by *Penicillium* species, particularly *P. roqueforti, P. commune* and related species (Spicher, 1984a, 1985; Filtenborg et al., 1996; Lund et al., 1996). Yeasts and yeast-like fungi can also be a problem in sliced rye bread (Filtenborg et al., 1996).

Penicillium species can cause spoilage of sponge and Madeira cakes. Sorbate or propionate may be added to these products to extent the shelf life, but as the pH is near 6.0, the preservatives are largely ineffective (Marín et al., 2004). Some Penicillia are capable of decarboxylating sorbate to 1,3-pentadiene (Daley et al., 1986), which produces a kerosene-like odour causing the cake to spoil even before mould growth is apparent. In one such spoilage incident investigated in our laboratory, *Penicillium crustosum, P. glabrum, P. commune, P. chrysogenum* and *P. brevicompactum* were isolated from the spoiled cakes, but the species responsible for the production of the 1,3-pentadiene was not identified.

We have also seen spoilage of icing on cakes from a xerophilic yeast which was causing liquefaction of small areas of the icing. Presumably *Zygosaccharomyces rouxii* was the cause, and cleaning of the factory, especially the equipment used in manufacturing the icing, the answer. Seiler (1980) and Legan and Voysey (1991) have discussed in detail the problems caused by yeasts in baked goods. Lanciotti et al. (1998) reported spoilage of cream-filled cakes by *Pichia anomala* (as *Hansenula anomala*) due to production of ethyl acetate and other flavour defects.

Modified atmosphere packaging and the use of oxygen-absorbing sachets can extend the shelf life of bakery products (Guynot et al., 2003a,b), but this technology is ineffective against yeast and chalk mould spoilage. Mould free shelf life can also be extended by the use of ethanol vapour generators (Powers and Berkowitz, 1990; Legan and Voysey, 1991; Smith, 1994; Franke et al., 2002).

Spoilage in fruit cakes will be discussed under concentrated foods.

12.1.5 Maize

Maize cobs and kernels are relatively large. Moist conditions at harvest often result in slow drying. In consequence both preharvest and postharvest fungi may become well established. Lichtwardt et al. (1958) and Barron and Lichtwardt (1959) carried out a very thorough study of the mycoflora of dried and stored maize in Iowa. Lichtwardt et al. (1958) identified the internal flora of surface disinfected maize grains both in sterile moist chambers and on malt salt agar (6% NaCl). In addition samples were ground and dilution plated. Approximately 50 genera were recognised. A combination of isolation methods enabled Barron and Lichtwardt (1959) to estimate the relative importance of the isolated genera in the spoilage of stored maize. *Eurotium* species, especially *E. rubrum, E. amstelodami* and *E. chevalieri*, were the most significant, together with *Aspergillus restrictus* and *Penicillium* species, especially *P. aurantiogriseum, P. viridicatum* and closely related species. In maize samples from Thailand, the most commonly encountered storage fungi were again *Eurotium* species (*E. chevalieri, E. rubrum* and *E. amstelodami*) but *Wallemia sebi, Aspergillus flavus*, *A. wentii, A. tamarii* and *A. niger* were also present in a significant number of samples (Pitt et al., 1993). Similar results were obtained from 148 samples of maize from the Philippines and 82 samples of maize from Indonesia, except that *W. sebi* was not isolated from the Indonesian samples (Pitt et al., 1998a). *Fusarium* species, particularly *F. verticillioides, F. semitectum* and *F. proliferatum*, persisted in high numbers in stored maize from all three countries (Pitt et al., 1993, 1998a). Wicklow et al. (1998) also noted predominance of *E. chevalieri* and *A. flavus* and the persistence of *F. verticillioides* in maize stored for up to 2 years under a range of temperature (10–40°C) and moisture (40–88% r.h.) conditions. A similar range of fungi was reported from maize in Venezuela (Mazzani et al., 2004)

Penicillium species occurring in maize both pre-harvest and in storage, and the factors which influenced their role as spoilage fungi, were investigated by Mislivec and Tuite (1970a,b). Some common pre-harvest species, such as *P. funiculosum,* were rarely isolated later; species such as *P. citrinum* and *P. oxalicum* were commonly present at all times; others again, such as *P. aurantiogriseum* and *P. viridicatum*, were almost exclusively associated with the stored grain. In Thai, Philippine and Indonesian maize, *P. citrinum* was the most frequently encountered *Penicillium* species, with *P. funiculosum, P. pinophilum, P. oxalicum* and *P. raistrickii* also commonly detected (Pitt et al., 1993, 1998a). *P. citrinum* was also the most commonly occurring *Penicillium* species in the storage study of Wicklow et al. (1998), who reported that *P. funiculosum* declined during storage.

Recent studies have emphasised the importance of *Aspergillus flavus* as a pre-harvest invader (see Chapter 11) and the spoilage of maize, not from fungal growth per se, but from the production of unacceptable levels of aflatoxins. Consequently, maize should always be checked for aflatoxins at marketing. AFPA can also be used as a monitor for *A. flavus.*

Difficulties in field drying of maize in the midwestern United States due to the onset of winter rains have led to attempts to store moist maize under refrigeration. However, many *Penicillium* species grow well below 5°C, and such maize may develop "blue eye" disease, with the production of high levels of penicillic acid (Ciegler and Kurtzman, 1970). High moisture maize (16.4%) stored in underground silos in Brazil developed populations of *Aspergillus candidus* and film forming yeasts in the upper layers, with *Fusarium* species persisting during the storage period of 8 months (Sartori et al., 1991). Auerbach et al. (1998) reported development of *Penicillium roqueforti* in whole crop maize silage, with roquefortine detected in some visibly mouldy samples. Mansfield et al. (2008) reported roquefortine, patulin, mycophenolic acid and cyclopiazonic acid in fresh and ensiled maize.

Fungi acquired in the field, particularly *Fusarium verticillioides, F. proliferatum, F. oxysporum* and *Aspergillus flavus,* can persist in maize and the moulds and their toxins may be carried through to maize products such as flour, grits, corn chips, tortillas, breakfast cereals, etc. (Pitt and Hocking, 1997). Other fungi reported in maize products include *Penicillium funiculosum, P. duclauxii* and *P. brevicompactum* in maize meal and maize starch in Brazil (Ribeiro et al., 2003), *Aspergillus parasiticus, P. citrinum, P. funiculosum* and *Alternaria alternata* in milled maize in Argentina (Broggi et al., 2002), and *A. flavus, A. sulphureus, Penicillium stoloniferum* and *P. aurantiogriseum* in maize flour in Kenya (Muriuki and Siboe, 1995). The presence of aflatoxins and fumonisins in maize is of particular concern for maize products, as both mycotoxins are very heat stable and survive the temperatures used for drying and processing maize (Mann et al., 1967; Stoloff and Trucksess, 1981; Dupuy et al., 1993). Ochratoxin A has also been reported in maize products (Muriuki and Siboe, 1995; Juan et al., 2007).

Fungi detected in processed maize products include *Aspergillus flavus, A. fumigatus, A. niger, Cladosporium sphaerospermum, Eurotium amstelodami, Penicillium chrysogenum, Fusarium* species and other *Aspergillus, Eurotium* and *Penicillium* species (Kamphuis et al., 1992; Zohri et al., 1995).

12.1.6 Soybeans, Mung Beans, other Beans and Chickpeas

Although soybeans have a strongly protective seed coat, they are still subject to fungal deterioration in storage. Mislivec and Bruce (1977) found the mycoflora of stored soybeans dominated by *Eurotium* species, *Aspergillus flavus, A. candidus, Penicillium aurantiogriseum, Alternaria* and *Cladosporium* species. In 49 samples of soybeans from Thailand, Pitt et al. (1994) also observed that after *A. flavus* (present in 67% of samples) the most common storage fungi were *Eurotium* species, particularly *E. rubrum* which was present in 51% of the samples. Other storage species were *E. chevalieri* (in 33% of samples), *E. amstelodami* (16%), *E. repens* (4%), *Aspergillus restrictus* (16%), *A. penicillioides* (6%) and *Eupenicillium cinnamopurpureum* (8%). In 30 samples of soybeans from the Philippines, *A. flavus* was the most commonly encountered species, present in 90% of the 30 samples examined, followed in frequency by *E. chevalieri* (80%), *E. rubrum* (50%), *Aspergillus niger* (47%), *Penicillium citrinum* (33%), *A. restrictus* (27%) and *P. olsonii* (20%) (Pitt et al., unpublished data). In soybeans from Indonesia, a similar range of fungi was encountered, but the

dominant *Eurotium* species was *E. rubrum* (present in 60% of 48 samples); there were less Penicillia, and an uncommon species *Scopulariopsis gracilis* Samson was isolated from 17% of samples (Pitt et al., 1998a).

The storage mycoflora of mung beans in Thailand, Indonesia and the Philippines was very similar to that detected in soybeans. *Aspergillus flavus* and *A. niger* were the most common of the Aspergilli, *Eurotium rubrum* and *E. chevalieri* the most common *Eurotium* species and *Penicillium citrinum* the most common *Penicillium* species. *P. olsonii* was present in some samples from Thailand and Indonesia (Pitt et al., 1994, 1998a). Barua et al. (2007) reported *A. flavus, A. niger, Fusarium oxysporum, F. verticillioides* (as *F. moniliforme*), *F. semitectum* and *Penicillium* spp. in stored mung beans in Bangladesh.

The storage fungi of kidney beans (*Phaseolus vulgaris*) in India included *Alternaria, Aspergillus, Cladosporium, Colletotrichum, Fusarium, Penicillium, Rhizoctonia, Stemphylium* and *Trichoderma* (Sud et al., 2005). A comparison of the mycoflora of similar beans from Canada and Taiwan found that *Aspergillus, Penicillium* and *Eurotium* spp. were common in Taiwanese beans but, in Canadian beans, the most prevalent fungi were *Alternaria, Fusarium* and *Rhizoctonia* (Tseng et al., 1995). On velvet beans, dominant fungi were *Eurotium rubrum, E. amstelodami, Aspergillus flavus, A. niger, Fusarium, Rhizopus, Mucor* and *Cladosporium* spp. and yeasts (Bhat et al., 2007).

Aspergillus flavus was found to dominate the mycoflora of Indian chickpeas after harvest (Singh and Ahmad, 1989), and storage fungi comprised mainly *A. flavus, A. niger, A. ochraceus, Emericella nidulans* and *Penicillium* species (Ahmad and Singh, 1991). In Australian chick peas, Sarantinos et al. (1996) found that the dominant fungi were *Stemphylium botryosum* and *Botrytis cinerea*, followed by *A. flavus, Alternaria alternata, Eurotium chevalieri* and *E. amstelodami*.

12.1.7 Nuts

As noted in the previous chapter, some nuts, such as macadamias, are well protected by a heavy shell during development and rarely suffer from mould invasion; others such as peanuts may be invaded preharvest by a very wide range of fungi.

Dried nuts are very susceptible to spoilage because their soluble carbohydrate content is low, so any increase in moisture content causes an appreciable rise in a_w. Such a rise can readily be caused by moisture movement due to uneven storage temperatures, as may happen in shipping containers. Also if refrigerated storage, widely used to retard the development of rancidity, is not efficiently dehumidified, increases in moisture can rapidly result.

If moisture does increase marginally, spoilage will result from growth of *Eurotium* species. However, nuts shipped in containers across the tropics can become a total loss from moisture movement due to unsuitable stowage, for example on deck or near engine rooms. Under these conditions we have seen rampant growth of *Aspergillus flavus* and very high aflatoxin levels, resulting in the complete loss of container loads of peanuts. We have also examined samples from a container load of hazelnuts lost to *Eurotium repens*.

12.1.8 Peanuts

The most significant papers on the mycoflora of stored peanuts are those by Joffe (1969), McDonald (1970) and Pitt et al. (1993). During a 5 year period, Joffe (1969) isolated fungi from freshly harvested peanuts and samples stored for up to 6 months. Over 400 samples of stored peanuts were examined. By far the most common species encountered was *Aspergillus niger*, isolated from a low of 8.4% of kernels in one year to a high of 71%. The other dominant members of the flora were *A. flavus* (0.2–8.4%); *Penicillium funiculosum* (2.6–16.2%); *P. purpurogenum* (1.6–7.8%); and *Fusarium solani* (0–9.1%). From our observations on Australian peanuts, and those of Joffe (1972) on peanuts in Israel, *A. niger* is a competitor of *A. flavus*, and the relative abundance of these two species is dependent on climatic and agricultural factors which at present are poorly understood.

In Thai peanuts, Pitt et al. (1993) found that after *Aspergillus flavus* (present in 95% of 109 samples), the most frequently isolated species was *A. niger* (in 86% of samples, at up to 100% infection rate within a sample). Other Aspergilli were also common: *A. tamarii* in 31% of samples, *A. wentii* 20% and *A. candidus* 4%. *Eurotium* species were an important component of the storage mycoflora,

with *E. rubrum* most common (in 51% of samples), followed by *E. chevalieri* (46%), *E. amstelodami* (9%) and *E. repens* (6%). *Wallemia sebi* was present in 12% of samples, with an infection rate of 98% observed in one sample. The most common *Penicillium* species was *P. citrinum* (in 46% of samples, at infection rates up to 60% within a sample), with *P. funiculosum,* (14% of samples, up to 92% infection within a sample), *P. olsonii* (in 8% of samples), *P. pinophilum* (4%), *P. glabrum* and *P. janthinellum* (3% each). Other Aspergilli and Penicillia were encountered at lower frequencies (Pitt et al., 1993).

In peanuts from Indonesia (256 samples) and the Philippines (132 samples), a similar range of fungi was encountered. The most common Aspergilli were *A. flavus* (present in 98% of Indonesian and 97% of Philippine samples), *A. niger* (80 and 90%, respectively) and *A. tamarii* (38 and 19%). The most common *Eurotium* species were *E. rubrum* and *E. chevalieri,* and of the Penicillia, *P. citrinum* was most common, present in 55% of Indonesian and 41% of Philippine peanuts, followed by *P. funiculosum* (4 and 12%, respectively) (Pitt et al., 1998a and unpublished data).

In an extensive study of peanuts from Uganda and Kenya, Ismail (2001) reported that the most frequently isolated species was *Aspergillus niger,* followed by *A. flavus*, *Macrophomina phaseolina* and *Eurotium* spp. (*E. repens, E. rubrum, E. amstelodami*). *Rhizopus stolonifer, Aspergillus parasiticus, Fusarium solani, Lasiodiplodia theobromae* and *Penicillium chrysogenum* were also common. Ihejirika et al. (2005) identified *A. flavus, A. niger* and *A. versicolor* as the major fungi responsible for storage rots of peanuts in Nigeria. Other studies have reported a similar range of fungi in stored peanuts (Oluma and Nwankiti, 2003; Gachomo et al., 2004; Mphande et al., 2004). Recent studies on the mycoflora of peanuts have concentrated on the presence of mycotoxigenic fungi, including those capable of producing ochratoxin A (Magnoli et al., 2006a, 2007b; Nakai et al., 2008).

One noteworthy point concerns the infection of peanuts by *Aspergillus parasiticus*. In most regions, including the United States, South America, Australia and some parts of Africa, *A. parasiticus* is commonly found in peanuts along with *A. flavus* and this has been documented in a number of papers. Indeed peanuts are regarded as one of the major habitats for this species, for example, Barros et al. (2006) reported *A. parasiticus* in peanuts from Argentina. However, *A. parasiticus* has rarely been reported from peanuts (or any other commodity) from Southeast Asian countries, and in some papers its absence has specifically been noted: in Thailand (Pitt et al., 2003), Indonesia (Pitt et al., 1998a), the Philippines (Pitt et al., unpublished) and Vietnam (Tran-Dinh et al., 2009). The reasons for this geographical difference from *A. flavus* is not known.

The great majority of the fungi present in peanuts are not capable of causing spoilage due to visible growth, but may cause discolouration, which from both the processor's and consumers' points of view is a type of spoilage. At least in developed countries, discoloured peanuts are sorted out by colour sorting machines. Colour sorting, introduced to eliminate discoloured nuts, has proved to be an effective way of removing nuts which contain aflatoxins also. Reject nuts may be used in the manufacture of peanut oil, where refining processes remove both fungi and aflatoxins. *Eurotium* species sometimes cause pitting and erosion in peanuts, rather than discolouration, but the conditions responsible have not been established (our observations).

12.1.9 Hazelnuts, Walnuts, Pecans and Almonds

Several studies on fungi in hazelnuts and walnuts (Senser, 1979; Abdel-Hafez and Saber, 1993; Sahin and Kalyoncuoglu, 1994) and on pecans (Schindler et al., 1974; Huang and Hanlin, 1975; Wells and Payne, 1976; Wells, 1980) have been published. Senser (1979) isolated 33 mould species from 149 samples of hazelnuts; the most commonly occurring were *Rhizopus stolonifer* and *Penicillium aurantiogriseum*. In Egyptian hazelnuts and walnuts, Abdel-Hafez and Saber (1993) detected 51 fungal species: the most commonly occurring were *Aspergillus flavus, A. fumigatus, A. niger, Cladosporium cladosporioides, C. herbarum, Penicillium chrysogenum, P. citrinum* and *P. oxalicum*. *Fusarium* species (*F. equiseti, F. moniliforme* and *F. oxysporum*) were recovered from walnuts at a moderate frequency. *Eurotium* species (*E. amstelodami, E. chevalieri, E. repens* and *E. rubrum*) were commonly isolated when a reduced a_w medium was used. Aflatoxins were detected in 90% of hazelnut samples, at levels of 25–175 μg/kg, and in 75% of walnut samples, with similar levels.

Zearalenone was detected in one walnut sample at a level of 125 μg/kg. In Turkish hazelnuts, *Penicillium* and *Aspergillus* species dominated the mycoflora, but some Zygomycetes (*Rhizopus stolonifer, Absidia corymbifera* and *Syncephalastrum racemosum*) were also common (Sahin and Kalyoncuoglu, 1994). Potentially aflatoxigenic fungi were detected in 47–79% of Turkish hazelnuts at harvest. Of the 5,564 strains isolated, 89% were *Aspergillus flavus* and 11% were *A. parasiticus*. Aflatoxins were only detected in nuts that had been in contact with the ground during drying (Ozay et al., 2008).

In walnuts in the USA, Bayman et al. (2002a) reported that the most commonly occurring fungi were *Aspergillus niger, Rhizopus* spp. and *Penicillium* spp., with *A. flavus* uncommon in surface disinfected nuts. In Indian walnuts, Singh and Shukla (2008) found the highest levels of fungi in nuts stored during the rainy season (July–October). *A. flavus* was present in 64% of kernels, with *Penicillium citrinum* in 32% and *A. niger* and *Rhizopus* present in 26% of kernels. *Chaetomium globosum, Alternaria alternata, Cladosporium herbarum, Syncephalastrum racemosum* and *Trichothecium roseum* were detected in 2–5% of kernels stored in both summer (April–June) and the rainy season, but were rare in the nuts stored over the winter months (November–February) (Singh and Shukla, 2008). Molyneux et al. (2007) reported that phenolic compounds present in walnuts, particularly tannic acid, reduced the probability of aflatoxin contamination. Mouldy walnuts were reported to have caused a tremorgenic mycotoxicosis in a dog in New Zealand. *Penicillium crustosum* was identified from mouldy walnuts that were collected from the ground where the dog had been, and penitrems A-F and roquefortine were isolated from the nuts (Munday et al., 2008).

From 37 samples of pecans, Huang and Hanlin (1975) isolated 119 species from 44 genera. As in other studies on stored dried foods, *Eurotium, Aspergillus* and *Penicillium* were the dominant genera. *Aspergillus* species accounted for 48% of the more than 1,300 isolates obtained; next came *Penicillium* (19%), *Eurotium* (18%) and *Rhizopus* (8%). The dominant species were *A. niger* and *A. flavus,* followed by *Eurotium repens, E. rubrum, A. parasiticus, Rhizopus oryzae* and *Penicillium expansum*.

Wells and Payne (1976) obtained an unusual distribution of genera from pecans which had been invaded by weevils in the field. Nearly half of 2,300 isolates from several hundred mouldy nuts were *Alternaria* or *Epicoccum* species. *Penicillium* species made up 25% of the total, and *Aspergillus* only 1.0%. In pecans taken from drying bins 4 months after harvest, Wells (1980) found that the mycoflora was dominated by *Cladosporium* (39% of isolates), *Penicillium* (21%), *Alternaria* and *Epicoccum* species (9% each).

Aspergillus niger and *Penicillium glabrum* were the most prevalent of 12 genera and 51 species identified during a survey of the mycoflora of almonds during harvest and storage (King and Schade, 1986). Species isolated after 1 month of storage at 25°C were *Eurotium* species, *Aspergillus flavus, A. wentii, Penicillium implicatum* and *P. rugulosum*. *Rhizopus stolonifer* and *R. oryzae,* which were common on freshly harvested nuts persisted during storage. In almonds collected in the field, Bayman et al., (2002a) found that *Penicillium* spp. were the most commonly occurring fungi, followed by *Aspergillus niger, A. ochraceus* and *Rhizopus* spp., whereas in retail almonds, the dominant fungi were *Rhizopus* spp., then *A niger* and *A. flavus*.

Kenjo et al. (2007) examined 30 samples of almond powder imported into Japan, the majority from the USA. They reported that the dominant fungi were *Aspergillus niger, A. flavus* and related species, *Penicillium, Cladosporium* and *Rhizopus*. *A. parasiticus* and *A. nomius* were also isolated.

12.1.10 Pistachio Nuts

The storage mycoflora of pistachios appears to comprise mainly *Aspergillus* species, although, as observed in Chapter 11, this reporting may be biased by the predisposition of this commodity to aflatoxin contamination, rather than the true composition of the storage mycoflora. In Iranian pistachios, *A. niger, A. flavus* and *Neosartorya fischeri* var. *spinosa* were most prevalent, followed by *A. terreus, A. tamarii* and *Emericella nidulans* (Mojtahedi et al., 1979). *Penicillium* species were also common, but the species detected were not specified. In pistachios from California, *A. niger, A. flavus, A. parasiticus, A. ochraceus* and *A. melleus* Yukawa were reported (Doster and Michailides, 1994). Bayman et al. (2002a) found that *A. niger* was dominant on field collected and retail Californian pistachios, with

A. nidulans, A. ochraceus, A. melleus, A. fumigatus, A. tamarii and *A. flavus* also present. *Penicillium* and *Rhizopus* spp. were also common. The mycoflora of stored Turkish pistachios was dominated by *Aspergillus, Penicillium, Cladosporium* and *Rhizopus* species, with *Ulocladium, Trichothecium, Aureobasidium* and *Eurotium* present in lower numbers (Heperkan et al., 1994).

12.1.11 Other Nuts

Storage fungi encountered in 45 samples of cashews from Thailand included *Aspergillus* species: *A. flavus* (in 60% of samples), *A. niger* (53%), *A. sydowii* (11%), *A. wentii* (9%) and *A. tamarii* (7%); *Eurotium* species: *E. chevalieri* (in 40% of samples), *E. rubrum* (31%) and *E. amstelodami* (16%); and *Penicillium* species: *P. citrinum* (in 29% of samples), *P. olsonii* (7%), *P. implicatum* and *P. solitum* (both in 4% of samples). *Wallemia sebi* was detected in 9% of samples (Pitt et al., 1993). Adebajo and Diyaolu (2003) reported *A. niger*, *A. flavus*, *A. restrictus* and *A. fumigatus* in retail cashews in Nigeria. In Brazilian cashews, *A. niger* was the most frequently isolated species, followed by *A. flavus*. *Penicillium brevicompactum* and *P. glabrum* were the most common Penicillia, and *Chaetomium globosum* was also recorded at a high level (Freire et al., 1999).

In Indonesian kemiri nuts (19 samples), the storage mycoflora was dominated by the same range of fungi encountered in other tropical stored commodities: *Aspergillus flavus, A. niger, A. wentii, A. tamarii, Eurotium rubrum, E. chevalieri* and *Penicillium citrinum*. Two less common species, *Penicillium aethiopicum* and *P. allii,* were detected in 16 and 10% of samples, respectively, comprising a significant proportion of the total mycoflora (Pitt et al., 1998a).

In Brazil nuts (four samples) the most common fungi were *Aspergillus flavus, Penicillium glabrum, Cunninghamella elegans, P. citrinum, Rhizopus oryzae, A. niger* and *Fusarium oxysporum* (Freire et al., 2000). Bayman et al. (2002a) reported that *Rhizopus* was the most prevalent fungus in Brazil nuts they purchased in California. *A. flavus* was the next most common, followed by *Penicillium* spp., *Aspergillus tamarii, A. niger, A. nidulans* and *A. fumigatus*. Olsen et al. (2008) isolated *A. nomius* as well as *A. flavus* from Brazil nuts and suggested that *A. nomius* may be an important source of aflatoxin in these nuts.

Overy et al. (2003) isolated fungi from chestnuts collected from seven Canadian provinces. The mycoflora of the chestnuts was dominated by *Penicillium* spp., the most frequently isolated being *P. crustosum* (76%), *P. glabrum*/*P. spinulosum* (19%) and *P. discolor* (18%). Aspergilli were rarely encountered, the most common being *Aspergillus ochraceus* (2%) (Overy et al., 2003).

12.1.12 Coconut

A wide range of fungi can be found on copra, as detailed in Chapter 11. Desiccated coconut is produced from copra and is also subject to fungal spoilage. Zohri and Saber (1993) investigated the mycoflora of coconut and reported a wide range of fungi, including *Aspergillus flavus, A. niger, A. sydowii, Penicillium chrysogenum, P. oxalicum, Cladosporium cladosporioides, Alternaria alternata* and *Eurotium chevalieri*. Aflatoxin B_1 was detected in 5 of 25 samples (15–25 μg/kg) and 3 samples were contaminated with ochratoxin A (50–205 μg/kg). Kinderlerer (1984b) isolated 28 species from coconut. *Eurotium* species were the principle group, but *Penicillium, Cladosporium, A. flavus* and *A. niger* were also common. *Chrysosporium farinicola* was also isolated. Ismail (2001) reported over 60 different fungal species from desiccated coconut samples collected in Kenyan and Ugandan markets. The mycoflora was dominated by *A. niger, A. flavus* and *P. chrysogenum*. On DRBC, *A. ochraceus, Penicillium waksmanii, P. variotii, P. islandicum* and *Rhodotorula mucilaginosa* were common, whereas on DG18, species of *Cladosporium, Chrysosporium* and *Eurotium* were more frequent. *Chrysosporium fastidium*, *C. farinicola* and *C. inops* were detected (Ismail, 2001).

Two distinct types of spoilage in coconut due to the action of xerophilic fungi have been reported: ketonic rancidity due to production of aliphatic methyl ketones and secondary alcohols by *Eurotium* species and cheesy butyric spoilage brought about by growth and metabolism of *Chrysosporium farinicola* (Kinderlerer, 1984a; Kinderlerer and Kellard, 1984). Fermentation of coconut by *Chrysosporium xerophilum* resulted in the production, after 9 months, of a

complex mixture of aliphatic methyl ketones, esters, secondary alcohols and medium chain length free fatty acids (Kinderlerer et al., 1988).

12.1.13 Spices

Because of their tropical origin, and the methods used in their production, spices are frequently heavily contaminated with xerophilic fungi. Counts on enumeration media may be very high, figures up to 10^9 per gram having been recorded in our laboratory (Hocking, 1981). *Aspergillus, Eurotium* and *Penicillium* species are often the dominant flora of dried, whole or ground spices (see Pitt and Hocking, 1997), with *E. halophilicum,* a rare species, reported from cardamom seeds (Hocking and Pitt, 1988). Pepper is often cited as a source of fungal contamination for foods and may carry significant fungal loads of up to $10–10^7$ cfu/g (King et al., 1981; Muhamad et al., 1986; Atanda et al., 1990). The most common species in 42 samples of black peppercorns from the Philippines were *Aspergillus flavus* (present in 95% of samples), *A. niger* (76%), *Eurotium rubrum* (74%), *Chaetomium globosum* (45%), *E. chevalieri* (38%) and *A. sydowii* (31%). Overall, 60 species of fungi from 24 genera were isolated from the peppercorns, including 16 species of *Aspergillus* and *Eurotium* and 9 species of *Penicillium* (Pitt et al., unpublished). Freire et al. (2000) reported 42 species of fungi from Brazilian black and white pepper. *A. flavus* was the predominant species, followed by *A. niger, C. globosum, Penicillium brevicompactum, Emericella nidulans, Eurotium* spp. and *P. glabrum*. Gatti et al. (2003) reported that *Eurotium* spp. (*E. chevalieri, E. rubrum* and *E. amstelodami*) were the most common fungi in 115 samples of Brazilian black pepper. *A. flavus* and *A. niger* were the next most common. *A. tamarii, A. carbonarius, A. fumigatus, A. sydowii, A. restrictus, A. ochraceus* and *A. parasiticus* were also isolated. Other genera included *Rhizopus, Penicillium, Curvularia, Cladosporium, Emericella* and *Paecilomyces*. Despite the prevalence of potentially mycotoxigenic fungi, no aflatoxins or ochratoxin A were detected in the pepper samples (Gatti et al., 2003).

We have isolated *Eurotium rubrum, E. chevalieri, Aspergillus penicillioides* and *Xeromyces bisporus* from mould affected nutmegs.

It is difficult to know what constitutes spoilage of a spice. So little is eaten that slight off-flavours may be irrelevant. Some concern has been expressed about the potential for mycotoxin contamination of foods from spices (Llewellyn et al., 1992), but the levels of aflatoxin contamination reported (40–160 μg/kg) are probably not high enough to represent a real health threat, given the dilution factors involved. The important point for food use is that a mouldy spice may contaminate other ingredients, and hence the final product. Where a heat process is not used, such as in processed meat manufacture, the spices should be sterilised before use (Hadlok, 1969). Gamma irradiation of spices is effective in reducing microbial contamination and is used in some countries (Muhamad et al., 1986; Llewellyn et al., 1992).

12.1.14 Coffee Beans

The possibility of ochratoxin A contamination in coffee beans was first raised many years ago (Levi et al., 1974; Tsubouchi et al., 1984; Micco et al., 1989; Studer-Rohr et al., 1995), and most recent studies on the mycoflora of green coffee beans have concentrated on the occurrence of potentially ochratoxigenic fungi. Mislivec et al. (1983) reported that *Aspergillus* species dominated the fungal flora of 944 coffee bean samples, and *A. ochraceus* was the most common toxigenic species isolated.

Urbano et al. (2001) investigated the sources of ochratoxin A in Brazilian coffee beans and reported that while only 10% of isolates were *Aspergillus ochraceus*, 88% of them were capable of producing ochratoxin. Levels of ochratoxin A were low, below EU limits. The mycoflora was dominated by *A. niger* (63% of isolates), with *A. ochraceus* and closely related species also common (31% of isolates) and *A. carbonarius* also present (6%). Most *A. ochraceus* (75%) and *A. carbonarius* (77%) isolates produced ochratoxin A in pure culture, but only 3% of *A. niger* isolates did (Taniwaki et al., 2003). They concluded that ochratoxin A in Brazilian coffee beans was due to growth of *A. ochraceus* (and related species) and *A. carbonarius*, and that better harvesting, drying and processing practices could minimise toxin production. Martins et al.

(2003) and Batista et al. (2003) also analysed the fungi in green Brazilian coffee beans, with similar results, though dilution plating used by these latter authors were less quantifiable than the direct plating techniques used by Urbano et al. (2001) and Taniwaki et al. (2003).

In coffee beans from Vietnam, Ilic et al. (2007) did not detect *Aspergillus ochraceus* or *A. carbonarius,* but found that 93% of samples contained *A. niger*. Eight of 92 *Aspergillus niger* isolates (8.7%) produced ochratoxin. Leong et al. (2007) examined Robusta (65 samples) and Arabica (11 samples) beans from southern and central Vietnam and found that Robusta beans were much more heavily infected than Arabica beans. They reported that *A. niger* infected 89% of Robusta beans, whereas *A. carbonarius* and *Aspergillus* section *Circumdati* isolates each infected 12–14% of beans. Ochratoxin was not produced by 98 *A. niger* isolates or 77 *A. ochraceus* isolates, but was detected in 110 of 113 isolates of *A. carbonarius*, 10 of 10 isolates of *A. westerdijkiae* and the single isolate of *A. steynii*. These results indicate that species from *Aspergillus* section *Nigri* may be largely responsible for ochratoxin contamination of coffee beans in Vietnam.

The revelation that a new species, *Aspergillus westerdijkiae,* and not *A. ochraceus,* is the main producer of ochratoxin in *Aspergillus* section *Circumdati* (Frisvad et al., 2004) sheds doubt on the identity of isolates from coffee reported as *A. ochraceus*. Many of these may actually be *A. westerdijkiae*. A method for detection and quantification of *A. westerdijkiae* in coffee beans, based on selective amplification of the β-tubulin gene by real-time PCR, has recently been published (Morello et al., 2007).

In summary, both *Aspergillus carbonarius* (and occasionally *A. niger*) and *A. westerdijkiae* (plus the less common *A. steynii*) are responsible for ochratoxin A formation in coffee beans, but the proportions of these species vary geographically, and perhaps with other unidentified factors.

In India, coffee may undergo a process known as monsooning, a solid state fermentation, resulting in flavour changes that impart characteristic aroma, flavour and cup quality. Tharappan and Ahmed (2006) followed mycological changes over 9 weeks of monsooning. The highest fungal populations occurred between 4 and 7 weeks and comprised mainly *Aspergillus niger, A. ochraceus, A. tamarii, A. candidus*, *A. versicolor*, *Penicillium* spp. (including *P. rugulosum* and *P. chermesinum*) and *Absidia heterospora*. *Wallemia sebi* was also detected in Arabica beans between 3 and 7 weeks fermentation. Taniwaki et al. (2005) also reported distinct flavour changes in green coffee beans, some of which were positive and some negative, depending on the fungi present. The most common fungi isolated from green coffee beans which still produced a good, clean beverage were *Cladosporium* spp., *Alternaria* spp., *Fusarium* spp., *Penicillium* spp., black moulds and yeasts. The most common fungi isolated from coffee beans which had a wood and 'Rio' taste were *Aspergillus niger*, *A. ochraceus*, *Eurotium* spp., *Fusarium* spp. and dematiaceous fungi.

Severe fungal infection of coffee beans can lead to a defect known as 'Rio flavour', which is caused by the formation in the beans of trichloroanisoles by fungal metabolism (Liardon et al., 1992). A number of species of *Eurotium, Aspergillus, Penicillium* and also *P. variotii* have been shown to be capable of methylation of chlorophenols to chloroanisoles (Hill et al., 1995).

12.1.15 Cocoa

Cocoa beans are subject to a microbial fermentation as the first stage of chocolate production. The first 2–3 days are characterised by a succession of various species of filamentous fungi, yeasts, lactic acid bacteria and acetic acid bacteria, with the latter stages of the fermentation dominated by *Bacillus* species (Ardhana and Fleet, 2003; Schwan and Wheals, 2003; Nielsen et al., 2007). Filamentous fungi may develop during the fermentation or later during storage of the beans, leading to deleterious changes. Guehi et al. (2007) examined cocoa beans from Ivory Coast with high free fatty acid content. They isolated *Absidia corymbifera, Rhizopus oryzae* and *Penicillium chrysogenum* from 80% of the beans, and *Aspergillus niger, A. flavus* and *A. tamarii* were present in 40–50% of samples. Dharmaputra et al. (1999) reported *A. flavus, Eurotium amstelodami, E. chevalieri* and *Penicillium citrinum* from insect damaged cocoa beans in Indonesia. Thermophilic fungi (*Aspergillus fumigatus, Rhizomucor pusillus* and *Thermoascus aurantiacus*) were isolated from fermenting cocoa beans with a temperature of 45–50°C (Ogundero, 1983).

Cocoa and cocoa products may contain ochratoxin A (Bonvehi, 2004; Tafuri et al., 2004; Jørgensen, 2005; Raters and Matissek, 2005; Mounjouenpou et al., 2008). Ochratoxin A is rarely found in fresh cocoa beans (Raters and Matissek, 2007), but toxin formation may occur during or after fermentation, is more common at the end of the harvesting season when levels of filamentous fungi are higher and is exacerbated by poor handling and storage practices in the countries of origin (Mounjouenpou et al., 2008). Mounjouenpou et al. (2008) found high levels of *Aspergillus niger* and *A. carbonarius* in cocoa in Cameroon, and although 70% of *A. niger* isolates tested produced ochratoxin A at low levels, they concluded that *A. carbonarius* was the main source of ochratoxin A. They also commonly isolated *A. fumigatus, A. tamarii, Penicillium sclerotiorum, P. paneum, P. crustosum* and *Mucor, Rhizopus, Fusarium* and *Trichoderma* spp. *A. ochraceus* was not reported (Mounjouenpou et al., 2008).

12.1.16 Dried Meat

Van der Riet (1976) studied the mycoflora of 20 samples of South African biltong. As would be expected *Eurotium* species were dominant, followed by *Aspergillus* and *Penicillium* species. A surprising number of other fungi were also isolated. Van der Riet (1976) reported that yeasts were also isolated from all of the samples and at least some were identified as lipolytic species. None were known xerophiles, and presumably all had grown during the drying period. If drying were prolonged, rancid spoilage could perhaps occur. As the samples were not surface disinfected, it is a matter of conjecture which of the filamentous fungi had grown in the meat during drying and which were merely aerial contaminants. Wolter et al. (2000) reported *Debaryomyces hansenii* and *Yarrowia lipolytica* were the most common yeasts isolated from biltong.

In samples of dried beef jerky and kangaroo biltong examined in our laboratory, *Eurotium* species were the most common spoilage fungi, but *Aspergillus candidus* and *Debaryomyces hansenii* were also isolated. As with other dried foods, the shelf life of such products is dictated by their water activity. In this case there is an added factor in spoilage due to fat rancidity, which may be induced by yeast or mould growth during drying, and continue in storage.

12.2 Low Water Activity Foods: Concentrated Foods

Concentrated foods are defined here as including both evaporated products and those to which sugars have been added. The list includes jams, dried fruit, fruit cakes, confectionery and fruit concentrates. Such foods are as susceptible to spoilage by the common xerophiles as are dried foods and in addition provide ideal habitats for the most xerophilic fungi known – *Xeromyces bisporus* and *Zygosaccharomyces rouxii*. As noted earlier in this chapter, the important point about these two fungi is that processing and handling systems must positively exclude them from concentrated foods, as no commercial product can be manufactured of an a_w sufficiently low to prevent them from growing.

12.2.1 Jams

Traditional jams and conserves, made almost entirely from fruit and sucrose, are boiled or evaporated down to 0.75 a_w or below and hot filled into jars before closing. Consequently they very rarely spoil. The answer to any spoilage problem with a traditional type of jam must rely on that basic premise: to produce jams which will not spoil, the water content of the product must be reduced to a safe a_w. However, many commercially prepared products are made by a much reduced cooking process and are heated for a shorter time, and their a_w is often closer to 0.80–0.82. At this a_w they are much more likely to support mould growth, and mould free shelf life depends on hot filling into jars with a reliable, airtight seal. Jams which have much lower levels of added sugars and consequently much higher water activities are manufactured for diabetics. Such products are usually stabilised by preservatives, but the precise recipes permitting stable products have rarely been published.

If jams spoil, *Eurotium* species, *Aspergillus restrictus* and related species are usually responsible, although we have seen xerophilic Penicillia,

especially *P. corylophilum,* from time to time. Because jams are hot filled, *Zygosaccharomyces rouxii* is unlikely to be a problem, but we have seen the occasional jar of jam bubbling over from an infection with that yeast, presumably from contamination after opening. The black yeast-like fungus *Trichosporonoides nigrescens* A.D. Hocking and Pitt has also been reported from spoiled, fermenting jam (Hocking and Pitt, 1981).

12.2.2 Dried Fruit

Some fruit, including apricots, peaches, pears and bananas, are dried after preservation with SO_2, which is essential to prevent browning from the Maillard reaction. The high levels of SO_2 also completely eliminate the microflora, even during prolonged storage. If the SO_2 levels decline to less than 1 g/kg during storage, these products may be spoiled by the xerophilic yeast *Zygosaccharomyces rouxii*, which is moderately resistant to preservatives, and xerophilic fungi such as *Xeromyces bisporus* and *Eurotium* species.

Prunes. Dried prunes, and most dried vine fruits, are not processed with SO_2 and are susceptible to spoilage by xerophiles. Pitt and Christian (1968) reported the isolation of nearly every known xerophilic fungus from Australian dried and high moisture prunes, which at that time relied on hot filling for microbial stability. The most common fungi isolated were *Eurotium* species, especially *E. herbariorum, Xeromyces bisporus* and xerophilic *Chrysosporium* species. El-Halouat and Debevere (1997) reported *Zygosaccharomyces rouxii, Eurotium amstelodami* and *Aspergillus niger* as the most prevalent spoilage fungi in hydrated prunes. Prunes are still a rich source of xerophiles: a new xerophile, *Monascus eremophilus* A.D. Hocking and Pitt, was isolated from Australian prunes in 1988 (Hocking and Pitt, 1988). Most countries now permit the addition of preservatives to high moisture prunes.

Dried vine fruits. Reports of ochratoxin A in dried vine fruits in the late 1990s (MacDonald et al., 1999; MAFF, 1999) focused attention on the incidence and significance of *Aspergillus* section *Nigri* species in grapes and grape products. In their 1998 survey of retail products for ochratoxin A, MAFF (1999) detected ochratoxin A in 286 of 301 samples of dried vine fruits (currants, sultanas and raisins), with 9% containing high levels (>10 μg/kg). MacDonald et al. (1999) examined 60 samples of dried vine fruit purchased in the UK and found that 88% contained ochratoxin A. Subsequently, the European Commission set a limit of 10 μg/kg for ochratoxin A in dried vine fruit (European Commission, 2002). Subsequent surveys and reviews have indicated that although ochratoxin A contamination of dried vine fruits is widespread, overall levels are low, with only a small proportion exceeding the EU limit (Lombaert et al., 2004; Meyvaci et al., 2005; Varga and Kozakiewicz, 2006; Aksoy et al., 2007; Trucksess and Scott, 2008).

Aspergillus carbonarius is probably the major contributor to ochratoxin A contamination of dried vine fruits (Abarca et al., 2003; Leong et al., 2004, 2006a; Magnoli et al., 2004; Tjamos et al., 2004; Chulze et al., 2006). *Aspergillus niger* may also contribute to ochratoxin A contamination as it is often present on dried vine fruits in much higher numbers, but the incidence of toxigenic strains is much lower (Abarca et al., 2003; Leong et al., 2004, 2006a; Magnoli et al., 2004; Tjamos et al., 2004; Iamanaka et al., 2005). Iamanaka et al. (2005) also isolated ochratoxigenic *Aspergillus ochraceus* strains from Brazilian dried vine fruit. Ochratoxin A contamination of dried vine fruit can be reduced by controlling infection by black Aspergilli in the immediate pre harvest period, rapid drying of fruit after harvest and removing mouldy fruit during processing (Leong et al., 2006a).

Aspergillus flavus and aflatoxins have been reported from sultanas in Brazil (Iamanaka et al., 2007) and Egypt (Youssef et al., 2000). Australian vine fruits which are sun dried and not preserved with SO_2 are always contaminated with *A. niger* and related species, which undoubtedly grow to some extent during drying and are presumably highly resistant to the very strong sunlight in the Australian inland irrigation areas (Leong et al., 2004). Other fungi, including *Penicillium citrinum,* other *Penicillium* species, *Alternaria, Epicoccum, Trichoderma, Rhizopus, Cladosporium* and yeasts also occur but are much less common (King et al., 1981). We have also observed spoilage of currants by *Xeromyces bisporus* on several occasions.

Mixed dried fruit usually contains sultanas, currants, raisins, glace citrus peel and glace cherries. The high price of glace cherries has led to the substitution of this product with imitation cherries

made from gelatine and sugar. These have a higher a_w and usually contain a preservative such as sorbic or benzoic acid. If insufficient preservative is added, these imitation cherries may act as a focus for infection by xerophilic fungi such as *Xeromyces bisporus*. We have seen several instances of *X. bisporus* spoilage of mixed dried fruit in recent years.

Figs. The seed cavities of mature figs are always contaminated by yeasts (Miller and Phaff, 1962; see Chapter 11). Spoilage of dried figs sometimes occurs if these contaminant yeasts include xerophilic species. *Aspergillus* species can occur in figs and may cause spoilage and form mycotoxins postharvest (Doster et al., 1996; Bayman et al., 2002b; Trucksess and Scott, 2008). Although *A. niger* and related species are common, infection by members of *Aspergillus* section *Flavi* has more serious consequences, with aflatoxin production a significant problem in figs from some countries (Sharman et al., 1991; Doster et al., 1996; Senyuva et al., 2005, 2007; Karaca and Nas, 2006). Aflatoxin contamination is much more likely if *A. parasiticus* rather than *A. flavus* is present (Doster et al., 1996). Ochratoxin A has been detected in figs (Doster et al., 1996; Bayman et al., 2002b; Senyuva et al., 2005). The potential source may be *A. ochraceus* and related species, or *A. alliaceus* (Doster et al., 1996; Bayman et al., 2002b). Patulin and low levels of *Fusarium* toxins (zearalenone, HT-2 toxin and fumonisin B_2) have also been reported from figs (Karaca and Nas, 2006; Senyuva and Gilbert, 2008). Mycotoxin contamination of figs can be reduced by the application of fungicides during growth and maturation (Tosun and Delen, 1998), improved handling and drying techniques (Ozay et al., 1995) and sorting of dried figs using BGY fluorescence (Steiner et al., 1988; Doster and Michailides, 1998; Trucksess and Scott, 2008).

Other fruits. Sharma and Sumbali (1999a, b) have reported the natural occurrence of aflatoxins and ochratoxin A in dried sliced quinces in India. *Aspergillus* species were the most commonly occurring fungi, with *A. niger* and *A. flavus* prevalent (Sumbali and Sharma, 1997) but *A. ochraceus* was also isolated (Sharma and Sumbali, 1999b).

Glace fruits are preserved by SO_2 which is added in the syrup with which the fruit are infused in a series of increasing concentrations. We have seen samples of partially prepared glace pineapple spoiling from the yeast *Schizosaccharomyces pombe,* which apparently possesses a unique combination of resistance to SO_2 and tolerance to reduced a_w, enabling it to grow at a particular point in the infusion process.

12.2.3 Fruit Cakes

Fruit cakes and similar puddings are concentrated foods because, as well as the fruit, the cake or pudding mix itself is high in sugar. Such cakes and puddings are expected to have quite a long shelf life, often 6 months, and therefore must be prepared and baked to give a final a_w of 0.75 or below. Under these conditions spoilage is not usually a problem, but we have seen very severe cases of spoilage in fruit cakes of 0.75 a_w caused by *Xeromyces bisporus*. In one instance, cakes showed patches of mould several centimetres across only a few weeks after manufacture, with mycelium penetrating deeply into the interior (Pitt and Hocking, 1982). The number of cakes undergoing spoilage was large, indicating a systemic contamination of the cakes in the factory, and in all probability the survival of ascospores of *Xeromyces* through the baking process. Because of this and the ability of *X. bisporus* to thrive at 0.75 a_w, fruit cakes cannot be made which will be resistant to spoilage by this fungus. Fortunately, *X. bisporus* is an uncommon species and, once eliminated from the factory by thorough cleaning, is unlikely to appear again. Tracing the source of *X. bisporus* contamination in the factory environment can be extremely difficult, if not impossible, as the more common xerophiles such as *Eurotium* species are usually present also and will overgrow *X. bisporus* on MY50G isolation plates. The time between production date and visible spoilage can often be quite long – from as little as 6 weeks up to 9 months. This delay can add to the difficulty of tracing the contamination source.

Fruit cakes are also subject to spoilage by *Eurotium* species, *Wallemia sebi, Aspergillus restrictus* and *A. penicillioides*. Control relies on good hygienic practices in the manufacture and packaging of these products. Preservatives (sorbate or propionate) may be added to some higher a_w cakes such as light fruit cakes to extend shelf life, but the pH of these products (5.5–6.5) renders the preservatives only marginally effective.

12.2.4 Confectionery

Most confectionery, including chocolates, jubes, gelatine confections and licorice, have high sugar contents and rely for stability on their low a_w. Formulations are usually traditional and are often prepared in small factories unaware of water activity and its implications, but usually well versed in the control of soluble solids by refractometry. Correctly made, such products are stable for long periods against normal xerophiles such as *Eurotium* species, but are at risk from the extreme xerophiles.

For example, *Zygosaccharomyces rouxii* causes spoilage of filled chocolates, such as caramel filled Easter eggs, chocolate coated marshmallow biscuits and strawberry cream chocolates (Hocking and Pitt, 1996). An infection of this yeast in a chocolate filling line can be impossible to detect, but will provide low level contamination of the final product. Even a few cells will eventually grow, produce gas and cause spoilage by splitting the chocolate casing. The characteristic symptom of this kind of spoilage is wet wrappers due to leaking fillings. Microscopic examination is usually sufficient to confirm the presence of this yeast. The problem is readily cured by cleaning of the filling lines, but losses may be high.

Chrysosporium and *Bettsia* species have been isolated from a number of different types of confections. *C. inops* caused spoilage of a range of Australian gelatine confectioneries, of the type made in a dry starch mould. The a_w of the 30 tonne batch of starch in use in that factory was maintained at what was believed to be an acceptably low level, but was sufficiently high to permit growth of *C. inops*. This species differentiates into large numbers of aleurioconidia and arthroconidia, even at very low a_w. The confectionery was thus systematically contaminated by spores in the starch during manufacture and rapidly spoiled despite having an a_w of 0.72. The diagnosis of the problem involved culture of both confectionery and starch on MY50G agar. The cure involved a long and very careful heat treatment of the starch, which eventually destroyed the fungus without generating an explosion in the starch.

Chrysosporium species can also spoil chocolate. We have isolated *Bettsia alvei* from spoiled hazelnut chocolate, and this species, along with its anamorph *C. farinicola* and other *Chrysosporium* species, has been isolated from spoiled compounded chocolate in the UK (J. Kinderlerer, unpublished). We found *C. xerophilum* causing spoilage of chocolate-coated peppermint patties. Spoilage of an Argentinean caramel jam filled chocolate confection by *Eurotium chevalieri* has been reported (Larumbe et al., 1991).

Xeromyces bisporus was originally isolated from licorice in this laboratory (Fraser, 1953). We have seen this mould on that product on several occasions since. Licorice appears to be safe from spoilage by most other fungi, but we have once seen spoilage due to *Eurotium* species and *Aspergillus penicillioides*. *X. bisporus* has also been isolated from spoiled gelatine confections and from low $a_w$fruit based snack bars in our laboratory. We have found *X. bisporus, A. penicillioides, Wallemia sebi* and *Penicillium implicatum* on spoiled licorice allsorts.

12.2.5 Fruit Concentrates

Fruit juices are shipped around the world in 200 litre drums and one tonne pallecons in the form of concentrates, of 65–80° Brix. Pasteurised, evaporated and hot filled, such concentrates are of low pH and low a_w and are as a rule microbiologically stable. The pasteurising step removes all but the most heat resistant fungal ascospores, i.e. *Byssochlamys* and *Neosartorya,* but these cannot grow at the reduced a_w of the product. Occasionally, however, the xerophilic yeast *Zygosaccharomyces rouxii* will enter the filling system downstream from the pasteuriser. *Z. rouxii* can grow and produce CO_2 down to 0.62 a_w and so is capable of spoiling any liquid, concentrated food. Growing slowly in the lines, it will contaminate the product with sufficient cells to eventually cause the containers to become swollen and even to explode. Spoilage is insidious, because the time to visible swelling may be many months. Losses can be very high. Concentrates undergoing spoilage can be recovered by dilution, pasteurisation and reconcentration, then refilling into drums through carefully cleaned lines. However, a useful short term expedient is to refrigerate the drums, which will stop growth and fermentation by the yeast and permit use or reprocessing.

Monitoring product for this yeast is difficult. The most effective technique of which we are aware of is to aseptically collect ca. 500 ml samples in sterile bottles, preferably 2 litre and made of plastic, add 500 ml sterile

water, mix gently and incubate at ca. 25°C. If gas is not produced when containers are shaken after a 7 day incubation period, the product is probably sound.

12.2.6 Honey and Syrups

Walker and Ayres (1970) discussed at length the various reports of spoilage in honey, generally due to *Zygosaccharomyces rouxii*. Honey can also be contaminated with the ascospores of *Ascosphaera apis* (Claussen) L.S. Olive and Spiltoir, the cause of chalkbrood disease of honey bees (Hale and Menapace, 1980). Modern technology for handling honey includes a heat treatment to prevent crystallisation of glucose, and this treatment also effectively sterilises it. Although many species of xerophilic yeast can be isolated from unprocessed honey (Poncini and Wimmer, 1986; Beckh et al., 2005), spoilage of commercially processed honey is now a very rare problem.

Glucose syrups and liquid malt extracts are susceptible to spoilage by xerophilic yeasts. Spoilage may be manifested by slow fermentation in the product itself or through foaming when the viscosity of the syrup is reduced by heating to allow easier handling. *Zygosaccharomyces rouxii* is usually the cause, but other xerophilic yeasts including *Z. bailii, Torulaspora delbrueckii* (Lindner) Lindner, *Candida lusitanae* Uden and Carmo Souza and *Schizosaccharomyces* spp. may also cause spoilage (Tilbury, 1980; Jermini et al., 1987; Fleet, 1992). In maple syrup, which has a higher a_w, we have seen spoilage by surface growth of *Wallemia sebi*.

12.3 Low Water Activity Foods: Salt Foods

The principal salted food which is susceptible to fungal spoilage is salt fish. This is a very widely distributed product, as salting is the most common way of preserving fresh fish in most tropical regions, and in some temperate zone countries as well. In temperate climates, *Wallemia sebi* is regarded as the principal spoilage fungus (Frank and Hess, 1941). In Brazil, Mok et al. (1981) reported the human pathogen *Hortaea werneckii* (Horta) Nishim. and Miyaji to be one fungus growing on salt fish.

In tropical countries, *Aspergillus* species often dominate the mycoflora of dried and salted fish. Townsend et al. (1971) reported that Vietnamese dried fish were contaminated by a wide variety of Aspergilli, especially *A. clavatus, A. flavus* and *A. niger*. On Malaysian dried fish, *A. niger* and related species were most common, with *A. flavus, A. fumigatus* and *A. ochraceus* also present. *Penicillium chrysogenum* was reported as common (Ito and Abu, 1985).

The principal fungus isolated during a study of fungi on salted and dried fish in Indonesia was *Polypaecilum pisce* (Pitt and Hocking, 1985). It was isolated from nearly 50% of the 60 samples of mouldy fish examined. In some cases growth was apparent over most of the fish surface. *Eurotium* species, particularly *E. rubrum, E. amstelodami* and *E. repens*, were also common, being found on about 30% of the fish. However, growth was usually less extensive than that of *P. pisce*. *Aspergillus* species were also quite frequently isolated. *A. penicillioides* (24% of samples), *A. niger* (20%), *A. flavus* (18%), *A. sydowii* (16%) and *A. wentii* (10%) were of most common occurrence. Apart from *A. penicillioides* and *A. wentii,* it is doubtful whether the *Aspergillus* species had actually grown on the fish. *Penicillium* species were less common, the most frequently isolated being *P. citrinum* and *P. thomii,* each of which was isolated from 18% of the fish examined. *Cladosporium cladosporioides* was also present on 18% of the fish. Of interest also was the isolation of *Basipetospora halophila.* Although encountered infrequently, the water relations of this fungus will clearly allow it to grow on fully dried fish.

In a survey of fungi occurring on Sri Lankan salt fish, 11 different species were isolated, of which *Aspergillus niger* was the most common (Atapattu and Samarajeewa, 1990). Other Aspergilli included *A. flavus, A. fumigatus, A. restrictus* and *Eurotium* spp. *Basipetospora halophila* was the second most commonly isolated species. An unusual *Penicillium* species *P. chalybeum* Pitt and A.D. Hocking, originally described from Indonesian salt fish (Pitt and Hocking, 1985), was encountered frequently on the Sri Lankan salt fish (Atapattu and Samarajeewa, 1990). *Basipetospora* was also isolated frequently from cured fish in Nigeria, but as with other studies, *Aspergillus* and *Eurotium* species were reported most commonly. *Scopulariopsis* species, including *S. brevicaulis,* were also present (Diyaolu and Adebajo, 1994). *Aspergillus penicillioides* and *A. terreus* were the most commonly

isolated species from dry-salted fish in Venezuela (Grau et al., 2003).

In smoke-dried fish from Sierra Leone, the only fungi isolated were four species of *Aspergillus: A. flavus, A. ochraceus, A. tamarii* and *A. niger*. The plating medium was MEA, so if halophilic fungi were present, they would not have been detected (Jonsyn and Lahai, 1992).

12.4 Intermediate Moisture Foods: Processed Meats

A wide variety of meat products of reduced a_w are manufactured around the world. They include bacon, hams and many types of dry fermented sausage products, including salamis. These products are more or less shelf stable, depending on the ingredients and process used, but are frequently contaminated by fungi. At first, interest in these fungi stemmed from consideration of possible beneficial effects on flavour, etc., of the cured products (e.g. Leistner and Ayres, 1968). However, it was soon realised that these moulds might be potentially mycotoxigenic, stimulating further interest. A number of studies were subsequently carried out, 12 of which were summarised by Leistner and Eckardt (1981). The most significant mycotoxin problem in these meat products is ochratoxin A contamination. If animals are fed grain containing ochratoxin, there may be residual ochratoxin A in the meat (usually pork meat) used in the manufacture of the hams, salamis, etc. (e.g. Krogh et al., 1976; Gareis, 1996; Gareis and Scheuer, 2000). Growth of *Penicillium nordicum* during maturation of processed meats can also result in ochratoxin A contamination, a problem that was unrecognised until relatively recently (e.g. Bogs et al., 2006; Pietri et al., 2006; Battilani et al., 2007).

The dominant mycoflora on cured meats are *Penicillium* species, probably because curing takes place at relatively cool temperatures (10–15°C) less suited to the development of Aspergilli. Leistner and Eckardt (1981) listed 50 species, the most frequently isolated being *P. chrysogenum, P. expansum, P. roqueforti, P. rugulosum, P. variabile* and *P. viridicatum*. *Eurotium* species were also quite common, the principal ones being *E. amstelodami, E. repens* and *E. rubrum*. With the exception of *A. flavus* and *A. versicolor*, *Aspergillus* species were relatively uncommon. Potentially mycotoxigenic species of both *Aspergillus* and *Penicillium* were isolated quite frequently by Leistner and Pitt (1977). These results have been confirmed by other studies (Grazia et al., 1986; Mutti et al., 1988; Andersen, 1995; López-Días et al., 2001; Ludemann et al., 2004; Papagianni et al., 2007; Sørensen et al., 2008) where *Penicillium* species, including *P. commune, P. solitum* and *P. olsonii*, have been found to be dominant on fermented sausages. The *Penicillium* species now most frequently isolated from fermented sausages is the starter culture *P. nalgiovense* (Andersen, 1995). Use of *P. nalgiovense* was developed by the Bundesanstalt für Fleishforschung, Kulmbach, Germany, who sought a desirable *Penicillium,* non toxigenic and with a persistently white appearance (Mintzlaff and Christ, 1973). Use of a starter culture appears to be the most satisfactory way to avoid overgrowth by undesirable moulds during the drying and curing of salamis and other semi-preserved meats. However, some strains of *P. nalgiovense* can produce penicillin (Laich et al., 1999), so strains need to be chosen carefully. Yeasts also form a considerable component of the mycoflora of fermented sausages, dominating during the early stages of fermentation (Andersen, 1995; Filtenborg et al., 1996). *Debaryomyces hansenii* and its anamorph *Candida famata* are by far the most frequently reported species (Comi and Cantoni, 1980; Grazia et al., 1986; Fleet, 1992).

Both yeasts and moulds can cause spoilage of ham during maturation. As with fermented sausages, yeasts occur mainly on the surface of ripening hams, where they may cause rancidity due to their lipolytic activity. *Debaryomyces hansenii, Candida saitoana* Nakase and M. Suzuki, *C. zeylanoides* (Castellani) Langeron and Guerra and *C. famata* are the species most commonly reported, with *Hansenula* (now *Pichia*) species, *Geotrichum candidum, Rhodotorula glutinis, Cryptococcus albidus* (Saito) C.E. Skinner and *Trichosporon* species also present (Comi and Cantoni, 1983; Comi et al., 1983; Monte et al., 1986; Molina et al., 1990; Nuñez et al., 1996; Simoncini et al., 2007). The mould flora of raw hams during ripening is dominated by *Aspergillus* and *Penicillium* species. The most commonly reported *Penicillium* species are *P. nordicum* (as *P. verrucosum*), *P. viridicatum*, *P. expansum*, *P. aurantiogriseum, P. chrysogenum, P. brevicompactum* and *P. commune* (Monte et al., 1986; Spotti et al., 1989;

Peintner et al., 2000). We have isolated *P. nordicum* from Australian prosciutto ham. Spoilage of the prosciutto occurred when pork legs were mechanically boned, leaving an air gap in the centre of the curing prosciutto, which allowed internal growth of *P. nordicum* and production of ochratoxin A. *P. commune* has been found to be responsible for "phenol defect" (production of a phenol-like odour) of ham during ripening (Spotti et al., 1988). At lower a_w values (less than 0.85 a_w) *Eurotium* and *Aspergillus* species, including *A. flavus, A. candidus, A. wentii, A. niger* and *A. fumigatus*, are generally dominant (Dragoni et al.,1980b; Monte et al., 1986; Spotti et al.,1989; Rojas et al., 1991; Comi et al., 2004). *E. halophilicum* has been reported from the surface of dry-salted Spanish ham in the latter stages (6–12 months) of the ripening period (Huerta et al., 1987).

Scopulariopsis species have been isolated from cured meats. *S. brevicaulis* was isolated from the surface of maturing smoked bacon (Dragoni et al., 1988) and *S. candida* (Gueg.) Vuill and *Penicillium chrysogenum* provided an optimum combination as a starter for the manufacture of rohwurst, a raw ripened German sausage (Hwang et al., 1993b). Growth of *P. chrysogenum* and *Debaryomyces hansenii* contributed to the flavour of dry-cured ham (Martín et al., 2004, 2006).

12.5 Heat Processed Acid Foods

Because bacterial spores are not a problem, heat processes for acid foods such as fruits and fruit products have traditionally been light. For most fruits, pasteurisation at temperatures of about 70–75°C is effective, as it inactivates most enzymes, yeasts and the conidia of common contaminant fungi. However, fungi producing ascospores are capable of surviving such processes and causing spoilage.

In practice, only a few ascosporogenous species have been isolated from fruit products after a heat process, and still fewer have been recorded as causing spoilage. The list of such species is headed by *Byssochlamys fulva* and *B. nivea*, which have been recorded as causing spoilage in strawberries in cans or bottles (Hull, 1939; Put and Kruiswijk, 1964; Richardson, 1965), blended juices with a passionfruit content, fruit gel baby foods (Hocking and Pitt, 1984), sugarcane juice (Ayesha and Viswanath, 2006) and apple concentrate (Salomão et al., 2008). In our laboratory, we have isolated *B. fulva* from spoiled grapefruit juice and from fruit juice jelly. We have isolated *B. nivea* from cream cheese and from a dairy-based beverage.

The revelation that *Paccilomyces variotii* is the anamorph of the heat resistant fungus *Byssochlamys spectabilis* (Houbraken et al., 2008) explains the frequent occurrence of *P. variotii* as a cause of spoilage of heat processed products in our laboratory. We have often isolated *P. variotii* from heat processed fruit juices (8 occasions), fruit purees (13), sports beverages (3), apple concentrate (1) and taco sauce (1).

Neosartorya fischeri has been repeatedly isolated from strawberries (Kavanagh et al., 1963; McEvoy and Stuart, 1970, and in our laboratory) and other products, including papaya fruits (Rajashekhara et al., 1996), apple concentrate (Salomão et al., 2008) and, in our laboratory, from a berry fruit puree used in fruit-based yoghurt. *Talaromyces trachyspermus* (Shear) Stolk and Samson has been reported from retail packaged canned and frozen pineapple juice (Enigl et al., 1993). In our laboratory we have seen this species causing spoilage in apple juice, heat processed pineapple slices, canned strawberries, fruit juice jellies and a tea-based beverage. *Talaromyces macrosporus, T. bacillisporus* (Swift) C.R. Benj. and *Eupenicillium* species are also potential causes of spoilage in heat processed products (Hocking and Pitt, 1984). *Talaromyces eburneus* (anamorph *Geosmithia argillacea*) has recently been reported as causing spoilage of a pasteurised pineapple product in Japan (Yaguchi et al., 2005).

Techniques for detection of heat resistant moulds have been outlined in Chapter 4. Raw materials which should be screened routinely for heat resistant moulds are grapes, passionfruit, pineapple and mango juices and pulps, strawberries and other berries, sugar syrups, indeed any raw material which may have come in contact with soil directly or as a result of rain splash.

12.6 Preserved Foods

Certain acid liquid foods are stabilised by the use of preservatives. Benzoic acid, sorbic acid and/or sulphur dioxide are usually added to fruit juices, soft

drinks, cordials and a variety of other products. The natural preservative acetic acid is used in products such as tomato sauce, pickled vegetables, mayonnaises and salad dressings. Ciders and wines are preserved by alcohol.

All of these products are susceptible to spoilage by preservative resistant yeasts, yeasts which are capable of growth in the presence of maximum levels of preservatives permitted in such products (Pitt and Richardson, 1973). Our observations and those of others (Couto et al. 1996; Deák and Beuchat, 1996; James and Stratford, 2003; Michels and Koning, 2000; Kurtzman and James, 2006) show that by far the most significant of these is *Zygosaccharomyces bailii* which is capable of spoiling all of the products listed above. Like *Xeromyces* and *Zygosaccharomyces rouxii, Z. bailii* cannot be excluded from products with normal food technological processes. If present in a final product, *Z. bailii* will cause spoilage of most preserved foods. We have isolated *Z. bailii* and the closely related species *Z. bisporus* from a wide range of acid preserved foods and beverages, including pickled onions, mayonnaise, salad dressings, chilli sauce, cordial concentrates, fruit preparations for bakery use and fruit-based drinks containing preservatives. Some particular product types are unlikely to be spoiled by *Z. bailii*, for example synthetic products such as soft drinks and water ices which lack a nitrogen source or are made with sucrose, which *Z. bailii* usually cannot assimilate.

Other species of yeast are also capable of causing spoilage in acid liquid foods. In products preserved with a combination of SO_2 and sorbate or benzoate, *Schizosaccharomyces pombe* may cause spoilage, as this yeast appears to be more resistant to SO_2 than does *Zygosaccharomyces bailii. Candida parapsilosis, Pichia anomala, P. membranaefaciens, Torulaspora delbrueckii* and *Z. bisporus* have been isolated from salad dressings and mayonnaise, all of which rely on the preservative effects of acetic acid. Recently we have seen several spoilage incidents caused by preservative resistant *Saccharomyces cerevisiae* in beverages containing sorbate or benzoate as preservatives.

Almost every factory in Australia which produces acid liquid products has had substantial losses on one or more occasions from preservative resistant yeasts. Other products, too, have been spoiled from time to time: the list in Australia includes cherries for cake manufacture, chocolate topping, mineral water with added fruit juice and water ices made with glucose as a proportion of the sugar.

Pasteurisation is an effective method for eliminating *Zygosaccharomyces bailii* and other preservative resistant yeasts from liquid products. The temperature required depends on the product – pH, sugar content and preservative level, in particular. The precise heat treatments which will be effective should be worked out for each product type. Temperatures around 65–70°C for more than a few seconds should eliminate low numbers of cells. Pasteurisation within the final closed container is to be preferred: if this is not possible, then scrupulous attention to regular cleaning of the lines and fillers downstream from the pasteuriser is essential.

Filter sterilisation is also an effective technique for ridding products of *Zygosaccharomyces bailii,* but its use is confined to clear, liquid products such as ciders and wines.

Like *Zygosaccharomyces rouxii, Z. bailii* can cause spoilage from very low inocula, which makes detection in the plant very difficult. The most effective quality control technique is to test for the presence of the yeast in the final product itself, using the techniques outlined in Chapter 4. For detection of preservative resistant yeasts in the factory or in raw materials, the use of TGY + 0.5% acetic acid or malt acetic agar is recommended.

12.7 Cheese

Cheese is very susceptible to mould growth and is normally kept under refrigeration. Many retail packs are vacuum packaged or gas flushed. Thus, spoilage is generally confined to moulds which are psychrotolerant and can grow under conditions of relatively low oxygen.

In Australasian Cheddar cheese, fungal growth may occur during maturation, causing a defect known as "thread mould". Cheeses manufactured by continuous forming systems such as the Wincanton tower, designed for the rapid processing of curd into 19–20 kg blocks, are particularly susceptible to this type of spoilage. The blocks are vacuum packaged into polyethylene-nylon bags and matured for up to 9–12 months at 8–12°C. Of 195 fungi isolated from thread mould defects, 44% were *Cladosporium,* with *C. cladosporioides* and *C. herbarum* most common, and

27% were *Penicillium* species, with *P. commune* and *P. glabrum* dominant. A Coelomycete, tentatively identified as a *Phoma* species, comprised 15% of isolates (Hocking and Faedo, 1992). Yeasts were also frequently encountered (19% of isolates), with *Candida intermedia* (Cif. and Ashford) Langeron and Guerra, *C. parapsilosis, C. multis-gemmis* (Buhagiar) S.A. Mey. and Yarrow and *Yarrowia lipolytica* most common. In a similar study in Argentina, Basílico et al. (2001) identified *Phoma glomerata* as the primary cause of thread mould defect in 5 kg blocks of hard cheese. Other fungi commonly isolated were *Penicillium commune, P. glabrum, Mucor hiemalis, Geotrichum candidum* and *Moniliella suaveolans*. *Phoma glomerata* isolated from factory air and from some of the cheese making and packaging equipment was relatively resistant to sanitisers (Basílico et al., 2001).

The range of fungi causing spoilage in Australian and New Zealand cheeses in the retail sector differs from that found on maturing Cheddar blocks. *Penicillium commune* and *P. roqueforti* were the most common spoilage species, but other Penicillia from subgenus *Penicillium* (*P. chrysogenum, P. expansum, P. solitum, P. viridicatum* and *P. brevicompactum*) were a significant proportion of the spoilage mycoflora (Hocking, 1994). *P. roqueforti* and *P. commune* (from which *P. camemberti* is derived) are particularly well suited to growth in the cheese environment, having been associated with cheese manufacture for centuries. Shredded cheese is particularly susceptible to spoilage by yeasts and moulds. We have seen yeast growth in shredded cheese cause 'blowing' of packs. *P. commune* and *P. roqueforti* have also been isolated from mouldy shredded cheese in our laboratory. Oyugi and Buys (2007) reported *P. solitum* as the main species causing spoilage in shredded cheese in South Africa. Spoilage of this type could be controlled by modified atmosphere packaging in 73% CO_2/27% N_2,with or without oxygen scavenging sachets (Oyugi and Buys, 2007).

In cheeses in Europe, *Penicillium* species are by far the most commonly reported spoilage fungi. In packaged cheeses, *P. commune* was the most common spoilage species, with *P. verrucosum, P. solitum, P. roqueforti* and *P. nalgiovense* also significant (Lund et al., 1995). In Norwegian semi-hard cheeses (Jarlsburg and Norvegia), dominant spoilage species were *P. roqueforti, P. commune, P. palitans, P. solitum* and *G. candidum* (Kure and Skaar, 2000; Kure et al., 2001). Factory air was identified as an important source of contamination (Kure et al., 2004, 2008). In Turkish Kuflu cheese, *Penicillium* species, including *P. commune, P. roqueforti* and *P. verrucosum*, were also identified as the dominant spoilage organisms, being isolated from over 70% of samples (Hayaloglu and Kirbag, 2007). In other studies on Turkish cheeses, *P. roqueforti* was reported as the most important spoilage species (Erdogan et al., 2003; Korukluoglu et al., 2005). Other *Penicillium* species, along with *Geotrichum, Aspergillus, Mucor, Rhizopus, Alternaria, Fusarium, Cladosporium* and *Scopulariopsis*, were also isolated from mouldy cheese samples (Erdogan et al., 2003; Korukluoglu et al., 2005). In a survey of Spanish cheeses, *Penicillium* species were dominant (63% of samples), with the most common species being *P. brevicompactum*, *P. granulatum* (= *P. glandicola*) and *P. verrucosum*. Other fungi reported were *Mucor* species (27% of samples), *Geotrichum candidum* (17%) and *Cladosporium herbarum* (10%) (Barrios et al., 1998). In Italian sheep and goat's milk cheeses, *P. commune, P. roqueforti, P. verrucosum* and *P. brevicompactum* were the most frequently encountered species. Other Penicillia, *Geotrichum, Aspergillus* and *Mucor* species were also reported (Montagna et al., 2004).

Yeasts and yeast-like fungi are often present in cheese and are important in the surface smears used as starters for soft-ripened cheeses because of their lipolytic and proteolytic activities. *Geotrichum candidum* is a normal part of the mycoflora of the smear of surface-ripened cheeses (Marcellino and Benson, 1992; Lefortier-Medvey, 1993; Lund et al., 1995), although it can also be present as a spoilage organism in other types of cheese (Gueguen, 1988). *Debaryomyces hansenii, Kluyveromyees* species, *Saccharomyces cerevisiae* and *Candida* species are common on the surface of St Nectaire, Camembert and blue-veined cheeses and may play an important role in the development of texture and flavour of these products (Vergeade et al., 1976; Schmidt et al., 1979; Schmidt and Lenoir, 1980; Nunez et al., 1981; Roostita and Fleet, 1996; Addis et al., 2001). *D. hansenii* and *Yarrowia lipolytica* may be responsible for brown defects that sometimes affect mould-ripened cheeses (Carreira et al., 1998; Ross et al., 2000). When present in Cheddar and Gouda cheeses, yeasts can cause taints and off-flavours due to their lipolytic and proteolytic activities and

the production of bitter compounds from lactose fermentation (Viljoen and Greying, 1995).

Sporendonema casei Desm. is an uncommon species that can occur on the surface of some types of cheese. *S. casei* and *Aspergillus candidus* were the dominant species on Provolone cheese after 4–5 months of ripening at 15°C and 85% ERH (Galli and Zambrini, 1978). On Rodez cheese (a Toscanello variety), *S. casei* is responsible for the production of reddish orange surface spots, giving the cheese its so-called 'sunburnt' colour at the end of the ripening period. *S. casei* grows optimally at 0.97 a_w, at 18°C and pH 7.0 (Ratomahenina et al., 1994). We have isolated *S. casei* from cheese affected by white surface mould.

Heat resistant moulds can occasionally cause spoilage in heat processed cheeses such as cream cheese. Ascospores of *Byssochlamys nivea* and other heat resistant species may be present in raw milk, and these ascospores easily survive the pasteurisation processes applied to milk and cream (Engel and Teuber, 1991). Prolonged storage and inadequate cooling (>12°C) can allow growth of heat resistant moulds in dairy products, and we have seen several such cases in Australia. Three separate spoilage incidents involving cream cheese were caused by *B. nivea, Talaromyces avellaneus, Neosartorya fischeri* var. *spinosa* and *Eupenicillium brefeldianum*. *T. avellaneus* also caused spoilage of UHT custard.

Although most cheeses are not permitted to contain preservatives, some types of cheese, and products such as cheese spreads, may contain sorbate or other mould inhibitors. A number of fungal species, including *Penicillium roqueforti* and a few other Penicillia, are capable of decarboxylating sorbate to *trans*-1,3-pentadiene, causing a flavour defect described as a "kerosene" flavour, a problem not restricted to cheese (Liewen and Marth, 1985; Daley et al., 1986; Kinderlerer and Hatton, 1990; Sensidoni et al,, 1994). Some fungi (particularly *Penicillium* species) can also reduce sorbic acid to 4-hexanol and 4-hexanoic acid (Kinderlerer and Hatton, 1990).

The factors enabling fungi to cause spoilage in cheese are the ability to grow at refrigeration temperatures, to grow in low oxygen concentrations, lipolytic activity, resistance to the preservative action of free fatty acids and growth at reduced a_w. *Penicillium roqueforti* and *P. commune* meet all these criteria and are thus the most successful spoilage moulds on cheese. Toxin production (roquefortine and PR toxin from *P. roqueforti* and cyclopiazonic acid from *P. commune*) is a definite, though probably small, hazard. PR-imine was detected in 50 of 60 samples of blue-vein cheese, but PR toxin was not (Siemens and Zawistowski, 1993). Roquefortine was detected in 1 of 10 samples of Valdeon cheese, a blue-mould ripened Spanish variety (López-Dias et al., 1996), in all 11 samples of European blue-mould cheeses purchased in Finland (Kokkonen et al., 2005a) and in all 30 samples of European blue-mould cheese examined in Italy (Finoli et al., 2001). The levels of roquefortine detected by Kokkonen et al. (2005b) ranged from 0.8 to 12 mg/kg, whereas levels reported by Finoli et al. (2001) were lower (0.08–1.47 mg/kg). Mycophenolic acid was detected in 4 of 12 samples of mouldy Spanish Manchego cheese (López-Dias et al., 1996) and 1 of 11 samples of blue-mould cheese in Finland (Kokkonen et al., 2005a). Ochratoxin A has recently been reported by Dall'Asta et al. (2008) in blue-mould cheeses from Italy (23 of 54 samples) and France (7 of 14 samples) at levels from 0.25 to 3.0 μg/kg. Cyclopiazonic acid detected in six samples of Italian Taleggio, a soft, smear-ripened cheese, was confined mainly to the rind (Finoli et al., 1999). Sterigmatocystin produced by *Aspergillus versicolor* was detected in the surface layer of hard cheeses in the Netherlands (Northolt et al., 1980). Mycotoxin levels reported in cheese are not usually considered to be of public health significance.

Mouldy cheese is unsuitable for sale and for manufacturing purposes. Protection from the Penicillia relies on clean production conditions, low temperature storage, low oxygen atmospheres, integrity of packaging materials, intact rinds, preservative impregnated wrappers and rapid turnover of stock.

Media Appendix

Aspergillus flavus and parasiticus agar (AFPA)

Peptone, bacteriological	10 g
Yeast extract	20 g
Ferric ammonium citrate	0.5 g
Chloramphenicol	100 mg
Agar	15 g
Dichloran (0.2% in ethanol, 1.0 ml)	2 mg
Water, distilled	1 l

After addition of all ingredients, sterilise by autoclaving at 121°C for 15 min. The final pH of this medium is 6.0–6.5.

Creatine sucrose neutral agar (CSN)

CS concentrate	10 ml
Sucrose	10 g
Creatine	5.0 g
KH_2PO_4	1.0 g
Bromocresol purple	0.05 g
Agar	15 g
Water, distilled to	1 l

Creatine sucrose (CS) concentrate

KCl	5 g
$MgSO_4 \cdot 7H_2O$	5 g
$FeSO_4 \cdot 7H_2O$	0.1 g
$ZnSO_4 \cdot 7H_2O$	0.1 g
$CuSO_4 \cdot 5H_2O$	0.05 g
Water, distilled to	100 ml

Sterilise by autoclaving at 121°C for 15 min. Final unadjusted pH is approximately 6.8. A pH between 5.5 and 6.8 is satisfactory. For identification of *Penicillium* subgenus *Penicillium* species.

Czapek concentrate

$NaNO_3$	30 g
KCl	5 g
$MgSO_4 \cdot 7H_2O$	5 g
$FeSO_4 \cdot 7H_2O$	0.1 g
Water, distilled	100 ml

Czapek concentrate will keep indefinitely without sterilisation. The precipitate of $Fe(OH)_3$ which forms in time can be resuspended by shaking before use.

Czapek iprodione dichloran agar (CZID)

Sucrose	30 g
Yeast extract	5 g
Chloramphenicol	100 mg
Dichloran (0.2% in ethanol, 1 ml)	2 mg
Czapek concentrate	10 ml
Trace metal solution	1 ml
Agar	15 g
Water, distilled	1 l
Iprodione (suspension)	1 ml

Add iprodione suspension [0.3 g Roval 50 WP (Rhone-Poulenc Agro-Chemie, Lyon, France) in 50 ml sterile water, shaken before addition to medium] after autoclaving. Sterilise by autoclaving at 121°C for 15 min. This formulation is an adaptation of the original published formulation (Abildgren et al., 1987) made from basic ingredients rather than using commercial Czapek–Dox broth. Chloramphenicol (100 mg/l) replaces the original combination of chlortetracycline (50 mg) and chloramphenicol (50 mg).

Czapek yeast extract agar (CYA)

K_2HPO_4	1 g
Czapek concentrate	10 ml
Trace metal solution	1 ml
Yeast extract, powdered	5 g
Sucrose	30 g

Agar	15 g
Water, distilled	1 l

Refined table grade sucrose is satisfactory for use in CYA provided it is free from sulphur dioxide. Sterilise by autoclaving at 121°C for 15 min. The final pH is 6.7.

Czapek yeast extract agar with 20% sucrose (CY20S)

K_2HPO_4	1 g
Czapek concentrate	10 ml
Yeast extract	5 g
Sucrose	200 g
Agar	15 g
Water, distilled	1 l

Sterilise by autoclaving at 121°C for 15 min. The final pH is 5.2.

Dichloran chloramphenicol malt extract agar (DCMA)

Malt extract	10 g
Dichloran	2 mg
(0.2% w/v in ethanol, 1 ml)	
Chloramphenicol	0.1 g
Agar	15 g
Water, distilled to	1 l

Sterilise by autoclaving at 121°C for 15 min. Recommended for identification of *Alternaria* species and some other dematiaceous Hyphomycetes. The final pH is 5.5–6.0.

Dichloran chloramphenicol peptone agar (DCPA)

Peptone	15 g
KH_2PO_4	1 g
$MgSO_4 \cdot 7H_2O$	0.5 g
Chloramphenicol	0.1 g
Dichloran	2 mg
(0.2% in ethanol, 1 ml)	
Agar	15 g
Water, distilled	1 l

After addition of all ingredients, sterilise by autoclaving at 121°C for 15 min. The final pH of this medium is 5.5–6.0.

Dichloran 18% glycerol agar (DG18)

Glucose	10 g
Peptone	5 g
KH_2PO_4	1 g
$MgSO_4 \cdot 7H_2O$	0.5 g
Glycerol, A.R.	220 g
Agar	15 g
Dichloran	2 mg
(0.2% w/v in ethanol, 1 ml)	
Chloramphenicol	100 mg
Water, distilled	1 l

To produce this medium, add minor ingredients and agar to ca. 800 ml distilled water. Steam to dissolve agar, then make to 1 l with distilled water. Add glycerol: note that the final concentration is 18% w/w, not w/v. Sterilise by autoclaving at 121°C for 15 min. The final a_w of this medium is 0.955 and pH is in the range 5.5–5.8.

Dichloran rose bengal chloramphenicol agar (DRBC)

Glucose	10 g
Peptone, bacteriological	5 g
KH_2PO_4	1 g
$MgSO_4 \cdot 7H_2O$	0.5 g
Agar	15 g
Rose bengal	25 mg
(5% w/v in water, 0.5 ml)	
Dichloran	2 mg
(0.2% w/v in ethanol, 1 ml)	
Chloramphenicol	100 mg
Water, distilled	1 l

After the addition of all ingredients, sterilise by autoclaving at 121°C for 15 min. The final pH is in the range 5.5–5.8. Store prepared media away from light; photoproducts of rose bengal are highly inhibitory to some fungi, especially yeasts. In the dark, the medium is stable for at least 1 month at 1–4°C. The stock solutions of rose bengal and dichloran need no sterilisation, and are also stable for very long periods. The chlortetracycline in the original formulation of King et al. (1979) has been replaced with chloramphenicol, an effective antibiotic originally recommended for mycological media by Put (1974). Media containing chloramphenicol are easier to prepare, are not affected by autoclaving and have greater long-term stability.

Dichloran rose bengal yeast extract sucrose agar (DRYS)

Yeast extract	20 g
Sucrose	150 g
Dichloran	2 mg
(0.2% in ethanol, 1 ml)	
Rose bengal	25 mg
(5% w/v in water, 0.5 ml)	
Chloramphenicol	50 mg
Agar	20 g

Water, distilled	to 1 l
Chlortetracycline	50 mg
(1% in water, filter sterilised, 5 ml)	

Sterilise all ingredients except chlortetracycline by autoclaving at 121°C for 15 min. Add chlortetracycline after tempering to 50°C. In our experience, chloramphenicol at twice the concentration specified (i.e. 100 mg/l) adequately controls bacteria in most situations, and this avoids the need for a second antibiotic which must be filter sterilised.

25% Glycerol nitrate agar (G25N)

K_2HPO_4	0.75 g
Czapek concentrate	7.5 ml
Yeast extract	3.7 g
Glycerol, analytical grade	250 g
Agar	12 g
Water, distilled	750 ml

Glycerol for G25N should be of high quality, with a low (1%) water content. If a lower grade is used, allowance should be made for the additional water. Sterilised by autoclaving at 121°C for 15 min. The final pH is 7.0.

Malt acetic agar (MAA)

To 100 ml sterile tempered malt extract agar, aseptically add 0.5 ml of glacial acetic acid, giving a final concentration of 0.5% acetic acid. Mix well before pouring. Note that MAA cannot be autoclaved or reheated as the low pH (approx. 3.2) causes the agar gel to break down if the medium is subjected to any further heat treatment after the addition of acetic acid. There is no need to sterilise the glacial acetic acid.

Malt extract agar (MEA)

Malt extract, powdered	20 g
Peptone	1 g
Glucose	20 g
Agar	20 g
Water, distilled	1 l

Commercial malt extract used for home brewing is satisfactory for use in MEA, as is bacteriological peptone. Sterilise by autoclaving at 121°C for 15 min. Do not sterilise for longer, as this medium will become soft on prolonged or repeated heating. The final pH is 5.6.

Malt extract yeast extract 50% glucose agar (MY50G)

Malt extract	10 g
Yeast extract	2.5 g
Agar	10 g
Water, distilled	to 500 g
Glucose, A.R.	500 g

Add the minor constituents and agar to ca. 450 ml distilled water and steam to dissolve the agar. Immediately make up to 500 g with distilled water. While the solution is still hot, add the glucose all at once and stir rapidly to prevent the formation of hard lumps of glucose monohydrate. If lumps do form, dissolve them by steaming for a few minutes. Sterilise by steaming for 30 min; note that this medium is of a sufficiently low a_w not to require autoclaving. Food grade glucose monohydrate (dextrose) may be used in this medium instead of analytical reagent grade glucose, but allowance must be made for the additional water present. Use 550 g of $C_6H_{12}O_6 \cdot H_2O$, and 450 g of the basal medium. As the concentration of water is unaffected by this procedure, the quantities of the minor ingredients are unaltered. The final a_w of this medium is 0.89. The final pH is 5.3.

Malt extract yeast extract 70% glucose fructose agar (MY70GF)

Malt extract	6 g
Yeast extract	1.5 g
Agar	6 g
Water, distilled	to 300 g
Glucose, A.R.	350 g
Fructose, A.R.	350 g

After steaming to dissolve agar, make the solution accurately to 300 g with water and, while it is still hot, add both sugars. Steam gently for up to 30 min to completely dissolve the sugars. Further sterilisation is unnecessary: contaminant micro-organisms of any kind are unable to grow on this medium, which is about 0.76 a_w. MY70GF will take some hours to gel, because of the low proportion of water and agar. If possible, allow 24 h after pouring for the medium to attain gel strength before use.

Malt extract yeast extract 5% (or 10%) salt 12% glucose agar (MY5-12 and MY10-12)

Malt extract	20 g
Yeast extract	5 g
NaCl	50 g
(100 g for MY10-12)	
Glucose	120 g
Agar	20 g
Water, distilled	to 1 l

Sterilise MY5-12 by autoclaving at 121°C for 10 min, and MY10-12 by steaming for 30 min. Overheating of these media will cause softening. The final a_w of MY5-12 is 0.93 and of MY10-12 is 0.88.

Oxytetracycline glucose yeast extract agar (OGY)

Glucose	20 g
Yeast extract	5 g
Agar	15 g
Water, distilled	1 l
Oxytetracycline	100 mg

Sterilise by autoclaving at 121°C for 15 min. After tempering to 50°C, add 10 ml of filter-sterilised oxytetracycline (Terramycin, Pfizer; 0.1% aqueous) per 100 ml of medium. The final pH is 6.8–7.2.

Potato dextrose agar (PDA)

Potatoes	250 g
Glucose	20 g
Agar	15 g
Water, distilled	to 1 l

PDA prepared from raw ingredients is more satisfactory than commercially prepared media. Wash the potatoes, which should not be of a red skinned variety, and dice or slice, unpeeled, into 500 ml of water. Steam or boil for 30–45 min. At the same time, melt the agar in 500 ml of water. Strain the potato through several layers of cheese cloth into the flask containing the melted agar. Squeeze some potato pulp through also. Add the glucose, mix thoroughly, and make up to 1 l with water if necessary. Sterilise by autoclaving at 121°C for 15 min.

Tap water agar (TWA)

Agar	15 g
Tap water	to 1 l

Sterilise by autoclaving at 121°C for 15 min. Natural substrates such as carnation leaf pieces, wheat straw, wheat or millet grains may be added to TWA plates after the agar is poured and before it sets. These can provide a substrate for growth and sporulation of plant pathogenic fungi like *Fusarium*, *Drechslera, Bipolaris* and some other dematiaceous Hyphomycetes.

Trace metal solution

$CuSO_4 \cdot 5H_2O$	0.5 g
$ZnSO_4 \cdot 7H_2O$	1 g
Water, distilled	100 ml

Keeps indefinitely without sterilisation.

Tryptone glucose yeast extract agar (TGY)

Glucose	100 g
Tryptone	5 g
Yeast extract	5 g
Chloramphenicol	0.1 g
Agar	15 g
Distilled water	to 1 l

Sterilise by autoclaving at 121°C for 10 min. Prolonged heating will cause browning of the medium. Chloramphenicol may be omitted if suppression of growth of bacteria is not required. The final pH is 5.5–6.0.

Tryptone glucose yeast extract acetic agar (TGYA)

Make as for malt acetic agar (MAA), but use TGY agar without chloramphenicol as the base rather than MEA. As with MAA, TGYA should not be reheated. The final pH is 3.8.

Tryptone glucose yeast extract broth (TGY broth)

Make as for TGY agar, but omit the agar from the formulation.

Tryptone glucose yeast extract acetic broth (TGYA broth)

Make as for TGY broth with the addition of glacial acetic acid to give a final concentration of 0.5%. Sterilise by steaming for 30 min. The final pH is 3.8.

V-8 Juice agar (V-8 J)

V-8 Juice, clarified	200 ml
$CaCO_3$	23 g
Agar	20 g
Water, distilled	800 ml

Clarify V-8 juice by straining through cheese cloth. Mix ingredients well and sterilise by autoclaving at 121°C for 15 min.

Glossary

A

acerose needle-like; shaped like a pine needle.

acervulus a flat- or cup-shaped fruiting body, usually embedded in the agar, containing conidiophores and conidia.

aleurioconidium a terminal conidium, usually thick-walled, blown out from the end of a sporogenous cell.

ampulliform flask-shaped.

anamorph the asexual or conidial form of a fungus.

annelide a conidiogenous cell which produces conidia in succession, each conidium being produced through the scar of the previous one, leaving a ring-like scar at the apex of the spore-bearing cell.

apical at the apex, e.g. of a hypha or phialide.

apiculate having a short projection at one end.

arthroconidium (pl. arthroconidia) conidia, often cylindrical, produced by fragmentation of hyphae into separate cells.

ascocarp a fruiting body in Ascomycetes—containing asci and ascospores.

ascospore a sexual spore formed in an ascus.

ascus (pl. asci) a thin-walled sac containing ascospores, usually eight, but in some cases one, two or four.

aseptate without any crosswalls; usually refers to hyphae.

asporogenous not having any spores.

B

basipetal describes the succession of conidia in which the youngest conidium is at the base of the chain.

biverticillate having two branching points; usually referring to a penicillus or similar spore-bearing structure with metulae and phialides.

C

clavate club-shaped.

chlamydoconidium (pl. chlamydoconidia) a thick-walled resting spore formed by the swelling and thickening of a single cell, usually within a hypha.

cleistothecium an ascocarp with a well-defined wall, but without a special opening (ostiole).

collula the necks of phialides or annelides.

columella in some Mucorales species, the swollen tip of the sporangiophore formed within the sporangium.

coelomycete a fungus–forming conidia in a closed body such as an acervulus.

conidioma (pl. conidiomata) any structure which bears conidia, including conidiophores, acervulae, pycnidia and sporodochia.

conidiophore a specialised hypha, either simple or branched, bearing conidiogenous cells and conidia.

conidium (pl. conidia) an asexually produced spore.

coremium (pl. coremia) an erect, compact, sometimes fused cluster of conidiophores, bearing conidia at the apex only, or on both apex and sides.

D

dendritic irregularly branched; tree-like.

denticle a smooth tooth-like projection, especially one on which a spore is borne.

doliiform barrel-shaped.

E

ellipsoidal elliptical in optical section.

exudate drops of liquid on the surface of fungal colonies; sometimes minute droplets adhering to hyphae.

F

fascicle a little group or bundle, especially of hyphae.

fimbriate fringed; delicately toothed; referring to colony margins.

floccose cottony, fluffy.

footcell in *Aspergillus*, the basal cell from which a stipe forms.

funicle a fine rope of hyphae.

funiculose aggregated into rope-like strands.

fusiform spindle-like; narrowing towards the ends.

G

geniculate bent like a knee.

gymnothecium an ascocarp having walls composed of hyphae.

H

holomorph referring to the whole fungus; both anamorph and teleomorph.

hyphomycete a fungus-bearing conidia on an aerial fruiting structure.

Hülle cells thick-walled cells surrounding ascocarps in, e.g. *Emericella nidulans*.

hyaline transparent or nearly so; colourless.

I

intercalary between the apex and the base.

M

macroconidium in *Fusarium*, the larger type of conidium, multicelled and more or less curved.

merosporangium in Mucorales, a cylindrical outgrowth from the swollen end of the sporangiophore, in which sporangiospores are produced.

metula (pl. metulae) apical branch of a stipe-bearing phialides, especially in *Penicillium* and *Aspergillus*.

microconidium in *Fusarium* species, a small, usually one-celled conidium, distinction from macroconidium.

N

nonseptate without any crosswalls; usually referring to hyphae.

O

oblate flattened at the poles.

ogival pointed at one end, rounded at the other.

ontogeny development (of fruiting structures or conidia).

ostiole a pore by which spores are freed from an ascocarp or other enveloping fruiting body.

P

papilla a small, rounded process.

pedicel a small stalk.

penicillus the structure which bears conidia in *Penicillium* and similar genera; consisting of phialides alone or in combination with metulae or other supportive elements, borne on a stipe.

perithecium (pl. perithecia) a subglobose or flask-shaped ascocarp, closed at maturity except for a narrow passage (ostiole) through which the ascospores are liberated.

phialide a conidiogenous cell which produces conidia in basipetal succession, without an increase in the length of the phialide itself.

pionnotes a spore mass with a mucoid or grease-like appearance (in *Fusarium*).

polyphialide conidiogenous cell with more than one opening, through which conidia are produced in basipetal succession.

pycnidium flask-shaped or spherical fruiting body superficially resembling a perithecium, but lined with conidiophores and conidia.

pyriform pear-shaped.

R

ramus (pl. rami) a specialised cell giving rise to a whorl of metulae and phialides.

reniform kidney-shaped.

rhizoid a root-like structure, usually acting as a holdfast or feeding organ for hyphae (in *Rhizopus*).

rugose with surface roughened.

S

sclerotium (pl. sclerotia) a resting body, usually globose, consisting of a compacted mass of mycelium, often very hard.

sclerotioid hard, like a sclerotium.

septum (pl. septa) a crosswall in a cell.

spinose spiny.

sporangiole a small sporangium.

sporangiophore a specialised hyphal branch which supports one or more sporangia.

sporangiospore an asexual spore borne within a sporangium.

sporangium (pl. sporangia) a closed unicellular structure, usually round, in which asexual spores are produced (e.g. in Mucorales).

s**porodochium (pl. sporodochia)** a cushion-like mass of conidiogenous cells producing conidia (e.g. macroconidia in *Fusarium*).

stipe a hypha supporting a fruiting structure, the whole forming a conidiophore.

stolon a "runner", as in *Rhizopus*.

striate marked with ridges, grooves or lines.
stroma (pl. stromata) a layer or matrix of vegetative hyphae bearing spores on very short conidiophores, or having perithecia or pycnidia embedded in it.

sympodial describes a mechanism of conidiogenous cell proliferation in which each new growing point appears just behind and to one side of the previous apex, producing a succession of fruiting structures.

sulcate furrowed or grooved.

T

teleomorph the ascosporic state of a fungus.

terverticillate refers to a penicillus with three branch points, i.e. bearing rami, metulae and phialides.

truncate ending abruptly, as if cut straight across.

U

umbonate having the central portion of the colony raised.

V

velutinous with a surface texture like velvet.

verticil a cluster of metulae or phialides with a common origin.

vesicle a swelling; the apical swelling of a stipe.

vesiculate terminating in a vesicle.

X

xerophile a fungus which is able to grow at or below a water activity of 0.85.

Z

zygospore a thick-walled sexual spore produced by Zygomycetes.

References

Aal, W.E.A., Abdel-Magied, M.M., Alian, A.M., Alla, E.A. M.A. and Hegazy, E.M. 1997. Histopathological change of sterigmatocystin in rat liver. Egypt. J. Vet. Sci. 31: 89–99.

Abarca, M.L., Bragulat, M.R., Castellá, G. and Cabañes, F.J. 1994. Ochratoxin A production by strains of *Aspergillus niger* var. *niger*. Appl. Environ. Microbiol. 60: 2650–2652.

Abarca, M.L., Accensi F., Bragulat, M.R., Castellá G. and Cabañes, F.J. 2003. *Aspergillus carbonarius* as the main source of ochratoxin A contamination in dried vine fruits from the Spanish market. J. Food Prot. 66: 504–506.

Abarca, M.L., Accensi, F., Cano, J. and Cabañes, F.J. 2004. Taxonomy and significance of black aspergilli. Antonie van Leeuwenhoek 86: 33–49.

Abbas, H.K. and Bosch, U. 1990. Evaluation of trichothecene and nontrichothecene mycotoxins produced by *Fusarium* in soybeans. Mycotoxin Res. 6: 13–20.

Abbas, H.K. and Mirocha, C.J. 1988. Isolation and purification of a hemorrhagic factor (wortmannin) from *Fusarium oxysporum* (N17B). Appl. Environ. Microbiol. 54: 1268–1274.

Abbas, H.K., Mirocha, C.J., Meronuck, R.A., Pokorny, J.D., Gould, S.L. and Kommedahl, T. 1988. Mycotoxins and *Fusarium* spp. associated with infected ears of corn in Minnesota. Appl. Environ. Microbiol. 54: 1930–1933.

Abbas, H.K., Mirocha, C.J. and Gunther, R. 1989a. Mycotoxins produced by toxic *Fusarium* isolates obtained from agricultural and nonagricultural areas (Arctic) of Norway. Mycopathologia 105: 143–151.

Abbas, H.K., Mirocha, C.J., Kommedahl, T., Vesonder, R.F. and Golinski, P. 1989b. Production of trichothecene and non-trichothecene mycotoxins by *Fusarium* species isolated from maize in Minnesota. Mycopathologia 108: 55–58.

Abbas, H.K., Mirocha, C.J. and Gunther, R. 1991. Production of zearalenone, nivalenol, moniliformin, and wortmannin from toxigenic cultures of *Fusarium* obtained from pasture soil samples collected in New Zealand. Mycotoxin Res. 7: 53–60.

Abbas, H.K., Mirocha, C.J., Shier, W.T. and Gunther, R. 1992. Bioassay, extraction, and purification procedures for wortmannin, the hemorrhagic factor produced by *Fusarium oxysporum* N17B grown on rice. J. AOAC Int. 75: 474–480.

Abbas, H.K., Ocamb, C.M., Xie W.P., Mirocha, C.J. and Shier, W.T. 1995. First report of fumonisin B1, B2 and B3 production by *Fusarium oxysporum* var. *redolens*. Plant Dis. 79: 968.

Abd-Elsalam, K.A., Schnieder, F., Asran-Amal, A., Khalil, M.S. and Verreet, J.A. 2003. Intra-species genomic groups in *Fusarium semitectum* and their correlation with origin and cultural characteristics. Zeitschr. Pflanzenkrankheiten Pflanzenschutz 110: 409–418.

Abdel-Hafez, S.I.I. and Saber, S.M. 1993. Mycoflora and mycotoxin of hazelnut (*Corylus avellana* L.) and walnut (*Juglans regia* L.) seeds in Egypt. Zentralbl. Mikrobiol. 148: 137–147.

Abdel-Hafez, S.I.I., El-Kady, I.A., Mazen, M.B. and El-Maghraby, A.D. 1987. Mycoflora and trichothecene toxins of paddy grains from Egypt. Mycopathologia 100: 103–112.

Abdel-Kader, M.I.A., Moubasher, A.H. and Abdel-Hafez, S.I.I. 1979. Survey of the mycoflora of barley grains in Egypt. Mycopathologia 69: 143–147.

Abe, A., Sujaya, I.N., Sone, T., Asano, K. and Oda, Y. 2004. Microflora and selected metabolites of potato pulp fermented with an Indonesian starter Ragi tape. Food Technol. Biotechnol. 42: 169–173.

Abe, A., Oda, Y., Asano, K. and Sone, T. 2006. The molecular phylogeny of the genus *Rhizopus* based on rDNA sequences. Biosci. Biotechnol. Biochem. 70: 2387–2393.

Abellana, M., Torres, L., Sanchis, V. and Ramos, A.J. 1997. Characterisation of different industrial bakery products. II. Study of mycoflora. Alimentaria 35(287): 51–56.

Abildgren, M.P., Lund, F., Thrane, U. and Elmholt, S. 1987. Czapek-Dox agar containing iprodione and dicloran as a selective medium for the isolation of *Fusarium*. Lett. Appl. Microbiol. 5: 83–86.

Aboagye-Nuamah, F., Offei, S.K., Cornellius, E.W. and Bancroft, R.D. 2005. Severity of spoilage storage rots of white yam (*Dioscorea rotundata* Poir.). Ann. Appl. Biol. 147: 183–190.

Abramson, D., Sinha, R.N. and Mills, J.T. 1980. Mycotoxin and odor formation in moist cereal grain during granary storage. Cereal Chem. 57: 346–351.

Abramson, D., Clear, R.M. and Nowicki, T.W. 1987. *Fusarium* species and trichothecene mycotoxins in suspect samples of 1985 Manitoba wheat. Can. J. Plant Sci. 67: 611–619.

Abramson, D., Clear, R.M. and Smith, D.M. 1993. Trichothecene production by *Fusarium* spp. isolated from Manitoba grain. Can. J. Plant Pathol. 15: 147–152.

Abramson, D., Clear, R.M., Gaba, D., Smith, D.M., Patrick, S.K. and Saydak, D. 2001. Trichothecene and moniliformin production by *Fusarium* species from Western Canadian wheat. J. Food Prot. 64: 1220–1225.

Abramson, D., McCallum, B., Tekauz, A. and Smith, D.M. 2004. HT-2 and T-2 toxins in barley inoculated with *Fusarium sporotrichioides*. Can. J. Plant Sci. 84: 1189–1192.

Abranches, J., Vital, M.J.S., Starmer, W.T., Mendonca-Hagler, L.C. and Hagler, A.N. 2000. The yeast community and mycocin producers of guava fruit in Rio de Janeiro, Brazil. Mycologia 92: 16–22.

Accensi, F., Abarca, M. L. and Cabañes, F. J. 2004. Occurrence of *Aspergillus* species in mixed feeds and component raw materials and their ability to produce ochratoxin A. Food Microbiol. 21: 623–627.

Adamek, P., Bergström, B., Börjesson, T. and Stöllman, U. 1992. Determination of volatile compounds for the detection of moulds. *In* Modern Methods in Food Mycology, eds R.A. Samson, A.D. Hocking, J.I. Pitt and A.D. King. Amsterdam: Elsevier. pp. 327–336.

Adaskaveg, J.E. and Ogawa, J.M. 1994. Penetration of iprodione into mesocarp fruit tissue and suppression of gray mold and brown rot of sweet cherries. Plant Dis. 78: 293–296.

Addis, E., Fleet, G.H., Cox, J.M., Kolak, D. and Leung, T. 2001. The growth, properties and interactions of yeasts and bacteria associated with the maturation of Camembert and blue-veined cheeses. Int. J. Food Microbiol. 69: 25–36.

Adebajo, L.O. 1994. Isolation and assay for the toxicity of fungi from kola nuts. Chem. Mikrobiol. Technol. Lebensm. 16: 25–28.

Adebajo, L.O. 2000. Fungal infection and aflatoxin production in kola nuts. Crop Res. 20: 469–475.

Adebajo, L.O. and Diyaolu, S.A. 2003. Mycology and spoilage of retail cashew nuts. Afr. J. Biotechnol. 2: 369–373.

Adebajo, L.O. and Oyesiku, O.O. 1994. Investigation on the toxicity of fungi from rootstock snacks. Nahrung 38: 26–31.

Adebajo, L.O. and Popoola, O.J. 2003. Mycoflora and mycotoxins in kolanuts during storage. Afr. J. Biotechnol. 2: 365–368.

Adebajo, L.O., Idowu, A.A. and Adesanya, O.O. 1994. Mycoflora and mycotoxin production in Nigerian corn and corn-based snacks. Mycopathologia 126: 183–192.

Adegoke, G.O., Akinnuoye, O.F.A. and Akanni, A.O. 1993. Effect of processing on the mycoflora and aflatoxin B_1 level of a cassava-based product. Plant Foods Hum. Nutr. 43: 191–196.

Adejumo, T.O., Hettwer, U. and Karlovsky, P. 2007. Occurrence of *Fusarium* species and trichothecenes in Nigerian maize. Int. J. Food Microbiol. 116: 350–357.

Adeniji, M.O. 1970a. Fungi associated with storage decay of yam in Nigeria. Phytopathology 60: 590–592.

Adeniji, M.O. 1970b. Influence of moisture and temperature on yam decay organisms. Phytopathology 60: 1698–1699.

Adisa, V.A. 1983. Fruit rots of *Capsicum annuum* L. and *C. frutescens* L. in Nigeria. III. Effect of post-harvest infection by two fusaria species on a few nutrients. Nahrung 27: 669–674.

Adisa, V.A. 1989. Pectic enzymes of *Aspergillus aculeatus* associated with post-harvest deterioration of *Citrus sinensis* fruit. J. Food Biochem. 13: 243–252.

Agnolucci, M., Scarano, S., Rea, F., Toffanin, A. and Nuti, M. 2007. Detection of *Dekkera/Brettanomyces bruxellensis* in pressed Sangiovese grapes by real time PCR. Ital. J. Food Sci. 19: 153–164.

Agut, M. and Calvo, M.A. 2004. *In vitro* germination of *Arthrinium areum* and *Arthrinium phaeospermum*. Mycopathologia 157: 363–367.

Ahamad, S., Srivastava, M. and Surulirajan, M. 2003. Fungi associated with rapeseed-mustard seeds, pathogenicity and chemical control. Annals Agric. Res. 24: 671–672.

Ahammed, S.K., Aggarwal, R., Gupta, V.K. and Singh, D.V. 2005. Optimising nutritional conditions for mass multiplication of *Chaetomium globosum* Kunze, an efficient biocontrol agent of *Bipolaris sorokiniana* (Sacc.) Shoemaker. Indian J. Plant Protection 33: 90–93.

Ahammed, S.K., Anandam, R.J., Babu, G.P., Munikrishnaiah, M. and Gopal, K. 2006. Studies on seed mycoflora of soybean and its effect on seed and seedling quality. Legume Res. 29: 186–190.

Ahmad, M.S. and Sinha, K.K. 2006. Mycotoxin contamination of mustard (*Brassica juncea* L.) seeds at the time of harvest. J. Mycopathol. Res. 40: 159–162.

Ahmad, R. and Magan, N. 2003. Monsooned coffee: impact of environmental factors on fungal community structure, enzyme production profiles and ochratoxin. Aspects Appl. Biol. 68: 161–168.

Ahmad, S.K. 1993. Mycoflora changes and aflatoxin production in stored blackgram seeds. J. Stored Prod. Res. 29: 33–36.

Ahmad, S.K. and Singh, P.L. 1991. Mycofloral changes and aflatoxin contamination in stored chickpea seeds. Food Addit. Contam. 8: 723–730.

Ahmed, E.K. and Abdel-Sater, M.A. 2003. Mycological quality of laban rayeb sold in Assiut City. Assiut Vet. Med. J. 49: 70–80.

Aja-Nwachukwu, J. and Emejuaiwe, S.O. 1994. Aflatoxin-producing fungi associated with Nigerian maize. Environ. Toxicol. Water Qual. 9: 17–23.

Aksoy, U., Eltem, R., Meyvaci, K.B., Altindisli, A. and Karabat, S. 2007. Five-year survey of ochratoxin A in processed sultanas from Turkey. Food Addit. Contam. 24: 292–296.

Alasoadura, S.O. 1970. Culture studies on *Botryodiplodia theobromae* Pat. Mycopathol. Mycol. Appl. 42: 153–160.

Alcorn, J.L. 1983. Generic concepts in *Drechslera, Bipolaris* and *Exserohilum*. Mycotaxon 17: 1–86.

Alderman, S.C. and Lacy, M.L. 1984. Influence of temperature and water potential on growth of *Botrytis allii*. Can. J. Bot. 62: 1567–1570.

Ali, S., Rivera, V.V. and Secor, G.A. 2005. First report of *Fusarium graminearum* causing dry rot of potato in North Dakota. Plant Dis. 89: 105.

Almela, L., Rabe, V., Sánchez, B., Torrella, F., López-Pérez, J.P., Gabaldón, J.A. and Guardiola, L. 2007. Ochratoxin A in red paprika: relationship with the origin of the raw material. Food Microbiol. 24: 319–327.

Al-Musallam, A. 1980. Revision of the black *Aspergillus* species. Ph.D. thesis. Utrecht, Netherlands: University of Utrecht.

Alonso-Calleja, C., Carballo, J., Capita, R., Bernardo, A. and García-López, M.L. 2002. Evaluation of the spiral plating method for the enumeration of microorganisms throughout the manufacturing and ripening of raw goat's milk cheese. J. Food Prot. 65: 339–344.

Alvarez-Rodriguez, M.L., Lopez-Ocana, L., Lopez-Coronado, J.M., Rodriguez, E., Martinez, M.J., Larriba, G. and Coque, J-J. R. 2002. Cork taint of wines: role of the filamentous fungi isolated from cork in the formation of 2,4,6-trichloroanisole by O methylation of 2,4,6-trichlorophenol. Appl. Environ. Microbiol. 68: 5860–5869.

Alves, I., Oliveira, N.G., Laires, A., Rodrigues, A.S. and Rueff, J. 2000. Induction of micronuclei and chromosomal aberrations by the mycotoxin patulin in mammalian cells: role of ascorbic acid as a modulator of patulin clastogenicity. Mutagenesis 5: 229–234.

Alves, A., Crous, P.W., Correia, A. and Phillips, A.J.L. 2008. Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. Fungal Divers. 28: 1–13.

Amiri, A. and Bompeix, G. 2005. Diversity and population dynamics of *Penicillium* spp. on apples in pre- and post-harvest environments: consequences for decay development. Plant Pathol. 54: 74–81.

Amusa, N.A. and Baiyewu, R.A. 1999. Storage and market disease of yam tubers in Southwestern Nigeria. Ogun J. Agric. Res. (Nigeria) 11: 211–225.

Amusa, N.A., Ashaye, O.A. and Oladapo, M.O. 2003. Biodeterioration of the African star apple (*Chrysophylum albidum*) in storage and the effect on its food value. Afr. J. Biotechnol. 2: 56–59.

Amusa, N.A., Ashaye, O.A. and Oladapo, M.O. 2005. Microbiological quality of ogi and soy-ogi (a Nigerian fermented cereal porridge) widely consumed and notable weaning food in southern Nigeria. J. Food Agric. Environ. 3: 81–83.

Anand, S. and Rati, E.R. 2006. An enzyme-linked immunosorbent assay for monitoring of *Aspergillus ochraceus* growth in coffee powder, chilli powder and poultry feed. Lett. Appl. Microbiol. 42: 59–65.

Ancasi, E.G., Carrillo, L. and Benitez Ahrendts, M.R. 2006. Moulds and yeasts in bottled water and soft drinks. Rev. Argentina Microbiol. 38: 93–96.

Andersen, B. and Frisvad, J.C. 2004. Natural occurrences of fungi and fungal metabolites in moldy tomatoes. J. Agric. Food Chem. 52: 7507–7513.

Andersen, B. and Hollensted, M. 2008. Metabolite production by different *Ulocladium* species. Int. J. Food Microbiol. 126: 172–179.

Andersen, B. and Thrane, U. 1996. Secondary metabolites produced by *Alternaria infectoria* and their use as chemotaxonomic markers. Mycotoxin Res. 12: 54–60.

Andersen, B., Solfrizzo, M. and Visconti, A. 1995. Metabolite profiles of common *Stemphylium* species. Mycol. Res. 99: 672–676.

Andersen, B., Thrane, U., Svendsen, A. and Rasmussen, I.A. 1996. Associated field mycobiota on malt barley. Can. J. Bot. 74: 854–858.

Andersen, B., Kroger, E. and Roberts, R.G. 2002. Chemical and morphological segregation of *Alternaria arborescens*, *A. infectoria* and *A. tenuissima* species-groups. Mycol. Res. 106: 170–182.

Andersen, S.J. 1995. Compositional changes in surface mycoflora during ripening of naturally fermented sausages. J. Food Prot. 58: 426–429.

Andersen, S.J. and Frisvad, J.C. 1994. Penicillin production by *Penicillium nalgiovense*. Lett. Appl. Microbiol. 19: 486–488.

Andrews, S. 1992a. Comparative study of WL Nutrient agar with DRBC and OGY for yeast enumeration in foods. *In* Modern Methods in Food Mycology, eds R.A. Samson, A.D. Hocking, J.I. Pitt and A.D. King. Amsterdam: Elsevier. pp. 61–65.

Andrews, S. 1992b. Differentiation of *Alternaria* species isolated from cereals on dichloran malt extract agar. *In* Modern Methods in Food Mycology, eds R.A. Samson, A.D. Hocking, J.I. Pitt and A.D. King. Amsterdam: Elsevier. pp. 351–355.

Andrews, S. and Pitt, J.I. 1986. Selective media for *Fusarium* species and dematiaceous Hyphomycetes from cereals. Appl. Environ. Microbiol. 51: 1235–1238.

Andrews, S. and Pitt, J.I. 1987. Further studies on the water relations of xerophilic fungi, including some halophiles. J. Gen. Microbiol. 133: 233–238.

Angayarkanni, J., Palaniswamy, M., Murugesan, S. and Swaminathan, K. 2002. Improvement of tea leaves fermentation with *Aspergillus* spp. pectinase. J. Biosci. Bioeng. 94: 299–303.

Anon. 1967. Unusual heat resistance mould in apple juice. Food Ind. S. Afr. 19: 55–56.

Antony, M., Shukla, Y. and Janardhanan, K.K. 2003. Potential risk of acute hepatotoxicity of kodo poisoning due to exposure to cyclopiazonic acid. J. Ethnopharmacol. 87: 211–214.

Aoki, T. and O'Donnell, K. 1999a. Morphological and molecular characterization of *Fusarium pseudograminearum* sp. nov., formerly recognized as the Group 1 population of *F. graminearum*. Mycologia 91: 597–609.

Aoki, T. and O'Donnell, K. 1999b. Morphological characterization of *Gibberella coronicola* sp. nov., obtained through mating experiments of *Fusarium pseudograminearum*. Mycoscience 40: 443–453.

Apinis, A.E. 1972. Mycological aspects of stored grain. *In* Biodeterioration of Materials, Vol. 2, eds A.H. Walters and E.H. Hueck-van der Plas. London: Applied Science Publishers. pp. 493–498.

Aran, N. and Eke, D. 1987. Mould mycoflora of some Turkish cereals and cereal products. J. Appl. Microbiol. Biotechnol. 3: 281–287.

Araujo, R. and Rodriges, A.G. 2004. Variability of germinative potential among pathogenic species of *Aspergillus*. J. Clin. Microbiol. 42: 4335–4337.

Ardhana, M.M. and Fleet, G.H. 2003. The microbial ecology of cocoa bean fermentations in Indonesia. Int. J. Food Microbiol. 86: 87–99.

Arjona, R.E.E., Albarracin, N.S. and de Rodrigez, O.S.A. 1996. Diagnostics of post-harvest fungal diseases in carrot roots (*Daucus carota* L.) in Venezuela. Rev. Mex. Fitopatol. 14: 59–64.

Armolik, N. and Dickson, J.G. 1956. Minimum humidity requirements for germination of conidia associated with storage of grain. Phytopathology 46: 462–465.

Arras, G. 1993. Inhibition of postharvest fungal pathogens by *Bacillus subtilis* strains isolated from citrus fruit. Adv. Hortic. Sci. 7: 123–127.

Arroyo-Lopez, F.N., Duran-Quintana, M.C., Ruiz-Barba, J. L., Querol, A. and Garrido-Fernandez, A. 2006. Use of molecular methods for the identification of yeast associated with table olives. Food Microbiol. 23: 791–796.

Arrus, K., Blank, G., Clear, R., Holley, R.A. and Abramson, D. 2005. Microbiological and aflatoxin evaluation of Brazil nut pods and the effects of unit processing operations. J. Food Prot. 68: 1060–1065.

Arsvoll, K. 1975. Fungi causing winter damage on cultivated grasses in Norway. Meld. Nor. Landbrukschoegsk. 54: 49.

Arya, A. 2004. Mycoflora associated with walnut fruit in Baroda. J. Mycol. Plant Pathol. 34: 128–129.

Asehraou, A., Peres, C., Brito, D., Faid, M. and Serhrouchni, M. 2000. Characterization of yeast strains isolated from bloaters of fermented green table olives during storage. Grasas Aceites. 51: 225–229.

Asgari, B., Zare, R. and Payghami, E. 2004. Hyphomycetous fungal community of barley phylloplane in East Azarbaijan Province with emphasis on new taxa for Iranian fungal flora. Rostaniha 5: 171–197.

Askun, T. 2006. Investigation of fungal species diversity of maize kernels. J. Biol. Sci. 6: 275–281.

Assante, G., Camarda, L., Locci, R., Merlini, L., Nasini, G. and Papadopoulos, E. 1981. Isolation and structure of red pigments from *Aspergillus flavus* and related species, grown on a differential medium. J. Agric. Food Chem. 29: 785–787.

Assawah, M.W. and Al-Zarari, A.J. 1984. Identification and study of fungi causing diseases and post-harvest rots of squash in Ninevah province, Iraq. Iraqi J. Agric. Sci. 2: 67–75.

Atanda, O.O., Akano, D.A. and Afolabi, J.F. 1990. Mycoflora of dry 'tatase' pepper (*Capsicum annuum* L.) stored for sale in Ibadan markets. Lett. Appl. Microbiol. 10: 35–37.

Atapattu, R. and Samarajeewa, U. 1990. Fungi associated with dried fish in Sri Lanka. Mycopathologia 111: 55–59.

Aubertine, C.L., Rivera, M., Rohan, S.M. and Larone, D.H. 2006. Comparative study of the new colorimetric VITEK 2 yeast identification card versus the older fluorometric card and of CHROMagar Candida as a source medium with the new card. J. Clin. Microbiol. 44: 227–228.

Auerbach, H., Oldenburg, E. and Weissbach, F. 1998. Incidence of *Penicillium roqueforti* and roquefortine C in silages. J. Sci. Food Agric. 76: 565–572.

Austwick, P.K.C. and Ayerst, G. 1963. Toxic products in groundnuts: groundnut microflora and toxicity. Chem. Ind. 1963: 55–61.

Avari, G.P. and Allsopp, D. 1983. The combined effect of pH, solutes, and water activity (a_w) on the growth of some xerophilic *Aspergillus* species. Biodeterioration 5: 548–556.

Aveskamp, M., de Gruyter, H. and Crous, P. 2006. *Phoma* barcoding: the next phase in taxonomic research in a complex fungal genus. Gewasbescherming 37: 266–269.

Aydin, A., Ulusoy, B.H. and Ergun, O. 2005. A survey of heat-resistant moulds in heat milk, milk products and fruit juices. Archiv. Lebensmittelhyg. 56: 58–60.

Ayerst, G. 1966. The influence of physical factors on deterioration by moulds. Soc. Chem. Ind. Monogr. 23: 14–20.

Ayerst, G. 1969. The effects of moisture and temperature on growth and spore germination in some fungi. J. Stored Prod. Res. 5: 669–687.

Ayesha, F. and Viswanath, P. 2006. *Byssochlamys* spp. in sugar cane juice and its significance. J. Food Sci. Tech. Mysore 43: 407–409.

Azeredo, L.A.I. de, Gomes, E.A.T., Mendonca-Hagler, L.C. and Hagler, A.N. 1998. Yeast communities associated with sugarcane in Campos, Rio de Janeiro, Brazil. Int. Microbiol. 1: 205–208.

Aziz, N.H., Mattar, Z.A. and Mahrous, S.R. 2006. Contamination of grains by mycotoxin-producing molds and mycotoxins and control by gamma irradiation. J. Food Saf. 26: 184–201.

Azziz-Baumgartner, E., Lindblade, K., Gieseker, K., Schurz Rogers, H., Kieszak, S., Njapau, H., Schleicher, R., McCoy, L.F., Misore, A., DeCock, K., Rubin, C., Slutsker, L. and the Aflatoxin Investigative Group. 2005. Case control study of an acute aflatoxicosis outbreak, Kenya, 2004. Environ. Health Perspect. 113: 1779–1783.

Back, W. and Anthes, S. 1979. Taxonomische untersuchungen an Limonadenschädlichen Hefen. Brauwissenschaft 32: 145–154.

Backhouse, D. and Burgess, L.W. 2002. Climatic analysis of the distribution of *Fusarium graminearum*, *F. pseudograminearum* and *F. culmorum* on cereals in Australia. Aust. Plant Pathol. 31: 321–327.

Bacon, C.W. and Nelson, P.E. 1994. Fumonisin production in corn by toxigenic strains of *Fusarium moniliforme* and *Fusarium proliferatum*. J. Food Prot. 57: 514–521.

Bacon, C.W., Porter, J.K., Norred, W.P. and Leslie, J.F. 1996. Production of fusaric acid by *Fusarium* species. Appl. Environ. Microbiol. 62: 4039–4043.

Baert, K., Valero, A., de Meullenaer, B., Samapundo, S., Ahmed, M.M., Bo, L., Debevre, J. and Devlieghere, F. 2007a. Modelling the effect of temperature on the growth rate and lag phase of *Penicillium expansum* in apples. Int. J. Food Microbiol. 118: 139–150.

Baert, K., Devlieghere, F., Flyps, H., Oosterlinck, M., Ahmed, M.M., Rajkovic, A.,Verlinden, B., Nicolai, B., Debevere, J. and Meulenaer, B. de. 2007b. Influence of storage conditions of apples on growth and patulin production by *Penicillium expansum*. Int. J. Food Microbiol. 119: 170–181.

Baggerman, W.I. and Samson, R.A. 1988. Heat resistance of fungal spores. *In* Introduction to Foodborne Fungi, 3rd edn, eds R.A. Samson and E.S. van Reenen-Hoekstra. Baarn, Netherlands: Centraalbureau voor Schimmelcultures. pp. 262–267.

Bailly, J.D., Tabuc, C. Quérin, A. and Guerre, P. 2005. Production and stability of patulin, ochratoxin A, citrinin and cyclopiazonic acid on dry cured ham. J. Food Prot. 68: 1516–1520.

Bainier, G. 1907a. Mycothece de l'École de Pharmacie. XI. Bull. Trimest. Soc. Mycol. Fr. 23: 26–27.

Bainier, G. 1907b. Mycothece de l'École de Pharmacie. XIV. Bull. Trimest. Soc. Mycol. Fr. 23: 98–105.

Bakan, B., Pinson, L., Cahagnier, B., Melcion, D., Semon, E. and Richard-Molard, D. 2001. Toxigenic potential of *Fusarium culmorum* strains isolated from French wheat. Food Addit. Contam. 18: 998–1003.

Balasubramanian, S., Panigrahi, S., Kottapalli, B. and Wolf-Hall, C.E. 2007. Evaluation of an artificial olfactory system for grain quality discrimination. LWT – Food Sci. Technol. 40: 1815–1825.

Baleiras Couto, M.M., Hartog, B.J., Huis in't Veld, J.H., Hofstra, H. and van der Vossen, J.M.B.M. 1996. Identification of spoilage yeasts in a food-production chain by microsatellite polymerase chain reaction fingerprinting. Food Microbiol. 13: 59–67.

Ballestra, P. and Cuq, J. 1998. Influence of pressurized carbon dioxide on the thermal inactivation of bacterial and fungal spores. Lebensm. Wiss. Technol. 31: 84–88.

Barbetti, M.J. 1980. Bunch rot of Rhine Riesling grapes in the lower south-west of Western Australia. Aust. J. Exp. Agric. Anim. Husb. 20: 247–251.

Barkai-Golan, R. 1974. Species of *Penicillium* causing decay of stored fruits and vegetables in Israel. Mycopathol. Mycol. Appl. 54: 141–145.

Barkai-Golan, R. 1980. Species of *Aspergillus* causing post-harvest fruit decay in Israel. Mycopathologia 71: 13–16.

Barnes, G.L. 1971. Mycoflora of developing peanut pods in Oklahoma. Mycopathol. Mycol. Appl. 45: 85–92.

Barnett, J.A., Payne, R.W. and Yarrow, D. 1990. The Yeasts: Characteristics and Identification, 2nd edn. Cambridge, UK: Cambridge University Press.

Barnett, J.A., Payne, R.W. and Yarrow, D. 1996. Yeast Identification PC Programme. Version 4. Norwich, UK: J.A. Barnett.

Barnett, J.A., Payne, R.W. and Yarrow, D. 2000. Yeasts: Characteristics and Identification, 3rd edn. Cambridge, UK: Cambridge University Press.

Barreiro, J.A., Vidaurreta, J.L., Boscan, L.A., Mendoza, S. and Saiz, E. 1981. Heat resistance of *Candida tropicalis* and *Rhodotorula rubra* in orange juice. Arch. Latinoam. Nutr. 31: 463–470.

Barreto, D., Carmona, M., Ferrazini, M., Zanelli, M. and Perez, B.A. 2004. Occurrence and pathogenicity of *Fusarium poae* in barley in Argentina. Cereal Res. Commun. 32: 53–60.

Barrios, M.J., Medina, L.M., Lopez, M.C. and Jordano, R. 1998. Fungal biota isolated from Spanish cheeses. J. Food Saf. 18: 151–157.

Barron, G.L. 1968. The Genera of Hyphomycetes from Soil. Baltimore, MD: Williams and Wilkins.

Barron, G.L. and Lichtwardt, R.W. 1959. Quantitative estimations of the fungi associated with deterioration of stored corn in Iowa. Iowa St. J. Sci. 34: 147–155.

Barros, G., Chiotta, M.L., Torres, A. and Chulze, S. 2006. Genetic diversity in *Aspergillus parasiticus* population from the peanut agroecosystem in Argentina. Lett. Appl. Microbiol. 42: 560–566.

Barua, J., Hossain, M.M., Hossain, I., Rahman, A.A.M.S. and Sahel, M.A.T. 2007. Control of mycoflora of farmer's stored seeds of mungbean. Asian J. Plant Sci. 6: 115–121.

Baruzzi, F., Matarante, A., Caputo, L. and Morea, M. 2006. Molecular and physiological characterization of natural microbial communities isolated from a traditional Southern Italian processed sausage. Meat Sci. 72: 261–269.

Basílico, J.C., deBasílico, M.Z., Chiericatti, C. and Vinderola, C.G. 2001. Characterization and control of thread mould in cheese. Lett. Appl. Microbiol. 32: 419–423.

Basu, M. and Mehrotra, B.S. 1976. Additions to the fungi in stored cereal grains in India. I. Nov. Hedwig. 27: 785–791.

Batista, L.R., Chalfoun, S.M., Prado, G., Schwan, R.F. and Wheals, A.E. 2003. Toxigenic fungi associated with processed (green) coffee beans (*Coffea arabica* L.). Int. J. Food Microbiol. 85: 293–300.

Batra, L.R. and Millner, P.D. 1976. Asian fermented foods and beverages. Dev. Ind. Microbiol. 17: 117–128.

Battilani, P. and Pietri, A. 2002. Ochratoxin A in grapes and wine. Eur. J. Plant Pathol. 108: 639–643.

Battilani, P., Chiusa, G., Cervi, C., Trevisan, M. and Ghebbioni, C. 1996. Fungal growth and ergosterol content in tomato fruits infected by fungi. Ital. J. Food Sci. 8: 283–289.

Battilani, P., Logrieco, A. and Magan, N., (eds). 2006. Black Aspergilli and ochratoxin A in grapes and wine. Int. J. Food Microbiol. 111 Suppl.1: S1–S98.

Battilani, P., Pietri, A., Giorni, P., Formenti, S., Bertuzzi, T., Toscani, T., Virgili, R. and Kozakiewicz, Z. 2007. *Penicillium* populations in dry-cured ham manufacturing plants. J. Food Prot. 70: 975–980.

Bau, M., Bragulat, M.R., Abarca, S., Minués, F.J. and Cabañes, F.J. 2005. Ochratoxigenic species from Spanish wine grapes. Int. J. Food Microbiol. 98: 125–130.

Bau, M., Bragula, R.M., Abarca, L., Mingez, S. and Cabañes, F.J. 2006. Ochratoxin A producing fungi from Spanish vineyards. *In* Advances in Food Mycology, eds A.D. Hocking, J.I. Pitt, R.A. Smason and U. Thrane. New York: Springer. pp. 173–179.

Baumgart, J., Weber, B. and Hanekamp, B. 1983. Mikrobiologische Stabilitaet von Feinkosterzeugnissen. Fleischwirtschaft 63: 93–94.

Baydar, T., Engin, A.B., Girgin, G., Aydin, S. and Sahin, G. 2005. Aflatoxin and ochratoxin in various types of commonly consumed retail ground samples in Ankara, Turkey. Ann. Agric. Environ. Med. 12: 193–197.

Bayman, P. and Baker, J.L. 2006. Ochratoxins: a global perspective. Mycopathologia 162: 215–223.

Bayman, P., Baker, J.L. and Mahoney, N.E. 2002a. *Aspergillus* on tree nuts: incidence and associations. Mycopathologia 155: 161–169.

Bayman, P., Baker, J.L., Doster, M.A., Michailides, T.J. and Mahoney, N.E. 2002b. Ochratoxin production by the *Aspergillus ochraceus* group and *Aspergillus alliaceus*. Appl. Environ. Microbiol. 68: 2326–2329.

Bayne, H.G. and Michener, H.D. 1979. Heat resistance of *Byssochlamys* ascospores. Appl. Environ. Microbiol. 37: 449–453.

Baysal, A.D.H. and Unluturk, A. 2007. Effects of selected chemicals on microbial stability of turkey meat. Sci Aliments 27: 59–71.

Beardall, J.M. and Miller, J.D. 1994. Diseases in humans with mycotoxins as possible causes. *In* Mycotoxins in Grain: Compounds other than Aflatoxin, eds J.D. Miller and H.L. Trenholm. St. Paul, MN: Eagan Press. pp. 487–539.

Beckh, G., Wessel, P. and Lullmann, C. 2005. A contribution to yeasts and their metabolism products as natural components of honey – Part 4: isolated and identified yeast species. Deutsche Lebensmittel-Rundschau 101: 338–344.

Begum, M., Rai, V.R. and Lokesh, S. 2003. Effect of mycoflora on the physico-chemical characteristics of oil obtained from the infected sunflower, toria and sesame seeds. J. Food Sci. Technol. Mysore 40: 626–628.

Beh, A.L. 2007. Investigation of yeasts and yeast-like fungi associated with Australian wine grapes using cultural and molecular methods. Ph.D. thesis, University of New South Wales, NSW, Australia.

Belisario, A., Forti, E., Balmas, V., Valier, A. and Corazza, L. 1999. Fusariosis of fruit, a disease of walnut. Inf. Agrario 55: 51–52.

Bellí, N., Ramos, A.J., Sanchis, V. and Marín, S. 2004. Incubation time and water activity effects on ochratoxin A production by *Aspergillus* section *Nigri* strains isolated from grapes. Lett. Appl. Microbiol. 38: 72–77.

Bellí, N., Ramos, A.J., Coronas, I., Sanchis, V. and Marín, S. 2005. *Aspergillus carbonarius* growth and ochratoxin A production on a synthetic grape medium in relation to environmental factors. J. Appl. Microbiol. 98: 839–844.

Bellí, N., Marín, S., Sanchis, V. and Ramos, A.J. 2006. Impact of fungicides on *Aspergillus carbonarius* growth and ochratoxin A production on synthetic grape-like medium and on grapes. Food Addit. Contam. 23: 1021–1029.

Bem, Z. and Leistner, L. 1970. Die Wasseraktivitätstoleranz der bei Pökelfleischwaren vorkommenden Hefen. Fleischwirtschaft 50: 492–493.

Benbough, J.E., Bennett, A.M. and Parks, S.R. 1993. Determination of the collection efficiency of a microbial air sampler. J. Appl. Bacteriol. 74: 170–173.

Bencheqroun, S.K., Baji, M., Massart, S., Labhilili, M., El Jaafari, S. and Jijaki, M.H. 2007. In vitro and in situ study of postharvest apple blue mold biocontrol by *Aureobasidium pullulans*: Evidence for the involvement of competition for nutrients. Postharvest Biol. Technol. 46: 128–135.

Beneke, E.S., White, L.S. and Fabian, F.W. 1954. The incidence and proteolytic activity of fungi isolated from Michigan strawberry fruits. Appl. Microbiol. 2: 253–258.

Benjamin, C.R. 1955. Ascocarps of *Aspergillus* and *Penicillium*. Mycologia 47: 669–687.

Benjamin, C.R. 1966. Review of 'The Genus *Aspergillus*', by K.B. Raper and D.I. Fennell. Mycologia 58: 500–502.

Benjamin, R.K. 1959. The merosporangiferous Mucorales. Aliso 4: 321–433.

Benkhemmar, O., Lahlou, H., Dupont, J., Bompiex, G., Boubekri, C. and El-Mniai, H. 1993. Identification of different species of *Penicillium* causing deterioration of Moroccan table grapes during storage. Mycopathologia 124: 27–30.

Bentley, R. 2000. Mycophenolic acid: a one hundred year odyssey from antibiotic to immunosuppressant. Chem. Rev. 100: 3801–3825.

Ben-Yehoshua, S., Rodov, V., D'Hallewin, G. and Dore, A. 2005. Elicitation of resistance against pathogens in citrus fruits by combined UV illumination and heat treatments. Proc. 5th Int. Postharvest Symp. Acta Horticulturae 682: 2013–2019.

Berbee, M.L. and Taylor, J.W. 1992. Two Ascomycete classes based on fruiting-body characters and ribosomal DNA sequences. Mol. Biol. Evol. 9: 278–284.

Berenguer, J.A., Calderon, V., Herce, M.D. and Sanchez, J.J. 1991. Spoilage of a bakery product (sobao pasiego) by isoprene-producing moulds. Rev. Agroquim. Tecnol. Aliment. 31: 580–583.

Berghofer, L.K., Hocking, A.D., Miskelly, D., Jansson, E. 2003. Microbiology of wheat and flour milling in Australia. Int. J. Food Microbiol. 85: 137–149.

Bertolini, P. and Tian, S.P. 1996. Low-temperature biology and pathogenicity of *Penicillium hirsutum* on garlic in storage. Postharvest Biol. Technol. 7: 83–89.

Beuchat, L.R. 1981a. Synergistic effects of potassium sorbate and sodium benzoate on thermal inactivation of yeasts. J. Food Sci. 46: 771–777.

Beuchat, L.R. 1981b. Influence of potassium sorbate and sodium benzoate on heat inactivation of *Aspergillus flavus*, *Penicillium puberulum* and *Geotrichum candidum*. J. Food Prot. 44: 450–454.

Beuchat, L.R. 1981c. Combined effects of solutes and food preservatives on rates of inactivation of and colony formation by heated spores and vegetative cells of molds. Appl. Environ. Microbiol. 41: 472–477.

Beuchat, L.R. 1986. Extraordinary heat resistance of *Talaromyces flavus* and *Neosartorya fischeri* ascospores in fruit products. J. Food Sci. 51: 1506–1510.

Beuchat, L.R. 1987. Traditional fermented food products. *In* Food and Beverage Mycology, 2nd edn, ed. L.R. Beuchat. Westport, CT: AVI Publishing Co. pp. 269–306.

Beuchat, L.R. 1988. Influence of organic acids on heat resistance characteristics of *Talaromyces flavus* ascospores. Int. J. Food Microbiol. 6: 97–105.

Beuchat, L.R. 1992. Enumeration of fungi in grain flours and meals as influenced by settling time in diluent and by the recovery medium. J. Food Prot. 55: 899–901.

Beuchat, L.R. and de Daza, M.S.T. 1992. Evaluation of chemicals for restricting colony spreading by a xerophilic mold, *Eurotium amstelodami*, on dichloran-18% glycerol agar. Appl. Environ. Microbiol. 58: 2093–2095.

Beuchat, L.R. and Jones, W.K. 1978. Effects of food preservatives and antioxidants on colony formation by heated conidia of *Aspergillus flavus*. Acta Aliment. 7: 373–384.

Beuchat, L.R. and Pitt, J.I. 1990a. Influence of solute, pH and incubation temperature on recovery of heat-stressed *Wallemia sebi* conidia. Appl. Environ. Microbiol. 56: 2545–2550.

Beuchat, L.R. and Pitt, J.I. 1990b. Influence of water activity and temperature on survival of and colony formation by heat-stressed *Chrysosporium farinicola* aleuriospores. Appl. Environ. Microbiol. 56: 2951–2956.

Beuchat, L.R. and Pitt, J. I. 2001. Detection and enumeration of heat resistant molds. *In* Compendium of Methods for the Microbiological Examination of Foods, eds F. P. Downes and K. Ito. Washington DC, American Public Health Association. pp. 217–222.

Beuchat, L.R. and Rice, S.L. 1979. *Byssochlamys* spp. and their importance in processed fruits. Adv. Food Res. 25: 237–288.

Beuchat, L.R. and Toledo, R.T. 1977. Behaviour of *Byssochlamys nivea* ascospores in fruit syrups. Trans. Br. Mycol. Soc. 68: 65–71.

Beuchat, L.R., Nielsen, P.V. and Frisvad, J.C. 1988. Environmental factors influencing fumitremorgin production by *Neosartorya fischeri*. *In* Mycotoxins and Phycotoxins '88, Proc. 7th Int. IUPAC Symp. Mycotox. Phycotox., Tokyo, Japan, eds S. Natori, K. Hashimoto and Y. Ueno. Amsterdam: Elsevier Science. pp. 7–12.

Beuchat, L.R., Nail, B.V., Brackett, R.E. and Fox, T.L. 1990. Evaluation of a culture film (Petrifilm™ YM) method for enumerating yeasts and molds in selected dairy and high-acid foods. J. Food Prot. 53: 864, 869–874.

Beuchat, L.R., Mann, D.A. and Gurtler, J.B. 2007. Comparison of dry sheet media and conventional agar media methods for enumerating yeasts and molds in food. J. Food Prot. 70: 2661–2664.

Bezuidenhout, S.C., Gelderblom, W.C.A., Gorst-Allman, C. P., Horak, R.M., Marasas, W.F.O., Spiteller, G. and Vleggaar, R. 1988. Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. J. Chem. Soc. Chem. Commun. 1988: 743–745.

Bhardwaj, S., Sutar, R., Bachhawat, A.K., Singhi, S. and Chakrabarti, A. 2007. PCR-based identification and strain typing of *Pichia anomala* using the ribosomal intergenic spacer region IGS1. J. Med. Microbiol. 56: 185–189.

Bhat, R., Sridhar, K.R. and Velmourougane, K. 2007. Microbial quality evaluation of velvet bean seeds (*Mucuna pruriens* L. DC.) exposed to ionizing radiation. Trop. Subtrop. Agroecosyst. 7: 29–40.

Bhat, R.V., Beedu, S.R., Ramakrishna, Y. and Munshi, K.L. 1989. Outbreak of trichothecene mycotoxicosis associated with the consumption of mould-damaged wheat products in Kashmir Valley, India. Lancet, January 7, I: 35–37.

Bhattacharya, K. and Raha, S. 2002. Deteriorative changes of maize, groundnut and soybean seeds by fungi in storage. Mycopathologia 155: 135–141.

Biffi, R., Munari, M., Dioguardi, L., Ballabio, C., Cattaneo, A., Galli, C.L. and Restani, P. 2004. Ochratoxin A in conventional and organic cereal derivatives: a survey of the Italian market, 2001–02. Food Addit. Contam. 21: 586–591.

Binzel, R.M. 1980. Das Proteinasebildungsvermögen von fleischhygienisch bedeutsamen Arten der Gattung *Eurotium* Link ex Fries. Ph.D. thesis. Giessen, Germany: Justus Liebig University.

Bigirwa, G., Kaaya, A.N., Sseruwu, G., Adipala, E. and Okanya, S. 2007. Incidence and severity of maize ear rots and factors responsible for their occurrence in Uganda. J. Appl. Sci. 7: 3780–3785.

Birzele, B., Meier, A., Hindorf, H., Kramer, J. and Dehne, H. W. 2002. Epidemiology of *Fusarium* infection and deoxynivalenol content in winter wheat in the Rhineland, Germany. Eur. J. Plant Pathol. 108: 667–673.

Bishop, R.H., Duncan, C.L., Evancho, G.M. and Young, H. 1982. Estimation of fungal contamination of tomato products by a chemical assay for chitin. J. Food Sci. 47: 437–439, 444.

Bisset, J. 1984. A revision of the genus *Trichoderma*. I. Section *Longibrachiatum* sect. nov. Can. J. Bot. 62: 924–931.

Bisset, J. 1991a. A revision of the genus *Trichoderma*. II. Infrageneric classification. Can. J. Bot. 69: 2357–2372.

Bisset, J. 1991b. A revision of the genus *Trichoderma*. III. Section *Pachybasium*. Can. J. Bot. 69: 2373–2417.

Biswal, P.K., Mishra, B.D. and Mohapatra, S.S. 2007. Management of fungi causing post harvest rotting of pineapples in Orissa. J. Plant Prot. Environ. 4: 103–105.

Björnberg, A. and Schnürer, J. 1993. Inhibition of the growth of grain-storage molds in vitro by the yeast *Pichia anomala* (Hansen) Kurtzman. Can. J. Microbiol. 39: 623–628.

Blais, L.A., ApSimon, J.W., Blackwell, B.A., Greenhalgh, R. and Miller, J.D. 1992. Isolation and characterization of enniatins from *Fusarium avenaceum* DAOM 196490. Can. J. Chem. 70: 1281–1287.

Blanc, P.J., Loret, M.O. and Goma, G. 1995a. Production of various species of *Monascus*. Biotechnol. Lett. 17: 291–294.

Blanc, P.J., Laussac, J.P., Le Bars, J., Le Bars, P., Loret, M.O., Pareilleux, A., Prome, D., Prome, J.C., Santerre, A.L. and Goma, G. 1995b. Characterization of monascidin A from *Monascus* as citrinin. Int. J. Food Microbiol. 27: 201–213.

Blancard, D., la Guerche, S., Chamont, S., Sauris, P., Dauphin, B. and Darriet, P. 2006. Grape berry rot and aromatic defects: initial observations in vineyards. Phytoma 592: 32–36.

Blaney, B.J. and Dodman, R.L. 1988. Production of the mycotoxins zearalenone, 4-deoxynivalenol and nivalenol by isolates of *Fusarium graminearum* Groups 1 and 2 from cereals in Queensland. Aust. J. Agric. Res. 39: 21–29.

Blaney, B.J. and Dodman, R.L. 2002. Production of zearalenone, deoxynivalenol, nivalenol, and acetylated derivatives by Australian isolates of *Fusarium graminearum* and *F. pseudograminearum* in relation to source and culturing conditions. Aust. J. Agric. Res. 53: 1317–1326.

Blaney, B.J., Ramsey, M.D. and Tyler, A.L. 1986. Mycotoxins and toxigenic fungi in insect-damaged maize harvested during 1983 in Far North Queensland. Aust. J. Agric. Res. 37: 235–244.

Blank, G., Shamsuzzaman, K. and Sohal, S. 1992. Use of electron beam irradiation for mold decontamination on Cheddar cheese. J. Dairy Sci. 75: 13–18.

Blaser, P. 1975. Taxonomische und physiologische Untersuchungen über die Gatung *Eurotium* Link ex Fries. Sydowia 28: 1–49.

Bleve, G., Rizzotti, L., Dellaglio, F. and Torriani, S. 2003. Development of reverse transcription (RT)-PCR and real-time RT-PCR assays for rapid detection and quantification of viable yeasts and molds contaminating yogurts and pasteurized food products. Appl. Environ. Microbiol. 69: 4116–4122.

Bleve, G., Grieco, F., Cozzi, G., Logrieco, A. and Visconti, A. 2006. Isolation of epiphytic yeasts with potential for biocontrol of *Aspergillus carbonarius* and *A. niger* on grape. Int. J. Food Microbiol. 108: 204–209.

Bockelmann, W., Willems, K.P., Neve, H. and Heller, K.H. 2005. Cultures for the ripening of smear cheeses. Int. Dairy J. 15: 719–732.

Boekhout, T. and Robert, V. (eds). 2003. Yeasts in Food. Cambridge, UK: Woodhead Publishing.

Boerema, G.H., de Gruyter, J., Noordeloos, M.E. and Hamers, M.E.C. 2004. *Phoma* Identification Manual: Differentiation of Specific and Intra-specific Taxa in Culture. Wallingford, UK: CABI Publishing.

Boeswinkel, H.J. 1976. Storage of fungal cultures in water. Trans. Br. Mycol. Soc. 66: 183–185.

Bogs, C., Battilani, P. and Geisen, R. 2006. Development of a molecular detection and differentiation system for ochratoxin A producing *Penicillium* species and its application to analyse the occurrence of *Penicillium nordicum* in cured meats. Int. J. Food Microbiol. 107: 39–47.

Bokhary, H.A., Parvez, S. and Shibl, A.M. 1990. Some spoilage microflora of desert truffles 'Al-Kamah' of the kingdom of Saudi Arabia. J. Food Prot. 53: 779–781.

Bolin, H.R., King, A.D., Stanley, W.L. and Jurd, L. 1972. Antimicrobial protection of moisturized Deglet Noir dates. Appl. Microbiol. 23: 799–802.

Bolkan, H.A., Dianese, J.C. and Cupertino, F.P. 1979. Pineapple flowers as principal infection sites for *Fusarium moniliforme* var. *subglutinans*. Plant Dis. Rep. 63: 655–657.

Bonestroo, M.H., de Wit, J.C., Kusters, B.J.M. and Rombouts, F.M. 1993. Inhibition of the growth of yeasts in fermented salads. Int. J. Food Microbiol. 17: 311–320.

Bonjar, G.H.S. 2004. Incidence of aflatoxin producing fungi in early split pistachio nuts of Kerman, Iran. J. Biol. Sci. 4: 199–202.

Bonjean, B. and Guillaume, L.D. 2003. Yeasts in bread and baking products. *In* Yeasts in Food, eds T. Boekhout and V. Robert. Cambridge, UK: Woodhead Publishing. pp. 289–307.

Bonvehi, J.S. 2004. Occurrence of ochratoxin A in cocoa products and chocolate. J. Agric. Food Chem. 52: 6347–6352.

Booth, C. 1971. The Genus *Fusarium*. Kew: Commonwealth Mycological Institute.

Booth, C. 1983. The *Fusarium* problem: historical, economic and taxonomic aspects. *In* The Applied Biology of *Fusarium*, eds M.O. Moss and J.E. Smith. Cambridge, UK: Cambridge University Press. pp. 1–13.

Booth, C. and Sutton, B.C. 1984. *Fusarium pallidoroseum*, the correct name for *F. semitectum*. Trans. Br. Mycol. Soc. 83: 702–704.

Börjesson, T., Stöllman, U., Adamek, P. and Kaspersson, A. 1989. Analysis of volatile compounds for detection of models in stored cereals. Cereal Chem. 66: 300–304.

Börjesson, T., Stöllman, U. and Schnürer, J. 1990. Volatile metabolites and other indicators of *Penicillium aurantiogriseum* growth on different substrates. Appl. Environ. Microbiol. 56: 3705–3710.

Börjesson, T., Stöllman, U. and Schnürer, J. 1992. Volatile metabolites produced by six fungal species compared with other indicators of fungal growth on cereal grains. Appl. Environ. Microbiol. 58: 2599–2605.

Boruah, P., Bhuyan, P.D. and Singh, R.S. 2004. Fungal spoilage of banana in Eastern Sub-Himalayan region. Sci. Hort. 9: 27–36.

Borve, J. and Vangdal, E. 2007. Fungal pathogens causing fruit decay on plum (*Persica domestica* L.) in Norway. Acta Horticulturae 734: 367–369.

Bosch, U. and Mirocha, C.J. 1992. Toxin production by *Fusarium* species from sugar beets and natural occurrence of zearalenone in beets and beet fibers. Appl. Environ. Microbiol. 58: 3233–3239.

Boshoff, W.H.P., Pretorius, Z.A. and Swart, W.J. 1998. *Fusarium* species in wheat grown from head blight infected seed. S. Afr. J. Plant Soil 15: 46–47.

Bothast, R.J. and Fennell, D.I. 1974. A medium for rapid identification and enumeration of *Aspergillus flavus* and related organisms. Mycologia 66: 365–369.

Bottalico, A. and Logrieco, A. 1998. Toxigenic *Alternaria* species of economic importance. *In* Mycotoxins in Agriculture, eds K.K. Sinha and D. Bhatnagar. New York: Marcel Dekker. pp. 65–108.

Bottalico, A. and Logrieco A. 2001. Occurrence of toxigenic fungi and mycotoxins in Italy. *In* Occurrence of Toxigenic Fungi and Mycotoxins in Plants, Foods and Feeds in Europe, ed. A. Logrieco. European Commission, COST Action 835, EUR 19695. pp. 69–104.

Bottalico, A. and Perrone, G. 2002. Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. Eur. J. Plant Pathol. 108: 611–624.

Bottalico, A., Visconti, A. and Lerario, P. 1982. Effect of temperature on zearalenone production by isolates of *Fusarium* from cereals, in Italy. Phytopathol. Mediterr. 21: 79–82.

Bottalico, A., Logrieco, A., Ritieni, A., Moretti, A., Randazzo, G. and Corda, P. 1995. Beauvericin and fumonisin B_1 in preharvest *Fusarium moniliforme* maize ear rot in Sardinia. Food Addit. Contam. 12: 599–607.

Böttcher, H. and Pohle, K. 1993. The development of various rot pathogens on dry garlic (*Allium sativum* L.) during long term storage. Arch. Phytopathol. Plant Prot. 28: 203–214.

Boudra, H., le Bars, P. and le Bars, J. 1995. Thermostability of ochratoxin A in wheat under two moisture conditions. Appl. Environ. Microbiol. 61: 1156–1158.

Bourdages, J.V., Marchand, S., Rioux, S. and Belzile, F.J. 2006. Diversity and prevalence of *Fusarium* species from Quebec barley fields. Can. J. Plant Pathol. 28: 419–425.

Bours, J. and Mossel, D.A.A. 1973. A comparison of methods for the determination of lipolytic properties of yeasts mainly isolated from margarine, moulds, and bacteria. Arch. Lebensmittelhyg. 24: 197–203.

Boutrou, R. and Gueguen, M. 2005. Interests in *Geotrichum candidum* for cheese technology. Int. J. Food Microbiol. 102: 1–20.

Boutrou, R., Kerriou, L. and Gassi, J.Y. 2006. Contribution of *Geotrichum candidum* to the proteolysis of soft cheese. Int. Dairy J. 16: 775–783.

Boyd, M.R. and Wilson, B.J. 1972. Isolation and characterization of 4-ipomeanol, a lung toxic furanoterpenoid produced by sweet potatoes (*Ipomoea batatas*). J. Agric. Food Chem. 20: 428–430.

Boysen, M., Skouboe, P., Frisvad, J. and Rossen, L. 1996. Reclassification of the *Penicillium roqueforti* group into three species on the basis of molecular genetic and biochemical profiles. Microbiology 142: 541–549.

Brackett, R.E. 1987. Vegetables and related products. *In* Food and Beverage Mycology, 2nd edn, ed. L.R. Beuchat. New York: Van Nostrand Reinhold. pp. 129–154.

Brackett, R.E. and Marth, E.H. 1979. Patulin in apple juice from roadside stands in Wisconsin. J. Food Prot. 42: 862–863.

Bradburn, N., Coker, R.D. and Blunden, G. 1994. The aetiology of Turkey "X" disease. Phytochemistry 35: 817.

Braendlin, N. and Cox, L. 1992. Immunoagglutination assay for rapid detection of *Aspergillus* and *Penicillium* contamination in food. *In* Modern Methods in Food Mycology, eds R.A. Samson, A.D. Hocking, J.I. Pitt and A.D. King. Amsterdam: Elsevier. pp. 233–240.

Bragulat, M.R., Martinez, E., Castellá, G. and Cabañes, F.J. 2004. Selective efficacy of culture media recommended for isolation and enumeration of *Fusarium* spp. J. Food Prot. 67: 207–211.

Breuer, U. and Harms, H. 2006. *Debaryomyces hansenii* – an extremophilic yeast with biotechnological potential. Yeast 23: 415–437.

Brinkmeyer, U., Danicke, S., Valenta, H. and Flachowsky, G. 2005. Progression of deoxynivalenol and zearalenone concentrations in straw of wheat infected artificially with *Fusarium culmorum*. Mycotoxin Res. 21: 97–99.

Broad Institute. 2003. *Fusarium graminearum* database. The Institute, Cambridge, MA. http://www.broad.mit.edu/annotation/genome/fusarium_graminearum/Home.html (accessed April 2008).

Broad Institute. 2008. Current FGI Sequence projects. http://www.broad.mit/edu (accessed July 2008).

Broders, K.D., Lipps, P.E., Paul, P.A. and Dorrance, A.E. 2007. Evaluation of *Fusarium graminearum* associated with corn and soybean seed and seedling disease in Ohio. Plant Dis. 91: 1155–1160.

Brodsky, M.H., Entis, P., Entis, M.P., Sharpe, A.N. and Jarvis, G.A. 1982. Determination of aerobic plate and yeast and mold counts in foods using an automated hydrophobic grid-membrane filter technique. J. Food Prot. 45: 301–304.

Broggi, L.E., Gonzalez, H.H.L., Resnik, S.L. and Pacin, A.M. 2002. Mycoflora distribution in dry-milled fractions of corn in Argentina. Cereal Chem. 79: 741–744.

Broggi, L.E., Gonzales, L.H.H., Resnik, S.L. and Pacin, A.M. 2007. *Alternaria alternata* prevalence in cereal grains and soy bean seeds from Entre Rios, Argentina. Rev. Iberoam. Micol. 24: 47–51.

Brook, P.J. 1992. *Botrytis* stem-end rot and other storage diseases of kiwifruit. Acta Hortic. 2: 545–550.

Brooks, F. and Hansford, C.G. 1923. Mould growth upon cold-stored meat. Trans. Br. Mycol. Soc. 8: 113–142.

Brown, A.D. 1974. Microbial water relations: features of the intracellular composition of sugar tolerant yeasts. J. Bacteriol. 118: 769–777.

Brown, G.E., Mawk, P. and Craig, J.O. 1988. Pallet treatment with benomyl on citrus fruit on trucks for control of diplodia stem-end rot. Proc. Fla State Hortic. Soc. 101: 187–190.

Brown, G.E., Miller, W.R. and McDonald, R.E. 1991. Control of green mold in Marsh grapefruit with vapor heat quarantine treatment. Proc. Fla State Hortic. Soc. 1991: 115–117.

Bruce, V.R., Stack, M.E. and Mislivec, P.B. 1984. Incidence of toxic *Alternaria* species in small grains from the USA. J. Food Sci. 49: 1626–1627.

Brul, S. and Coote, P. 1999. Preservative agents in foods. Mode of action and microbial resistance mechanisms. Int. J. Food Microbiol. 50: 1–17.

Bruton, B.D., Redlin, S.C., Collins, J.K. and Sams, C.E. 1993. Postharvest decay of cantaloupe caused by *Epicoccum nigrum*. Plant Dis. 77: 1060–1062.

Bucheli, P. and Taniwaki, M.H. 2002. Research on the origin, and on the impact of post-harvest handling and manufacturing on the presence of ochratoxin A in coffee. Food Addit. Contam. 19: 655–665.

Buchta, V., Slavikova, E., Vadkartiova, R., Alt, S. and Jilek, P. 1996. *Zygosaccharomyces bailii* a potential spoiler of mustard. Food Microbiol. 13: 133–135.

Buhagiar, R.W.M. and Barnett, J.A. 1971. The yeasts of strawberries. J. Appl. Bacteriol. 34: 727–739.

Bullerman, L.B. 1983. Effects of potassium sorbate on growth and aflatoxin production by *Aspergillus parasiticus* and *Aspergillus flavus*. J. Food Prot. 46: 940–942.

Bullerman, L.B. 1984. Effects of potassium sorbate on growth and patulin production by *Penicillium patulum* and *Penicillium roqueforti*. J. Food Prot. 47: 312–315, 320.

Bullerman, L.B. 1985. Effects of potassium sorbate on growth and ochratoxin production by *Aspergillus ochraceus* and *Penicillium* species. J. Food Prot. 48: 162–165.

Bullerman, L.B. and Tsai, W-Y.J. 1994. Incidence and levels of *Fusarium moniliforme*, *Fusarium proliferatum* and fumonisins in corn and corn-based foods and feeds. J. Food Prot. 57: 541–546.

Burgess, L.W. and Summerell, B.A. 2000. Taxonomy of *Fusarium*: *Fusarium armeniacum* stat. & comb. nov. Mycotaxon 75: 347–348.

Burgess, L.W., Dodman, R.L., Pont, W. and Mayers, P. 1981. *Fusarium* diseases of wheat, maize and grain sorghum in Eastern Australia. *In Fusarium*: Diseases, Biology and Taxonomy, eds P.E. Nelson, T.A. Toussoun and R.J. Cook. University Park, PA: Pennsylvania State University Press. pp. 64–76.

Burgess, L.W., Summerell, B.A., Bullock, S., Gott, K.P. and Backhouse, D. 1994. Laboratory Manual for *Fusarium* Research, 3rd edn. Sydney, NSW: University of Sydney.

Burnside, J.E., Sippel, W.L., Forgacs, J., Carll, W.L., Atwood, M.B. and Doel, E.R. 1957. A disease in swine and cattle caused by eating moldy corn. II. Experimental production with pure cultures of moulds. Am. J. Vet. Res. 18: 817–824.

Butinar, L., Sonjak, S., Zalar, P., Plemenitas, A. and Gunde-Cimerman, N. 2005a. Melanized halophilic fungi are eukaryotic members of microbial communities in hypersaline waters of solar salterns. Bot. Marina 48: 73–79.

Butinar, L., Zalar, P., Frisvad, J. C. and Gunde-Cimerman, N. 2005b. The genus *Eurotium* – members of indigenous fungal community in hypersaline waters of salterns. FEMS Microbiol. Ecol. 51: 155–166.

Butler, E.E. 1960. Pathogenicity and taxonomy of *Geotrichum candidum*. Phytopathology 50: 665–672.

Butler, E.E., Webster, R.K. and Eckert, J.W. 1965. Taxonomy, pathogenicity, and physiological properties of the fungus causing sour rot of citrus. Phytopathology 55: 1262–1268.

Buttner, M.P. and Stetzenbach, L.D. 1993. Monitoring airborne fungi in an experimental indoor environment to evaluate sampling methods and the effects of human activity on air sampling. Appl. Environ. Microbiol. 59: 219–226.

Butz, P., Funtenberger, S., Haberditzl, T. and Tauscher, B. 1996. High pressure inactivation of *Byssochlamys nivea* ascospores and other heat resistant moulds. Lebensm. Wiss. Technol. 29: 404–410.

Cabañes, F.J., Alonso, J.M., Castellá, G., Alegre, F., Domingo, M. and Pont, S. 1997. Cutaneous hyalohyphomycosis caused by *Fusarium solani* in a loggerhead sea turtle (*Caretta caretta* L.). J. Clin. Microbiol. 35: 3343–3345.

Cabañes, F.J., Accensi, F., Bragulat, M.R., Abarca, M.L., Castellá, G., Minguez, S. and Pons, A. 2002. What is the source of ochratoxin A in wine? Int. J. Food Microbiol. 79: 213–215.

Cabral, D. and Fernandez Pinto, V.E. 2002. Fungal spoilage of bottled mineral water. Int. J. Food Microbiol. 72: 73–76.

Caggia, C., Restuccia, C., Pulvirenti, A. and Giudici, P. 2001. Identification of *Pichia anomala* isolated from yoghurt by RFLP of the ITS region. Int. J. Food Microbiol. 71: 71–73.

Cahagnier, B., Lesage, L. and Richard-Molard, D. 1993. Mould growth and conidiation in cereal grains as affected by water activity and temperature. Lett. Appl. Microbiol. 17: 7–13.

Cairns-Fuller, V. 2004. Dynamics and control of ochratoxigenic strains of *Penicillium verrucosum* and *Aspergillus ochraceus* in the stored grain ecosystem. Ph.D. thesis. Cranfield University, Silsoe, Bedford MK45 4DT, UK.

Cairns-Fuller, V., Aldred, D. and Magan, N. 2005. Water, temperature and gas composition interactions affect growth and ochratoxin A production by isolates of *Penicillium verrucosum* on wheat grain. J. Appl. Microbiol. 99: 1215–1221.

Caldas, E.D., Silva, S.C. and Oliveira, J.N. 2002. Aflatoxins and ochratoxin A in food and risks to human health. Rev. Saude Publica 36: 319–323.

Câmara, M.P.S., O'Neill, M.R. and van Berkum, P. 2002. Phylogeny of *Stemphylium* spp. based on ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. Mycologia 94: 660–672.

Camili, E.C. and Benato, E.A. 2005. Diseases of grapes. Inf. Agropec. 26(228): 50–55.

Campano, S.G., Kotula, A.W. and Kinsman, D.M. 1985. Antibacterial nature of molds isolated from aged beef. J. Food Prot. 48: 699–701.

Campbell, B.C., Molyneux, R.J. and Schatzki, T.F. 2003. Current research on reducing pre- and post-harvest aflatoxin contamination of U.S. almond, pistachio, and walnut. J. Toxicol. Toxin. Rev. 22: 225–266.

Candlish, A.A.G., Wibowo, M.S. and Smith, J.E. 1997. Immunoassay identification of *Aspergillus flavus* using monoclonal antibodies raised to the whole cell extracts. Biotechnol. Tech. 11: 21–24.

Cannon, P.F., Abdullah, S.K. and Abbas, B.A. 1995. Two new species of *Monascus* from Iraq, with a key to known species of the genus. Mycol. Res. 99: 659–662.

Cantalejo, M.J., Carrasco, J.M. and Hernández, E. 1997. Mycoflora of seeds and feeds from the Spanish market. J. Basic Microbiol. 37: 395–402.

Cantoni, C., Cozzi, M., Iacumin, L. and Comi, G. 2003. Some alterations of fresh soft cheeses. Ind. Aliment. 42: 15–19.

Cantoni, C., Comi, G., Chiesa, L. and Iacumin, L. 2007. Moulds and ochratoxin A on dry salami surfaces. Ind. Aliment. 46: 10–12, 19.

Carels, M. and Shepherd, D. 1977. The effect of different nitrogen sources on pigment production and sporulation of *Monascus* species in submerged, shaken culture. Can. J. Microbiol. 23: 1360–1372.

Carlton, W.W., Tuite, J. and Caldwell, R. 1973. *Penicillium viridicatum* toxins and mold nephritis. J. Am. Med. Vet. Assoc. 163: 1295–1297.

Carlton, W.W., Stack, M.E. and Eppley, R.M. 1976. Hepatic alterations produced in mice by xanthomegnin and viomellein, metabolites of *Penicillium viridicatum*. Toxicol. Appl. Pharmacol. 38: 455–459.

Carmichael, J.W. 1955. Lactofuchsin: a new medium for mounting fungi. Mycologia 47: 611.

Carreira, A., Paloma, L. and Loureiro, V. 1998. Pigment producing yeasts involved in the brown surface discoloration of ewes' cheese. Int. J. Food Microbiol. 41: 223–230.

Carrillo, L. 1995. *Penicillium ulaiense* Hsieh, Su & Tzean, a post-harvest pathogen of citrus fruits in the Argentine north-west. Rev. Argent. Microbiol. 27: 107–113.

Cartwright, P. and Hocking, A.D. 1984. *Byssochlamys* in fruit juices. Food Technol. Aust. 36: 210–211.

Casas, E., Valderrama, M.J. and Peinado, J.M. 1999. Sorbate detoxification by spoilage yeasts isolated from marzipan products. Food Technol. Biotechnol. 37: 87–91.

Casas, E., de Ancos, B., Valderrama, M.J., Cano, P. and Peinado, J. M. 2004. Pentadiene production from potassium sorbate by osmotolerant yeasts. Int. J. Food Microbiol. 94: 93–96.

Casella, M.L.A., Matasci, F. and Schmidt-Lorenz, W. 1990. Influence of age, growth medium, and temperature on heat resistance of *Byssochlamys nivea* ascospores. Lebensm. Wiss. Technol. 23: 404–411.

Casey, G.D. and Dobson, A.D.W. 2003. Molecular detection of *Candida krusei* contamination in fruit juice using the citrate synthase gene *cs1* and a potential role for this gene in the adaptive response to acetic acid. J. Appl. Microbiol. 95: 13–22.

Casey, G.D. and Dobson, A.D.W. 2004. Potential of using real-time PCR-based detection of spoilage yeast in fruit juice – a preliminary study. Int. J. Food Microbiol. 91: 327–335.

Castellá, G., Bragulat, M.R., Rubiales, M.V. and Cabañes, F.J. 1997. Malachite green agar, a new selective medium for *Fusarium* spp. Mycopathologia 137: 173–178.

Castellá, G., Cano, J., Guarro, J. and Cabañes, F.J. 1999a. DNA fingerprinting of *Fusarium solani* isolates related to a cutaneous infection in a sea turtle. Med. Mycol. 37: 223–226.

Castellá, G., Munkvold, G.P., Imerman, P. and Hyde, W.G. 1999b. Effects of temperature, incubation period and substrate on production of fusaproliferin by *Fusarium subglutinans* ITEM 2404. Nat. Toxins 7: 129–132.

Castillo, M.D., Gonzalez, H.H.L., Martinez, E.J., Pacin, A. M. and Resnik, S.L. 2004. Mycoflora and potential for mycotoxin production of freshly harvested black bean from the Argentinean main production area. Mycopathologia 158: 107–112.

CBS (Centraalbureau voor Schimmelcultures). 2007. Culture collection databases, accessible at http://www.cbs.knaw.nl/databases/index.htm.

Cerny, G. and Granzer, R. 1984. Schutzgasverpackung von Pommes frites. I. Grundlegende Untersuchungen zur mikrobiellen Stabilisierung vorfritierter Pommes frites durch CO_2-Begasung. Verpack. Rundsch. 35(8, Tech. wiss. Beil.): 49–52.

Chabalier, C., Vivier, D., Ratomahenina, R., Dieu, B. and Galzy, P. 1997. Contribution to the study of surface microflora on Cantral cheese. Acta Microbiol. Immunol. Hung. 44: 147–153.

Chakrabarti, N., Chattopadhyay, N.C. and Nandi, B. 1977. A new fruit disease of Singapuri variety of banana. Curr. Sci. 46: 93.

Champaco, E.R., Martyn, R.D. and Miller, M.E. 1993. Comparison of *Fusarium solani* and *F. oxysporum* as

causal agents of fruit rot and root rot of muskmelon. HortScience 28: 1174–1177.
Chan, Z.L. and Tian, S.P. 2005. Interaction of antagonistic yeasts against postharvest pathogens of apple fruit and possible mode of action. Postharvest Biol. Technol. 36: 215–223.
Chandler, E.A., Simpson, D.R., Thomsett, M.A. and Nicholson, P. 2003. Development of PCR assays to Tri7 and Tri13 trichothecene biosynthetic genes, and characterisation of chemotypes of *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium cerealis*. Physiol. Mol. Plant Pathol. 62: 355–367.
Chang, S.-C., Lu, K.-L. and Yeh, S.-F. 1993. Secondary metabolites resulting from degradation of PR toxin by *Penicillium roqueforti*. Appl. Environ. Microbiol. 59: 981–986.
Chapman, B., Winley, E., Fong, A.S.W., Hocking, A.D., Stewart, C.M. and Buckle, K.A. 2007. Ascospores inactivation and germination by high pressure processing is affected by ascospore age. Innov. Food Sci. Emerg Technol. 8: 531–534.
Chapman, E.S. and Fergus, C.L. 1975. Germination of ascospores of *Chaetomium globosum*. Mycologia 67: 1048–1052.
Char, C.D., Guerrero, S.N., Gonzalez, L. and Alzamora, S. M. 2005. Response of *Eurotium chevalieri*, *Aspergillus fumigatus* and *Penicillium brevicompactum* in argentine milk jam. Food Sci. Tech. Int. 11: 297–305.
Char, C.D., Guerrero, S.N. and Alzamora, S.M. 2006. Growth of *Eurotium chevalieri* in milk jam: influence of pH, potassium sorbate and water activity. J. Food Saf. 27: 1–16.
Charnock, C., Finsrud, T. and Foss, C. 2005. Controlling the proliferation of *Zygosaccharomyces bailii* in ephedrine anti-phlegm cough mixture. J. Clin. Pharm. Therapeut. 30: 329–335.
Chaturbhuj, M. and Rai, P.K. 2005. Epidemiology of post harvest fruit rot of tomato by *Fusarium pallidoroseum*. Ann. Agric. Res. 26: 8–12.
Chaturvedi, V.C., Gupta, S., Gupta, R. and Shukla, D.N. 2003. Effect of temperature on growth and sporulation of some *Fusarium* species. Bioved 14: 33–35.
Chelack, W.S., Borsa, J., Marquardt, R.R. and Frohlich, A. A. 1991. Role of the competitive microbial flora in the radiation-induced enhancement of ochratoxin production by *Aspergillus alutaceus* var. *alutaceus* NRRL 3174. Appl. Environ. Microbiol. 57: 2492–2496.
Chelkowski, J., Zawadzki, M., Zajkowski, P., Logrieco, A. and Bottalico, A. 1990. Moniliformin production by *Fusarium* species. Mycotoxin Res. 6: 41–45.
Chelkowski, J., Ritieni, A., Wisniewska, H., Mule, G. and Logrieco, A. 2007. Occurrence of toxic hexadepsipeptides in preharvest maize ear rot infected by *Fusarium poae* in Poland. J. Phytopathol. 155: 8–12.
Chen, A.W. 1966. Soil physical factors and the ecology of fungi. 5. Further studies in relatively dry soils. Trans. Br. Mycol. Soc. 49: 419–426.
Cherian, T.T. 2007. Effect of temperature, relative humidity and injury on development and spread of post harvest rot of banana (*Musa paradisiaca* L.). J. Plant Dis. Sci. 2: 187–189.
Chorin, M. and Rotem, J. 1961. Experiments on the control of tip rot in banana fruits. Israel J. Agric. Res. 11: 185–188.
Chourasia, H.K. 1995. Mycobiota and mycotoxins in herbal drugs of Indian pharmaceutical industries. Mycol. Res. 99: 697–703.
Christensen, C.M. 1963. Influence of small differences in moisture content upon the invasion of hard red winter wheat by *Aspergillus restrictus* and *A. repens*. Cereal Chem. 40: 385–390.
Christensen, C.M. 1965. Deterioration of stored grains by molds. Wallerstein Lab. Commun. 19: 31–48.
Christensen, C.M. 1978a. Storage fungi. *In* Food and Beverage Mycology, ed. L.R. Beuchat. Westport, CT: AVI Publishing Co. pp. 173–190.
Christensen, C.M. 1978b. Moisture and seed decay. *In* Water Deficits and Plant Growth, vol. 5, Water and Plant Diseases, ed. T.T. Koslowski. New York: Academic Press. pp. 199–219.
Christensen, C.M. 1987. Field and storage fungi. *In* Food and Beverage Mycology, 2nd ed., L.R. Beuchat. New York: Van Nostrand Reinhold. pp. 211–232.
Christensen, M. 1981. A synoptic key and evaluation of species in the *Aspergillus flavus* group. Mycologia 73: 1056–1084.
Christensen, C.M. and Kaufmann, H.H. 1965. Deterioration of stored grains by fungi. Annu. Rev. Phytopathol. 3: 69–84.
Christensen, C.M. and Kaufmann, H.H. 1969. Grain Storage – the Role of Fungi in Quality Loss. Minneapolis, MN: University of Minnesota Press.
Christensen, C.M., Papavisas, G.C. and Benjamin, C.R. 1959. A new halophilic species of *Eurotium*. Mycologia 51: 636–640.
Christensen, M. and Raper, K.B. 1978. Synoptic key to *Aspergillus nidulans* group species and related *Emericella* species. Trans. Br. Mycol. Soc. 71: 177–191.
Christensen, M. and States, J.S. 1982. *Aspergillus nidulans* group: *Aspergillus navahoensis* and a revised synoptic key. Mycologia 74: 226–235.
Chrystopher, R.K. and Theivendirarajah, K. 1988. Palmyrah palm wine. 1: Microbial and biochemical changes. J. Natl. Sci. Counc. Sri Lanka 16: 131–141.
Chuku, E.G., Ogbalu, O.K. and Osakwe, J.A. 2007. Fungi of coconut (*Cocos nucifera* L.) – their deteriorative ability, quality stability and the role of the fungus-eating insects. J. Appl. Sci. 7: 3106–3110.
Chulze, S.N., Magnoli, C.E. and Dalcero, A.M. 2006. Occurrence of ochratoxin A in wine and ochratoxigenic mycoflora in grapes and dried vine fruits in South America. Int. J. Food Microbiol. 111 (S1): S5–S9.
Chupp, C. and Sherf, A.F. 1960. Vegetable Diseases and their Control. New York: Ronald Press.
Cia, P., Pascholati, S F., Benato, E.A., Camili, E.C. and Santos C.A. 2007. Effects of gamma and UV-C irradiation on the postharvest control of papaya anthracnose. Postharvest Biol. Technol. 43: 366–373.
Ciegler, A., Fennell, D.I., Sansing, G.A., Detroy, R.W. and Bennett, G.A. 1973. Mycotoxin-producing strains of *Penicillium viridicatum*: classification into subgroups. Appl. Microbiol. 26: 271–278.
Cichowicz, S.M. and Eisenberg, W.V. 1974. Collaborative study of the determination of *Geotrichum* mold in selected canned fruits and vegetables. J. Assoc. Off. Anal. Chem. 57: 957–960.

Ciegler, A. 1972. Bioproduction of ochratoxin A and penicillic acid by members of the *Aspergillus ochraceus* group. Can. J. Microbiol. 18: 631–636.

Ciegler, A. and Kurtzman, C.P. 1970. Penicillic acid production by blue-eye fungi on various agricultural commodities. Appl. Microbiol. 20: 761–764.

Ciegler, A., Vesonder, R.F. and Jackson, L.K. 1977. Production and biological activity of patulin and citrinin from *Penicillium expansum*. Appl. Environ. Microbiol. 33: 1004–1006.

Ciegler, A., Hayes, A.W. and Vesonder, R.F. 1980. Production and biological activity of secalonic acid D. Appl. Environ. Microbiol. 39: 285–287.

Ciegler, A., Lee, L.S. and Dunn, J.J. 1981. Production of naphthoquinone mycotoxins and taxonomy of *Penicillium viridicatum*. Appl. Environ. Microbiol. 42: 446–449.

Ciolfi, G. 1991. Ecology of wine yeasts. Vini Ital. 33: 41–46.

Clark, C.A. 1980. End rot, surface rot, and stem lesions caused on sweet potato by *Fusarium solani*. Phytopathology 70: 109–112.

Clark, C.A. 1992. Postharvest diseases of sweet potatoes and their control. Postharvest News Inf 3: 75 N–79 N.

Clark, H.A. and Snedeker, S.M. 2006. Ochratoxin A: its cancer risk and potential for exposure. J. Toxicol. Environ. Health B 9: 265–296.

Clear, R.M., Nowicki, T.W. and Daun, J.K. 1989. Soybean seed discoloration by *Alternaria* spp. and *Fusarium* spp., effects on quality and production of fusariotoxins. Can. J. Plant Pathol. 11: 308–312.

Clear, R.M., Patrick, S.K., Gaba, D. Abramson, D. and Smith, D.M. 2005. Prevalence of fungi and fusariotoxins on hard red spring and amber durum wheat seed from western Canada, 2000 to 2002. Can. J. Plant Pathol. 27: 528–540.

Clerk, G.C. and Caurie, M. 1968. Biochemical changes caused by some *Aspergillus* species in root tuber of cassava (*Manihot esculenta* Crantz). Trop. Sci., Mysore 10: 149–154.

Cockrum, P.A., Culvenor, C.C.J., Edgar, J.A. and Payne, A. L. 1979. Chemically different tremorgenic mycotoxins in isolates of *Penicillium paxilli* from Australia and North America. J. Nat. Prod. 42: 534–536.

Cocolin, L., Aggio, D., Manzano, M., Cantoni, C. and Comi, G. 2002. An application of PCR-DGGE analysis to profile the yeast populations in raw milk. Int. Dairy J. 12: 407–411.

Colavita, G., Miotti Scapin, R., Ciocca, R.M. and Giaccone, V. 1999. Bacterial and fungal microflora of fresh, modified atmosphere-packaged pasta products. Ig. Mod. 112: 17–29.

Cole, G.T. and Kendrick, W.B. 1968. Conidium ontogeny in hyphomycetes: the imperfect state of *Monascus ruber* and its meristem arthrospores. Can. J. Bot. 46: 987–992.

Cole, R.J. 1981a. Fungal tremorgens. J. Food Prot. 44: 715–722.

Cole, R.J. 1981b. Tremorgenic mycotoxins: an update. *In* Antinutrients and Natural Toxicants in Foods, ed. R.L. Ory. Westport, CT: Food and Nutrition Press. pp. 17–33.

Cole, R.J. 1986. Etiology of Turkey "X" disease in retrospect: a case for the involvement of cyclopiazonic acid. Mycotoxin Res. 2: 3–7.

Cole, R.J. and Cox, R.H. 1981. Handbook of Toxic Fungal Metabolites. New York: Academic Press.

Cole, R.J. and Dorner, J.W. 1986. Role of fungal tremorgens in animal disease. *In* Mycotoxins and Phycotoxins, eds P.S. Steyn and R. Vleggaar. Amsterdam: Elsevier. pp. 501–511.

Cole, R.J., Kirksey, J.W., Moore, J.H., Blankenship, B.R., Diener, U.L. and Davis, N.D. 1972. Tremorgenic toxin from *Penicillium verruculosum*. Appl. Microbiol. 24: 248–256.

Cole, R.J., Kirksey, J.W., Cutler, H.G., Doupnik, B.L. and Peckham, J.C. 1973. Toxin from *Fusarium moniliforme*: effects on plants and animals. Science 179: 1324–1326.

Cole, R.J., Dorner, J.W., Cox, R.H. and Raymond, L.W. 1983. Two classes of alkaloid mycotoxins produced by *Penicillium crustosum* Thom isolated from contaminated beer. J. Agric. Food Chem. 31: 655–657.

Coley-Smith, J.R., Verhöff, K. and Jarvis, W.R. (eds). 1980. The Biology of *Botrytis*. London: Academic Press.

Collins, M.A. and Buick, R.K. 1989. Effect of temperature on the spoilage of stored peas by *Rhodotorula glutinis*. Food Microbiol. 6: 135–141.

Combrink, J.C., Kotze, J.M., Wehner, F.C. and Grobbelaar, C.J. 1985. Fungi associated with core rot of Starking apples in South Africa. Phytophylactica 17: 81–83.

Comi, G. and Cantoni, C. 1980. Yeasts on sausage casing surfaces. Ind. Aliment. (Pinerolo, Italy) 19: 563–569.

Comi, G. and Cantomi, C. 1983. Yeasts in dry Parma hams. Ind. Aliment. (Pinerolo, Italy) 22: 102–104.

Comi, G., D'Aubert, S. and Cantoni, C. 1982. Changes in fruit yoghurt. Latte 7: 543–546.

Comi, G., Drago, G., Fagnani, V., Gaggero, L., Rossi, E. and Cantoni, C. 1983. Lipolytic activity of yeasts from raw ham. Tecnol. Aliment. 6: 12–16.

Comi, G., Cantoni, C., Rosa, M. and Cattaneo, P. 1984. Yeasts and fish deterioration. Ristorazione Coll. 9: 65–73.

Comi, G., Manzoni, P., Provana, L. and Cantoni, C. 1992. Effects of physicochemical parameters on development of *Zygosaccharomyces rouxii* in ice cream mixes. Ind. Aliment. (Pinerolo, Italy) 31: 439–446.

Comi, G., Orlic, S., Redzepovic, S., Urso, R. and Iacumin, L. 2004. Moulds isolated from Istrian dried ham at the preripening and ripening level. Int. J. Food Microbiol. 96: 29–34.

Conner, R.L., Hwang, S.F. and Stevens, R.R. 1996. *Fusarium proliferatum*: a new causal agent of black point in wheat. Can. J. Plant Pathol. 18: 419–423.

Conway, W.S., Gross, K.C., Boyer, C.D. and Sams, C.E. 1988. Inhibition of *Penicillium expansum* polygalacturonase activity by increased apple cell wall calcium. Phytopathology 78: 1052–1055.

Conway W.S., Janisiewicz, W.J., Leverentz, B., Saftner, R.A. and Camp, M.J. 2007. Control of blue mold of apple by combining controlled atmosphere, an antagonist mixture and sodium bicarbonate. Postharvest Biol. Technol. 45: 326–332.

Cooney, D.G. and Emerson, R. 1964. Thermophilic Fungi. San Francisco, CA: W.H. Freeman.

Corpas-Hervias, C., Melero-Vara, J.M., Molinero-Ruiz, M. L., Zurera-Munoz, C. and Basallote-Ureba, M.J. 2006. Characterization of isolates of *Fusarium* spp. obtained from asparagus in Spain. Plant Dis. 90: 1441–1451.

Corry, J.E.L. 1976. The effect of sugars and polyols on the heat resistance and morphology of osmophilic yeasts. J. Appl. Bacteriol. 40: 269–276.

Corte, L., Rellini, P., Lattanzi, M., Picchetta, C., Fatichenti, F. and Cardinali, G. 2006. Diversity of salt responses among yeasts. Ann. Microbiol. 56: 363–368.

Cosic, J., Jurkovic, D., Vrandecic, K. and Simic, B. 2007. Pathogenicity of *Fusarium* species to wheat and barley ears. Cereal Res. Commun. 35: 529–532.

Cossentine, J.E., Sholberg, P.L., Jensen, L.B.J., Bedford, K.E. and Shephard, T.C. 2004. Fumigation of empty fruit bins with carbon dioxide to control diapausing codling moth larvae and *Penicillium expansum* Link. ex Thom spores. Hortscience 39: 429–432.

Coton, E., Coton, M., Levert, D., Casaregola, S. and Sohier, D. 2006. Yeast ecology in French cider and black olive natural fermentations. Int. J. Food Microbiol. 108: 130–135.

Cotty P.J. 1989. Virulence and cultural characteristics of two *Aspergillus flavus* strains pathogenic on cotton. Phytopathology 79: 808–814.

Cousin, M.A. 1996. Chitin as a measure of mold contamination of agricultural commodities and foods. J. Food Prot. 59: 73–81.

Cousin, M.A., Zeidler, C.S. and Nelson, P.E. 1984. Chemical detection of mold in processed foods. J. Food Sci. 49: 439–445.

Cousin, M.A., Dufrenne, J., Rombouts, F.M. and Notermans, S. 1990. Immunological detection of *Botrytis* and *Monascus* species in food. Food Microbiol. 7: 227–235.

Couto, J.A., Neves, F., Campos, F. and Hogg, T. 2005. Thermal inactivation of the wine spoilage yeasts *Dekkera/Brettanomyces*. Int. J. Food Microbiol. 104: 337–344.

Couto, M.M.B., Hartog, B.J., Huis in't Veld, J.H.J., Hofstra, H. and van der Vossen, J.M.B.M. 1996. Identification of spoilage yeasts in a food-production chain by microsatellite polymerase chain reaction fingerprinting. Food Microbiol. 13: 59–67.

Craven, H.M., Broome, M.C., Chandler, R.E. and Jensen, N. 2001. Dairy products. *In* Spoilage of Processed Foods: Causes and Diagnosis, eds C.J. Moir, C. Andrew-Kabilafkas, G. Arnold, B.M., Cox, A.D. Hocking and I. Jenson. Sydney, Australia: AIFST Inc. NSW Branch, pp. 147–163.

Crisan, E.V. 1973. Current concepts of thermophilism and the thermophilic fungi. Mycologia 65: 1171–1198.

Crous, P.W., Braun, U., Schubert, K. and Groenewald, J.Z. 2007. The genus *Cladosporium* and similar dematiaceous hyphomycetes. Stud. Mycol. 58: 1–253.

Cruickshank, R.H. and Pitt, J.I. 1987. Identification of species in *Penicillium* subgenus *Penicillium* by enzyme electrophoresis. Mycologia 79: 614–620.

Cuero, R.G., Smith, J.E. and Lacey, J. 1987. Interaction of water activity, temperature and substrate on mycotoxin production by *Aspergillus flavus*, *Penicillium viridicatum* and *Fusarium graminearum* on irradiated grains. Trans. Br. Mycol. Soc. 89: 221–226.

Cunningham. N.M. and Taverner, P.D. 2007. Efficacy of integrated postharvest treatments against mixed inoculations of *Penicillium digitatum* and *Geotrichum citri-aurantii* in 'Leng' navel oranges (*Citrus sinensis*). N.Z. J. Crop Hort. Sci. 35: 187–192.

Curtis, P.J. 1969. Anaerobic growth of fungi. Trans. Br. Mycol. Soc. 53: 299–302.

Curtis, R.F., Dennis, C., Gee, J.M., Gee, M.G., Griffiths, N.M., Land, D.G., Peel, J.L. and Robinson, D. 1974. Chloroanisoles as a cause of musty taint in chickens and their microbiological formation from chlorophenols in broiler house litters. J. Sci. Food Agric. 25: 811–828.

Cvetnic, Z. 1994. Cyclopiazonic acid and aflatoxin production by cultures of *Aspergillus flavus* isolated from dried beans and maize. Nahrung 38: 21–25.

da Silva, J.B., Dilkin, P., Fonseca, H. and Corrêa, B. 2004. Production of aflatoxins by *Aspergillus flavus* and of fumonisins by *Fusarium* species isolated from Brazilian sorghum. Braz. J. Microbiol. 35: 182–186.

Dakin, J.C. and Day, P.M. 1958. Yeasts causing spoilage in acetic acid preserves. J. Appl. Bacteriol. 21: 94–96.

Dakin, J.C. and Stolk, A.C. 1968. *Moniliella acetoabutans*: some further characteristics and industrial significance. J. Food Technol. 3: 49–53.

Daley, J.D., Lloyd, G.T., Ramshaw, E.H. and Stark, W. 1986. Off-flavours related to the use of sorbic acid as a food preservative. CSIRO Food Res. Q. 46: 59–63.

Dall'Asta, C., Lindner, J.D., Galaverna, G., Dossena, A., Neviani, E. and Marchelli, R. 2008. The occurrence of ochratoxin A in blue cheese. Food Chem. 106: 729–734.

Dallyn, H. and Everton, J.R. 1969. The xerophilic mould, *Xeromyces bisporus*, as a spoilage organism. J. Food Technol. 4: 399–403.

Dalton, H.K., Board, R.G. and Davenport, R.R. 1984. The yeasts of British fresh sausage and minced beef. Antonie van Leeuwenhoek 50: 227–248.

Daly, N.M., Lee, T.H. and Fleet, G.H. 1984. Growth of fungi on wine corks and its contribution to corky taints in wine. Food Technol. Aust. 36: 22–24.

Damare, S., Raghukumar, C., Muraleedharan, U.D. and Raghukumar, S. 2006. Deep-sea fungi as a source of alkaline and cold-tolerant proteases. Enz. Microbial Technol. 39: 172–181.

Damayanti, M., Sharma, G.J. and Kundu, S.C. 1992. Gamma radiation influences postharvest disease incidence of pineapple fruits. HortScience 27: 807–808.

Damoglou, A.P. and Campbell, D.S. 1986. The effect of pH on the production of patulin in apple juice. Lett. Appl. Microbiol. 2: 9–11.

Dang, J.K. and Singh, J.P. 1982. Fungal rot of stored onion bulbs in Haryana. Haryana Agric. Univ. J. Res. 12: 509–513.

Dantigny, P., Tchobanov, I., Bensoussan, M. and Zwietering, M.H. 2005. Modelling the effect of ethanol vapour on the germination time of *Penicillium chrysogenum*. J. Food Prot. 68: 1203–1207.

Davé, B., Sales, M. and Walia, M. 1989. Resistance of different strains of *Penicillium digitatum* to imazalil treatment in California citrus packinghouses. Proc. Fla State Hortic. Soc. 102: 178–179.

Dawar, S., Syed, F. and Gaffar, A. 2007. Seedborne fungi associated with chickpea in Pakistan. Pak. J. Botan. 39: 637–643.

De Bock, R., Gyssens, I., Peetermans, M. and Nolard, N. 1989. *Aspergillus* in pepper. Lancet ii: 331–332.

De Hoog, G.S. 1979. Taxonomic review of *Moniliella, Trichosporonoides* and *Hyalodendron*. Stud. Mycol. (Baarn) 19: 1–36.

De Hoog, G.S. and Horré, R. 2002. Molecular taxonomy of the *Alternaria* and *Ulocladium* species from humans and their identification in the routine laboratory. Mycoses 45: 249–276.

De Hoog, G.S. and Yurlova, N.A. 1994. Conidiogenesis, nutritional physiology and taxonomy of *Aureobasidium* and *Hormonema*. Antonie van Leeuwenhoek 65: 41–54.

De Hoog, G.S., Smith, M.Th. and Guého, E. 1986. A revision of the genus *Geotrichum* and its teleomorphs. Stud. Mycol. (Baarn) 29: 1–130.

De Hoog, G.S., Guarro, J., Gené. J. and Figueras, M.J. 2000. Atlas of Clinical Fungi, 2nd edn. Utrecht: Centraalbureau voor Schimmelcultures.

De Jesus, A.E., Steyn, P.S., van Heerden, F.R. and Vleggaar, R. 1984. Structure elucidation of the janthitrems, novel tremorgenic mycotoxins from *Penicillium janthinellum*. J. Chem. Soc., Perkin. Trans. 1, 1984: 697–701.

De Jong, J. and Put, H.M.C. 1980. Enumeration of yeasts in the presence of moulds. *In* Biology and Activities of Yeasts, eds F.A. Skinner, S.M. Passmore and R.R. Davenport. London: Academic Press. pp. 289–292.

De Nijs, M., Rombouts, F. and Notermans, S. 1996. *Fusarium* molds and their mycotoxins. J. Food Saf. 16: 15–58.

De Vries, G.A. 1952. Contribution to the knowledge of the genus *Cladosporium*. Dissertation. Utrecht, Netherlands: Univ. Utrecht. [Reprint, Lehre: J. Cramer, 1967].

Deák, T. 1992. Experiences with, and further improvement to, the Deák and Beuchat simplified identification scheme for foodborne yeasts. *In* Modern Methods in Food Mycology, eds R.A. Samson, A.D. Hocking, J.I. Pitt, and A.D. King. Amsterdam: Elsevier. pp. 47–54.

Deák, T. and Beuchat, L.R. 1987. Identification of foodborne yeasts. J. Food Prot. 50: 243–264.

Deák, T. and Beuchat, L.R. 1993. Yeasts associated with fruit juice concentrates. J. Food Prot. 56: 777–782.

Deák, T. and Beuchat, L.R. 1996. Handbook of Food Spoilage Yeasts. Boca Raton, FL: CRC Press.

Deiana, P., Fatichenti, F., Farris, G.A., Mocquot, G., Lodi, R., Todesco, R. and Cecchi, L. 1984. Metabolization of lactic and acetic acids in Pecorino Romano cheese made with a combined starter of lactic acid bacteria and yeast. Lait 64: 380–394.

Delfini, C., Gaia, P. and Bardi, L. 1990. Degree of contamination of rectified, concentrated musts (MCR) during the rectification, concentration and storage stages of production. Study of preventive and remedial steps. I. Riv. Viti. Enol. 43: 55–71.

Demirci, A.S. and Arici, M. 2006. Isolation of heat resistant moulds in margarine and determination of their heat resistance. J. Tekirdag Agric. Fac. 3: 269–273.

Demyttenaere, J.C.R., Morina, R.M., Kimpe, N. de and Sandra, P. 2004. Use of headspace solid-phase microextraction and headspace sorptive extraction for the detection of the volatile metabolites produced by toxigenic *Fusarium* species. J. Chromatogr. A 1027: 147–154.

Dennis, C. 1983. Yeast spoilage of fruit and vegetable products. Indian Food Packer 37: 38–53.

Dennis, C., Davis, R.P., Harris, J.E., Calcutt, L.W. and Cross, D. 1979. The relative importance of fungi in the breakdown of commercial samples of sulphited strawberries. J. Sci. Food Agric. 30: 959–973.

Desjardins, A.E. 2006. *Fusarium* Mycotoxins. Chemistry, Genetics and Biology. St Paul, MN, The American Phytopathological Society. 260 pp.

Desjardins, A.E. and Busman, M. 2006. Mycotoxins in developing countries: a case study of maize in Nepal. Mycotox. Res. 22: 92–95.

Desjardins, A.E., Hohn, T.M. and McCormick, S.P. 1993. Trichothecene biosynthesis in *Fusarium* species: Chemistry, genetics and significance. Microbiol. Rev. 57: 595–604.

Desjardins, A.E., Manandhar, H.K., Plattner, R.D., Manandhar, G.G., Poling, S.M. and Maragos, C.M. 2000a. *Fusarium* species from Nepalese rice and production of mycotoxins and gibberellic acid by selected species. Appl. Environ. Microbiol. 66: 1020–1025.

Desjardins, A.E., Plattner, R.D. and Gordon, T.R. 2000b. *Gibberella fujikuroi* mating population A and *Fusarium subglutinans* from teosinte species and maize from Mexico and Central America. Mycol. Res. 104: 865–872.

Desjardins, A.E., Maragos, C.M. and Proctor, R.H. 2006. Maize ear rot and moniliformin contamination by cryptic species of *Fusarium subglutinans*. J. Agric. Food Chem. 54: 7383–7390.

Desjardins, A.E., Busman, M., Proctor, R.H. and Stessman, R. 2007. Wheat kernel black point and fumonisin contamination by *Fusarium proliferatum*. Food Addit. Contam. 24: 1131–1137.

Devoyod, J.J. 1988. Microbial accidents in cheese making due to *Mucor*. Microbiol. Aliments Nutr. 6: 25–29.

Dewey, F.M., MacDonald, M.M., Phillips, S.I. and Priestley, R.A. 1990. Development of monoclonal-antibody-ELISA and -DIP-STICK immunoassays for *Penicillium islandicum* in rice grains. J. Gen. Microbiol. 136: 753–760.

Dewey, F.M., Twiddy, D.R., Phillips, S.I., Grose, M.J. and Wareing, P.W. 1992. Development of a quantitative monoclonal antibody-based immunoassay for *Humicola lanuginosa* in rice grains and comparison with conventional assays. Food Agric. Immunol. 4: 153–167.

D'Hallewin, G., Arras, G., Venditti, T., Rodov, V. and Ben-Yehoshua, S. 2005. Combination of ultraviolet-C irradiation and biocontrol treatments to control decay caused by *Penicillium digitatum* in "Washington navel" orange fruit. Proc. 5th Int. Postharvest Symp. Acta Horticulturae 682: 2007–2012.

Dharam, V. 1977. Post-harvest fungal deterioration of banana fruits. Curr. Sci. 46: 92.

Dharmaputra, O.S. and Retnowati, I. 1996. Fungi isolated from groundnuts in some locations of West Java. Biotropia 9: 15–25.

Dharmaputra, O.S., Sunjaya, Amad, M., Retnowati, I. and Wahyudi, T. 1999. The occurrence of insects and moulds in stored cocoa beans at South Sulawesi. Biotropia 12: 1–18.

Dhulipala, V.C., Maddali, K.K., Welshons, W.V. and Reddy, C.S. 2005. Secalonic acid D blocks embryonic palatal mesenchymal cell-cycle by altering the activity of CDK2 and the expression of p21 and cyclin E. Birth Defects Res. 74: 233–242.

Di Menna, M.E. and Mantle, P.G. 1978. The role of Penicillia in ryegrass staggers. Res. Vet. Sci. 24: 347–351.

Di Menna, M.E., Lauren, D.R. and Wyatt, P.A. 1986. Effect of culture conditions on tremorgen production by some *Penicillium* species. Appl. Environ. Microbiol. 51: 821–824.

Diaz-Borras, M.A., Vila-Aguilar, R. and Hernandez-Gimenez, E. 1987. Resistance to SOPP, benomyl, TBZ and CGA-64251 in strains of *Penicillium digitatum* isolated from Spanish citrus packinghouses. Rev. Agroquim. Tecnol. Aliment. 27: 439–445.

Diener, U.L. and Davis, N.D. 1967. Limiting temperature and relative humidity for growth and production of aflatoxin and free fatty acids by *Aspergillus flavus* in sterile peanuts. J. Am. Oil Chem. Soc. 44: 259–263.

Diener, U.L., Cole, R.J., Sanders, T.H., Payne, G.A., Lee, L.S. and Klich, M.A. 1987. Epidemiology of aflatoxin formation by *Aspergillus flavus*. Annu. Rev. Phytopathol. 25: 249–270.

Dijksterhuis, J. and Samson, R.A. 2006. Activation of ascospores by novel food preservation techniques. *In* Advances in Food Mycology, eds A.D. Hocking, J.I. Pitt, R.A. Samson and U. Thrane eds. New York: Springer, pp: 247–260.

Dijksterhuis, J. and Teunissen, P.G.M. 2004. Dormant ascospores of *Talaromyces macrosporus* are activated to germinate after treatment with ultra high pressure. J. Appl. Microbiol. 96: 162–169.

Dijksterhuis, J., van Driel, K.G.A., Sanders, M.G., Molenaar, D., Houbraken, J., Samson, R.A. and Kets, E.P.W. 2002. Trehalose degradation and glucose efflux precede cell ejection during germination of heat-resistant ascospores of *Talaromyces macrosporus*. Arch. Microbiol. 178: 1–7.

Dillon, V.M and Board, R.G. 1991. Yeasts associated with red meats. J. Appl. Bacteriol. 71: 93–108.

Dimici, L. and Wada, S. 1994. Lipid changes in bonito meat in the katsuobushi processing and quality assessment of the commercial product based on lipid composition. Abura Kagaku (J. Jpn. Oil Chem. Soc.) 43: 470–477.

Diyaolu, S.A. and Adebajo, L.O. 1994. Effects of sodium chloride and relative humidity on growth and sporulation of moulds isolated from cured fish. Nahrung 38: 311–317.

Djokoto, D., Dzogbefia, V.P. and Oldham, J.H. 2006. Rapid extraction of pawpaw juice with the application of locally produced pectic enzymes from *Saccharomyces cerevisiae* ATCC 51712. Food Biotechnol. 20: 31–41.

Do Amaral, A. L., Dal Soglio, F. K., De Carli, M. L. and Neto, J. F. B. 2005. Pathogenic fungi causing symptoms similar to *Phaeosphaeria* leaf spot of maize in Brazil. Plant Dis. 89: 44–49.

Dodds, G.T., Brown, J.W. and Ludford, P.M. 1991. Surface color changes of tomato and other solanaceous fruit during chilling. J. Am. Soc. Hortic. Sci. 116: 482–490.

Dombrink-Kurtzman, M.A. and Blackburn, J.A. 2005. Evaluation of several culture media for production of patulin by *Penicillium* species. Int. J. Food Microbiol. 98: 241–248.

Domsch, K.H., Gams, W. and Anderson, T.-H. 1980. Compendium of Soil Fungi, 2 Vol. London: Academic Press.

Donald, W.W. and Mirocha, C.J. 1977. Chitin as a measure of fungal growth in stored corn and soybean seed. Cereal Chem. 54: 466–474.

Dong, Y.H., Steffenson, B.J. and Mirocha, C.J. 2006. Analysis of ergosterol in single kernel and ground grain by gas chromatography-mass spectrometry. J. Agric. Food Chem. 54: 4121–4125.

Dorner, J.W. 1983. Production of cyclopiazonic acid by *Aspergillus tamarii* Kita. Appl. Environ. Microbiol. 46: 1435–1437.

Dorner, J.W. and Horn, B.W. 2007. Separate and combined applications of nontoxigenic *Aspergillus flavus* and *A. parasiticus* for biocontrol of aflatoxin in peanuts. Mycopathologia 163: 215–223.

Dorner, J.W., Cole, R.J. and Hill, R.A. 1984. Tremorgenic mycotoxins produced by *Aspergillus fumigatus* and *Penicillium crustosum* isolated from molded corn inplicated in a natural intoxication of cattle. J. Agric. Food Chem. 32: 411–413.

Dorner, J.W., Cole, R.J., Erlington, D.J., Suksupath, S., McDowell, G.H. and Bryden, W.L. 1994. Cyclopiazonic acid residues in milk and eggs. J. Agric. Food Chem. 42: 1516–1518.

Doster, M.A. and Michailides, T.J. 1994. *Aspergillus* molds and aflatoxins in pistachio nuts in California. Phytopathology 84: 583–590.

Doster, M.A. and Michailides, T.J. 1998. Production of bright greenish yellow fluorescence in figs infected by *Aspergillus* species in California orchards. Plant Dis. 82: 669–673.

Doster, M.A. and Michailides, T.J. 2007. Fungal decay of first-crop and main-crop figs. Plant Dis. 91: 1657–1662.

Doster, M.A., Michailides, T.J. and Morgan, D.P. 1996. *Aspergillus* species and mycotoxins in figs from California orchards. Plant Dis. 80: 484–489.

Doupnik, B. and Bell, D.K. 1971. Toxicity to chicks of *Aspergillus* and *Penicillium* species isolated from moldy pecans. Appl. Microbiol. 21: 1104–1106.

Doupnik, B., Jones, O.H. and Peckham, J.C. 1971. Toxic Fusaria isolated from moldy sweet potatoes involved in an epizootic of atypical interstitial pneumonia in cattle. Phytopathology 61: 890.

Doyle, M.P. and Marth, E.H. 1975a. Thermal inactivation of conidia from *Aspergillus flavus* and *Aspergillus parasiticus*. I. Effects of moist heat, age of conidia, and sporulation medium. J. Milk Food Technol. 38: 678–682.

Doyle, M.P. and Marth, E.H. 1975b. Thermal inactivation of conidia from *Aspergillus flavus* and *Aspergillus parasiticus*. 2. Effects of pH and buffers, glucose, sucrose, and sodium chloride. J. Milk Food Technol. 38: 750–758.

Dragoni, I. and Cantoni, C. 1979. Reddening of flour-based foods by *Epicoccum purpurascens*. Tec. Molitoria 30: 84–86.

Dragoni, I. and Cantoni, C. 1988. Mould spoilage of modified atmosphere packaged ravioli. Tec. Molitoria 39: 8–12.

Dragoni, I. and Marino, C. 1979. Description and classification of *Penicillium* species isolated from raw ripened sausages. Arch. Vet. Ital. 30: 142–176.

Dragoni, I., Comi, G., Corti, S. and Marino, C. 1979. Sulla presenza di muffe potenzialmente tossinogene in alimenti con crusca. Sel. Tec. Molitoria 30: 569–576.

Dragoni, I., Marino, C. and Ravenna, R. 1980a. Moulds in flours used for industrial breadmaking. Tec. Molitoria 31: 235–241.

Dragoni, I., Ravenna, R. and Marino, C. 1980b. Descrizione e classificazione delle specie di *Aspergillus* isolate dalla superficie di prosciutti stagionati di Parma e San Daniele. Arch. Vet. Ital. 31(Suppl. 5): 1–56.

Dragoni, I., Assente, G., Comi, G., Marino, C. and Ravenna, R. 1980c. Sull'ammuffimento del pane industriale confezionato: *Monilia* (*Neurospora*) *sitophila* e altre specie responsabili. Tecnol. Aliment. 3: 17–26.

Dragoni, I., Marino, C. and Cantoni, C. 1988. Moulds on smoked bacon. Ind. Aliment. (Pinerolo, Italy) 27: 353–355.

Dragoni, I., Balzaretti, C. and Ravarotto, R. 1989. Mycoflora seasonal variability in a confectionery production line. Ind. Aliment. (Pinerolo, Italy) 28: 481–486, 491.

Dragoni, I., Balzaretti, C., Puzzi, E. and Papa, A. 1990. The fungicidal action of microwaves. Tec. Molitoria 41: 1035–1041.

Dragoni, I., Papa, A. and Vallone, L. 1997. Mycological standards in Taleggio cheese. Microbiol. Aliment. Nutr. 15: 185–190.

Droby, S., Chalutz, E., Wilson, C.L. and Wisniewski, M. 1989. Characterization of the biological control activity of *Debaryomyces hansenii* in the control of *Penicillium digitatum* on grapefruit. Can. J. Microbiol. 35: 794–800.

Droby, S., Wisniewski, M., El-Ghaouth, A. and Wilson, C. 2003. Influence of food additives on the control of postharvest rots of apple and peach and efficacy of the yeast-based biocontrol product Aspire. Postharvest Biol. Technol. 27: 127–135.

Druvefors, U.A., Passoth, V. and Schnürer, J. 2005. Nutrient effects on biocontrol of *Penicillium roqueforti* by *Pichia anomala* J121 during airtight storage of wheat. Appl. Environ. Microbiol. 71: 1865–1869.

Duarte, F.L., Pais, U., Spencer-Martins, I. and Leao, C. 2004. Isoenzyme patterns: a valuable molecular tool for the differentiation of *Zygosaccharomyces* species and detection of misidentified isolates. Syst. Appl. Microbiol. 27: 436–442.

Duckworth, R.B. (ed.) 1975. Water Relations of Foods. London: Academic Press.

Duenas, M., Irastorza, A., Fernandez, K., Bilbao, A. and Huerta, A. 1994. Microbial populations and malolactic fermentation of apple cider using traditional and modified methods. J. Food Sci. 59: 1060–1064, 1085.

Dufosse, L., Galaup, P., Yaron, A., Arad, S.M., Blanc, P.J., Murthy, K.N.C. and Ravishankar, G.A. 2005. Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality? Trends Food Sci. Technol. 16: 389–406.

Dugan, F.M. and Roberts, R.G. 1994. Morphological and reproductive aspects of *Cladosporium macrocarpum* and *C. herbarum* from Bing cherry fruits. Mycotaxon 52: 513–522.

Dugan, F.M., Hellier, B.C. and Lupien, S.L. 2003. First report of *Fusarium proliferatum* causing rot of garlic bulbs in North America. Plant Pathol. 52: 426.

Dugan, F.M., Hellier, B.C. and Lupien, S.L. 2007. Pathogenic fungi in garlic seed cloves from the United States and China, and efficacy of fungicides against pathogens in garlic germplasm in Washington State. J. Phytopathol. 155: 437–445.

Duncan, B. 1973. Nutrition and fat production in submerged cultures of a strain of *Penicillium lilacinum*. Mycologia 65: 211–214.

Dupuy, J., Le Bars, P., Boudra, H. and Le Bars, J. 1993. Thermostability of fumonisin B_1, a mycotoxin from *Fusarium moniliforme*, in corn. Appl. Environ. Microbiol. 59: 2864–2867.

Duran, M.C., Garcia, P., Brenes, M. and Garrido, A. 1994. *Lactobacillus plantarum* survival in aerobic, directly brined olives. J. Food Sci. 59: 1197–1201.Easter, M. 2007. Hygiene monitoring in support of food safety – methods and trends. Food Sci. Technol. 21: 52–53.

Dyer, S.K. and McCammon, S. 1994. Detection of toxigenic isolates of *Aspergillus flavus* and related species on coconut cream agar. J. Appl. Bacteriol. 76: 75–78.

Easter, M. 2007. Hygiene monitoring in support of food safety – methods and trends. Food Sci. Technol. 21: 52–53.

Ebenezer, P., Murugan, M. and Muthumary, J. 2002. Effect of chemical environment on growth and sporulation in *Pestalotiopsis* spp. Indian J. Microbiol. 42: 303–307.

Echemendia, M. 2005. Searching of fungus diseases in *Sorghum halepense* (L.) Pers. in different locations in Cuba. Rev. Prot. Vegetal 20: 32–38.

Echerenwa, M.C. and Umechuruba, C.I. 2004. Post-harvest fungal diseases of pawpaw (*Carca papaya* L.) fruits and seeds in Nigeria. Global J. Pure Appl. Sci. 10: 69–73.

Eckert, J.W., Rubio, P.P., Mattoo, A.K. and Thompson, A.K. 1975. Diseases of tropical fruits and their control. *In* Postharvest Physiology, Handling and Utilization of Tropical and Subtropical Fruits and Vegetables, ed. E.B. Pantastico. Westport, CT: AVI Publishing Co. pp. 415–443.

Edwards, D.G. 1993. The nutritional evaluation of mycoprotein. Int. J. Food Sci. Nutr. 44 (Suppl. 1): S37–S43.

Edwards, S.G., O'Callaghan, J. and Dobson, A.D.W. 2002. PCR-based detection and quantification of mycotoxigenic fungi. Mycol. Res. 106: 1005–1025.

Ehrlich, K.C., Lee, L.S., Ciegler, A. and Palmgren, M.S. 1982. Secalonic acid D: natural contaminant of corn dust. Appl. Environ. Microbiol. 44: 1007–1008.

Ehrlich , K.C., Kobbeman, K., Montalbano, B.G. and Cotty, P.J. 2007. Aflatoxin-producing *Aspergillus* species from Thailand. Int. J. Food Microbiol. 114: 153–159.

Eikenes, M., Hietala, A.M., Alfredsen, G., Fossdal, C.G. and Solheim, H. 2005. Comparison of quantitative real-time PCR, chitin and ergosterol assays for monitoring colonization of *Trametes versicolor* in birch wood. Holzforschung 59: 568–573.

Eisenberg, W.V. and Cichowicz, S.M. 1977. Machinery mold – indicator organism in food. Food Technol., Champaign 31: 52–56.

Ekblad, A. and Nasholm, T. 1996. Determination of chitin in fungi and mycorrhizal roots by an improved HPLC analysis of glucosamine. Plant Soil 178: 29–35.

Ekblad, A., Wallander, H. and Nasholm, T. 1998. Chitin and ergosterol combined to measure total and living fungal biomass in ectomycorrhizas. New Phytol. 138: 143–14.

Ekundayo, C.A. 1987. Mycoflora and vitamin content of sun-dried food condiments in Nigeria. Plant Foods Hum. Nutr. 37: 247–252.

Ekundayo, J.A. and Daniel, T.M. 1973. Cassava rot and its control. Trans. Br. Mycol. Soc. 61: 27–32.

El-Aasar, S.A. 2006. Cultural conditions studies on kojic acid production by *Aspergillus parasiticus*. Int. J. Agric. Biol. 8: 468–473.

Elad, Y., Williamson, B., Tudzynski, P. and Delen, N. 2004. *Botrytis*: Biology, Pathology and Control. Dordrecht, Netherlands: Kluwer Academic Publishers.

El-Banna, A.A., Fink-Gremmels, J. and Leistner, L. 1987a. Investigation of *Penicillium chrysogenum* isolates for their suitability as starter cultures. Mycotoxin Res. 3: 77–83.

El-Banna, A.A., Pitt, J.I. and Leistner, L. 1987b. Production of mycotoxins by *Penicillium* species. Syst. Appl. Microbiol. 10: 42–46.

Elez-Martinez, P., Escola-Hernandez, J., Soliva-Fortuny, R. C. and Martin-Belloso, O. 2004. Inactivation of *Saccharomyces cerevisiae* suspended in orange juice using high-intensity pulsed electric fields. J. Food Prot. 67: 2596–2602.

El-Ghaouth, A., Wilson, C.L. and Wisniewski, M.E. 1995. Sugar analogs as potential fungicides for postharvest pathogens of apple and peach. Plant Dis. 79: 254–258.

El-Geddawy, M.A.H. 2005. Heat resistance and control measures of *Byssochlamys fulva* in some juices. Assiut J. Agric. Sci. 36: 1–8.

El-Habbaa, G. M. 2003. Polygalacturonase isoenzymes and cell wall degrading enzymes secreted by *Botrytis cinerea* the causal of grey mould disease on vegetables. Egypt. J. Phytopathol. 31: 87–101.

El-Halouat, A. and Debevere, J.M. 1996. Influence of modified atmosphere and preservatives on the growth of *Zygosaccharomyces rouxii* isolated from dried fruits. Int. J. Food Microbiol. 33: 219–229.

El-Halouat, A. and Debevere, J.M. 1997. Molds and yeasts isolated from hydrated prunes and raisins having different water activities. Sci. Aliments 17: 539–545.

El-Hassan, K.I., El-Saman, M.G., Mosa, A.A. and Mostafa, M.H. 2004. Interaction between non-pathogenic *Fusarium* isolates and *Fusarium* species causing dry rot of potato tubers. Ann. Agric. Sci. (Cairo) 49: 759–771.

El-Khoury, A., Rizk, T., Lteif, R., Azouri, H., Delia, M.L. and Lebrihi, A. 2006. Occurrence of ochratoxin A- and aflatoxin B_1-producing fungi in Lebanese grapes and ochratoxin A content in musts and finished wines during 2004. J. Agric. Food Chem. 54: 8977–8982.

Ellis, J.J. 1985. Species and varieties in the *Rhizopus arrhizus – Rhizopus oryzae* group as indicated by their DNA complementarity. Mycologia 77: 243–247.

Ellis, J.J. and Hesseltine, C.W. 1966. Species of *Absidia* with ovoid sporangiospores. Sabouraudia 5: 59–77.

Ellis, M.B. 1971. Dematiaceous Hyphomycetes. Kew: Commonwealth Mycological Institute.

El-Magraby, O.M.O. and El-Maraghy, S.S.M. 1988. Mycoflora and mycotoxins of peanut (*Arachis hypogaea* L.) seeds in Egypt. III. Cellulose-decomposing and mycotoxin-producing fungi. Mycopathologia 104: 19–24.

Elmer, W.H. 1996. Fusarium root rot of pumpkin in Connecticut. Plant Dis. 80: 131–135.

Elmer, W.H. 2000. Incidence of infection of asparagus spears marketed in Connecticut by *Fusarium* spp. Plant Dis. 84: 831–834.

Elmer, W.H. 2001. Fusarium diseases of asparagus. *In Fusarium*. Paul E. Nelson Memorial Symposium, eds. B.A. Summerell, J.F. Leslie, D. Backhouse, W.L. Bryden and L.W. Burgess. St Paul, MN, APS Press. pp. 248–262.

Elshafie, A.E., Al-Rashdi, T.A., Al-Bahry, S.N. and Bakheit, C.S. 2002. Fungi and aflatoxins associated with spices in the Sultanate of Oman. Mycopathologia 155: 155–160.

El-Shanawany, A.A., Mostafa, M.E. and Barakat, A. 2005. Fungal populations and mycotoxins in silage in Assiut and Sohag governorates in Egypt, with a special reference to characteristic Aspergilli toxins. Mycopathologia 159: 281–289.

El-Tabiy, A.A. 2006. Mycological study on some processed meat products exposed for sale in markets. Assiut Vet. Med. J. 52 (110): 121–131.

El-Tobshy, Z.M., Abdel-Sattar, M.A. and Baraka, M.A. 1979. Sour rot, a post-harvest disease of certain vegetables in the A.R.E. Agric. Res. Rev. 57: 39–46.

Empey, W.A. and Scott, W.J. 1939. Investigations on chilled beef. I. Microbial contamination acquired in the meatworks. Bull. Counc. Sci. Ind. Res. Melbourne 126: 1–71.

Engel, G. 1986. Hefen und Schimmelpilze in Futtermitteln, Rohmilch und Milchprodukten. Dtsch. Molk. Ztg. 107: 1286, 1288, 1290–1292.

Engel, G. 1988. Hefen in Quark. Dtsch. Milchwirtsch. 39: 512–514.

Engel, G. and Teuber, M. 1978. Simple aid for the identification of *Penicillium roqueforti* Thom. Eur. J. Appl. Microbiol. Biotechnol. 6: 107–111.

Engel, G. and Teuber, M. 1980. Bildung und Verteilung von Sterigmatocystin in Käse nach Beimpfung mit *Aspergillus versicolor* und *Aspergillus nidulans*. Milchwissenschaft 35: 721–724.

Engel, G. and Teuber, M. 1991. Heat resistance of ascospores of *Byssochlamys nivea* in milk and cream. Int. J. Food Microbiol. 12: 225–234.

Engel, G., Rösch, N. and Heller, K.J. 1994. Heat tolerance of yeasts. Hitzetoleranz von Hefen. Kiel. Milchwirtsch. Forschungsber. 46: 81–90.

English, M.P. 1954. The physiology of *Saccharomyces rouxii*. J. Gen. Microbiol. 10: 328–336.

Enigl, D.C., King, A.D. and Török, T. 1993. *Talaromyces trachyspermus*, a heat resistant mold isolated from fruit juice. J. Food Prot. 12: 1039–1042.

Entis, P. 1996. Two-day hydrophobic grid membrane filter method for yeast and mold enumeration in foods using YM-11 agar: collaborative study. J. AOAC Int. 79: 1069–1082.

Erdogan, A. and Sert, S. 2004. Mycotoxin-forming ability of two *Penicillium roqueforti* strains in blue mouldy Tulum cheese ripened at various temperatures. J. Food Prot. 67: 533–535.

Erdogan, A., Gurses, M. and Sert, S. 2003. Isolation of moulds capable of producing mycotoxins from blue mouldy Tulum cheeses produced in Turkey. Int. J. Food Microbiol. 85: 83–85.

Erickson, J.P. 1993. Hydrophobic membrane filtration methods for the selective recovery and differentiation of *Zygosaccharomyces bailii* in acidified ingredients. J. Food Prot. 56: 234–238.

Esfahani, M.N. 2006. Present status of Fusarium dry rot of potato tubers in Isfahan (Iran). Indian Phytopathol. 59: 142–147.

Esteban, A., Abarca, M.L., Bragulat, M.R. and Cabañes, F.J. 2004. Effects of temperature and incubation time on

production of ochratoxin A by black aspergilli. Res. Microbiol. 155: 861–866.
Esteban, A., Abarca, M.L., Bragulat, M.R. and Cabañes, F. J. 2005. Influence of pH and incubation time on ochratoxin A production by *Aspergillus carbonarius* in culture media. J. Food Prot. 68: 1435–1440.
Esteban, A., Abarca, M.L., Bragulat, M.R. and Cabañes, F. J. 2006a. Effect of water activity on ochratoxin A production by *Aspergillus niger* aggregate species. Int. J. Food Microbiol. 108: 188–195.
Esteban, A., Abarca, M.L., Bragulat, M.R. and Cabañes, F. J. 2006b. Effects of pH on ochratoxin A production by *Aspergillus niger* aggregate species. Food Addit. Contam. 23: 616–622.
Esterio, M., Auger, J. Ramos, C. and García, H. 2007. First report of fenhexamid resistant isolates of *Botrytis cinerea* on grapevine in Chile. Plant Dis. 91: 768.
Esteve-Zarzoso, B., Zorman, T., Belloch, C. and Querol, A. 2003. Molecular characterisation of the species of the genus *Zygosaccharomyces*. Syst. Appl. Microbiol. 26: 404–411.
Esuruoso, O.F. 1974. Fungi associated with kernel rot disease of cashew (*Anacardium occidentale* L.) in Nigeria. Int. Biodeterior. Bull. 10: 57–59.
Etchells, J.L., Costilow, R.N. and Bell, T.A. 1952. Identification of yeasts from commerical cucumber fermentations in northern bringing areas. Farlowia 4: 249–264.
Etchells, J.L., Bell, T.A. and Jones, I.D. 1953. Morphology and pigmentation of certain yeasts from brines and the cucumber plant. Farlowia 4: 265–304.
Etcheverry, M., Chulze, S., Dalcero, A., Varsavsky, E. and Magnoli, C. 1994. Effect of water activity and temperature on tenuazonic acid production by *Alternaria alternata* on sunflower seeds. Mycopathologia 126: 179–182.
Etcheverry, M., Torres, A., Ramirez, M.L., Chulze, S. and Magan, N. 2002. In vitro control of growth and fumonisin production by *Fusarium verticillioides* and *F. proliferatum* using antioxidants under different water availability and temperature regimes. J. Appl. Microbiol. 92: 624–632.
Etebarian H.R., Sholberg, P.L., Eastwell K.C. and Sayler, R. J. 2005. Biological control of apple blue mold with *Pseudomonas fluorescens*. Can. J. Microbiol. 51: 591–598.
Ethiraj, S. and Suresh, E.R. 1988. *Pichia membranaefaciens*: a benzoate resistant yeast from spoiled mango pulp. J. Food Sci. Technol. India 25: 63–66.
EU (European Union). 2002. Commission Regulation (EC) No. 472/2002 of 12 March 2002 amending Regulation (EC) No. 466/2001 setting maximum limits as regards certain contaminants in foodstuffs. Off. J. Eur. Union 77: 18–20, 42.
EU (European Union). 2004. Commission Regulation (EC) No. 455/2004 of 11 March 2004 amending Regulation (EC) No. 466/2001 as regards patulin. Off. J. Eur. Union L74/11.
Evancho, G.M., Sveum, W. H., Moberg, L.J. and Frank, J. H. 2001. Microbiological monitoring of the food processing environment. *In* Compendium of Methods for the Microbiological Examination of Foods, eds F. P. Downes and K. Ito. Washington DC, American Public Health Association. pp. 25–35.
Evans, H.A.V. 1982. A note on two uses for impedimetry in brewing microbiology. J. Appl. Bacteriol. 53: 423–426.
Evans, H.C. 1971. Thermophilous fungi of coal spoil tips. II. Occurrence, distribution and temperature relationships. Trans. Br. Mycol. Soc. 57: 255–266.
Everett, K.R. and Timudo-Torrevilla, O.E. 2007. *In vitro* fungicide testing for control of avocado fruit rots. N.Z. Plant Prot. 60: 99–103.
Eyles, M.J., Moss, R. and Hocking, A.D. 1989. The microbiological status of Australian flour and the effects of milling procedures on the microflora of wheat and flour. Food Aust. 41: 704–708.
Fahrasmane, L., Ganou-Parfait, B. and Parfait, A. 1988. Yeast flora of Haitian rum distilleries. Mircen J. Appl. Microbiol. Biotechnol. 4: 239–241.
Faid, M. and Tantaoui-Elaraki, A. 1989. Production of toxic metabolites by *Penicillium italicum* and *P. digitatum* isolated from citrus fruits. J. Food Prot. 52: 194–197.
Faid, M., Akhartouf, R. and Asehraou, A. 1994. Microorganisms associated with postharvest green olives deteriorations in Morocco. Grasas Aceites 45: 313–317.
Fakhrunnisa, Hashmi, M. H. and Ghaffar, A. 2006. Seedborne mycoflora of wheat, sorghum and barley. Pak. J. Bot. 38: 185–192.
Fapohunda, S.O. and Ogundero, V.W. 1990. Physiology of fungi associated with fast foods in Nigeria. Int. Biodeterior. 26: 23–32.
Fajola, A.O. 1979. The post-harvest fruit rots of tomato (*Lycopersicum esculentum*) in Nigeria. Nahrung 23: 105–109.
Fan, Q. and Tian, S.P. 2000. Postharvest biological control of *Rhizopus* rot of nectarine fruits by *Pichia membranaefaciens*. Plant Dis. 84: 1212–1216.
Fang, S.Y. and Peng, F.C. 2003. Factors affecting territrem b production by *Aspergillus terreus* CCRC 32111 in potato-dextrose medium. J. Food Drug Anal. 11: 27–31.
FAO (Food and Agriculture Organization). 2003. Worldwide regulations for mycotoxins in food and feed. http://www.fao.org/docrep/007/y5499e/y5499e07.htm, accessed August 2007. Farber, J.M. and Sanders, G.W. 1986. Production of fusarin C by *Fusarium* spp. J. Agric. Food Chem. 34: 963–966.
Farghaly, R.M., Gherbawy, Y.A.M.H. and Yosef, M.S. 2004. Contamination of meat stored in home refrigerators in Qena (Egypt). Czech Mycol. 56: 53-62.
Feinberg, B. and MacLaughlin, C.S. 1989. Biochemical mechanism of action of trichothecene mycotoxins. *In* Trichothecene Mycotoxins: Pathophysiological Effects. Vol. 1. Ed. V.R. Beasley. Boca Raton, FL: CRC Press. pp. 27–36.
Feng, X.M., Larsen, T.O. and Schnürer, J. 2007. Production of volatile compounds by *Rhizopus oligosporus* during soybean and barley tempeh fermentation. Int. J. Food Microbiol. 113: 133–141.
Fennell, D.I. 1973. Review of "The Genus *Talaromyces*: Studies on *Talaromyces* and related Genera". Mycologia 65: 1221–1223.
Fernandes, A., Ratola, N., Cerdeira, A., Alves, A. and Venancio, A. 2007. Changes in ochratoxin A concentration during winemaking. Am. J. Enol. Viticult. 58: 92–96.
Ferrati, A.R., Tavolaro, P., Destro, M.T., Landgraf, M. and Franco, B.D.G.M. 2005. A comparison of ready-to-use systems for evaluating the microbiological quality of acidic fruit juices using non-pasteurized orange juice as an experimental model. Int. Microbiol. 8: 49–53.

Ferreira, A.D. and Viljoen, B.C. 2003. Yeasts as adjunct starters in matured Cheddar cheese. Int. J. Food Microbiol. 86: 131–140.
Filtenborg, O., Frisvad, J.C. and Thrane, U. 1996. Moulds in food spoilage. Int. J. Food Microbiol. 33: 85–102.
Fink-Gremmels, J., El-Banna, A.A. and Leistner, L. 1988. Developing mould starter cultures for meat products. Fleischwirtschaft 68: 1292–1294.
Finol, M.L., Marth, E.H. and Lindsay, R.C. 1982. Depletion of sorbate from different media during growth of *Penicillium* species. J. Food Prot. 45: 398–404, 409.
Finoli, C., Vecchio, A., Galli, A. and Franzetti, L. 1999. Production of cyclopiazonic acid by molds isolated from Taleggio cheese. J. Food Prot. 62: 1198–1202.
Finoli, C., Vecchio, A., Galli, A. and Dragoni, I. 2001. Roquefortine C occurrence in blue cheese. J. Food Prot. 64: 246–251.
Fisher, W.S., Nilson, E.H., Steenbergen, J.F. and Lightner, D.V. 1978. Microbial diseases of cultured lobsters: a review. Aquaculture 14: 115–140.
Flannigan, B. 1969. Microflora of dried barley grain. Trans. Br. Mycol. Soc. 53: 371–379.
Flannigan, B. 1970. Comparison of seed-borne mycofloras of barley, oats and wheat. Trans. Br. Mycol. Soc. 55: 267–276.
Flannigan, B. 1986. *Aspergillus clavatus* – an allergenic, toxigenic deteriogen of cereals and cereal products. Int. Biodeterior. 22: 79–89.
Flannigan, B., Day, S.W., Douglas, P.E. and McFarlane, G. B. 1984. Growth of mycotoxin-producing fungi associated with malting of barley. *In* Toxigenic Fungi – Their Toxins and Health Hazard, eds H. Kurata and Y. Ueno. Amsterdam: Elsevier. pp. 52–60.
Fleet, G.H. 1990. Yeasts in dairy products. J. Appl. Bacteriol. 68: 199–211.
Fleet, G.H. 1992. Spoilage yeasts. Crit. Rev. Biotechnol. 12: 1–44.
Fleet, G.H. 2003a. Yeasts in fruit and fruit products. *In* Yeasts in Food, eds T. Boekhout and V. Robert. Cambridge, UK: Woodhead Publishing. pp. 267–287.
Fleet, G.H. 2003b. Yeast interactions and wine flavour. Int. J. Food Microbiol. 86: 11–22.
Fleet, G.H. 2006. *Saccharomyces* and related genera. *In* Food Spoilage Microorganisms, ed. C. de W. Blackburn. Cambridge, UK: Woodhead Publishing. pp. 306–335.
Fleet, G.H. and Heard, G.M. 1992. Yeasts – growth during fermentation. *In* Wine Microbiology and Biotechnology, ed. G.H. Fleet. Chur, Switzerland: Harwood Academic Publishers. pp. 27–54.
Fleet, G.H. and Mian, M.A. 1987. The occurrence and growth of yeasts in dairy products. Int. J. Food Microbiol. 4: 145–155.
Fleet, G.H., Lafon-Lafourcade, S. and Ribereau-Gayon, P. 1984. Evolution of yeasts and lactic acid bacteria during fermentation and storage of Bordeaux wines. Appl. Environ. Microbiol. 48: 1034–1038.
Fleet, G.H., Lafon-Lafourcade, S. and Ribereau-Gayon, P. 1984. Evolution of yeasts and lactic acid bacteria during fermentation and storage of Bordeaux wines. Appl. Environ. Microbiol. 48: 1034–1038. Fleet, G.H., Prakitchaiwattana, C., Beh, A.L. and Heard, G. 2002. The yeast ecology of wine grapes *In* Biodiversity and Biotechnology of Wine Yeasts, ed. M. Ciani. Kerala, India: Research Singpost. pp 1–17.
Flesch, P., Stockinger, G. and Voigt-Scheuermann, I. 1986. Isolierung und Identifizierung von Trichothecenen und diterpenlactonen aus Kulturen des Pilzes *Trichothecium roseum*. Wein-Wiss. 41: 182–189.
Flood, M.T. and Kondo, M. 2003. Safety evaluation of lipase produced from *Rhizopus oryzae*: summary of toxicological data. Reg. Toxicol. Pharmacol. 37: 293–304.
Fogle, M.R., Douglas, D.R., Jumper, C.A. and Straus, D.C. 2007. Growth and mycotoxin production by *Chaetomium globosum*. Mycopathologia 164: 49–56.
Follstad, M.N. 1966. Mycelial growth rate and sporulation of *Alternaria tenuis*, *Botrytis cinerea*, *Cladosporium herbarum*, and *Rhizopus stolonifer* in low-oxygen atmospheres. Phytopathology 56: 1098–1099.
Fomicheva, G.M., Vasilenko, O.V. and Marfenina, O.E. 2006. Comparative, morphological ecological and molecular studies of *Aspergillus versicolor* (Vuill.) Tiraboschi strains isolated from different ecotopes. Microbiology 75: 186–191.
Food Science Australia. 2006. On-line culture collection database. http://www.foodscience.csiro.au/fcc/index.htm.
Forgacs, J. and Carll, W.T. 1952. Mycotoxicoses. Adv. Vet. Sci. 7: 273–382.
Forgacs, J., Kock, H., Carll, W.T. and White-Stevens, R.H. 1958. Additional studies on the relationship of mycotoxicosis to the poultry hemorrhagic syndrome. Am. J. Vet. Res. 19: 744–753.
Foschino, R., Garzaroli, C. and Ottogalli, G. 1993. Microbial contaminants cause swelling and inward collapse of yoghurt packs. Lait 73: 395–400.
Fourie, J.F. and Holz, G. 1985. Postharvest fungal decay of stone fruit in the south-western Cape. Phytophylactica 17: 175–177.
Fourie, J.F. and Holz, G. 1987. Post-harvest fungicidal treatment of stone fruit for the control of *Monilinia laxa*, *Botrytis cinerea* and *Rhizopus stolonifer*. Phytophylactica 19: 71–76.
Fourie, J.F. and Holz, G. 1995. Initial infection by *Botrytis cinerea* on nectarine and plum fruit and the development of decay. Phytopathology 85: 82–87.
Fox, R.L. 1994. Fungal foes in your garden. 26. Blue mould rot of apples and pears. Mycologist 8: 177.
Francis, R.G. and Burgess, L.W. 1977. Characteristics of two populations of *Fusarium roseum* 'Graminearum' in Eastern Australia. Trans. Br. Mycol. Soc. 68: 421–427.
Frank, M. and Hess, E. 1941. Studies on salt fish. V. Studies on *Sporendonema epizoum* from dun salt fish. J. Fish. Res. Board Can. 5: 276–286.
Franke, I., Wijma, E. and Bouma, K. 2002. Shelf life extension of pre-baked buns by an ACTIVE PACKAGING ethanol emitter. Food Addit. Contam. 19: 314–322.
Fraser, L. 1953. A new genus of the Plectascales. Proc. Linn. Soc. N.S.W. 78: 241–246.
Fravel, D.R. 2005. Commercialization and implementation of biocontrol. Annu. Rev. Plant Biol. 43: 337–359.
Fredj, S.M.B., Chebil, S., Lebrihi, A., Lasram, S., Ghorbel, A. and Mliki, A. 2007. Occurrence of pathogenic fungal species in Tunisian vineyards. Int. J. Food Microbiol. 113: 245–250.
Fredlund, E., Druvefors, U., Boysen, M.E., Lingsten, K.J. and Schnürer, J. 2002. Physiological characteristics of the biocontrol yeast *Pichia anomala* J121. FEMS Yeast Res. 2: 395–402.

Freire, F.C.O. and Kozakiewicz, Z. 2005. Filamentous fungi, bacteria and yeasts associated with cashew kernels in Brazil. Rev. Cien. Agron. 36: 249–254.

Freire, F.C.O., Kozakiewicz, Z. and Paterson, R.R.M. 1999. Mycoflora and mycotoxins of Brazilian cashew kernels. Mycopathologia 145: 95–103.

Freire, F.C.O., Kozakiewicz, Z. and Paterson, R.R.M. 2000. Mycoflora and mycotoxins in Brazilian black pepper, white pepper and Brazil nuts. Mycopathologia 149: 13–19.

Frengova, G., Simova, E., Pavlova, K., Beshkova, D. and Grigorova, D. 1994. Formation of carotenoids by *Rhodotorula glutinis* in whey ultrafiltrate. Biotechnol. Bioeng. 44: 888–894.

Frevel, H.J., Engel, G. and Teuber, M. 1985. Schimmelpilze in Silage und Rohmilch. Milchwissenschaft 40: 129–132.

Frisvad, J.C. 1981. Physiological criteria and mycotoxin production as aids in identification of common asymmetric Penicillia. Appl. Environ. Microbiol. 41: 568–579.

Frisvad, J.C. 1983. A selective and indicative medium for groups of *Penicillium viridicatum* producing different mycotoxins in cereals. J. Appl. Bacteriol. 54: 409–416.

Frisvad, J.C. 1985. Creatine sucrose agar, a differential medium for mycotoxin producing terverticillate *Penicillium* species. Lett. Appl. Microbiol. 1: 109–113.

Frisvad, J.C. 1989. The connection between the Penicillia and Aspergilli and mycotoxins with special reference to misidentified isolates. Arch. Environ. Contam. Toxicol. 18: 452–467.

Frisvad, J.C. 1993. Modifications on media based on creatine for use in *Penicillium* and *Aspergillus* taxonomy. Lett. Appl. Microbiol. 16: 154–157.

Frisvad, J.C. and Filtenborg, O. 1983. Classification of terverticillate Penicillia based on profiles of mycotoxins and other secondary metabolites. Appl. Environ. Microbiol. 46: 1301–1310.

Frisvad, J.C. and Filtenborg, O. 1989. Terverticillate Penicillia: chemotaxonomy and mycotoxin production. Mycologia 81: 837–861.

Frisvad, J.C. and Samson, R.A. 1991. Mycotoxins produced by species of *Penicillium* and *Aspergillus* occurring in cereals. *In* Cereal Grain: Mycotoxins, Fungi and Quality in Drying and Storage, ed. J. Chelkowski. Amsterdam: Elsevier. pp. 441–476.

Frisvad, J.C. and Samson R.A. 2004. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. Studies in Mycology 49: 1–173.

Frisvad, J.C. and Thrane, U. 2004. Mycotoxin production by common filamentous fungi. *In* Introduction to Food and Airborne Fungi, eds R.A. Samson; E.S. Hoekstra and J. C. Frisvad. Utrecht: CBS, pp 321–331.

Frisvad, J.C., Samson, R.A. and Stolk, A.C. 1990a. Chemotaxonomy of *Eupenicillium javanicum* and related species. *In* Modern Concepts in *Penicillium* and *Aspergillus* Classification, eds R.A. Samson and J.I. Pitt. New York: Plenum Press. pp. 445–453.

Frisvad, J.C., Filtenborg, O., Samson, R.A. and Stolk, A.C. 1990b. Chemotaxonomy of the genus *Talaromyces*. Antonie van Leeuwenhoek 57: 179–189.

Frisvad, J.C., Hawksworth, D.L., Kozakiewicz, Z., Pitt, J.I., Samson, R.A. and Stolk, A.C. 1990c. Proposals to conserve important names in *Aspergillus* and *Penicillium*. *In* Modern Concepts in *Penicillium* and *Aspergillus* Classification, eds R. A. Samson and J.I. Pitt. New York: Plenum Press. pp. 83–89.

Frisvad, J.C., Filtenborg, O., Lund, F. and Thrane, U. 1992. New selective media for the detection of toxinogenic fungi in cereal products, meat and cheese. *In* Modern Methods in Food Mycology, eds R.A.Samson, A.D. Hocking, J.I. Pitt and A.D. King. Amsterdam: Elsevier Science Publishers. pp. 275–285.

Frisvad, J.C. Frank, J.M., Houbraken, J.A.M.P., Kuipers, A.F.A. and Samson, R.A. 2004. New ochratoxin A producing species of *Aspergillus* section *Circumdati*. Stud Mycol. (Utrecht) 50: 23–43.

Frisvad, J.C., Skouboe, P. and Samson, A.R. 2005a. Taxonomic comparison of three different groups of aflatoxin producers and a new efficient producer of aflatoxin B_1, sterigmatocystin and 3-*O*-methylsterigmatocystin, *Aspergillus rambellii* sp. nov. Syst. Appl. Microbiol. 28: 442–453.

Frisvad, J.C., Lund, F. and Elmholt, S. 2005b. Ochratoxin A producing *Penicillium verrucosum* isolates from cereals reveal large AFLP fingerprinting variability. J. Appl. Microbiol. 98: 684–692.

Frisvad, J.C., Thrane, U., Samson, R.A. and Pitt, J.I. 2006a. Important mycotoxins and the fungi which produce them. *In* Advances in Food Mycology – Advances in Experimental Medicine and Biology. Vol. 571, eds A.D. Hocking, J.I. Pitt, R.A. Samson and U. Thrane. Berlin: Springer-Verlag pp. 3–31.

Frisvad, J.C., Nielsen, K.F. and Samson, R.A. 2006b. Recommendations concerning the chronic problem of misidentification of mycotoxigenic fungi associated with foods and feeds. *In* Advances in Food Mycology – Advances in Experimental Medicine and Biology. Vol. 571, eds A.D. Hocking, J.I. Pitt, R.A. Samson and U. Thrane. Berlin: Springer-Verlag pp. 33–46.

Frisvad, J.C., Smedsgaard, J., Samson, R.A., Larsen, T.O. and Thrane, U. 2007. Fumonisin B2 production by *Aspergillus niger*. J. Agric. Food Chem. 55: 9727–9732.

Frobish, R.A., Bradley, B.D., Wagner, D.D., Long-Bradley, P.E. and Hairston, H. 1986. Aflatoxin residues in milk of dairy cows after ingestion of naturally contaminated grain. J. Food Prot. 49: 781–785.

Frölich-Wyder, M.-T. 2003. Yeasts in dairy products. *In* Yeasts in Food, eds T. Boekhout and V. Robert. Cambridge, UK: Woodhead Publishing. pp. 209–237.

Fuhrman, M.P., Charney, P. and Mueller, C.M. 2004. Hepatic proteins and nutrition assessment. J. Am. Diet. Assoc. 104: 1258–1264.

Fujii, K., Kurata, H., Odashima, S. and Hatsuda, Y. 1976. Tumor induction by a single subcutaneous injection of sterigmatocystin in newborn mice. Cancer Res. 36: 1615–1618.

Fullerton, R.A. 1982. Brown spot of passionfruit on Niue Island caused by *Alternaria alternata*. N.Z. J. Agric. Res. 25: 421–423.

Gachomo, E.W., Mutitu, E.W. and Kotochoni, O.S. 2004. Diversity of fungal species associated with peanuts in storage and the levels of aflatoxins in infected samples. Int. J. Agric. Biol. 6: 955–959.

Gallagher, R.T., Latch, G.C.M. and Keogh, R.G. 1980. The janthitrems: fluorescent tremorgenic toxins produced by

Penicillium janthinellum isolates from ryegrass pastures. Appl. Environ. Microbiol. 39: 272–273.

Galli, A. and Ottogalli, G. 1973. Microflora of the sour dough of Panettone cake. Ann. Microbiol. Enzimol. 23: 39–49.

Galli, A. and Zambrini, A. 1978. Surface microflora of Provolone cheese. Ind. Latt. 14: 3–12.

Galli, A., Franzetti, L. and Fortina, M.G. 1988. Isolation and identification of sour dour microflora. Microbiol., Aliments, Nutr. 6: 345–351.

Galloway, L.D. 1935. The moisture requirements of mold fungi with special reference to mildew of textiles. J. Text. Inst. 26: 123–129.

Gams, W. 1971. *Cephalosporium*-artige Schimmelpilze (Hyphomycetes). Stuttgart: G. Fischer.

Gams, W. and Samson, R.A. 1985. Typification of *Aspergillus* and related teleomorph genera. *In* Advances in *Penicillium* and *Aspergillus* Systematics, eds R.A. Samson and J.I. Pitt. New York: Plenum Press. pp. 23–29.

Gams, W., Christensen, M., Onions, A.H., Pitt, J.I. and Samson, R.A. 1985. Infrageneric taxa of *Aspergillus*. *In* Advances in *Penicillium* and *Aspergillus* Systematics, eds R.A. Samson and J.I. Pitt. New York: Plenum Press. pp. 55–62.

Gao, C. and Fleet, G.H. 1988. The effects of temperature and pH on the ethanol tolerance of the wine yeasts, *Saccharomyces cerevisiae*, *Candida stellata* and *Kloeckera apiculata*. J. Appl. Bacteriol. 65: 405–409.

Gao, C. and Fleet, G.H. 1995. Degradation of malic and tartaric acids by high density cell suspensions of wine yeasts. Food Microbiol. 12: 65–71.

García, S. and Heredia, N. 2006. Mycotoxins in Mexico: epidemiology, management, and control strategies. Mycopathologia 162: 255–264.

García, T., Mayoral, B., González, I., López-Calleja, I., Sanz, A., Hernández, P.E. and Martín, R. 2004. Enumeration of yeasts in dairy products: a comparison of immunological and genetic techniques. J. Food Prot. 67: 357–364.

García-Armesto, M.R., Otero, A., Rúa, J., Moreno, B. and García-López, M.L. 2002. Evaluation of the spiral plating system for the routine assessment of indicator microorganisms in raw ewe's milk. J. Food Prot. 65: 1281–1286.

Gardner, J.W. and Bartlett, P.N. 1994. A brief history of electronic noses. Sens. Actuators B 18: 211–220.

Gareis, M. 1996. Fate of ochratoxin A on processing of meat products. Food Addit. Contam. 13(Suppl.): 35–37.

Gareis, M. and Gareis, E.-M. 2007. Guttation droplets of *Penicillium nordicum* and *Penicillium verrucosum* contain high concentrations of the mycotoxins ochratoxin A and B. Mycopathologia 163: 207–214.

Gareis, M. and Scheuer, R. 2000. Ochratoxin A in meat and meat products. Arch. Lebensmittelhyg. 51(4/5): 102–104.

Gargi and Roy, A.N. 1988. Prevention and control of some post harvest fungal diseases of garlic bulbs. Pesticides 22: 11–15.

Garrido, N.S., Iha, M.H., Santos Ortolani, M.R. and Duarte Fávaro, R.M. 2003. Occurrence of aflatoxins M1 and M2 in milk commercialized in Ribeirão Presto-SP, Brazil. Food Addit. Contam. 20: 70–73.

Gatti, M.J., Fraga, M.E., Magnoli, C., Dalcero, A.M. and da Rocha Rosa, C.A. 2003. Mycological survey for potential aflatoxin and ochratoxin producers and their toxicological properties in harvested Brazilian black pepper. Food Addit. Contam. 20: 1120–1126.

Gaur, A. and Chenulu, V.V. 1981. Two unrecorded storage disorders of *Citrus reticulata* L. and *Solanum tuberosum* L. Curr. Sci. 50: 322–323.

Gauthier, B., Guiraud, J., Vincent, J.C., Parvais, J.P. and Galzy, P. 1977. Study of the yeast flora during Ivory Coast traditional fermentation of cocoa beans. Rev. Ferment. Ind. Aliment. 32: 160–163.

Geeta, S., Mehrotra, R.S. and Aneja, K.R. 1979. An unrecorded postharvest rot of apple. Curr. Sci. 48: 996.

Geisen, R. 2007. Molecular detection and monitoring. *In* Food Mycology. A Multifaceted Approach to Fungi and Food, eds J. Dijksterhuis and R.A. Samson. Boca Raton FL: CRC Press. pp. 255–278.

Geiser, D.M., Frisvad, J.C. and Taylor, J.W. 1998. Evolutionary relationships in *Aspergillus* section *Fumigata* inferred from partial beta-tubulin and hydrophobin DNA sequences. Mycologia 90: 831–845.

Geiser, D.M., Klich, M.A., Frisvad, J.C., Peterson, S.W., Varga, J. and Samson, R.A. 2007. The current status of species recognition and identification in *Aspergillus*. Stud. Mycol. 59: 1–10.

Gelderblom, W.C.A., Jaskiewicz, K., Marasas, W.F.O., Theil, P.G., Horak, R.M., Vleggaar, R. and Kriek, N.P.J. 1988. Fumonisins – novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Appl. Environ. Microbiol. 54: 1806–1811.

Gelderblom, W.C.A., Kriek, N.P.J, Marasas, W.F.O. and Theil, P.G. 1991. Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite fumonisin B_1 in rats. Carcinogenesis 12: 1247–1251.

Gente, S., Larpin, S., Cholet, O., Gueguen, M., Vernoux, J. P. and Desmasures, N. 2007. Development of primers for detecting dominant yeasts in smear-ripened cheeses. J. Dairy Res. 74: 137–145.

Ghazi, I., Fernandez-Arrojo, L, Garcia-Arellano, H., Ferrer, M., Ballesteros, A. and Plou, F. J. 2007. Purification and kinetic characterization of a fructosyltransferase from *Aspergillus aculeatus*. J. Biotech. 128: 204–211.

Gherbawy, Y.A.M.H., Maghraby, T.A. and Shebany, Y.M. 2006. Seasonal variations of *Fusarium* species in wheat fields in Upper Egypt. Arch. Phytopathol. Plant Prot. 39: 365–377.

Ghiasian, S.A., Kord-Bacheh, P., Rezayat, S.M., Maghsood, A.H. and Heshmatallah Taherkhani. 2004. Mycoflora of Iranian maize harvested in the main production areas in 2000. Mycopathologia 158: 113–121.

Ghiasian, S.A., Maghsood, A.H., Yazdanpanah, H., Shephard, G.S., Westhuizen, L. van der, Vismer, H.F., Rheeder, J.P. and Marasas, W.F.O. 2006. Incidence of *Fusarium verticillioides* and levels of fumonisins in corn from main production areas in Iran. J. Agric. Food Chem. 54: 6118–6122.

Ghosh, B.C., Bockelmann, W. and Heller, K. J. 2006. Enzymatic studies on predominant micro-organisms of surface-ripened cheeses. Aust. J. Dairy Technol. 61: 238–243.

Gibson, A.M., Baranyi, J., Pitt, J.I., Eyles, M.J. and Roberts, T.A. 1994. Predicting fungal growth: the effect of water activity on *Aspergillus flavus* and related species. Int. J. Food Microbiol. 23: 419–431.

Gibson, B. 1973. The effect of high sugar concentrations on the heat resistance of vegetative micro-organisms. J. Appl. Bacteriol. 36: 365–376.

Gill, C.O. and Lowry, P.D. 1982. Growth at sub-zero temperatures of black spot fungi on meat. J. Appl. Bacteriol. 52: 245–250.

Gill, C.O., Lowry, P.D. and Di Menna, M.E. 1981. A note on the identities of organisms causing black spot spoilage of meat. J. Appl. Bacteriol. 51: 183–187.

Gilman, G.A. 1969. An examination of fungi associated with groundnut pods. Trop. Sci. 11: 38–48.

Giorni, P., Magan, N., Pietri, A., Bertuzzi, T. and Battilani, P. 2007. Studies on *Aspergillus* section *Flavi* isolated from maize in northern Italy. Int. J. Food Microbiol. 113: 330–338.

Girardin, H., Monod, M. and Latgé, J.P. 1995. Molecular characterization of the food-borne fungus *Neosartorya fischeri* (Malloch and Cain). Appl. Environ. Microbiol. 61: 1378–1383.

Giray, B., Girgin, G., Engin, A.B., Ayddin, S. and Sahin, G. 2007. Aflatoxin levels in wheat samples consumed in some regions of Turkey. Food Control 18: 23–29.

Giridhar, P. and Reddy, S.M. 2001. Mycoflora and mycotoxins of almond and cardamom collected from Andhra Pradesh. Adv. Plant Sci. 14: 601–603.

Giudici, P. 1990. Inhibitory action of acetic acid on osmophilic yeasts isolated from traditional balsamic vinegar. Ind. Bevande 19: 475–477, 480.

Glahn, R.P., Shapiro, R.S., Vena, V.E., Wideman, R.F. and Huff, W.E. 1989. Effects of chronic ochratoxin A and citrinin toxicosis on kidney function of single comb White Leghorn pullets. Poultry Sci. 68: 1205–1212.

Gleason, F.H. 1971. Alcohol dehydrogenase in Mucorales. Mycologia 63: 906–910.

Gnonlonfin, G.J.B., Hell, K., Fandohan, P. and Siame, A.B. 2008. Mycoflora and natural occurrence of aflatoxins and fumonisin B_1 in cassava and yam chips from Benin, West Africa. Int. J. Food Microbiol. 122: 140–147.

Gobbetti, M., Corsetti, A., Rossi, J., la Rosa, F. and de Vincenzi, S. 1994. Identification and clustering of lactic acid bacteria and yeasts from wheat sourdoughs of central Italy. Ital. J. Food Sci. 6: 85–94.

Gock, M.A., Hocking, A.D., Pitt, J.I. and Poulos, P.G. 2003. Influence of temperature, water activity and pH on growth of some xerophilic fungi. Int. J. Food Microbiol. 81: 11–19.

Gogoi, B. K., Bezbaruah, K. R., Pillai, K. R. and Baruah, J. N. 1987. Production, purification and characterization of an alpha-amylase produced by *Saccharomycopsis fibuligera*. J. Appl. Bacteriol. 63: 373–379.

Goh, E.L.C., Hocking, A.D. Stewart, C.M., Buckle, K.A. and Fleet, G.H. 2007. Baroprotective effect of increased solute concentrations on yeast and moulds during high pressure processing. Innov. Food Sci. Emerg. Technol. 8: 535–542.

Gola, S., Maggi, A., Rovere, P., Carpi, G., Dall'Aglio, G. and Rossi, I. 1994. Microbial inactivation in apricot nectar and model systems by high-pressure treatment. Ind. Conserve 69: 194–198.

Golding, N.S. 1940a. The gas requirements of molds. II. The oxygen requirements of *Penicillium roquefortii* (three strains originally isolated from blue veined cheese) in the presence of nitrogen as diluent and the absence of carbon dioxide. J. Dairy Sci. 23: 879–889.

Golding, N.S. 1940b. The gas requirements of molds. III. The effect of various concentrations of carbon dioxide on the growth of *Penicillium roquefortii* (three strains originally isolated from blue veined cheese) in air. J. Dairy Sci. 23: 891–898.

Golding, N.S. 1945. The gas requirements of molds. IV. A preliminary interpretation of the growth rates of four common mold cultures on the basis of absorbed gases. J. Dairy Sci. 28: 737–750.

Gonçalves, A.B., Paterson, R.R.M. and Lima, N. 2006. Survey and significance of filamentous fungi from tap water. Int. J. Hyg. Environ. Health 209: 257–264.

Gonzalez, M. del C. 1995. *Fusarium solani* is the causative agent of brown spot disease in captive wild broodstock of the white shrimp *Penaeus vannamei*. Rev. Mex. Micol. 11: 175–178.

Gonzalez, G., Hinojo, M.J., Mateo, R., Medina, A. and Jimenez, M. 2005. Occurrence of mycotoxin producing fungi in bee pollen. Int. J. Food Microbiol. 105: 1–9.

Gonzalez, H.H.L., Resnik, S.L. and Vaamonde, G. 1988. Influence of temperature on growth rate and lag phase of fungi isolated from Argentine corn. Int. J. Food Microbiol. 6: 179–183.

González, L., Juan, C., Soriano, J.M., Moltó and Mañes, J. 2006. Occurrence and daily intake of ochratoxin A of organic and non-organic rice and rice products. Int. J. Food Microbiol. 107: 223–227.

González-Aguilar, G.A, Zavaleta-Gatica, R. and Tiznado-Hernández, M.E. 2007. Improving postharvest quality of mango 'Haden' by UV-C treatment. Postharvest Biol. Technol. 45: 108–116.

Goodman, T., Scott, P.M. and Watanabe, H. 1987. Risk assessment of the mycotoxin zearalenone. Regul. Toxicol. Pharmacol. 7: 253–306.

Gori, S., Pellegrini, G., Filipponi, F., Della Capanna, S., Biancofiore, G., Mosca, F. and Lofaro, A. 1998. Pulmonary aspergillosis caused by *Neosartorya fischeri* (*Aspergillus fischerianus*) in a liver transplant recipient. J. Mycol. Med. 8: 105–107.

Gossmann, M., Kleta, S., Humpf, H.U. and Buttner, C. 2005. Investigation on contamination of asparagus (*Asparagus officinalis* L.) spears with *Fusarium proliferatum* (Matsushima) Nirenberg during main harvest. Gesunde Pflanz. 57: 53–58.

Gourama, H. and Bullerman, L.B. 1988. Effects of potassium sorbate and natamycin on growth and penicillic acid production by *Aspergillus ochraceus*. J. Food Prot. 51: 139–144.

Gowdar, S.B., Babu, H.N.R., Reddy, N.A., Rajeshwari, N. and Krishnappa, M. 2007. Seed-borne mycoflora associated with sunflower seeds. Res. Crops 8: 469–473.

Goyal, S.K. and Jain, M.P. 1998. Storage mycoflora of blackgram (*Vigna mungo* L. Hepper) and their pathogenicity. Int. J. Trop. Plant Dis. 16: 195–202.

Gradel, A. and Müller, G. 1985. Ergebnisse von Untersuchungen des Schimmelpilzbefalls an Gärgutträgern in der Brötchen- und Spezial-Toast- Produktion. Bäker Konditor 33: 133–135.

Grau, C., Elguezabal, L., Vallenilla, O. and Zerpa, A. 2003. Evaluation of halophile microbial flora spoiling dry-salted fish processed in Sucre state. Rev. Cien. Fac. Cien. Vet., Univ. Zulia 13: 319–325.

Gräu, C., Sánchez, D., Zerpa, A. and García, N. 2007. Influence of water activity, pH and temperature on the growth of *Aspergillus penicillioides* and *A. terreus*, isolated from dry and salted skipjack tuna (*Katsuwonus pelamis*) meat. Rev. Cientif. Fac. Ciencias Vet. 17: 193–199.

Graves, R.R. and Hesseltine, C.W. 1966. Fungi in flour and refrigerated dough products. Mycopathol. Mycol. Appl. 29: 277–290.

Gray, W.D. 1970. The Use of Fungi as Food and in Food Processing. Cleveland, OH: CRC Press.

Grazia, L., Romano, P., Bagni, A., Roggiani, D. and Guglielmi, G. 1986. The role of moulds in the ripening process of salami. Food Microbiol. 3: 19–25.

Green, M.D. and Ibe, S.N. 1987. Yeasts as primary contaminants in yogurts produced commercially in Lagos, Nigeria. J. Food Prot. 50: 193–198, 205.

Greuter, W., Barrie, F.R., Burdet, H.M., Chaloner, W.G., Demoulin, V., Hawksworth, D.L., Jørgensen, P.M., Nicolson, D.H., Silva, P.C., Trehane, P. and McNeill, J. 1994. International Code of Botanical Nomenclature (Tokyo Code). Königstein, Germany: Koeltz Scientific Books.

Griffin, D.M. 1963. Soil moisture and the ecology of soil fungi. Biol. Rev., Cambridge 38: 141–166.

Gros, J.B., Roquebert, M.F., Berdague, J.L., Laroche, C., Fontanille, P. and Sirami, J. 2003. Selection of mould strains from the surface flora of French saucissons and study of their biocatalytic behaviour. Sci. Aliments 23: 150–153.

Grovel, O., Pouchus, Y.F. and Verbist, J-F. 2003. Accumulation of gliotoxin, a cytotoxic mycotoxin from *Aspergillus fumigatus*, in blue mussel (*Mytilus edulis*). Toxicon 42: 297–300.

Gubler, W.D. and Bernhardt, E.A. 1992. Cavity rot of winter melons caused by *Verticillium dahliae*. Plant Dis. 76: 416–417.

Gueguen, M. 1988. Moisissures responsables de defauts d'affinage en fromagerie (à l'exclusion des Mucoraceae). Microbiol. Aliments Nutr. 6: 31–35.

Guehi, T.S., Dingkuhn, M., Cros, E., Fourny, G., Ratomahenina, R., Moulin, G. and Vidal, A.C. 2007. Identification and lipase-producing abilities of moulds isolated from Ivorian raw cocoa beans. Res. J. Agric. Biol. Sci. 3: 838–843.

Guillet, M.H., Kauffmann-Lacroix, C., Dromer, F., Larsen, C. and Guillet, G. 2003. Urticaria and anaphylactic shock due to food allergy to *Penicillium italicum*. Rev. Fr. Allergol. Immunol. Clin. 43: 520–523.

Gunnell, P.S. and Gubler, W.D. 1992. Taxonomy and morphology of *Colletotrichum* species pathogenic to strawberry. Mycologia 84: 157–165.

Gunther, R., Abbas, H.K. and Mirocha, C.J. 1989. Acute pathological effects on rats of orally administered wortmannin-containing preparations and purified wortmannin from *Fusarium oxysporum*. Food Chem. Toxicol. 27: 173–179.

Guo, Y., Liu, L., Zhao, J. and Bi, Y. 2007. Use of silicon oxide and sodium silicate for controlling *Trichothecium roseum* postharvest rot in Chinese cantaloupe (*Cucumis melo* L.). Int. J. Food Sci. Technol. 42: 1012–1018.

Gupta, S.L. 1956. Occurrence of *Aspergillus carbonarius* (Bainier) Thom causing grape rot in India. Sci. Cult. 22: 167–168.

Gürses, M. 2006. Mycoflora and aflatoxin content of hazelnuts, walnuts, peanuts, almonds and roasted chick-peas (leblebi) sold in Turkey. Int. J. Food Prop. 9: 395–399.

Gutierrez, S.A., Mazzanti de Castanon, M.A. and Cundom, M.A. 2002. Fungi isolated from rice seed in Argentina. Fitopatologia 37: 156–163.

Gutiérrez-Sánchez, G., Roussos, S. and Augur, C. 2003. Effect of the nitrogen source on caffeine degradation by *Aspergillus tamari*. Lett. Appl. Microbiol. 38: 50–55.

Guynot, M.E., Marin, S., Sanchis, V. and Ramos, A.J. 2003a. Modified atmosphere packaging for prevention of mold spoilage of bakery products with different pH and water activity levels. J. Food Prot. 66: 1864–1872.

Guynot, M.E., Sanchis, V., Ramos, A.J. and Marin, S. 2003b. Mold-free shelf-life extension of bakery products by active packaging. J. Food Sci. 68: 2547–2552.

Guynot, M.E., Marín, S., Sanchis, V. and Ramos, A.J. 2005. An attempt to optimize potassium sorbate use to preserve low pH (4.5–5.5) intermediate moisture bakery products by modelling *Eurotium* spp., *Aspergillus* spp. and *Penicillium corylophilum* growth. Int. J. Food Microbiol. 101: 169–177.

Gyllang, H. and Martinson, E. 1976. *Aspergillus fumigatus* and *Aspergillus amstelodami* as causes of gushing. J. Inst. Brew. 82: 182–183.

Hadley, W.K. and Senyk, G. 1975. Early detection of metabolism and growth by measurement of electrical impedance. Microbiology 1975: 12–21.

Hadlok, R. 1969. Schimmelpiltzkontamination von Fleischerzeugnissen durch naturbelassene Gewrze. Fleischwirtschaft 49: 1601–1609.

Hadlok, R. and Schipper, M.A.A. 1974. Schimmelpilze und Fleisch: Reihe Mucorales. Fleischwirtschaft 54: 1796–1800.

Hagler, W.M., Towers, N.R., Mirocha, C.J., Eppley, R.M. and Bryden, W.L. 2001. Zearalenone: mycotoxin or mycoestrogen ? *In Fusarium*: Paul E. Nelson Memorial Symposium, eds B.A. Summerell, J.F. Leslie, D. Backhouse, W.L. Bryden and L.W. Burgess. St. Paul, MN: APS Press. pp. 321–331.

Hajjaj, H., Klaebe, A. Goma, G., Blanc, P.J., Barbier, E. and Francois, J. 2000. Medium-chain fatty acids affect citrinin production in the filamentous fungus *Monascus ruber*. Appl. Environ. Microbiol. 65: 1120–1125.

Hajjaji, A., El Otmani, M., Bouya, D., Bouseta A., Mathieu, F., Collin, S. and Lebrihi, A. 2006. Occurrence of mycotoxins (ochratoxin A, deoxynivalenol) and toxigenic fungi in Moroccan wheat grains: impact of ecological factors on the growth and ochratoxin A production. Mol. Nutr. Food Res. 50: 494–499.

Hald, B., Christensen, D.H. and Krogh, P. 1983. Natural occurrence of the mycotoxin viomellein in barley and the associated quinone-producing Penicillia. Appl. Environ. Microbiol. 46: 1311–1317.

Hale, P.J. and Menapace, D.M. 1980. Effect of time and temperature on the viability of *Ascosphaera apis*. J. Invertebr. Pathol. 36: 429–430.

Hall, E.G. and Scott, K.J. 1977. Storage and Market Diseases of Fruit. Melbourne, Australia: Commonwealth Scientific and Industrial Research Organisation.

Halt, M., Kovačević, D., Pavlović, H. and Jukić, J. 2004. Contamination of pasta and the raw materials for its production with mould of the genera *Aspergillus*. Czech J. Food Sci. 22: 67–72.

Hammad, A.M., Allah, H.M.H.G. and Ragheb, R.R. 2006. Mycological and mycotoxical evaluation of turkey carcasses marketed at Sharkia Province. Vet. Med. J. Giza 54: 405–412.

Handoyo, T., Maeda, T., Urisu, A., Adachi, T and Morita, N. 2006. Hypoallergenic buckwheat flour preparation by *Rhizopus oligosporus* and its application to soba noodle. Food Res. Int. 39: 598–605.

Hansen, I.D. and Pedersen, J.G. 1991. Ergosterol – et nyttigt værktNj ved vurdering af svampebelastning. Kolding, Denmark: Biotechnol. Inst. Rep. No. 91-2-4.

Hari, R.P.K., Patel, T. and Martin, A. 1992. A new strain of *Rhodotorula rubra* isolated from yogurt. J. Ind. Microbiol. 11: 43–51.

Harper, K.A., Beattie, B.B., Pitt, J.I. and Best, D.J. 1972. Texture changes in canned apricots following infection of the fresh fruit with *Rhizopus stolonifer*. J. Sci. Food Agric. 23: 311–320.

Harris, J.E. and Dennis, C. 1980. Distribution of *Mucor piriformis*, *Rhizopus sexualis* and *R. stolonifer* in relation to their spoilage of strawberries. Trans. Br. Mycol. Soc. 75: 445–450.

Harris, N.D., Karahadian, C. and Lindsay, R.C. 1986. Musty aroma compounds produced by selected molds and actinomycetes on agar and whole wheat bread. J. Food Prot. 49: 964–970.

Harrold, C.E. 1950. Studies on the genus *Eremascus*. I. The rediscovery of *Eremascus albus* Eidam and some new observations concerning its life history and cytology. Ann. Bot. (London) 14: 127–148.

Hartill, W.F.T. and Broadhurst, P.G. 1989. *Fusarium avenaceum* as a pathogen of stonefruit in New Zealand. N.Z. J. Crop Hort. Sci. 17: 293–295.

Harwig, J., Chen, Y.-K., Kennedy, B.P.C. and Scott, P.M. 1973. Occurrence of patulin and patulin-producing strains of *Penicillium expansum* in natural rots of apple in Canada. Can. Inst. Food Sci. Technol. J. 6: 22–25.

Harwig, J., Scott, P.M., Stoltz, D.R. and Blanchfield, B.J. 1979. Toxins of molds from decaying tomato fruit. Appl. Environ. Microbiol. 38: 267–274.

Hashmi, M.H. and Ghaffar, A. 1991. Seed-born mycoflora of *Coriandrum sativum* L. Pak. J. Bot. 23: 165–172.

Hashmi, M.H. and Thrane, U. 1990. Mycotoxins and other secondary metabolites in species of *Fusarium* isolated from seeds of capsicum, coriander and fenugreek. Pak. J. Bot. 22: 106–116.

Hasija, S.K. 1970. Physiological studies of *Alternaria citri* and *A. tenuis*. Mycologia 62: 289–295.

Hatcher, W.S., Weihe, J.L., Murdock, D.I., Folinazzo, J.F., Hill, E.C. and Albrigo, L.G. 1979. Growth requirements and thermal resistance of fungi belonging to the genus *Byssochlamys*. J. Food Sci. 44: 118–122.

Hatem, N.L., Hassab, H.M.A., El-Deeb, S.A. and Ahmed, R.L.E. 2005. Prevalence of aflatoxins in blood and urine of Egyptian infants with protein-energy malnutrition. Food Nutr. Bull. 26: 49–56.

Hawksworth, D.L. 1991. Biological diversity in fungi, bacteria and viruses. Biol. Educ. 8: 57–62.

Hawksworth, D.L. and Pitt, J.I. 1983. A new taxonomy for *Monascus* based on cultural and microscopical characters. Aust. J. Bot. 31: 51–61.

Hawthorn, B.T. 1988. Fungi causing storage rots on fruit of *Cucurbita* spp. N.Z. J. Exp. Agric. 16: 151–157.

Hayakawa, K., Ueno, Y., Nakanishi, S., Honda, Y., Komuro, H., Kikushima, S. and Shou, S. 1993. Production of fish sauce from fish meal treated with koji-mould. Seibutsu Kogaku Kaishi (J. Bioeng. Soc.) 71: 245–251.

Hayaloglu, A.A. and Kirbag, S. 2007. Microbial quality and presence of moulds in Kuflu cheese. Int. J. Food Microbiol. 115: 376–380.

Hayashi, N., Arai, R., Tada, S., Taguchi, H. and Ogawa, Y. 2007. Detection and identification of *Brettanomyces/Dekkera* sp. yeasts with a loop-mediated isothermal amplification method. Food Microbiol. 24: 778–785.

Haznedari, S. 1976. Microorganisms in sour dough breadmaking. I. Yeasts. Ann. Microbiol. Enzymol. 26: 83–88.

Headley, J.V., Peru, K.M., Verma, B. and Robarts, R.D. 2002. Mass spectrometric determination of ergosterol in a prairie natural wetland. J. Chromatogr. A 958: 149–156.

Heard, G.M. and Fleet, G.H. 1986a. Occurrence and growth of yeast species during the fermentation of some Australian wines. Food Technol. Aust. 38: 22–25.

Heard, G.M. and Fleet, G.H. 1986b. Evaluation of selective media for enumeration of yeasts during wine fermentation. J. Appl. Bacteriol. 60: 477–481.

Heard, G.M. and Fleet, G.H. 1988a. The effects of temperature and pH on the growth of yeast species during the fermentation of grape juice. J. Appl. Bacteriol. 65: 23–28.

Heard, G.M. and Fleet, G.H. 1988b. The effect of sulphur dioxide on yeast growth during natural and inoculated wine fermentation. Aust. N.Z. Wine Ind. J. 3: 57–60.

Heaton, J.B. 1980. Control of brown rot and transit rot of peaches with post-harvest fungicidal dips. Queensl. J. Agric. Anim. Sci. 37: 155–159.

Hee, K.J., Hyu, L.W., Soo, C.S., Sik, C.J., Jeong, R. and Geun, C.Y. 2002. Identification and characteristics of *Penicillium* spp. isolated from postharvest decay of pear. Res. Plant Dis. 8: 107–112.

Heenan, C.N., Shaw, K.J. and Pitt, J.I. 1998. Ochratoxin A production by *Aspergillus carbonarius* and *A. niger* isolates and detection using coconut cream agar. J. Food Mycol. 1: 67–72.

Heidarian, R., Nikkhah, M.J., Peyambari, M. and Ormaz, B. 2006. Study on fungal contamination of pistachio seeds in Kerman province, Iran and some new fungi for Iranian pistachio mycoflora. Proc. 4th Int. Symposium Pistachios Almonds 726: 615–617.

Heimann, M.F. 1994. First report of *Phytophthora* rot of cabbage caused by *Phytophthora porri* Foister in Wisconsin. Plant Dis. 78: 1123.

Heintzeler, I. 1939. Das Wachstum der Schimmelpilze in Abhangigkeit von der Hydraturverhaltnissen unter verschiedenen Aussenbedingungen. Arch. Mikrobiol. 10: 92–132.

Hendrickse, R.G., Coulter, J.B.S., Lamplugh, S.M., Macfarlane, S.B.J., Williams, T.E., Omer, M.I.A. and Suliman, G.I. 1982. Aflatoxins and kwashiorkor: a study in Sudanese children. Br. Med. J. 285: 843–846.

Heperkan, D., Aran, N. and Ayfer, M. 1994. Mycoflora and aflatoxin contamination in shelled pistachio nuts. J. Sci. Food Agric. 66: 273–278.

Heperkan, D., Meric, B.E., Sismanoglu, G., Dalkiliç and Güler, F.K. 2006. Mycobiota, mycotoxigenic fungi, and citrinin production in black olives. *In* Advances in Food Mycology – Advances in Experimental Medicine and Biology, eds Hocking, A.D., Pitt, J.I., Samson, R.A. and Thrane, U., Vol. 571, Berlin: Springer-Verlag. pp. 203–210.

Heresztyn, T. 1986. Metabolism of volatile phenolic compounds from hydroxycinnamic acids by *Brettanomyces* yeast. Arch. Microbiol. 146: 96–98.

Hermanides-Nijhof, E.J. 1977. *Aureobasidium* and allied genera. Stud. Mycol. (Baarn) 15: 141–177.

Hernandez, A., Martin, A., Aranda, E., Perez-Nevado, F. and Cordoba, M.G. 2007. Identification and characterization of yeast isolated from the elaboration of seasoned green table olives. Food Microbiol. 24: 346–351.

Hesseltine, C.W. 1965. A millennium of fungi and fermentation. Mycologia 57:49–197.

Hesseltine, C.W. 1991. Zygomycetes in food fermentations. Mycologist 5: 162–169.

Hesseltine, C.W. and Anderson, P. 1956. The genus *Thamnidium* and a study of the formation of its zygospores. Am. J. Bot. 43: 696–703.

Hesseltine, C.W., Rogers, R.F. and Shotwell, O.L. 1981. Aflatoxin and mold flora in North Carolina in 1977 corn crop. Mycologia 73: 216–228.

Hesseltine, C.W., Featherston, C.L., Lombard, G.L. and Dowell, V.R. 1985. Anaerobic growth of molds isolated from fermentation starters used for foods in Asian countries. Mycologia 77: 390–400.

Hestbjerg, H., Nielsen, K.F., Thrane, U. and Elmholt, S. 2002. Production of trichothecenes and other secondary metabolites by *Fusarium culmorum* and *Fusarium equiseti* on common laboratory media and a soil organic matter agar: an ecological interpretation. J. Agric. Food Chem. 50: 7593–7599.

Heussner, A.H., Dietrich, D.R. and O'Brien, E. 2006. In vitro investigation of individual and combined cytotoxic effects of ochratoxin A and other selected mycotoxins on renal cells. Toxicol. Vitro 20: 332–341.

Hide, G.A., Boorer, K.J. and Hall, S.M. 1994. Controlling potato tuber blemish diseases on cv. Estima with chemical and non-chemical methods. Ann. Appl. Biol. 124: 253–265.

Hill, J.E., Lomax, L.G., Cole, R.J. and Dorner, J.W. 1986. Toxicological and immunologic effects of sublethal doses of cyclopiazonic acid in rats. Am. J. Vet. Res. 47: 1174–1177.

Hill, J.L., Hocking, A.D. and Whitfield, F.B. 1995. The role of fungi in the production of chloranisoles in general purpose freight containers. Food Chem. 54: 161–166.

Hill, R.A. and Lacey, J. 1983. The microflora of ripening barley grain and the effects of pre-harvest fungicide application. Ann. Appl. Biol. 102: 455–465.

Hill, R.A. and Lacey, J. 1984. *Penicillium* species associated with barley grain in the U.K. Trans. Br. Mycol. Soc. 82: 297–303.

Ho, M.H.-M., Castenada, R.F., Dugan, F. and Jong, S.C. 1999. *Cladosporium* and *Cladophialophora* in culture: descriptions and an expanded key. Mycotaxon 72: 115–157.

Hocking, A.D. 1981. Improved media for enumeration of fungi from foods. CSIRO Food Res. Q. 41: 7–11.

Hocking, A.D. 1986. Effects of water activity and culture age on the glycerol accumulation patterns of five fungi. J. Gen. Microbiol. 132: 269–275.

Hocking, A.D. 1990. Responses of fungi to modified atmospheres. *In* Fumigation and Controlled Atmosphere Storage of Grain, eds B.R. Champ, E. Highley and H.J. Banks. ACIAR Proceedings No. 25. Canberra, Australia: Australian Centre for International Agricultural Research. pp 70–82.

Hocking, A.D. 1994. Fungal spoilage of high-fat foods. Food Aust. 46: 30–33.

Hocking, A.D. 1996. Media for preservative resistant yeasts: a collaborative study. Int. J. Food Microbiol. 29: 167–175.

Hocking, A.D. 2003. Microbiological facts and fictions in grain storage. *In* Stored Grain in Australia 2003, eds E.J. Wright, M.C. Webb and E. Highley, Proceedings of the Australian Postharvest Technical Conference, Canberra, June 2003. Canberra, ACT: Stored Grain Research Laboratory, CSIRO. pp. 55–58.

Hocking, A.D. and Andrews, S. 1987. Dichloran chloramphenicol peptone agar as an identification medium for *Fusarium* species and some dematiaceous Hyphomycetes. Trans. Br. Mycol. Soc. 89: 239–244.

Hocking, A.D. and Banks, H.J. 1991. Effects of phosphine fumigation on survival and growth of storage fungi in wheat. J. Stored Prod. Res. 27: 115–120.

Hocking, A.D. and Faedo, M. 1992. Fungi causing thread mould spoilage of vacuum packaged Cheddar cheese during maturation. Int. J. Food Microbiol. 16: 123–130.

Hocking, A.D. and Miscamble, B.F. 1995. Water relations of some Zygomycetes isolated from food. Mycol. Res. 99: 1113–1118.

Hocking, A.D. and Norton, R.S. 1983. Natural-abundance ^{13}C nuclear magnetic resonance studies on the internal solutes of xerophilic fungi. J. Gen. Microbiol. 129: 2915–2925.

Hocking, A.D. and Pitt, J.I. 1979. Water relations of some *Penicillium* species at 25°C. Trans. Br. Mycol. Soc. 73: 141–145.

Hocking, A.D. and Pitt, J.I. 1980. Dichloran-glycerol medium for enumeration of xerophilic fungi from low moisture foods. Appl. Environ. Microbiol. 39: 488–492.

Hocking, A.D. and Pitt, J.I. 1981. *Trichosporonoides nigrescens* sp. nov., a new xerophilic yeast-like fungus. Antonie van Leeuwenhoek 47: 411–421.

Hocking, A.D. and Pitt, J.I. 1984. Food spoilage fungi. II. Heat resistant fungi. CSIRO Food Res. Q. 44: 73–82.

Hocking, A.D. and Pitt, J.I. 1988. Two new species of xerophilic fungi and a further record of *Eurotium halophilicum*. Mycologia 80: 82–88.

Hocking, A.D. and Pitt, J.I. 1996. Fungi and mycotoxins in foods. *In* Fungi of Australia, Vol. 1B, Introduction – Fungi in the Environment, ed. A.E. Orchard. Canberra, Australia: Australian Biological Resources Study. pp. 315–342.

Hocking, A.D., Pitt, J.I., Samson, R.A. and King, A.D. 1992. Recommendations from the closing session of SMMEF II. *In* Modern Methods in Food Mycology, eds R.A. Samson, A.D. Hocking, J.I. Pitt and A.D. King. Amsterdam: Elsevier. pp. 359–364.

Hocking, A.D., Miscamble, B.F. and Pitt, J.I. 1994. Water relations of *Alternaria alternata*, *Cladosporium cladosporioides*, *Cladosporium sphaerospermum*, *Curvularia lunata* and *Curvularia pallescens*. Mycol. Res. 98: 91–94.

Hocking, A.D., Bull, M.K. and Charley, N.J. 1996. A sensitive enrichment technique for low numbers of preservative resistant yeasts in liquid, acid products. Abstract no. P12–17. Program and Abstracts, 9th International Symposium on Yeasts. Sydney, Australia, August 25–30, 1996. Sydney, Australia: Australian Biotechnology Association. pp 75–76.

Hocking, A.D., Pitt, J.I., Samson, R.A. and Thrane, U. (eds). 2006a. Advances in Food Mycology. Springer, New York.

Hocking, A.D., Begum, M. and Stewart, C.M. 2006b. Inactivation of fruit spoilage yeasts and moulds using high pressure processing. *In* Advances in Food Mycology, eds A.D. Hocking, J.I. Pitt, R.A. Samson and U. Thrane. New York: Springer. pp. 239–246.

Hocking, A.D., Leong, S.L., Kazi, B.A., Emmett, R.W. and Scott, E.S. 2007. Fungi and mycotoxins in vineyards and grape products. Int. J. Food Microbiol. 119: 84–88.

Holmes, G.J., Eckert, J.W. and Pitt, J.I. 1993. A new postharvest disease of citrus in California caused by *Penicillium ulaiense*. Plant Dis. 77: 537.

Holmes, G.J., Eckert, J.W. and Pitt, J.I. 1994. Revised description of *Penicillium ulaiense* and its role as a pathogen of citrus fruits. Phytopathology 84: 719–727.

Hood, M.A. 1983. Effects of harvesting waters and storage conditions on yeast populations in shellfish. J. Food Prot. 46: 105–108.

Hoogerwerf, S.W., Kets, E.P.W. and Dijksterhuis, J. 2002. High-oxygen and high-carbon dioxide containing atmospheres inhibit growth of food associated moulds. Lett. Appl. Microbiol. 35: 419–422.

Hope, R. and Magan, N. 2003. Two-dimensional environmental profiles of growth, deoxynivalenol and nivalenol production by *Fusarium culmorum* on a wheat-based substrate. Lett. Appl. Microbiol. 37: 70–74.

Hope, R., Aldred, D. and Magan, N. 2005. Comparison of environmental profiles for growth and deoxynivalenol production by *Fusarium culmorum* and *F. graminearum* on wheat grain. Lett. Appl. Microbiol. 40: 295–300.

Hori, M., Yamamoto, T., Ozawa, A., Matsuki, Y., Hamaguchi, A. and Soraoka, H. 1954. Studies on a fungus species isolated from malt feed causing mass death of cows. III. Classification, mechanism of toxin production, and chemical structure of the toxin. Nippon Saikingaku Zasshi (Jpn. J. Bacteriol.) 9: 1105–1111.

Horie, Y. 1995. Productivity of ochratoxin A of *Aspergillus carbonarius* in *Aspergillus* section *Nigri*. Nippon Kingakukai Kaiho 36: 73–76.

Horn, B.W. 2003. Ecology and population biology of aflatoxigenic fungi in soil. J. Toxicol. Toxin Rev. 22: 355–383.

Horn, B.W. 2007. Biodiversity of *Aspergillus* section *Flavi* in the United States: a review. Food Addit. Contam. 24: 1088–1101.

Horn, B.W., Dorner, J.W., Greene, R.L., Blankenship, P.D. and Cole, R.J. 1994. Effect of *Aspergillus parasiticus* soil inoculum on invasion of peanut seeds. Mycopathologia 125: 179–191.

Houbraken, J. and Samson, R.A. 2006. Standardization of methods for detecting heat resistant fungi. *In* Advances in Food Mycology, eds A. D. Hocking, J.I. Pitt, R.A. Samson and U. Thrane. New York: Springer. pp. 107–111.

Houbraken, J., Samson, R.A. and Frisvad, J.C. 2006. *Byssochlamys*: Significance of heat resistance and mycotoxin production. *In* Advances in Food Mycology. A.D. Hocking, J.I. Pitt, R.A. Samson and U. Thrane eds. New York: Springer, pp: 211–224.

Houbraken J., Varga, J., Rico-Munoz, E., Johnson, S. and Samson, R.A. 2008. Sexual reproduction as the cause of heat resistance in the food spoilage fungus *Byssochlamys spectabilis* (anamorph *Paecilomyces variotii*). Appl. Environ. Microbiol. 74: 1613–1619.

Hounhouigan, D.J., Nout, M.J.R., Nago, C.M., Houben, J. H. and Rombouts, F.M. 1994. Microbiological changes in mawe during natural fermentation. World J. Microbiol. Biotechnol. 10: 410–413.

Hsieh, H.-M., Su, H.-J. and Tzean, S.-S. 1987. The genus *Penicillium* in Taiwan. I. Two new taxa of synnematous *Penicillium*. Trans. Mycol. Soc. ROC 2: 157–168.

Huang, L.H. and Hanlin, R.T. 1975. Fungi occurring in freshly harvested and in-market pecans. Mycologia 67: 689–700.

Huang, J.-C., Yang, J.-W., Lai, W.-D., Song, M.-D., Deng, F. and Lin J.-Y. 2003. Impedance method for rapid detection of total counts of bacteria and fungi in bottled purified water. J. AOAC Int. 86: 719–721.

Huang, Y., Deverall, B.J., Morris, S.C. and Wild, B.L. 1993. Biocontrol of postharvest orange diseases by a strain of *Pseudomonas cepacia* under semi-commercial conditions. Postharvest Biol. Technol. 3: 293–304.

Hudec, K. 2007. Influence of harvest date and geographical location on kernel symptoms, fungal infestation and embryo viability of malting barley. Int. J. Food Microbiol. 113: 125–132.

Huerta, T., Sanchis, V., Hernandez, J. and Hernandez, E. 1987. Mycoflora of dry-salted Spanish ham. Microbiol. Aliments Nutr. 5: 247–252.

Hull, R. 1939. Study of *Byssochlamys fulva* and control measures in processed fruits. Ann. Appl. Biol. 26: 800–822.

Hunter, P.R., Hornby, H., Campbell, C.K. and Browne, K. F. 1994. Isolation of food spoilage yeasts from salads purchased from delicatessens. Br. Food J. 96: 23–25.

Hur, J.-K., Shin, J.-G., Kim, S.-K., Baek, Y.-J. and Chung, D.-H. 1992. Studies on the yeasts isolated from stirred yoghurts. II. Isolation and identification of yeasts from stirred yoghurts. Han'guk Naknong Hakhocci (Korean J. Dairy Sci.) 14: 283–291.

Hur, J.-K., Lee, S.-J., Oh, S.-J., Kim, S.-K. and Baek, Y.-J. 1993. Physiological characteristics and heat resistance of yeasts isolated from stirred yogurts. Han'guk Naknong Hakhocci (Korean J. Dairy Sci.) 15: 56–65.

Hussein, F.N., Abd-Elrazik, A., Darweish, F.A. and Rushdi, M.H. 1977. Survey of storage diseases of onions and their incitants in upper Egypt. Egypt. J. Phytopathol. 9: 15–21.

Hussein, H.M., Baxter, M., Andrew, I.G. and Franich, R.A. 1991. Mycotoxin production by *Fusarium* species isolated

from New Zealand maize fields. Mycopathologia 113: 35–40.

Hussein, H.M., Christensen, M.J. and Baxter, M. 2002. Occurrence and distribution of *Fusarium* species in maize fields in New Zealand. Mycopathologia 156: 25–30.

Hwang, H.-J., Vogel, R.F. and Hammes, W.P. 1993a. Development of mould cultures for sausage fermentation – characterisation and toxicological assessment. Fleischwirtschaft 73: 89–92.

Hwang, H.-J., Vogel, R.F. and Hammes, W.P. 1993b. Entwicklung von Schimmelpilzkulturen fuer die Rohwurstherstellung. Technologische Eignung der Stämme und sensorische Bewertung der Produkte. Fleischwirtschaft 73: 327–328, 331–332.

Iamanaka, B.T., Taniwaki, M.H., Castle de Menezes, H. and Vincente, E. 2005. Incidence of toxigenic fungi and ochratoxin A in dried fruits sold in Brazil. Food Addit. Contam. 22: 1258–1263.

Iamanaka, B.T., Menezes, H.C., Vicente, E., Leite, R.S.F., and Taniwaki, M.H. 2007. Aflatoxigenic fungi and aflatoxins occurrence in sultanas and dried figs commercialized in Brazil. Food Control. 18: 454–457.

IARC (International Agency for Research on Cancer). 1993. Some naturally occurring substances: food items and constituents, heterocyclic amines and mycotoxins. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 56. IARC, Lyon, France.

IARC (International Agency for Research on Cancer). 2002. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 82. IARC, Lyon, France.

Ichinoe, M., Kurata, H., Sugiura, Y. and Ueno, Y. 1983. Chemotaxonomy of *Gibberella zea* with special reference to production of trichothecenes and zearalenone. Appl. Environ. Microbiol. 46: 1364–1369.

ICMSF (International Commission on Microbiological Specifications for Foods). 1986. Microorganisms in Foods. 2. Sampling for Microbiological Analysis: Principles and Specific Applications, 2nd edn. Toronto, Ontario: University of Toronto Press.

ICMSF (International Commission on Microbiological Specifications for Foods). 1996a. Toxigenic fungi: *Aspergillus*. *In* Microorganisms in Foods. 5. Characteristics of Food Pathogens. London: Blackie Academic and Professional. pp. 347–381.

ICMSF (International Commission on Microbiological Specifications for Foods). 1996b. Toxigenic fungi: *Penicillium*. *In* Microorganisms in Foods. 5. Characteristics of Food Pathogens. London: Blackie Academic and Professional. pp. 397–413.

ICMSF (International Commission on Microbiological Specifications for Foods). 2002. Microorganisms in Foods 7: Microbiological Testing in Food Safety Management. New York: Kluwer Academic/Plenum Publishers.

Iglesias, H.H. and Chirife, J. 1982. Handbook of Food Isotherms. New York: Academic Press.

Ihejirika, G.O., Nwufo, M.I., Durugbo, C.I., Ibeawuchi, I.I., Onyia, V.H., Onweremadu, E.U. and Chikere, P.N. 2005. Identification of fungi associated with storage rot of groundnut in Imo State, southeastern Nigeria. Plant Pathol. J. 4: 110–112.

Iizuka, H. 1957. Studies on the microorganisms found in Thai rice and Burma rice. J. Gen. Appl. Microbiol. 3: 146–161.

Iizuka, H. 1958. Studies on the microorganisms found in Thai rice and Burma rice. Part II. On the microflora of Burma rice. J. Gen. Appl. Microbiol. 4: 108–119.

Ilic, Z., Bui, T., Tran-Dinh, N., Dang, M.H.V., Kennedy, I. and Carter, D. 2007. Survey of Vietnamese coffee beans for the presence of ochratoxigenic Aspergilli. Mycopathologia 163: 177–182.

Immerzeel, T.D. van, Mukasa, O. and Moorman, P.W. 2006. Morbidity and mortality on a paediatric ward in rural Tanzania. Trop. Doctor 36: 171–173.

Inagaki, N. 1962. On some fungi isolated from foods. I. Trans. Mycol. Soc. Jpn 4: 1–5.

Inch, A.J. 1978. Passion fruit diseases. Queensl. Agric. J. 104: 479–484.

Incze, K. and Mihalyi, V. 1976. Besteht eine Mykotoxingefahr bei der ungarischen Salami? Fleischwirtschaft 56: 1616–1618.

Infantes, M. and Schmidt, J.L. 1992. Characterization of the yeast flora of natural sourdoughs located in various French areas. Sci. Aliments 12: 271–287.

Ioos, R., Belhadj, A. and Menez, M. 2004. Occurrence and distribution of *Microdochium nivale* and *Fusarium* species isolated from barley, durum and soft wheat grains in France from 2000 to 2002. Mycopathologia 158: 351–362.

Ishitani, T., Kojo, T. and Yanai, S. 1981. Effects of microwave irradiation of mould spores. Shokuryo Kenkyusho Kenkyu Hokoku (Bull. Nat. Inst. Hyg. Sci.) 1981: 102–105.

Ishrat, Niaz, Jalaluddin, M. and Kazmi, S.A.R. 2005. Determination of suitable medium and optimum temperature required for the growth of seed borne fungi of sorghum. Int. J. Biol. Biotech. 2: 1013–1014.

Ismail, M.A. 2001. Deterioration and spoilage of peanuts and desiccated coconuts from two sub-Saharan tropical East African countries due to the associated mycobiota and their degradative enzymes. Mycopathologia 150: 67–84.

Ismail, M.A. and Zaky, Z.M. 1999. Evaluation of the mycological status of luncheon meat with special reference to aflatoxigenic moulds and aflatoxin residues. Mycopathologia 146: 147–154.

Ismail, M.E. and Abdalla, H.M. 2005. The fungus *Chaetomium globosum* a new pathogen to pear fruits in Egypt. Assiut J. Agric. Sci. 36: 177–188.

Ito, H. and Abu, M.Y. 1985. Study of microflora in Malaysian dried fishes and their decontamination by gamma-irradiation. Agric. Biol. Chem. 49: 1047–1051.

Ito, P.J., Kunimoto, R. and Ko, W.H. 1979. Transmission of *Mucor* rot of guava fruits by three species of fruit flies. Trop. Agric. 56: 49–52.

Iwasawa, S., Ueda, M., Miyata, N., Hirota, T. and Ahiko, K. 1982. Identification and fermentation character of kefir yeast. Agric. Biol. Chem. 46: 2631–2636.

Iwata, T. 2006. Mucor rot of long Chinese yam (*Dioscorea batatas*) caused by *Mucor piriformis*. Ann. Rept. Soc. Plant Prot. N. Jpn 57: 33–37.

Iyer, M.S. and Cousin, M.A. 2003. Immunological detection of *Fusarium* species in cornmeal. J. Food Prot. 66: 451–456.

Jacobsen, B.J., Harlin, K.S., Swanson, S.P., Lambert, R.J., Beasley, V.R., Sinclair, J.B. and Wei, L.S. 1995. Occurrence of fungi and mycotoxins associated with field mold damaged soybeans in the midwest. Plant Dis. 79: 86–89.

Jain, B.L. 1975. *Trichoconiella* gen. nov. Kavaka 3: 37–39.

Jain, S.K., Saxena, A.K. and Saksena, S.B. 1982. Two new postharvest diseases of fruits in India. Curr. Sci. 51: 1071.

Jaklitsch, W.M., Samuals, G.J., Dodd, S.L., Lu B.S. and Druzhinina, I.S. 2006. *Hypocrea rufa*/*Trichoderma viride:* a reassessment, and description of five closely related species with and without warted conidia. Stud. Mycol. 56: 135–177.

James, S.A. and Stratford, M. 2003. Spoilage yeasts with emphasis on the genus *Zygosaccharomyces*. *In* Yeasts in Food, eds T. Boekhout and V. Robert. Cambridge, UK: Woodhead Publishing. pp. 171–191.

James, S.A., Collins, M.D. and Roberts, I.N. 1994a. The genetic relationship of *Lodderomyces elongisporus* to other ascomycete yeast species as revealed by small-subunit rRNA gene sequences. Lett. Appl. Microbiol. 19: 308–311.

James, S.A., Collins, M.D. and Roberts, I.N. 1994b. Genetic interrelationship among species of the genus *Zygosaccharomyces* as revealed by small-subunit rRNA gene sequences. Yeast 10: 871–881.

Janardhanan, K.K., Sattar, A. and Husain, A. 1984. Production of fumigaclavine A by *Aspergillus tamarii* Kita. Can. J. Microbiol. 30: 247–250.

Janda, K. and Ulfig, K. 2005. Study on the quantitative and qualitative composition of fungi in dried medicinal plants. Roczniki Panstwowego Zakladu Higieny 56: 331–338.

Janisiewicz, W.J. 1994. Enhancement of biocontrol of blue mold with the nutrient analog 2-deoxy-D-glucose on apples and pears. Appl. Environ. Microbiol. 60: 2671–2676.

Janisiewicz, W.J. and Bors, B. 1995. Development of a microbial community of bacteria and yeast antagonists to control wound-invading postharvest pathogens of fruits. Appl. Environ. Microbiol. 61: 3261–3267.

Janisiewicz, W.J. and Korsten, L. 2002. Biological control of postharvest diseases of fruits. Annu. Rev. Phytopathol. 40: 411–441.

Janisiewicz, W.J., Peterson, D.L. and Bors, R. 1994. Control of storage decay of apples with *Sporobolomyces roseus*. Plant Dis. 78: 466–470.

Jarvis, B. 1973. Comparison of an improved rose bengal-chlortetracycline agar with other media for the selective isolation and enumeration of moulds and yeasts in foods. J. Appl. Bacteriol. 36: 723–727.

Jarvis, B. 1977. A chemical method for the estimation of mould in tomato products. J. Food Technol. 12: 581–591.

Jarvis, B. 1983. Mould and mycotoxins in mouldy cheeses. Microbiol., Aliments, Nutr. 1: 187–191.

Jarvis, B., Seiler, D.A.L., Ould, A.J.L. and Williams, A.P. 1983. Observations on the enumeration of moulds in food and feedingstuffs. J. Appl. Bacteriol. 55: 325–336.

Jarvis, W.R. 1977. *Botryotinia* and *Botrytis* species: taxonomy, physiology, and pathogenicity. Can. Dept Agric. Res. State Harrow Monogr. 15:195.

Jarvis, W.R. and Traquair, J.A. 1984. Bunch rot of grapes caused by *Aspergillus aculeatus*. Plant Dis. 68: 718–719.

Javaid, A., Bajwa, R., Javaid, A. and Anjum, T. 2005. Fungi associated with seeds of pulses collected from Lahore and their effect on seed germination. Mycopathologia 3 (1/2): 13–16.

Jayaraman, P. and Kalyanasundaram, I. 1994. Changes in storage mycoflora of parboiled rice through different stages of processing. J. Food Sci. Technol. 31: 219–224.

JCVI (J. Craig Venter Institute). 2008. http://www.jcvi.org/cms/research/projects/msc/overview/ Accessed July 2008.

JECFA (Joint FAO/WHO Expert Committee on Food Additives) 2001. Safety Evaluation of certain Mycotoxins in Food. WHO Food Additive Series No. 47. Geneva: World Health Organisation. Fumonisins, pp. 103–279; Ochratoxin, pp. 281–415; Deoxynivalenol, pp. 419–555; T-2 and HT-2 Toxins, pp. 557–652.

Jeewon, R., Liew, E.C.Y. and Hyde K.D. 2004. Phylogenetic evaluation of species nomenclature of *Pestalotiopsis* in relation to host association. Fungal Divers. 17: 39–55.

Jenkins, P., Poulos, P.G., Cole, M.B., Vandeven, M.H. and Legan, J.D. 2000. The boundary for growth of *Zygosaccharomyces bailii* in acidified products described by models for time to growth and probability of growth. J. Food Prot. 63: 222–230.

Jennessen, J., Schnürer, J., Olsson, J., Samson, R.A. and Dijksterhuis, J. 2008. Morphological characteristics of the sporangiospores of the tempe fungus *Rhizopus oligosporus* differentiate it from other taxa of the *R. microsporus* group. Mycol. Res. 112: 547–563.

Jennings, P., Coates, M.E., Turner, J.A., Chandler, E.A. and Nicholson, P. 2004. Determination of deoxynivalenol and nivalenol chemotypes of *Fusarium culmorum* isolates from England and Wales by PCR assay. Plant Pathol. 53: 182–190.

Jermini, M.F.G. and Schmidt-Lorenz, W. 1987a. Growth of osmotolerant yeasts at different water activity values. J. Food Prot. 50: 404–410.

Jermini, M.F.G. and Schmidt-Lorenz, W. 1987b. Cardinal temperatures for growth of osmotolerant yeasts in broths at different water activity values. J. Food Prot. 50: 473–478.

Jermini, M.F.G. and Schmidt-Lorenz, W. 1987c. Heat resistance of vegetative cells and asci of two *Zygosaccharomyces* yeasts in broths at different water activity values. J. Food Prot. 50: 835–841.

Jermini, M.F.G. and Schmidt-Lorenz, W. 1987d. Activity of Na-benzoate and ethyl-paraben against osmotolerant yeasts at different water activity values. J. Food Prot. 50: 920–927, 932.

Jermini, M.F.G., Geiges, O. and Schmidt-Lorenz, W. 1987. Detection, isolation and identification of osmotolerant yeasts from high-sugar products. J. Food Prot. 50: 468–472, 478.

Jermini, M., Conedera, M. Sieber, T. N., Sassella, A., Scharer, H., Jelmini, G. and Hohn, E. 2006. Influence of fruit treatments on perishability during cold storage of sweet chestnuts. J. Sci. Food Agric. 86: 877–885.

Jesenská, Z., Piecková, E and Bernát, D. 1992. Heat-resistant fungi in the soil. Int. J. Food Microbiol. 16: 209–214.

Jespersen, L., Halm, M., Kpodo, K. and Jakobsen, M. 1994. Significance of yeasts and moulds occurring in maize

dough fermentation for "kenkey" production. Int. J. Food Microbiol. 24: 239–248.

Jespersen, L., Nielsen, D.S., Hønholt, S. and Jakobsen, M. 2005. Occurrence and diversity of yeasts involved in the fermentation of West African cocoa beans. FEMS Yeast Res. 5: 441–453.

Ji, Y., Zhu, K., Quian, H. and Zhou, H. 2007. Effect of water activity and temperature on growth of *Penicillium citrioviride* and *Penicillium citrinium* on MiGao (rice cake). Can. J. Microbiol. 53: 231–236.

Jijakli, M.H. and Lepoivre, P. 1998. Characterization of an exo-ß-1,3-glucanase produced by *Pichia anomala* strain K, antagonist of *Botrytis cinerea* on apples. Phytopathology 88: 335–343.

Jiménez, M. and Mateo, R. 2001. Occurrence of toxigenic fungi and mycotoxins in agricultural commodities in Spain. *In* Occurrence of Toxigenic Fungi and Mycotoxins in Plants, Food and Feeds in Europe, ed. A. Logrieco. European Commission, COST Action 835, EUR 19695, pp. 173–190.

Jiménez, M., Logrieco, A. and Bottalico, A. 1993. Occurrence and pathogenicity of *Fusarium* species in banana fruits. J. Phytopathol. 137: 214–220.

Jiménez, M., Heurta, T. and Mateo, R. 1997. Mycotoxin production by *Fusarium* species isolated from bananas. Appl. Environ. Microbiol. 63: 364–369.

Joffe, A.Z. 1962. Biological properties of some toxic fungi isolated from over-wintered cereals. Mycopathol. Mycol. Appl. 16: 201–221.

Joffe, A.Z. 1969. The mycoflora of fresh and stored groundnut kernels in Israel. Mycopathol. Mycol. Appl. 39: 255–264.

Joffe, A.Z. 1972. *Aspergillus niger* on groundnuts and in groundnut soil in Israel. Oleagineux 27: 489–491.

Joffe, A.Z. 1978. *Fusarium poae* and *F. sporotrichioides* as principal causal agents of alimentary toxic aleukia. *In* Mycotoxigenic Fungi, Mycotoxins, Mycotoxicoses: an Encyclopedic Handbook, Vol. 3, eds T.D. Wyllie and L. G. Morehouse. New York: Marcel Dekker. pp. 21–86.

Johnson, G.I., Sangchote, S. and Cooke, A.W. 1990. Control of stem end rot (*Dothiorella dominicana*) and other postharvest diseases of mangoes (cv. Kensington Pride) during short- and long-term storage. Trop. Agric. 67: 183–187.

Johnson, G.I., Mead, A.J., Cooke, A.W. and Dean, J.R. 1991. Mango stem end rot pathogens – infection levels between flowering and harvest. Ann. Appl. Biol. 119: 465–473.

Jonsyn, F.E. and Lahai, G.P. 1992. Mycotoxic flora and mycotoxins in smoke-dried fish from Sierra Leone. Nahrung 36: 485–489.

Joosten, H.M.L.J., Goetz, J., Pittet, A., Schellenberg, M. and Bucheli, P. 2001. Production of ochratoxin A by *Aspergillus carbonarius* on coffee cherries. Int. J. Food Microbiol. 65: 39–44.

Jørgensen, K. 2005. Occurrence of ochratoxin A in commodities and processed food – A review of EU occurrence data. Food Addit. Contam. Suppl. 1: 26–30.

Juan, C., Lino, C.M., Pena, A., Molto, J.C., Manes, J. and Silveira, I. 2007. Determination of ochratoxin A in maize bread samples by LC with fluorescence detection. Talanta 73: 246–250.

Julian, A.M., Wareing, P.W., Phillips, S.I., Medlock, V.F.P., MacDonald, M.V. and Delrio, L.E. 1995. Fungal contamination and selected mycotoxins in pre- and postharvest maize in Honduras. Mycopathologia 129: 5–16.

Jurjevic, Z., Wilson, J.P., Wilson, D.M. and Casper, H.H. 2007. Changes in fungi and mycotoxins in pearl millet under controlled storage conditions. Mycopathologia 164: 229–239.

Jussila, J., Komulainen, H., Kosma, V.M., Nevalainen, A., Pelkonen, J. and Hirvonen, M.R. 2002. Spores of *Aspergillus versicolor* isolated from indoor air of a moisture-damaged building provoke acute inflammation in mouse lungs. Inhalat. Toxicol. 14: 1261–1277.

Juven, B.J., Kanner, J. and Weisslowicz, H. 1978. Influence of orange juice composition on the thermal resistance of spoilage yeasts. J. Food Sci. 43: 1074–1076, 1080.

Kadakal, C., Tagi, S. and Artik, N. 2004. Effect of tomato decay proportion on ergosterol level and some tomato pulp properties. J. Food Qual. 27: 255–263.

Kadakal, C., Nas, S. and Ekinci, R. 2005. Ergosterol as a new quality parameter together with patulin in raw apple juice produced from decayed apples. Food Chem. 90: 95–100.

Kadau, R., Huyskens-Keil, S. Gossmann, M. and Buttner, C. 2005. Effect of film packaging on phytopathological and physiological processes of fresh-cut white asparagus (*Asparagus officinalis* L.) during storage. Acta Hort. 682: 1865–1869.

Kakker, R.K. and Mehrotra, B.R. 1971. Studies on imperfect fungi. 3. Influence of temperature. Sydowia 26: 119–127.

Kalafatoglu, H. and Karapinar, M. 1991. Spoilage microflora in two apple cultivars during storage. Ege Univ. Ziraat Fak. Derg. 26: 347–356.

Kalathenos, P., Sutherland, J.P. and Roberts, T.A. 1995. Resistance of some wine spoilage yeasts to combinations of ethanol and acids present in wine. J. Appl. Bacteriol. 78: 245–250.

Kaminarides, S.E. and Laskos, N.S. 1993. Yeasts in factory brine of Feta cheese. Aust. J. Dairy Technol. 47: 68–71.

Kaminski, E. and Wacowicz, E. 1991. The usage of volatile compounds produced by moulds as indicators of grain deterioration. *In* Cereal Grain: Mycotoxins, Fungi and Quality in Drying and Storage, ed. J. Chelkowski. Amsterdam: Elsevier. pp. 229–258.

Kaminski, E., Stawicki, S. and Wasowicz, E. 1974. Volatile flavor compounds produced by molds of *Aspergillus*, *Penicillium* and Fungi Imperfecti. Appl. Microbiol. 27: 1001–1004.

Kaminski, E., Stawicki, S. and Wasowicz, E. 1975. Volatile flavor substances produced by moulds on wheat grain. Acta Aliment. Pol. 1: 153–164.

Kamphuis, H.J., Notermans, S., Veeneman, G.H., Van Boom, J.H. and Rombouts, F.M. 1989. A rapid and reliable method for the detection of molds in foods: using the latex agglutination assay. J. Food Prot. 52: 244–247.

Kamphuis, H.J., van der Horst, M.I., Samson, R.A., Rombouts, F.M. and Notermans, S. 1992. Mycological conditions of maize products. Int. J. Food Microbiol. 16: 237–245.

Kang, K.-H., Lee, K.-H., Choe, Y.-J. and Lee, J.-Y. 1980. Studies of the making of yeast cheese with *Debaryomyces hansenii* and *Saccharomyces fragilis*. Han'guk Ch'uksan Hakhoechi (Korean J. Anim. Sci.) 22: 101–107.

Kang, X.H., He, X.S. and Zhang, L. 2002. Studies on disease of *Agaricus bisporus* infected by *Fusarium pallidoroseum*. Plant Prot. 28: 15.

Karabulut O.A., Cohen, L., Wiess, B., Daus, A., Lurie, S. and Droby, S. 2002. Control of brown rot and blue mold of peach and nectarine by short hot water brushing and yeast antagonists. Postharvest Biol. Technol. 24:103–111.

Karabulut, O.A., Tezcan, H., Daus, A., Cohen, L., Wiess, B. and Droby, S. 2004. Control of preharvest and postharvest fruit rot in strawberry by *Metschnikowia fructicola*. Biocontrol Sci. Technol. 14: 513–521.

Karaca, H. and Nas, S. 2006. Aflatoxins, patulin and ergosterol contents of dried figs in Turkey. Food Addit. Contam. 23: 502–508.

Karaca, H. and Velioglu, Y.S. 2007. Ozone applications in fruit and vegetable processing. Food Rev. Int. 23: 91–106.

Karlshøj, K. and Larsen, T.O. 2005. Differentiation of species from the *Penicillium roqueforti* group by volatile metabolite profiling. J. Agric. Food Chem. 53: 708–715.

Karlshøj, K., Nielsen, P.V. and Larsen, T.O. 2007a. Differentiation of closely related fungi by electronic nose analysis. J. Food Sci. 72: M187–M192.

Karlshøj, K., Nielsen, P.V. and Larsen, T.O. 2007b. Prediction of *Penicillium expansum* spoilage and patulin concentration in apples used for apple juice production by electronic nose analysis. J. Agric. Food Chem. 55: 4289–4298.

Kassim, M.Y. 1987. Chemical control of post-harvest diseases of some vegetable fruits in Saudi Arabia. J. Coll. Sci. King Saud Univ. 18: 43–49.

Katta, S.K., Eskridge, K.M. and Bullerman, L.B. 1995. Mold content of commercial popcorn. J. Food Prot. 58: 1014–1017.

Kavanagh, J., Larchet, N. and Stuart, M. 1963. Occurrence of a heat resistant species of *Aspergillus* in canned strawberries. Nature (London) 198: 1322.

Kayali, H.A. and Tarhan, L. 2005. Role of pyruvate and ascorbate production in regulation of antioxidant enzymes and membrane LPO levels in *Fusarium acuminatum*. Appl. Biochem. Biotechnol. 120: 15–27.

Kebede, A., Viljoen, B.C., Gadaga, H., Narvhus, J.A. and Lourens-Hattingh, A. 2007. The effect of incubation temperature on the survival and growth of yeasts in Sethemi, South African naturally fermented milk. Food Technol. Biotechnol. 45: 21–26.

Keblys, M., Bernhoft, A., Höfer, C.C., Morrision, E., Larsen, H.J.S. and Flåøyen, A. 2004. The effects of the *Penicillium* mycotoxins citrinin, cyclopiazonic acid, ochratoxin A, patulin, penicillic acid, and roquefortine C on in vitro proliferation of porcine lymphocytes. Mycopathologia 158: 317–324.

Kendrick, B., (ed). 1971. Taxonomy of Fungi Imperfecti. Toronto, Ontario: University of Toronto Press.

Kenjo, T., Ishide, Y., Aoyama, K. and Ichinoe, M. 2007. Fungal population and distribution of aflatoxigenic fungi in commercial almond powder products. J. Food Hyg. Soc. Japan 48: 90–96.

Kesari, R., Mishra, D.P., Garg, G.K. and Kumar, A. 2004. Suitability of dyed latex bead agglutination test for immunodiagnosis of Karnal bunt (*Tilletia indica*) teliospores in a single seed of wheat. Food Agric. Immunol. 16: 73–81.

Keshri, G. and Magan, N. 2000. Detection and differentiation between mycotoxigenic and non-mycotoxigenic strains of two *Fusarium* spp. using volatile production profiles and hydrolytic enzymes. J. Appl. Microbiol. 89: 825–833.

Keshri, G., Magan, N. and Voysey, P. 1998. Use of an electronic nose for the early detection and differentiation between spoilage fungi. Lett. Appl. Microbiol. 27: 261–264.

Khan, A., Williams, K. and Nevalainen, H. 2003. Testing the nematophagous biological control strain *Paecilomyces lilacinus* 251 for paecilotoxin production. FEMS Microbiol. Lett. 227: 107–111.

Khanna, K.K. and Chandra, S. 1975. A new disease of apple fruit. Plant Dis. Rep. 59: 329–330.

Kilpatrick, J.A. and Chilvers, G.A. 1981. Variation in the natural population of *Epicoccum purpurascens*. Trans. Br. Mycol. Soc. 77: 497–508.

Kim, J.-C. and Lee, Y.-W. 1994. Sambutoxin, a new mycotoxin produced by toxic *Fusarium* isolates obtained from rotted potato tubers. Appl. Environ. Microbiol. 60: 4380–4386.

Kim, J.-C., Kang, H.-J., Lee, D.-H., Lee, Y.-W. and Yoshizawa, T. 1993. Natural occurrence of *Fusarium* mycotoxins (trichothecenes and zearalenone) in barley and corn in Korea. Appl. Environ. Microbiol. 59: 3798–3802.

Kinderlerer, J.L. 1984a. Spoilage in desiccated coconut resulting from growth of xerophilic fungi. Food Microbiol. 1: 23–28.

Kinderlerer, J.L. 1984b. Fungi in desiccated coconut. Food Microbiol. 1: 205–207.

Kinderlerer, J.L. 1987. Ethanol production in table jelly by two species of *Chrysosporium*. J. Appl. Bacteriol. 63: 395–399.

Kinderlerer, J.L. 1995. Czapek casein 50% glucose (CZC50G): a new medium for the identification of food-borne *Chrysosporium* spp. Lett. Appl. Microbiol. 21: 131–136.

Kinderlerer, J.L. 1996. The effect of age on the thermal resistance of arthroconidia from *Chrysosporium inops*. Lett. Appl. Microbiol. 23: 359–362.

Kinderlerer, J.L. 1997. *Chrysosporium* species, potential spoilage organisms of chocolate. J. Appl. Microbiol. 83: 771–778.

Kinderlerer, J.L. and Hatton, P.V. 1990. Fungal metabolites of sorbic acid. Food Addit. Contam. 7: 657–669.

Kinderlerer, J.L. and Hatton, P.V. 1991. The effect of temperature, water activity and sorbic acid on ketone rancidity produced by *Penicillium crustosum* Thom on coconut and palm kernel oils. J. Appl. Bacteriol. 70: 502–506.

Kinderlerer, J.L. and Kellard, B. 1984. Ketonic rancidity in coconut due to xerophilic fungi. Phytochemistry 23: 2847–2849.

Kinderlerer, J.L., Hatton, P.V., Chapman, A.J. and Rose, M. E. 1988. Essential oil produced by *Chrysosporium xerophilum* in coconut. Phytochemistry 27: 2761–2763.

King, A.D. and Halbrook, W.U. 1987. Ascospore heat resistance and control measures for *Talaromyces flavus* isolated from fruit juice concentrate. J. Food Sci. 52: 1252–1254, 1266.

King, A.D. and Schade, J.E. 1986. Influence of almond harvest, processing and storage on fungal population and flora. J. Food Sci. 51: 202–205, 215.

King, A.D. and Whitehand, L.C. 1990. Alteration of *Talaromyces flavus* heat resistance by growth conditions and heating medium composition. J. Food Sci. 55: 830–832, 836.

King, A.D., Michener, H.D. and Ito, K.A. 1969. Control of *Byssochlamys* and related heat-resistant fungi in grape products. Appl. Microbiol. 18: 166–173.

King, A.D., Hocking, A.D. and Pitt, J.I. 1979. Dichloran-rose bengal medium for enumeration and isolation of molds from foods. Appl. Environ. Microbiol. 37: 959–964.

King, A.D., Hocking, A.D. and Pitt, J.I. 1981. The mycoflora of some Australian foods. Food Technol. Aust. 33: 55–60.

King, A.D., Pitt, J.I., Beuchat, L.R. and Corry, J.E.L. (eds). 1986. Methods for the Mycological Examination of Food. New York: Plenum Press.

Kirk, P.M., Cannon, P.F., David, J.C. and Stalpers, J.A. 2001. Dictionary of the Fungi. 9th edn. Wallingford, UK: CAB International.

Kirsop, B.E. 1984. Maintenance of yeasts. *In* Maintenance of Microorganisms. A Manual of Laboratory Methods, eds B.E. Kirsop and J.J.S. Snell. London: Academic Press. pp. 109–130.

Kisko, G., Stegeman, H. and Farkas, J. 1998. Detection of moulds in paprika powder by enzyme-linked immunosorbent assay. Acta Aliment. (Budapest) 27: 97–103.

Kivanç, M. 1992. Fungal contamination of Kashar cheese in Turkey. Nahrung 36: 578–583.

Kivanç, M. and Akguel, A. 1990. Mould growth on black table olives and prevention by sorbic acid, methyleugenol and spice essential oil. Nahrung 34: 369–373.

Klich, M.A. 1993. Morphological studies of *Aspergillus* section *Versicolores* and related species. Mycologia 85: 100–107.

Klich, M.A. 2002. Identification of Common *Aspergillus* Species. Utrecht: Centraalbureau voor Schimmelcultures.

Klich, M.A. and Pitt, J.I. 1985. The theory and practice of distinguishing species of the *Aspergillus flavus* group. *In* Advances in *Penicillium* and *Aspergillus* Systematics, eds R.A. Samson and J.I. Pitt. New York: Plenum Press. pp. 211–220.

Klich, M.A. and Pitt, J.I. 1988. Differentiation of *Aspergillus flavus* from *A. parasiticus* and other closely related species. Trans. Br. Mycol. Soc. 91: 99–108.

Knan, T.N. 1971. Effects of light on sporulation of *Drechslera tritici-repentis*. Trans. Br. Mycol. Soc. 56: 309–311.

Knight, M.T., Newman, M.C., Benzinger, M.J.Jr., Neufang, K.L., Agin, J.R., McAllister, J.S. and Ramos, M. 1997. Comparison of the Petrifilm dry rehydratable film and conventional culture methods for enumeration of yeasts and molds in foods: collaborative study. J. AOAC Int. 80: 806–823.

Ko, S.D. and Hesseltine, C.W. 1979. Tempe and related foods. *In* Economic Microbiology, Vol. 4, Microbial Biomass, ed. A.H. Rose. London: Academic Press. pp. 115–140.

Ko, Y., Sun, S.K. and Chang, C.F. 1994. Postharvest fruit rot of apples caused by *Alternaria alternata* and *Stemphylium herbarum*. Plant Path. Bull. 3: 128–131.

Kobatake, M., Kreger-van Rij, N.J.W., Plácidot, M.T.L.C. and van Uden, N. 1992. Isolation of proteolytic psychrotrophic yeasts from fresh raw seafoods. Lett. Appl. Microbiol. 14: 37–42.

Koch, M. and Taanami, Z. 1995. Occurrence of *Fusarium* rot of stored garlic in Israel. Plant Dis. 79: 426.

Koehler, B. 1938. Fungus growth in shelled corn as affected by moisture. J. Agric. Res. 56: 291–307.

Kogika, M.M., Hagiwara, M.K. and Mirandola, R.M.S. 1993. Experimental citrinin nephrotoxicosis in dogs – renal function evaluation. Vet. Hum. Toxicol. 35: 136–140.

Koizumi, T. 2001. Mystery of fermented foods – there are friendly molds too! Jap. J. Med. Mycol. 42: 1–5.

Kokkonen, M., Jestoi, M. and Rizzo, A. 2005a. The effect of substrate on mycotoxin production of selected *Penicillium* strains. Int. J. Food Microbiol. 99: 207–214.

Kokkonen, M., Jestoi, M. and Rizzo, A. 2005b. Determination of selected mycotoxins in mould cheeses with liquid chromatography coupled to tandem with mass spectrometry. Food Addit. Contam. 22: 449–456.

Kolařík, M., Kubátová, A., Pažoutová, S. and Šrůtka, P. 2004. Morphological and molecular characterisation of *Geosmithia putterillii*, *G. pallida* comb. nov. and *G. flava* sp. nov., associated with subcorticolous insects. Mycol. Res. 108: 1053–1069.

Konishi, K., Iida, A., Kaneko, M., Tomioka, K., Tokuda, H., Nishino, H. and Kumeda, Y. 2003. Cancer preventive potential of trichothecenes from *Trichothecium roseum*. Bioorg. Med. Chem. 11: 2511–2518.

Kononeko, G.P., Soboleva, N.A., Leonov, A.N. and Malinovskaya, L.S. 1993. Screening of *Fusarium* isolates as effective enniatin producers. Appl. Biochem. Microbiol. 29: 23–27.

Korpi, A., Kasanen, J.-P., Raunio, P. and Pasanen, A-.L. 2003. Acute effects of *Aspergillus versicolor* aerosols on murine airways. Indoor Air 13: 260–266.

Kornerup, A. and Wanscher, J.H. 1978. Methuen Handbook of Colour, 3rd edn. London: Eyre Methuen.

Korukluoglu, M., Yigit, A. and Sahan, Y. 2005. Mycoflora of some cheese samples in Bursa, Turkey. Indian Vet. J. 82: 340–341.

Kosiak, B., Torp, M., Skjerve, E. and Thrane, U. 2003. The prevalence and distribution of *Fusarium* species in Norwegian cereals: a survey. Acta Agric. Scand. Sect. B 53: 168–176.

Kosiak, B., Torp, M., Skjerve, E. and Andersen, B. 2004. *Alternaria* and *Fusarium* in Norwegian grains of reduced quality – a matched pair sample study. Int. J. Food Microbiol. 93: 51–62.

Kosiak, E.B., Holst-Jensen, A., Rundberget, T., Gonzalez Jaen, M.T. and Torp, M. 2005. Morphological, chemical and molecular differentiation of *Fusarium equiseti* isolated from Norwegian cereals. Int. J. Food Microbiol. 99: 195–206.

Kosse, D., Seiler, H., Amann, R., Ludwig, W. and Scherer, S. 1997. Identification of yoghurt-spoiling yeasts with 18S rRNA-targeted oligonucleotide probe. Syst. Appl. Microbiol. 20: 468–480.

Kostecki, M., Wisniewska, H., Perrone, G., Ritieni, A., Golinski, P., Chelkowski, J. and Logrieco, A. 1999. The effects of cereal substrate and temperature on production of beauvericin, moniliformin and fusaproliferin by *Fusarium subglutinans* ITEM-1434. Food Addit. Contam. 16: 361–365.

Kotzekidou P. 1997a. Heat resistance of *Byssochlamys nivea*, *Byssochlamys fulva* and *Neosartorya fischeri* isolated from canned tomato paste. J. Food Sci. 62: 410–412.

Kotzekidou, P. 1997b. Identification of yeasts from black olives in rapid system microtitre plates. Food Microbiol. 14: 609–616.

Kouadio, A.I., Agbo, N.G., Lebrihi, A. Mathieu, F. and Dosso, M. 2006. Effect of the frequency of the mixing of coffee cherries put out for drying on the kinetics of drying and the relationship to ochratoxin A production. Food Addit. Contam. 23: 295–304.

Kouyeas, V. 1964. An approach to the study of moisture relations of soil fungi. Plant Soil 20: 351–363.

Kovics, G.J., Pandey, A.K. and Rai, M.K. 2005. *Phoma* Saccardo and related genera: some new perspectives in taxonomy and biotechnology. *In* Biodiversity of Fungi: their Role in Human Life, eds S.K. Deshmukh and M.K. Rai. Enfield, NH: Science Publishers. pp. 129–153.

Kozakiewicz, Z. 1989. *Aspergillus* species on stored products. Mycol. Papers 161: 1–188.

Kozakiewicz, Z., Frisvad, J.C., Hawksworth, D.L., Pitt, J.I., Samson, R.A. and Stolk, A.C. 1992. Proposals for nomina specifica conservanda and rejicienda in *Aspergillus* and *Penicillium* (Fungi). Taxon 41: 109–113.

Kreger-van Rij, N.J.W., (ed). 1984. The Yeasts: A Taxonomic Study, 3rd edn. Amsterdam: Elsevier.

Krejci, M.E., Bretz, N.S. and Koechel, D.A. 1996. Citrinin produces acute adverse changes in renal function and ultrastructure in pentobarbital-anesthetized dogs without concomitant reductions in $[potassium]_{plasma}$. Toxicology 106: 167–177.

Kriek, N.P.J. and Wehner, F.C. 1981. Toxicity of *Penicillium italicum* to laboratory animals. Food Cosmet. Toxicol. 19: 311–315.

Krishnamachari, K.A.V.R., Bhat, R.V., Nagarajan, V. and Tilak, T.B.G. 1975. Investigations into an outbreak of hepatitis in parts of Western India. Indian J. Med. Res. 63: 1036–1048.

Krogh, P., Hald, B. and Pedersen, E.J. 1973. Occurrence of ochratoxin and citrinin in cereals associated with mycotoxic porcine nephropathy. Acta Pathol. Microbiol. Scand. Sect. B 81: 689–695.

Krogh, P., Elling, F., Hald, B., Larsen, A.E., Lillehoj, E.B., Madsen, A. and Mortensen, H.P. 1976. Time-dependent disappearance of ochratoxin A residues in tissues of bacon pigs. Toxicology 6: 235–242.

Krotje, D. 1992. Starter cultures. New developments in meat products. Int. Food. Ingred. 1992(6): 14–18.

Kuehn, H.H. and Gunderson, M.F. 1963. Psychrophilic and mesophilic fungi in frozen food products. Appl. Microbiol. 11: 352–356.

Kuiper-Goodman, T. 1991. Approaches to the risk assessment of mycotoxins in foods: aflatoxins. Pennington Cent. Nutr. Ser. 1: 65–86.

Kuku, F.O. 1980. Some biodeteriorative effects of lipolytic moulds on vegetable oils. Ann. Rep. Nigerian Stored Prod. Res. Inst. 1977/1978: 23–29.

Kumar, H., Jha, Y.K. and Chauhan, G.S. 2002. Detection and estimation of aflatoxin in food grains of Tarai regions and effect of heat treatments on its inactivation. J. Food Sci. Tech. Mysore 39: 479–483.

Kumaresan, G., Nareshkumar, C., Mani, K. and Saravanakumar, K. 2003. Aflatoxigenic moulds in cheese and curd. Cheiron 32 (1/2): 6–9.

Kurata, H. and Ichinoe, M. 1967. Studies on the population of toxigenic fungi in foodstuffs. I. Fungal flora of flour-type foodstuffs. Shokuhin Eiseigaku Zasshi (J. Food Hyg. Soc. Jpn) 8: 237–246.

Kure, C.F. and Skaar, I. 2000. Mould growth on the Norwegian semi-hard cheeses Norvegia and Jarlsberg. Int. J. Food Microbiol. 62: 133–137.

Kure, C.F., Wasteson, Y., Brendehaug, J. and Skaar, I. 2001. Mould contaminants on Jarlsberg and Norvegia cheese blocks from four factories. Int. J. Food Microbiol. 70: 21–27.

Kure, C.F., Skaar, I. and Brendehaug, J. 2004. Mould contamination in production of semi-hard cheese. Int. J. Food Microbiol. 93: 41–49.

Kure, C.F., Borch, E., Karlsson, I., Homleid, J.P. and Langsrud, S. 2008. Use of the selective agar medium CREAD for monitoring the level of airborne spoilage moulds in cheese production. Int. J. Food Microbiol. 122: 29–34.

Kurtzman, C.P. 1984. Synonomy of the yeast genera *Hansenula* and *Pichia* demonstrated through comparisons of deoxyribonucleic acid relatedness. Antonie van Leeuwenhoek 50: 209–217.

Kurtzman, C.P. 2003. Phylogenetic circumscription of *Saccharomyces*, *Kluyveromyces* and other members of the Saccharomycetaceae, and the proposal of the new genera *Lachancea*, *Nakaseomyces*, *Naumovia*, *Vanderwaltozyma* and *Zygotorulaspora*. FEMS Yeast Res. 4: 233–245.

Kurtzman, C.P. and Fell, J.W. 1998. The Yeasts, A Taxonomic Study, 4th edn. Amsterdam: Elsevier.

Kurtzman, C.P. and James, S.A. 2006. *Zygosaccharomyces* and related genera. *In* Food Spoilage Microorganisms, ed. C. de W. Blackburn. Cambridge, UK: Woodhead Publishing. pp. 289–305.

Kurtzman, C.P. and Robnett, C.J. 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie van Leeuwenhoek 73: 331–371.

Kurtzman, C.P. and Robnett, C.J. 2003. Phylogenetic relationships among yeasts of the '*Saccharomyces* complex' determined from multigene sequence analyses. FEMS Yeast Res. 3: 417–432.

Kurtzman, C.P., Wickerham, L.J. and Hesseltine, C.W. 1970. Yeasts from wheat and flour. Mycologia 6: 542–547.

Kurtzman, C.P., Horn, B.W. and Hesseltine, C.W. 1986a. *Aspergillus nomius*, a new aflatoxin-producing species related to *Aspergillus flavus* and *Aspergillus tamarii*. Antonie van Leeuwenhoek 53: 147–158.

Kurtzman, C.P., Smiley, M.J., Robnett, C.J. and Wicklow, D.T. 1986b. DNA relatedness among wild and domesticated species in the *Aspergillus flavus* group. Mycologia 78: 955–959.

Kurtzman, C.P., Boekhout, T., Robert, V., Fell, J.W. and Deak, T. 2003. Methods to identify yeasts. *In* Yeasts in Food, eds T. Boekhout and V. Robert. Cambridge, UK: Woodhead Publishing. pp. 69–121.

Kushalappa, A.C., Lui, L.H., Chen, C.R. and Lee, B. 2002. Volatile fingerprinting (SPME-GC-FID) to detect and

discriminate diseases of potato tubers. Plant Dis. 86: 131–137.
Kusum, B. 1992. Tomato as a new host for three species of *Fusarium*. Plant Dis. Rep. 7: 91–92.
Kusum, B. and Geeta, S. 1990. Note: new reports on diseases of sapodilla (*Achras sapota* L.) fruits. Philipp. Agric. 73: 359–363.
Kusters-van Sommeren, M.A., Samson, R.A. and Visser, J. 1991. The use of RFLP analysis in classification of the black Aspergilli: reinterpretation of the *Aspergillus niger* aggregate. Curr. Genet. 19: 21–26.
Kuthubutheen, A.J. 1979. Thermophilic fungi associated with freshly harvested rice seeds. Trans. Br. Mycol. Soc. 73: 357–359.
Kuti, J.O. and Boehm, E.C. 1994. Preharvest foliar calcium treatments for reduction of postharvest *Myrothecium* fruit rot of muskmelon. Report – Cucurbit Genetics Cooperative 17: 78–80. [Dept Agron. Resource Sci., Texas A&M University, Kingsville, Texas].
Kvashnina, E.S. 1976. (Physiological and ecological characteristics of *Fusarium* species Sect. *Sporotrichiella*). Mikol. Fitopatol. 10: 275–281.
Kwasna, H., Ward, E. and Bateman, G.L. 2006. Phylogenetic relationships among Zygomycetes from soil based on ITS1/2 rDNA sequences. Mycol. Res. 110: 501–510.
La Guerche, S., Dauphin, B., Pons, M., Blancard, D. and Darriet, P. 2006. Characterization of some mushroom and earthy off-odors microbially induced by the development of rot on grapes. J. Agric. Food Chem. 54: 9193–9200.
Labuda, R. and Tančinová, D. 2003. *Eupenicillium ochrosalmoneum*, a rare species isolated from a feed mixture in Slovakia. Biologia, Bratislava 58: 1123–1126.
Lacey, J. 1980. Colonization of damp organic substrates and spontaneous heating. *In* Microbial Growth and Survival in Extreme Environments, eds G.W. Gould and J.E.L. Corry. London: Academic Press. pp. 53–70.
Lahali, R., Serrhini, M.N. and Jijakli, M.H. 2004. Efficacy assessment of *Candida oleophila* (strain O) and *Pichia anomala* (strain K) against major postharvest diseases of citrus fruits in Morocco. Commun. Appl. Biol. Sci. Ghent Univ. 69: 601–609.
Lahali, R., Serrhini, M.N. and Jijakli, M.H. 2005. Studying and modelling the combined effect of temperature and water activity on the growth rate of *P. expansum*. Int. J. Food Microbiol. 103: 315–322.
Lahlali, R., Serrhini, M.N., Friel, D. and Jijakli, M.H. 2007. Predictive modelling of temperature and water activity (solutes) on the *in vitro* radial growth of *Botrytis cinerea* Pers. Int. J. Food Microbiol. 114: 1–9.
Laich, F., Fierro, F., Cardoza, R.E. and Martin, J.F. 1999. Organization of the gene cluster for biosynthesis of penicillin in *Penicillium nalgiovense* and antibiotic production in cured dry sausages. Appl. Environ. Microbiol., 65: 1236–1240.
Lamper, C., Teren, J., Bartok, T., Komoroczy, R., Mesterhazy, A. and Sagi, F. 2000. Predicting DON contamination in *Fusarium*-infected wheat grains via determination of the ergosterol content. Cereal Res. Commun. 28: 337–344.
Lanciotti, R., Sinigaglia, M., Gardini, F. and Guerzoni, M.E. 1998. *Hansenula anomala* as spoilage agent of cream-filled cakes. Microbiol. Res. 153: 145–148.
Landers, K.E., Davis, N.D. and Diener, U.L. 1967. Influence of atmospheric gases on aflatoxin production by *Aspergillus flavus* in peanuts. Phytopathology 57: 1086–1090.
Langseth, W., Bernhoft, A., Rundberget, T., Kosiak, B. and Gareis, M. 1999. Mycotoxin production and cytotoxicity of *Fusarium* strains isolated from Norwegian cereals. Mycopathologia 144: 103–113.
Langvad, F. 1980. A simple and rapid method for qualitative and quantitative study of the fungal flora of leaves. Can. J. Microbiol. 26: 666–670.
LaRocco, K.A., Galligan, P., Littel, K.J. and Spurgash, A. 1985. A rapid bioluminescent ATP method for determining yeast contamination in a carbonated beverage. Food Technol. 39: 49–52.
Laroche, C., Fine, F and Gervais, P. 2005. Water activity affects heat resistance of microorganisms in fine powders. Int. J. Food Microbiol. 97: 307–315.
Larsen, T.O. and Frisvad, J.C. 1995. Characterization of volatile metabolites from 47 *Penicillium* taxa. Mycol. Res. 99: 1153–1166.
Larsen, T.O., Gareis, M., Frisvad, J.C. 2002. Cell cytotoxicity and mycotoxin and secondary metabolite production by common Penicillia on cheese agar. J. Agric. Food Chem. 50: 6148–6152.
Larumbe, A., Gonzalez, H.H.L., Resnik, S.L. and Chirife, J. 1991. Moisture migration and mold growth in a composite chocolate product. Lebensm. Wiss. Technol. 24: 307–309.
Las Heras-Vazquez, F.J., Mingorance-Cazorla, L., Clemente-Jimenez, J.M. and Rodriguez-Vico, F. 2003. Identification of yeast species from orange fruit and juice by RFLP and sequence analysis of the 5.8s rRNA gene and the two internal transcribed spacers. FEMS Yeast Res. 3: 3–9.
Lasram, S., Bellí, N., Chebil, S., Nahla, Z., Mliki, A., Snachis, V. and Ghorbel, A. 2007. Occurrence of ochratoxigenic fungi and ochratoxin A in grapes from a Tunisian vineyard. Int. J. Food Microbiol. 114: 376–379.
Latorre-Garcia, L., del Castillo-Agudo, L. and Polaina, J. 2007. Taxonomical classification of yeasts isolated from kefir based on the sequence of their ribosomal RNA genes. World J. Microbiol. Biotechnol. 23: 785–791.
Lau, A.P.S., Lee, A.K.Y., Chan, C.K. and Fang Ming. 2006. Ergosterol as a biomarker for the quantification of the fungal biomass in atmospheric aerosols. Atmos. Environ. 40: 249–259.
Lauren, D.R., Sayer, S.T. and Di Menna, M.E. 1992. Trichothecene production by *Fusarium* species isolated from grain and pasture throughout New Zealand. Mycopathologia 120: 167–176.
Laurent, D., Platzer, N., Kohler, F., Sauviat, M.P. and Pellegrin, F. 1989. Macrofusine et micromoniline: deux nouvelles mycotoxines isolees de mais infeste par *Fusarium moniliforme* Sheld. Microbiol. Alim. Nutr. 7: 9–16.
Le, V.K., Hatai, K., Yuasa, A. and Sawada, K. 2005. Morphology and molecular phylogeny of *Fusarium solani* isolated from kuruma prawn *Penaeus japonicus* with black gills. Gyobyo Kenkyu = Fish Pathology 40: 103–109.
Lee, C.Z., Liou, G.Y. and Yuan, G.F. 2004. Comparison of *Aspergillus flavus* and *Aspergillus oryzae* by amplified fragment length polymorphism. Bot. Bull. Acad. Sinica 45: 61–68.

Lee, T.-S. and Lee, S.-K. 1970. Studies on yeasts for soy sauce brewing. I. Isolation, identification, and classification of yeasts in soy sauce koji. Han'guk Nonghwa Hakhoechi (J. Korean Agric. Chem. Soc.) 13: 97–103.

Lee, U.-S., Jang, H.-S., Tanaka, T., Toyasaki, N., Sugiura, Y., Oh, Y.-J., Cho, C.-M. and Ueno, Y. 1986. Mycological survey of Korean cereals and production of mycotoxins by *Fusarium* isolates. Appl. Environ. Microbiol. 52: 1258–1260.

Lee. Y.J. and Hagler, W.M. 1991. Aflatoxin and cyclopiazonic acid production by *Aspergillus flavus* isolated from contaminated maize. J. Food Sci. 56: 871–872.

Lefortier-Medvey. M. 1993. Unsere praktischen Erfahrungen in der Anwendung von Mikroorganismen für die Käseoberfläche. Milchwirtsch. Ber. Bundesanst. Wolfpassing Rotholz 114: 35–36.

Legan, J.D. and Voysey, P.A. 1991. Yeast spoilage of bakery products and ingredients. J. Appl. Bacteriol. 70: 361–371.

Leifert, C., Sigee, D.C., Stanley, R., Knight, C. and Epton, H.A.S. 1993. Biocontrol of *Botrytis cinerea* and *Alternaria brassicicola* on Dutch white cabbage by bacterial antagonists at cold-store temperatures. Plant Pathol. 42: 270–279.

Leistner, L. 1990. Mould-fermented foods: recent developments. Food Biotechnol. 4: 433–441.

Leistner, L. and Ayres, J.C. 1968. Molds and meats. Fleischwirtschaft 48: 62–65.

Leistner, L. and Eckardt, C. 1981. Schimmelpilze und Mykotoxine in Fleisch und Fleischerzeugnissen. *In* Mykotoxine in Lebensmitteln, ed. J. Reiss. Stuttgart, Germany: Gustav Fischer Verlag. pp. 297–341.

Leistner, L. and Pitt, J.I. 1977. Miscellaneous *Penicillium* toxins. *In* Mycotoxins in Human and Animal Health, eds J.V. Rodricks, C.W. Hesseltine and M.A. Mehlman. Park Forest South, IL: Pathotox Publishers. pp. 639–653.

Leistner, L. and Rödel, W. 1976. Inhibition of micro-organisms in foods by water activity. *In* Inhibition and Inactivation of Vegetative Microbes, eds F.A. Skinner and W.B. Hugo. London: Academic Press. pp. 219–237.

Lennox, C.L., Spotts, R.A. and Booyse, M. 2004. Incidence of Postharvest decay of d'Anjou pear and control with a thiabendazole drench. Plant Dis. 88: 474–478.

Lenovich, L.M., Buchanan, R.L., Worley, N.J. and Restaino, L. 1988. Effect of solute type on sorbate resistance in *Zygosaccharomyces rouxii*. J. Food Sci. 53: 914–916.

Leonard, K.J. and Suggs, E.G. 1974. *Setosphaeria prolata*, the ascigerous state of *Exserohilum prolatum*. Mycologia 66: 281–297.

Leong S.L., Hocking A.D. and Pitt, J.I. 2004. Occurrence of fruit rot fungi (*Aspergillus* section *Nigri*) on some drying varieties of irrigated grapes. Aust. J. Grape Wine Res. 10: 83–88.

Leong S.L., Hocking A.D., Pitt, J.I., Kazi, B.A., Emmett, R. W. and Scott, E.S. 2006a. Australian research on ochratoxigenic fungi and ochratoxin A. Int. J. Food Microbiol. 111 Suppl. 1: S10–S17.

Leong S.L., Hocking, A.D. and Scott E.S. 2006b. Effect of temperature and water activity on growth and ochratoxin A production by Australian *Aspergillus carbonarius* and *A. niger* isolates on a simulated grape juice medium. Int. J. Food Microbiol. 110: 209–216.

Leong, S.L., Hocking, A.D., Varelis, P., Giannikopoulos, G. and Scott, E.S. 2006c. Fate of ochratoxin A during vinification of Semillon and Shiraz grapes. J. Agric. Food Chem. 54: 6460–6464.

Leong, S.L., Hocking, A.D. and Scott, E.S. 2006d. The effect of juice clarification, static or rotary fermentation and fining on ochratoxin A in wine. Aust. J. Grape Wine Res. 12: 245–251.

Leong, S.L., Hocking, A.D. and Scott, E.S. 2006e. Effects of water activity and temperature on the survival of *Aspergillus carbonarius* spores *in vitro*. Lett. Appl. Microbiol. 42: 326–330.

Leong, S.L., Hien, L.T., An, T.V., Trang, N.T., Hocking, A. D. and Scott, E.S. 2007. Ochratoxin A-producing Aspergilli in Vietnamese green coffee. Lett. Appl. Microbiol. 45: 301–306.

Leonov, A.N., Kononenko, G.P. and Soboleva, N.A. 1990. Production of DON-related trichothecenes by *Fusarium graminearum* Schw. from Krasnodarski krai of the USSR. Mycotoxin Res. 6: 54–60.

Leonov, A.N., Malinovskaya, L.S., Kononenko, G.P., Soboleva, N.A. and Putina, T.G. 1993. Production of fusarin C by fungi causing fusariosis of grain cultures. Appl. Biochem. Microbiol. 29: 28–31.

Leslie, J.F. and Summerell, B.A. 2006. The *Fusarium* Laboratory Manual. Ames, IA: Blackwell Publishing.

Leslie, J.F., Zeller, K.A., Logrieco, A., Mulè, G., Moretti, A. and Ritieni, A. 2004. Species diversity of and toxin production by *Gibberella fujikuroi* species complex strains isolated from native prairie grasses in Kansas. Appl. Environ. Microbiol. 70: 2254–2262.

Leslie, J.F., Zeller, K.A. Lamprecht, S.C., Rheeder, J.P. and Marasas, W.F.O. 2005. Toxicity, pathogenicity, and genetic differentiation of five species of *Fusarium* from sorghum and millet. Phytopathology 95: 275–283.

Leslie, J.F., Anderson, L.L., Bowden, R.L. and Lee YinWon. 2007. Inter- and intra-specific genetic variation in *Fusarium*. Int. J. Food Microbiol. 119: 25–32.

Levi, C.P., Trenk, H.L. and Mohr, H.K. 1974. Study of the occurrence of ochratoxin A in green coffee beans. J. Assoc. Off. Anal. Chem. 57: 866–870.

Lew, H., Adler, A. and Edinger, W. 1991. Moniliformin and the European corn borer (*Ostrinia nubilalis*). Mycotoxin Res. 7: 71–76.

Lewis, P.R., Donoghue, M.B., Hocking, A.D. and Cook, L. 2005. Tremor syndrome associated with a fungal toxin: sequelae of food contamination. Med. J. Aust. 182: 582–584.

Li, J.R., Liang X.G., Deng G. and Joyce, D. 2002. Isolation and identification of spoilage fungi from red bay berry. Postharvest News Inf. 13(4): 49 N–52 N.

Li, S.Z., Marquardt, R.R. and Abramson, D. 2000. Immunochemical detection of molds: a review. J. Food Prot. 63: 281–291.

Liardon, R., Braendlin, N. and Spadone, J.C. 1992. Biogenesis of Rio flavour impact compound: 2,4,6-trichloroanisole. *In* Proc. 14th Int. Conf. Coffee Sci., San Francisco, 14–19 July 1991. Paris: Assoc. Sci. Int. Cafe. pp. 608–614.

Lichtwardt, R.W., Barron, G.L. and Tiffany, L.H. 1958. Mold flora associated with shelled corn in Iowa. Iowa State Coll. J. Sci. 33: 1–11.

Liewen, M.B. and Marth, E.H. 1984. Inhibition of Penicillia and Aspergilli by potassium sorbate. J. Food Prot. 47: 554–556.

Liewen, M.B. and Marth, E.H. 1985. Use of gas chromatography and mass spectroscopy to identify and determine 1,3-pentadiene in cheese or mold cultures. Z. Lebensm. Unters. Forsch. 180: 45–47.

Lillehoj, E.B. 1983. Effect of environmental and cultural factors on aflatoxin contamination of developing corn kernels. *In* Aflatoxin and *Aspergillus flavus* in Corn, eds U.L. Diener, R.L. Asquith and J.W. Dickens. Auburn, AL: Alabama Agricultural Experiment Station. pp. 27–34.

Lillehoj, E.B., Fennell, D.I. and Kwolek, W.F. 1976a. *Aspergillus flavus* and aflatoxin in Iowa corn before harvest. Science 193: 495–496.

Lillehoj, E.B., Kwolek, W.F., Peterson, R.E., Shotwell, O.L. and Hesseltine, C.W. 1976b. Aflatoxin contamination, fluorescence, and insect damage in corn infected with *Aspergillus flavus* before harvest. Cereal Chem. 53: 505–512.

Lillehoj, E.B., Kwolek, W.F., Horner, E.S., Widstrom, N.W., Josephson, L.M., Franz, A.O. and Catalano, E.A. 1980. Aflatoxin contamination of preharvest corn: role of *Aspergillus flavus* inoculum and insect damage. Cereal Chem. 57: 255–257.

Lim, W.H. 1983. *Penicillium funiculosum* isolates associated with fruit blemishes of pineapple (cv. Masmerah) in Peninsular Malaysia. MARDI Res. Bull. 11: 179–186.

Lima, V.M.G., Krieger, N., Sarquis, M.I.M., Mitchell, D.A., Ramos, L.P. and Fontana, J.D. 2003. Effect of nitrogen and carbon sources on lipase production by *Penicillium aurantiogriseum*. Food Tech. Biotech. 41: 105–110.

Lin, C.-F. 1975. Studies on the *Monascus* isolated from the starter of kaoliang brandy. Chin. J. Microbiol. 8: 152–160.

Lin, H.H. and Cousin, M.A. 1985. Detection of mold in processed foods by high performance liquid chromatography. J. Food Prot. 48: 671–678.

Lin, H.H. and Cousin, M.A. 1987. Evaluation of enzyme-linked immunosorbent assay for detection of molds in foods. J. Food Sci. 52: 1089–1096.

Lin, H.H., Lister, R.M. and Cousin, M.A. 1986. Enzyme-linked immunosorbent assay for detection of mold in tomato puree. J. Food Sci. 51: 180–183.

Linfield, C.A., Kenny, S.R. and Lyons, N.F. 1995. A serological test for detecting *Botrytis allii*, the cause of neck rot of onion bulbs. Ann. Appl. Biol. 126: 259–268.

Ling, K.H. 1994. Territrems, tremorgenic mycotoxins isolated from *Aspergillus terreus*. J. Toxicol. Toxin Rev. 13: 243–252.

Lioliou, K., Litopoulou-Tzanetaki, E., Tzanetakis, N. and Robinson, R.K. 2001. Changes in the microflora of manouri, a traditional Greek whey cheese, during storage. Int. J. Dairy Technol. 54: 100–106.

Liou, G.-Y., Chen, S.-R., Wei, Y.-H., Lee, F.-L., Fu, H.-M., Yuan, G.-F. and Stalpers, A. 2007. Polyphasic approach to the taxonomy of the *Rhizopus stolonifer* group. Mycol. Res. 111: 196–203.

Liu, B.-H., Wu, T.-S., Yu, F.-Y. and Su, C.-C. 2007a. Induction of oxidative stress response by the mycotoxin patulin in mammalian cells. Toxicol. Sci. 95: 340–347.

Liu, C.L., Xu, W.N., Liu, F.M. and Jiang, S.R. 2007b. Fumonisins production by *Fusarium proliferatum* strains isolated from asparagus crown. Mycopathologia 164: 127–134.

Liu, C.-H., Hsu, W.-H. Lee, F.-L. and Liao, C.-C. 1996. The isolation and identification of microbes from a fermented tea beverage, Haipao, and their interactions during Haipao fermentation. Food Microbiol. 13: 407–415. 1996.

Liu, X.Y., Huang, H. and Zheng, R.Y. 2001. Relationships within *Cunninghamella* based on sequence analysis of ITS rDNA. Mycotaxon 80: 77–95.

Llewellyn, G.C., Mooney, R.L., Cheatle, T.F. and Flannigan, B. 1992. Mycotoxin contamination of spices – an update. Int. Biodeter. Biodegr. 29: 111–121.

Llorens, A., Mateo, R., Hinojo, M.J., Logrieco, A. and Jimenez, M. 2004a. Influence of the interactions among ecological variables in the characterization of zearalenone producing isolates of *Fusarium* spp. Syst. Appl. Microbiol. 27: 253–260.

Llorens, A., Mateo, R., Hinojo, M.J., Valle-Algarra, F.M. and Jimenez, M. 2004b. Influence of environmental factors on the biosynthesis of type B trichothecenes by isolates of *Fusarium* spp. from Spanish crops. Int. J. Food Microbiol. 94: 43–54.

LoBuglio, K.F., Pitt, J.I. and Taylor, J.W. 1993. Phylogenetic analysis of two ribosomal DNA regions indicates multiple independent losses of a sexual *Talaromyces* state among asexual *Penicillium* species in subgenus *Biverticillium*. Mycologia 58: 592–604.

Lofti, A., Abd-El-Rahman, H.A., Hefnawy, Y. and Youssef, H. 1983. Studies on the mycological status of sausage in upper Egypt. Fleischwirtschaft 63: 595–596, 633–634.

Logrieco, A., Bottalico, A. and Altomare, C. 1988. Chemotaxonomic observations on zearalenone and trichothecene production by *Gibberella zeae* from cereals in southern Italy. Mycologia 80: 892–895.

Logrieco, A., Visconti, A. and Bottalico, A. 1990a. Mandarin fruit rot caused by *Alternaria alternata* and associated mycotoxins. Plant Dis. 74: 415–417.

Logrieco, A., Bottalico, A., Solfrizzo, M. and Mule, G. 1990b. Incidence of *Alternaria* species in grains from Mediterranean countries and their ability to produce mycotoxins. Mycologia 82: 501–505.

Logrieco, A., Altomare, C., Moretti, A. and Bottalico, A. 1992. Cultural and toxigenic variability in *Fusarium acuminatum*. Mycol. Res. 96: 518–523.

Logrieco, A., Moretti, A., Altomare, C., Bottalico, A. and Torres, E.C. 1993a. Occurrence and toxicity of *Fusarium subglutinans* from Peruvian maize. Mycopathologia 122: 185–190.

Logrieco, A., Moretti, A., Ritieni, A., Chelkowski, J., Altomare, C., Bottalico, A. and Randazzo, G. 1993b. Natural occurrence of beauvericin in preharvest *Fusarium subglutinans* infected corn ears in Poland. J. Agric. Food Chem. 41: 2149–2152.

Logrieco, A., Moretti, A., Ritieni, A., Bottalico, A. and Corda, P. 1995. Occurrence and toxigenicity of *Fusarium proliferatum* from preharvest maize ear rot, and associated mycotoxins, in Italy. Plant Dis. 79: 727–731.

Logrieco, A., Moretti, A., Castella, G., Kostecki, M., Golinski, P., Ritieni, A. and Chelkowski, J. 1998a. Beauvericin production by *Fusarium* species. Appl. Environ. Microbiol. 64: 3084–3088.

Logrieco, A., Doko, B., Moretti, A., Frisullo, S. and Visconti, A. 1998b. Occurrence of fumonisin B1 and B2 in

Fusarium proliferatum infected asparagus plants. J. Agric. Food Chem. 46: 5201–5204.

Logrieco, A., Mulè, G., Moretti, A. and Bottalico, A. 2002. Toxigenic *Fusarium* species and mycotoxins associated with maize ear rot in Europe. Eur. J. Plant Pathol. 108: 597–609.

Logrieco, A., Bottalico, A., Mule, G, Moretti, A. and Perrone, G. 2003. Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops. Eur. J. Plant Pathol. 109: 645–667.

Loiveke, H., Ilumae, E. and Laitamm, H. 2004. Microfungi in grain and grain feeds and their potential toxicity. Agron. Res. 2: 195–205.

Lomax, L.G., Cole, R.J. and Dorner, J.W. 1984. The toxicity of cyclopiazonic acid in weaned pigs. Vet. Pathol. 21: 418–424.

Lombaert, G.A., Pellaers, P., Neumann, G., Kitchen, D., Huzel, V., Trelka, R., Kotello, S. and Scott, P.M. 2004. Ochratoxin A in dried vine fruits on the Canadian retail market. Food Addit. Contam. 21: 578–585.

Lopandic, K., Zelger, S, Branszky, L. K., Eliskases-Lechner, F. and Prillinger, H. 2006. Identification of yeasts associated with milk products using traditional and molecular techniques. Food Microbiol. 23: 341–350.

López-Díaz, T.M., Román Blanco, C., García Arias, M.T., García Fernández, M.C. and García López, M.L. 1996. Mycotoxins in two Spanish cheese varieties. Int. J. Food Microbiol. 30: 391–395.

López-Díaz, T-M., Santos, J-A., García-López, M-L. and Otero, A. 2001. Surface mycoflora of a Spanish fermented meat sausage and toxigenicity of *Penicillium* isolates. Int. J. Food Microbiol. 68: 69–74.

López-Malo, A. and Palou, E. 2000. Modeling the growth/no-growth interface of *Zygosaccharomyces bailii* in mango puree. J. Food Sci. 65: 516–520.

Lötzsch, R. and Trapper, D. 1978. Bildung von Aflatoxinen und Patulin in Abhängigkeit von der Wasseraktivität (a_w-Wert). Fleischwirtschaft 58: 2001–2007.

Loureiro, V and Malfeito-Ferreira, M. 2006. *Dekkera*/*Brettamomyces* spp. *In* Food Spoilage Microorganisms, ed. C. de W. Blackburn. Cambridge, UK: Woodhead Publishing. pp. 354–398.

Lowings, P.H. 1956. The fungal contamination of Kentish strawberry fruits in 1955. Appl. Microbiol. 4: 84–88.

Lowry, P.D. and Gill, C.O. 1982. Microbiological considerations in cold storage of beef. Sci. Technol. Froid 1982: 93–97.

Lu, P., Marquardt, R.R., Frohlich, A.A. and Mills, J.T. 1995. Development of solid-phase immunoassays for the detection and quantification of *Aspergillus ochraceus* in wheat grain. Food Agric. Immunol. 7: 81–93.

Lubulwa, A.S.G. and Davis, J.S. 1994. Estimating the social costs of the impacts of fungi and aflatoxins in maize and peanuts. *In* Stored Product Protection, eds E. Highley, E. Wright, H.J. Banks and B.R. Champ. Proc. 6th Int. Working Conf. Stored Prod. Protect. Wallingford, UK: CAB International. pp. 1017–1042.

Ludemann, V., Pose, G.N., Pollio, M.L. and Segura, J.A. 2004. Surface mycoflora of Argentinean dry fermented sausages and toxigenicity of *Penicillium* isolates. J. Food Technol. 2: 288–292.

Lugauskas, A., Repeckiene, J. and Novosinskas, H. 2005. Micromycetes, producers of toxins, detected on stored vegetables. Ann. Agric. Environ. Med. 12: 253–260.

Lugauskas, A., Raila, A., Railiene, M. and Raudoniene, V. 2006. Toxic micromycetes in grain raw material during its processing. Ann. Agric. Environ. Med. 13: 147–161.

Lund, F. 1995a. Differentiating *Penicillium* species by detection of indole metabolites using a filter paper method. Lett. Appl. Microbiol. 20: 228–231.

Lund, F. 1995b. Diagnostic characterization of *Penicillium palitans*, *P. commune* and *P. solitum*. Lett. Appl. Microbiol. 21: 60–64.

Lund, F. 1996. Direct identification of the common cheese contaminant *Penicillium commune* in factory air samples as an aid to factory hygiene. Lett. Appl. Microbiol. 22: 339–341.

Lund, F. and Frisvad, J.C. 1994. Chemotaxonomy of *Penicillium aurantiogriseum* and related species. Mycol. Res. 98: 481–492.

Lund, F. and Frisvad, J.C. 2003. *Penicillium verrucosum* in wheat and barley indicates presence of ochratoxin A. J. Appl. Microbiol. 95: 1117–1123.

Lund, F., Filtenborg, O. and Frisvad, J.C. 1995. Associated mycoflora of cheese. Food Microbiol. 12: 173–180.

Lund, F., Filtenborg, O., Westall, S. and Frisvad, J.C. 1996. Associated mycoflora of rye bread. Lett. Appl. Microbiol. 23: 213–217.

Lutchmeah, R.S. 1993. Common field and post-harvest diseases of passion fruit (*Passiflora edulis* f. *flavicarpa*) and the associated fungi in Mauritius. Rev. Agric. Sucr. Ile Maurice 72: 55–59.

Ma, F., Misumi, J., Zhao, W., Aoki, K. and Kudo, M. 2003. Long-term treatment with sterigmatocystin, a fungus toxin, enhances the development of intestinal metaplasia of gastric mucosa in *Helicobacter pylori*-infected Mongolian gerbils. Scand. J. Gastroenterol. 38: 360–369.

McCallum, J.L., Tsao, R. and Zhou, T. 2002. Factors affecting patulin production by *Penicillium expansum*. J. Food Prot. 65: 1937–1942.

McDonald, D. 1970. Fungal infection of groundnut fruit after maturity and during drying. Trans. Br. Mycol. Soc. 54: 461–472.

MacDonald, S., Wilson, P., Barnes, K., Damant, A., Massey, R., Mortby, E. and Shepherd, M.J. 1999. Ochratoxin A in dried vine fruit: method development and survey. Food Addit. Contam. 16: 253–260.

McEvoy, I.J. and Stuart, M.R. 1970. Temperature tolerance of *Aspergillus fischeri* var. *glaber* in canned strawberries. Ir. J. Agric. Res. 9: 59–67.

McGinnis, M.R. 1980. Recent taxonomic developments and changes in medical mycology. Annu. Rev. Microbiol. 34: 109–135.

McGuire, R.G. 1994. Application of *Candida guillermondii* in commercial citrus coatings for biocontrol of *Penicillium digitatum* on grapefruits. Biol. Control 4: 1–7.

McNeill, J., Barrie, F. R., Burdet, H.M., Demoulin, V., Hawksworth, D.L., Marhold, K., Nicolson, D.H., Prado, J., Silva, P.C., Skog, J.E., Wiersema, J.H. and Turland, N.J. 2006. International Code of Botanical

Nomenclature (Vienna Code). Ruggell, Liechtenstein: A. R.G. Ganter Verlag.

Madhyastha, S.M., Marquardt, R.R., Frohlich, A.A., Platford, G. and Abramson, D. 1990. Effects of different cereal and oilseed substrates on the growth and production of toxins by *Aspergillus alutaceus* and *Penicillium verrucosum*. J. Agric. Food Chem. 38: 1506–1510.

MAFF (Ministry of Agriculture, Fisheries and Food, U.K.). 1999. 1998 survey of retail products for ochratoxin A. Food Surveillance Information Sheet, No. 185: 1–36.

Magan, N. and Evans, P. 2000. Volatiles as an indicator of fungal activity and differentiation between species, and the potential use of electronic nose technology for early detection of grain spoilage. J. Stored Prod. Res. 36: 319–340.

Magan, N. and Lacey, J. 1984a. Effect of water activity, temperature and substrate on interactions between field and storage fungi. Trans. Br. Mycol. Soc. 82: 83–93.

Magan, N. and Lacey, J. 1984b. Effects of gas composition and water activity on growth of field and storage fungi and their interactions. Trans. Br. Mycol. Soc. 82: 305–314.

Magan, N. and Lacey, J. 1984c. Water relations of some *Fusarium* species from infected wheat ears and grain. Trans. Br. Mycol. Soc. 83: 281–285.

Magan, N., Cayley, G.R. and Lacey, J. 1984. Effect of water activity and temperature on mycotoxin production by *Alternaria alternata* in culture and on wheat grain. Appl. Environ. Microbiol. 47: 1113–1117.

Magan, N., Jenkins, N.E. and Howarth, J. 1993. Lipolytic activity and degradation of rapeseed oil and rapeseed by spoilage fungi. Int. J. Food Microbiol. 19: 217–227.

Maggi, A., Gola, S., Spotti, E., Rovere, P. and Mutti, P. 1994. High-pressure treatments of ascospores of heat-resistant moulds and patulin in apricot nectar and water. Ind. Conserve 69: 26–29.

Magnani, M., Fernandes, T., Prete, C.E.C., Homechim, M., Sataque Ono, E.Y., Vilas-Boas, L.A., Furlaneto, M.C. and Fungaro Pelegrinelli, M.H. 2005. Molecular identification of *Aspergillus* spp. isolated from coffee beans. Sci. Agric. 62: 45–49.

Magnoli C., Dalcero A.M., Chiacchiera S.M., Miazzo, R. and Saenz, M.A. 1998. Enumeration and identification of *Aspergillus* group and *Penicillium* species in poultry feeds from Argentina. Mycopathologia 142: 27–32.

Magnoli C., Astoreca A., Ponsone L., Combina M., Palacio G., Rosa C.A.R. and Dalcero A.M. 2004. Survey of mycoflora and ochratoxin A in dried vine fruits from Argentina markets. Lett. Appl. Microbiol. 39: 326–331.

Magnoli C., Hallak, C., Astoreca A., Ponsone L., Chiacchiera S.M., Palacio, G. and Dalcero A.M. 2005. Surveillance of toxigenic fungi and ochratoxin A in feedstuffs from Córdoba Province, Argentina. Vet. Res. Commun. 29: 431–445.

Magnoli C., Astoreca A., Ponsone L., Fernández-Juri M., Chiacchiera S. and Dalcero A.M. 2006a. Ochratoxin A and the occurrence of ochratoxin A-producing black aspergilli in stored peanut seeds from Córdoba, Argentina. J. Sci. Food Agric. 86: 2369–2373.

Magnoli C., Hallak C., Chiacchiera S. and Dalcero A.M. 2006b. Occurrence of ochratoxin A-producing fungi in commercial corn kernels in Argentina. Mycopathologia 161: 53–58.

Magnoli C., Astoreca A., Chiacchiera S. and Dalcero A.M. 2007a. Occurrence of ochratoxin A and ochratoxigenic mycoflora in corn and corn based foods and feeds in some South American countries. Mycopathologia 163: 249–260.

Magnoli, C., Astoreca, A., Ponsone, M.L., Fernandez-Juri, M.G., Barberis, C. and Dalcero, A.M. 2007b. Ochratoxin A and *Aspergillus* section *Nigri* in peanut seeds at different months of storage in Cordoba, Argentina. Int. J. Food Microbiol. 119: 213–218.

Mahmoody, B. 1998. *Fusarium oxysporum* associated with garlic rot in Khorasan province. Iran. J. Plant Pathol. 34: 235–236.

Majumder, S.K. 1974. Control of Microflora and Related Production of Mycotoxins in Stored Sorghum, Rice and Groundnut. Mysore, India: Wesley Press.

Makun, H.A., Gbodi, T.A., Akanya, O.H., Salako, E.A. and Ogbadu, G.H. 2007a. Fungi and some mycotoxins contaminating rice (*Oryza sativa*) in Niger State, Nigeria. Afr. J. Biotechnol. 6: 99–108.

Makun, H.A., Gdobi, T.A., Tijani, A.S., Abai, A. and Kadiri, G.U. 2007b. Toxicological screening of fungi isolated from millet (*Pennisetum* spp.) during the rainy and dry harmattan seasons in Niger state, Nigeria. Afr. J. Biotechnol. 6: 34–40.

Mallick, A.K. and Nandi, B. 1981. Research: rice – results of India experiments. Rice J. 84: 8–13, 19–23.

Malloch, D. and Cain, R.F. 1972. New species and combinations of cleistothecial Ascomycetes. Can. J. Bot. 50: 61–72.

Mamede, M.E.O. and Pastore, G.M. 2006. Study of methods for the extraction of volatile compounds from fermented grape must. Food Chem. 96: 586–590.

Manabe, M., Shinshi, E., Goto, T., Misawa, Y. and Tanaka, K. 1984. Studies on the fluorescent compound in fermented foods. VI. HPLC analytical system for kojic acid. Shokuhin Sogo Kenkyusho Kenkyo Hokoku (Rep. Food Res. Inst.) 46: 337–341.

Manandhar, K.L. and Apinis, A.E. 1971. Temperature relations in *Monascus*. Trans. Br. Mycol. Soc. 57: 465–472.

Mandeel, Q.A. 2005. Fungal contamination of some imported spices. Mycopathologia 159: 291–298.

Manicom, B., Ruggiero, B., Ploetz, R.C. and Goes, A.A. 2003. Diseases of passionfruit. *In*: Diseases of tropical fruit crops, ed. R.C. Ploetz. Wallingford, UK: CABI Publishing. pp. 413–441.

Manilal, V.B., Narayanan, C.S. and Balagopalan, C. 1991. Cassava starch effluent treatment with concomitant SCP production. World J. Microbiol. Biotechnol. 7: 185–190.

Mann, G.E., Codifer, L.P. and Dollear, F.G. 1967. Effect of heat on aflatoxins in oilseed meals. Agric. Food Chem. 15: 1090–1092.

Manninen, M.T., Fung, D.Y.C. and Hart, R.A. 1991. Spiral system and laser colony scanner for enumeration of microorganisms. J. Food Saf. 11: 177–187.

Mansfield, M.A., Jones, A.D. and Kuldau, G.A. 2008. Contamination of fresh and ensiled maize by multiple *Penicillium* mycotoxins. Phytopathology 98: 330–336.

Marakis, S. 1995. Fungi and yeasts isolated from Greek tannery liquid wastes. Cryptogamie Mycol. 16: 111–119.

Marasas, W.F.O. 2001. Discovery and occurrence of the fumonisins: a historical perspective. Environ. Health Perspect. 109: 239–243.

Marasas, W.F.O. and Vismer, H.F. 2003. Food for thought about mycotoxins, organic and genetically modified foods. *In*: Advances in Stored Product Protection. Proceedings of the 8th International Working Conference on Stored Product Protection, York, UK, 22–26 July 2002: 423–427.

Marasas, W.F.O., Kriek, N.P.J., Steyn, M., van Rensburg, S.J. and van Schalkwyk, D.J. 1978. Mycotoxicological investigations on Zambian maize. Food Cosmet. Toxicol. 16: 39–45.

Marasas, W.F.O., van Rensburg, S.J. and Mirocha, C.J. 1979. Incidence of *Fusarium* species and the mycotoxins, deoxynivalenol and zearalenone, in corn produced in esophageal cancer areas in Transkei. J. Agric. Food Chem. 27: 1108–1112.

Marasas, W.F.O., Nelson, P.E. and Tousson, T.A. 1984. Toxigenic *Fusarium* Species. University Park, PA: Pennsylvania State University.

Marasas, W.F.O., Thiel, P.G., Rabie, C.J., Nelson, P.E. and Toussoun, T.A. 1986. Moniliformin production in *Fusarium* Section *Liseola*. Mycologia 78: 242–247.

Marasas, W.F.O., Kellerman, T.S., Gelderblom, W.C.A., Coetzer, J.A., Thiel, P.G. and van der Lugt, J.J. 1988. Leucoencephalomalacia in a horse induced by fumonisin B_1 isolated from *Fusarium moniliforme*. Onderstepoort J. Vet. Res. 55: 197–203.

Maravalhas, N. 1966. Mycological deterioration of cocoa beans during fermentation and storage in Bahia. Rev. Int. Choc. 21: 375–378.

Marcellino, N. and Benson, D.R. 1992. Scanning electron and light microscopic study of microbial succession on Bethlehem St. Nectaire cheese. Appl. Environ. Microbiol. 58: 3448–3454.

Marchi, A., Folchi, A. and Pratella, G.C. 1994. *Phialophora* spp. agent of epidermic lesions on kiwifruits in cold storage. Riv. Frutticoltura Ortofloricoltura 56: 73–74.

Marfleet, I., Magan, N. and Lacey, J. 1991. The relationship between fungal biomass, ergosterol and grain spoilage. *In* Proceedings of the Fifth International Working Conference on Stored Product Protection, eds F. Fleurat-Lesard and P. Ducom. Bordeaux, France: Institut National de la Recherche Agronomique, Paris. pp. 405–412.

Mari, M., Bertolini, P. and Pratella, G. C. 2003. Non-conventional methods for the control of post-harvest pear diseases. J. Appl. Microbiol. 94: 761–766.

Marin, D.H., Sutton, T.B., Blankenship, S.M. and Swallow, W.H. 1996. Pathogenicity of fungi associated with crown rot of bananas in Latin America on Grande Naine and disease-resistant hybrid bananas. Plant Dis. 80: 525–528.

Marín, S., Sanchis, V., Vinas, I., Canela, R. and Magan, N. 1995. Effect of water activity and temperature on growth and fumonisin B_1 and B_2 production by *Fusarium proliferatum* and *F. moniliforme* on maize grain. Lett. Appl. Microbiol. 21: 298–301.

Marín, S., Sanchis, V., Teixido, A., Saenz, R., Ramos, A.J., Vinas, I. and Magan, N. 1996. Water and temperature relations and microconidial germination of *Fusarium moniliforme* and *Fusarium proliferatum* from maize. Can. J. Microbiol. 42: 1045–1050.

Marín, S., Magan, N., Belli, N., Ramos, A.J., Canela, R. and Sanchis, V. 1999. Two-dimensional profiles of fumonisin B1 production by *Fusarium moniliforme* and *Fusarium proliferatum* in relation to environmental factors and potential for modelling toxin formation in maize grain. Int. J. Food Microbiol. 51: 159–167.

Marín, S., Guynot, M. E., Sanchis, V. and Ramos, A. J. 2003. Hydrolytic enzyme activities as indicators of fungal spoilage in bakery products. J. Sci. Food Agric. 83: 685–691.

Marín, S., Abellana, M., Rull, F., Sanchis, V. and Ramos, A.J. 2004. Efficacy of propionates and benzoates on the control of growth of *Eurotium* species in bakery products with near neutral pH. J. Sci. Food Agric. 84: 1147–1152.

Marín, S., Ramos, A.J. and Sanchis, V. 2005. Comparison of methods for the assessment of growth of food spoilage moulds in solid substrates. Int. J. Food Microbiol. 99: 329–341.

Marín, S., Bellí, N., Lasrom, S., Chebil, S., Ramos, A.J., Ghorbel, A. and Sanchis, V. 2006. Kinetics of ochratoxin a production and accumulation by *Aspergillus carbonarius* on synthetic grape medium at different temperature levels. J. Food Sci. 71: M196–M200.

Marín, S., Vinaixa, M., Brezmes, J., Llobet, E., Vilanova, X., Correig, X., Ramos, A.J. and Sanchis, V. 2007a. Use of a MS-electronic nose for prediction of early fungal spoilage of bakery products. Int. J. Food Microbiol. 114: 10–16.

Marín, S., Ramos, A.J., Vazquez, C. and Sanchis, V. 2007b. Contamination of pine nuts by fumonisin produced by strains of *Fusarium proliferatum* isolated from *Pinus pinea*. Lett. Appl. Microbiol. 44: 68–72.

Marín, S., Cuevas, D., Ramos, A.J. and Sanchis, V. 2008. Fitting of colony diameter and ergosterol as indicators of food borne mould growth to known growth models in solid medium. Int. J. Food Microbiol. 121: 139–149.

Marquina, D., Peres, C., Caldas, F.V., Marques, J.F., Peinado, J.M. and Spencer-Martins, I. 1992. Characterisation of the yeast populations in olive brines. Lett. Appl. Microbiol. 14: 279–283.

Martelleto, L.A.P., Castilho, A.M.C. and de Goes, A. 1998. Influence of incubation temperature on mycelial growth, sporulation and pathogenicity of *Fusarium subglutinans*, the causing agent of Fusarium wilt in the pineapple plant. Summa Phytopathol. 24: 242–246.

Marth, E.H. 1978. Dairy products. *In* Food and Beverage Mycology, ed. L.R. Beuchat. Westport, CT: AVI Publishing Co. pp. 145–172.

Martín, A., Córdora, J.J., Núñez, F., Benito, M.J. and Asensio, M.A. 2004. Contribution of a selected fungal population to proteolysis on dry-cured ham. Int. J. Food Microbiol. 94: 55–66.

Martín, A., Aranda, E., Benito, M.J., Pérez-Nevado, F. and Córdora, M.G. 2005. Identification of fungal contamination and determination of mycotoxigenic moulds by micellar electrokinetic capillary chromatography in smoked paprika. J. Food Prot. 68: 815–822.

Martín, A., Córdora, J.J., Aranda, E., Córdora, M.G. and Asensio, M.A. 2006. Contribution of a selected fungal population to the volatile compounds on dry-cured ham. Int. J. Food Microbiol. 110: 8–18.

Martin, A.M., Chun, L. and Patel, T.R. 1993. Growth parameters for the yeast *Rhodotorula rubra* grown in peat extracts. J. Ferment. Bioeng. 76: 321–325.

Martínez-Culebras, P.V. and Ramón, D. 2007. An ITS-RFLP method to identify black *Aspergillus* isolates responsible for OTA contamination in grapes and wine. Int. J. Food Microbiol. 113: 147–153.

Martínez-Romero, D., Guillén, F., Valverde, J.M., Bailén, G., Zapata, P., Serrano, M., Castillo, S. and Valero, D. 2007. Influence of carvacrol on survival of *Botrytis cinerea* inoculated in taple grapes. Int. J. Food Microbiol. 115: 144–148.

Martins, M.L., Martins, H.M. and Gimeno, A. 2003. Incidence of microflora and of ochratoxin A in green coffee beans (*Coffea arabica*). Food Addit. Contam. 20: 1127–1131.

Martorell, P., Fernández-Espinar, T. and Querol, A. 2005a. Molecular monitoring of spoilage yeasts during the production of candied fruit nougats to determine food contamination sources. Int. J. Food Microbiol. 101: 293–302.

Martorell, P., Querol, A. and Fernandez-Espinar, M.T. 2005b. Rapid identification and enumeration of *Saccharomyces cerevisiae* cells in wine by real-time PCR. Appl. Environ. Microbiol. 71: 6823–6830.

Martorell, P., Stratford, M., Steels, H., Fernandez-Espinar, M.T. and Querol, A. 2007. Physiological characterization of spoilage strains of *Zygosaccharomyces bailii* and *Zygosaccharomyces rouxii* isolated from high sugar environments. Int. J. Food Microbiol. 114: 234–242.

Marziano, F., Nanni, B., Scalcione, M. and Noviello, C. 1992. *Fusarium solani* and *F. avenaceum* as causal agents of a post harvest rot of carrots. Inf. Fitopatol. 42: 57–63.

Masih, E.I. and Paul, B. 2002. Secretion of beta-1,3-glucanases by the yeast *Pichia membranaefaciens* and its possible role in the biocontrol of *Botrytis cinerea* causing grey mould disease of the grapevine. Curr. Microbiol. 44: 391–395.

Masih, E.I., Alie, I. and Paul, B. 2000. Can the grey mould disease of the grape-vine be controlled by yeast? FEMS Microbiol. Lett. 189: 233–237.

Masoud, W. and Jespersen, L. 2006a. Pectin degrading enzymes in yeasts involved in fermentation of *Coffea arabica* in East Africa. Int. J. Food Microbiol. 110: 291–296.

Masoud, W. and Kaltoft, C.H. 2006b. The effects of yeasts involved in the fermentation of *Coffea arabica* in East Africa on growth and ochratoxin A (OTA) production by *Aspergillus ochraceus*. Int. J. Food Microbiol. 106: 229–234.

Masoud, W., Cesar, L.B., Jespersen, L. and Jakobsen, M. 2004. Yeast involved in fermentation of *Coffea arabica* in East Africa determined by genotyping and by direct denaturating gradient gel electrophoresis. Yeast 21: 549–556.

Masuda, T. and Ueno, Y. 1984. Microsomal transformation of emodin into a direct mutagen. Mutat. Res. 125: 135–144.

Matcham, S.E., Jordan, B.R. and Wood, D.A. 1985. Estimation of fungal biomass in a solid substrate by three independent methods. Appl. Microbiol. Biotechnol. 21: 108–112.

Matheny, P.B., Gossmann, J.A., Zalar, P., Kumar, T.K.A. and Hibbett, D.S. 2006. Resolving the phylogenetic position of the Wallemiomycetes: an enigmatic major lineage of Basidiomycota. Can. J. Bot. 84: 1794–1805.

Matos, A.P. de, Cabral, J.R.S., Sanches, N.F. and Caldas, R. C. 2000. Effect of temperature and rainfall on the incidence of *Fusarium subglutinans* on pineapple fruits. Acta Hort. 529: 265–272.

Matos, A.P. de, Ferreira, D.M.V. and Cordeiro, Z.J. 2005. Disease of pineapple. Inf. Agropec. 26: 7–11.

Matossian, M.K. 1989. Poisons of the Past: Molds, Epidemics, and History. New Haven, CT: Yale University Press.

Mawhinney, I., Woodger, N., Trickey, S. and Payne, J. 2008. Suspected sweet potato poisoning in cattle in the UK. Vet. Rec. 162: 62–63.

Maximay, S., Duncan, E.J. and Brathwaite, C.W.D. 1992. Internally seedborne fungi of pigeon pea [*Cajanus cajan* (L.) Millsp.] in Trinidad: isolation and occurrence. Trop. Agric. 69: 260–262.

Mayoral, M.B., Martin, R., Hernandez, P.E., Gonzalez, I. and Garcia, T. 2006. A reverse transcriptase PCR technique for the detection and viability assessment of *Kluyveromyces marxianus* in yoghurt. J. Food Prot. 69: 2210–2216.

Mazigh, D. 1994. Microbiology of chocolate. The application of HACCP in the processing of cocoa. Int. Food Ingredients 1994: 34–39.

Mazzani, C., Luzon, O., Beomont, P. and Chavarri, M. 2004. Mycobiota associated to maize kernels in Venezuela and in vitro aflatoxigenity of the *Aspergillus flavus* isolates. Fitopatol. Venezolana 17: 19–23.

Medina, A., Valle-Algarra, F.M., Mateo, R., Gimeno-Adelantado, J.V., Mateo, F. and Jiménez, M. 2006. Survey of the mycobiota of Spanish malting barley and evaluation of the mycotoxin producing potential of species of *Alternaria*, *Aspergillus* and *Fusarium*. Int. J. Food Microbiol. 108: 196–203.

Mehdi, N.A.Q., Carlton, W.W. and Tuite, J. 1981. Citrinin mycotoxicosis in broiler chickens. Food Cosmet. Toxicol. 19: 723–733.

Mehdi, N.A.Q., Carlton, W.W. and Tuite, J. 1984. Mycotoxicoses produced in ducklings and turkeys by dietary and multiple doses of citrinin. Avian Pathol. 13: 37–50.

Meirelles, P.G., Ono, M.A., Ohe, M.C.T., Maroneze, D.M., Itano, E.N., Garcia, G.T., Sugiura, Y., Ueno, Y., Hirooka, E.Y. and Ono, E.Y.S. 2006. Detection of *Fusarium* sp. contamination in corn by enzyme-linked immunosorbent assay. Food Agric. Immunol. 17: 79–89.

Melcion, D., Cahagnier, B., Bakan, B. and Richard-Molard, D. 1998. Influence of temperature on fumonisin B1 production on maize grain by *Fusarium proliferatum*. Sci. Aliments 18: 301–311.

Menke, M., Lederer, M., Muno-Bender, J., Grossmann, M. and von Wallbrunn, C. 2007. Microbiological quality control of wine – A survey of wines traded in Germany. Deutsche Lebensmittel-Rundschau 103: 197–202.

Menzies, J.G., Ehret, D.L., Koch, C., Hall, J.W., Seifert, K. A., Bisset, J. and Barr, D.J.S. 2005. Fungi associated with roots of cucumber grown in different greenhouse root substrates. Can. J. Bot. 83:80–92.

Mercier, J. and Wilson, C.L. 1995. Effect of wound moisture on the biocontrol by *Candida oleophila* of gray mold rot (*Botrytis cinerea*) of apple. Postharvest Biol. Technol. 6: 9–15.

Mercier, J., Makhlouf, J. and Martin, R.A. 1991. *Fusarium avenaceum,* a pathogen of stored broccoli. Can. Plant Dis. Surv 71: 161–162.

Meroth, C.B., Hammes, W.P. and Hertel, C. 2003. Identification and population dynamics of yeasts in sourdough fermentation processes by PCR-denaturing gradient gel electrophoresis. Appl. Environ. Microbiol. 69: 7453–7461.

Meyer, D., Weipert, D. and Mielke, H. 1986. Bëinflussung der Qualitaet von Weizen durch den Befall mit *Fusarium culmorum*. Getreide, Mehl Brot, 40: 35–39.

Meyvaci, K.B., Altindisli, A., Aksoy, U., Eltem, R., Turgut, H., Arasiler, Z. and Kartal, N. 2005. Ochratoxin A in sultanas from Turkey I: survey of unprocessed sultanas from vineyards and packing-houses. Food Addit. Contam. 22: 1138–1143.

Mheen, T.I., Cheig, H.S., Ragunathan, A.N. and Majundar, K.S. 1982. Fungi in stored rice. Korean J. Appl. Microbiol. Bioeng. 10: 191–196.

Mian, M.A., Fleet, G.H. and Hocking, A.D. 1997. Effect of diluent type on viability of yeasts enumerated from foods or pure culture. Int. J. Food Microbiol. 35: 103–107.

Micco, C., Grossi, M., Miragli, M. and Brera, C. 1989. A study of the contamination by ochratoxin A of green and roasted coffee beans. Food Addit. Contam. 6: 333–339.

Michailides, T.J. 1991. Characterisation and comparative studies of *Mucor* isolates from stone fruits from California and Chile. Plant Dis. 75: 373–380.

Michailides, T.J. and Morgan, D.P. 1998. Spread of endosepsis in Calimyrna fig orchards. Phytopathology 88: 637–647.

Michailides, T.J. and Ogawa, J.M. 1989. Effects of high temperatures on the survival and pathogenicity of propagules of *Mucor piriformis*. Phytopathology 79: 547–554.

Michailides, T.J. and Spotts, R.A. 1990. Postharvest diseases of pome and stone fruits caused by *Mucor piriformis* in the Pacific Northwest and California. Plant Dis. 74: 537–543.

Michailides, T.J. and Thomidis, T. 2007. First report of *Aspergillus flavus* causing fruit rot of peaches in Greece. Plant Pathol. 56: 352.

Michels, M.J.M. and Koning, W. 2000. Mayonnaise, dressings, mustard, mayonnaise-based salads, and acid sauces. *In* The Microbiological Safety and Quality of Food, Vol. 1, eds B.M. Lund, T.C. Barid-Parker and G. W. Gould. Gaithersberg, Maryland: Aspen Publishers Inc. pp. 807–835.

Michener, H.D. and Elliott, R.P. 1964. Minimum growth temperatures for food-poisoning, fecal-indicator, and psychrophilic micro-organisms. Adv. Food Res. 13: 349–396.

Middelhoven, W.J. 1998. The yeast flora of maize silage. Food Technol. Biotechnol. 36: 7–11.

Mielniczuk, E. 2001. The occurrence of *Fusarium* spp. on panicles of oat (*Avena sativa* L.). J. Plant Prot. Res. 41: 173–180.

Mikami, Y., Yazawa, K., Fukushima, K., Arai, T., Udagawa, S. and Samson, R.A. 1989. Paecilotoxin production in clinical or terrestrial isolates of *Paecilomyces lilacinus* strains. Mycopathologia 108: 195–199.

Mikata, K., Yamauchi, S. and Banno, I. 1983. Preservation of yeast cultures by L-drying: viabilities of 1710 yeasts after drying and storage. Res. Commun. Inst. Ferment. Osaka 11: 25–46.

Miller, D.D. and Golding, N.S. 1949. The gas requirements of molds. V. The minimum oxygen requirements for normal growth and for germination of six mold cultures. J. Dairy Sci. 32: 101–110.

Miller, J.D. 1994a. Epidemiology of *Fusarium* ear diseases of cereals. *In* Mycotoxins in Grain: Compounds other than Aflatoxins, eds J.D. Miller and L.H. Trenholm. St. Paul, MN: Eagan Press. pp. 19–36.

Miller, J.D. 1994b. Fungi and indoor air quality. Aust. Mycotoxin Newslett. 5(4): 1.

Miller, J.D. 1995. Fungi and mycotoxins in grains: implications for stored products research. J. Stored Prod. Res. 31: 1–16.

Miller, J.D. 2001. Factors that affect the occurrence of fumonisin. Environ. Health Perspect. 109 Suppl. 2: 321–324.

Miller, J.D. 2008. Mycotoxins in small grains and maize: old problems, new challenges. Food Addit. Contam. 25: 219–230.

Miller, J.D. and Trenholm, L.H., eds. 1994. Mycotoxins in Grain: Compounds Other than Aflatoxins. St. Paul, MN: Eagan Press.

Miller, J.D. and Young, J.C. 1997. The use of ergosterol to measure exposure to fungal propagules in indoor air. Am. Ind. Hyg. Assoc. J. 58: 39–43.

Miller, J.D., Greenhalgh, R., Wang, Y.-Z. and Lu, M. 1991. Trichothecene chemotypes of three *Fusarium* species. Mycologia 83: 121–130.

Miller, J.D., ApSimon, J.W., Blackwell, B.A., Greenhalgh, R. and Taylor, A. 2001. Deoxynivalenol: a 25 year perspective on a trichothecene of agricultural importance. *In Fusarium*: Paul E. Nelson Memorial Symposium, eds. B. A. Summerell, J.F. Leslie, D. Backhouse, W.L. Bryden and L.W. Burgess. St Paul, MN: APS Press. pp. 310–320.

Miller, M.W. and Mrak, E.M. 1953. Yeasts associated with dried-fruit beetles in figs. Appl. Microbiol. 1: 174–178.

Miller, M.W. and Phaff, H.J. 1958. A comparative study of the apiculate yeasts. Mycopathol. Mycol. Appl. 10: 113–141.

Miller, M.W. and Phaff, H.J. 1962. Successive microbial populations of Calimyrna figs. Appl. Microbiol. 10: 394–400.

Miller, S.S., Reid, L.M. and Harris, L.J. 2007. Colonization of maize silks by *Fusarium graminearum*, the causative organism of gibberella ear rot. Can. J. Bot. 85: 369–376.

Mills, J.T. 1990. Mycotoxins and toxigenic fungi on cereal grains in western Canada. Can. J. Physiol. Pharmacol. 68: 982–986.

Mills, J.T. and Abramson, D. 1986. Production of sterigmatocystin by isolates of *Aspergillus versicolor* from western Canadian stored barley and rapeseed/canola. Can. J. Plant Pathol. 8: 151–153.

Mills, J.T. and Wallace, H.A.H. 1979. Microflora and condition of cereal seeds after a wet harvest. Can. J. Plant Sci. 59: 645–651.

Mills, J.T., Seifert, K.A., Frisvad, J.C. and Abramson, D. 1995. Nephrotoxigenic *Penicillium* species occurring on

farm-stored cereal grains in western Canada. Mycopathologia 130: 23–28.
Minervini, F., Monaci, L., Montagna, M.T. and Dragoni, I. 2002. Assessment of mycotoxin production by *Aspergillus* and *Penicillium* fungi isolated from dairy products. Ind. Alimen. 41: 1336–1340.
Minoura, K., Morinaga, T and Muroi, T. 1975. Some Ascomycetes isolated from soil of Nepal. Rep. Tottori Mycol. Inst. (Jpn) No. 12: 171–185.
Mintzlaff, H.-J. and Christ, W. 1973. *Penicillium nalgiovensis* als Starterkultur für Sudtiroler Bauernspeck. Fleischwirtschaft 53: 864–867.
Mirete, S., Vazquez, C., Mule, G., Jurado, M. and Gonzalez-Jaen, M.T. 2004. Differentiation of *Fusarium verticillioides* from banana fruits by IGS and EF-1 alpha sequence analyses. Eur. J. Plant Pathol. 110: 515–523.
Mirocha, C.J., Abbas, H.K., Kommedahl, T. and Jarvis, B. B. 1989a. Mycotoxin production by *Fusarium oxysporum* and *Fusarium sporotrichioides* isolated from *Baccaris* sp. from Brazil. Appl. Environ. Microbiol. 55: 254–255.
Mirocha, C.J., Abbas, H.K., Windels, C.E. and Xie, W. 1989b. Variation in deoxynivalenol, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol, and zearalenone production by *Fusarium graminearum* isolates. Appl. Environ. Microbiol. 55: 1315–1316.
Mislivec, P.B. and Bruce, V.R. 1977. Incidence of toxic and other mold species and genera in soybeans. J. Food Prot. 40: 309–312.
Mislivec, P.B. and Tuite, J. 1970a. Species of *Penicillium* occurring in freshly-harvested and in stored dent corn kernels. Mycologia 62: 67–74.
Mislivec, P.B. and Tuite, J. 1970b. Temperature and relative humidity requirements of species of *Penicillium* isolated from yellow dent corn kernels. Mycologia 62: 75–88.
Mislivec, P.B., Bruce, V.R. and Gibson, R. 1983. Incidence of toxigenic and other molds in green coffee beans. J. Food Prot. 46: 969–973.
Mitchell, D., Parra, R., Aldred, D and Magan, N. 2004. Water and temperature relations of growth and ochratoxin A production by *Aspergillus carbonarius* strains from grapes in Europe and Israel. J. Appl. Microbiol. 97: 439–445.
Miyaki, K., Yamazaki, M., Horie, Y. and Udagawa, S. 1970. Toxigenic fungi growing on stored rice. Shokuhin Eiseigaku Zasshi (J. Food Hyg. Soc. Japan) 11: 373–380.
Miyake, T., Kong, I., Nozarki, N. and Sammoto, H. 2008. Analysis of pigment compositions in various *Monascus* cultures. Food Sci. Technol. Res. 14: 194–197.
Moalemiyan, M., Vikram, A., Kushalappa, A.C. and Yaylayan, V. 2006. Volatile metabolite profiling to detect and discriminate stem-end rot and anthracnose diseases of mango fruits. Plant Pathol. 55: 792–802.
Mohamed, A.A and Hussein, N.A. 2004. Proteolytic and lipolytic activity of fungi isolated from luncheon meat and poultry in Assiut City. Assiut Vet. Med. J. 50: 100–113.
Mojtahedi, H., Rabie, C.J., Lübben, A., Steyn, M. and Danesh, D. 1979. Toxic Aspergilli from pistachio nuts. Mycopathologia 67: 123–127.
Mok, W.Y., Castelo, F.P. and Barreto da Silva, M.S. 1981. Occurrence of *Exophiala werneckii* on salted freshwater fish *Osteoglossum bicirrhosum*. J. Food Technol. 16: 505–512.
Molina, I., Silla, H. and Flores, J. 1990. Study of the microbial flora in dry-cured ham. IV. Yeasts. Fleischwirtschaft 70: 74–76.
Molto, G.A., Gonzalez, H.H.L., Resnik, S.L. and Pereyra Gonzalez, A. 1997. Production of trichothecenes and zearalenone by isolates of *Fusarium* spp. from Argentinian maize. Food Addit. Contam. 14: 263–268.
Molyneux, R.J.; Mahoney, N.; Kim, J.H. and Campbell, B. C. 2007. Mycotoxins in edible tree nuts. Int. J. Food Microbiol. 119: 72–78.
Mondal, G.C. and Nandi, B. 1984. Role of fungi on oil quality of stored seeds of sesame, rape and linseed. J. Food Sci. 49: 1394–1395, 1400.
Montagna, M.T., Santacroce, M.P., Spilotros, G., Napoli, C., Minervini, F., Papa, A. and Dragoni, I. 2004. Investigation of fungal contamination in sheep and goat cheeses in southern Italy. Mycopathologia 158: 245–249.
Monte, E., Villanueva, J.R. and Dominguez, A. 1986. Fungal profiles of Spanish country-cured hams. Int. J. Food Microbiol. 3: 355–359.
Moore, R.T. 1986. A note on *Wallemia sebi*. Antonie van Leeuwenhoek 52: 183–187.
Montealegre, J.R. and Palma, J.F. 1994. Aetiology of the main postharvest rots in green asparagus in Chile. Fitopatologia 29: 102–105.
Monteil, D., Dickinson, M.J., Lee, H.A., Dyer, P.S., Jeenes, D.J., Roberts, I.N., James, S., Fuller, L.J., Matsuchima, K. and Archer, D.B. 2003. Genetic differentiation of the *Aspergillus* section *Flavi* complex using AFLP fingerprints. Mycol. Res. 107: 1427–1434.
Moosawi-Jorf, S.A., Farrokhi-Nejad, R., Azimi, S. and Afarin, S. 2007. Study of Fusarium Head Blight of wheat in Khuzestan province in Iran and reporting of *Fusarium xylaroides* as a new causal agents for disease. J. Agron. 6: 212–215.
Moraes, R.J.Q., Almeida, C.A.A., Dilkin, P., Kowalski, C. H., Murmann, L. and Mallmann, C.A. 2003. Dosage of ergosterol as an indicator of fungi contamination of stored maize. Arquiv. Inst. Biol. (Sao Paulo) 70: 483–489.
Moraes, W. da S., Zambolim, L. and Lima, J.D. 2006. Incidence of fungi in post harvest of bananas (*Musa* spp.) 'Prata Ana' (AAB). Summa Phytopathol. 32: 67–70.
Morales, H., Marín, S., Rovira, A., and Sanchis, V. 2006. Patulin accumulation in apples by *Penicillium expansum* during postharvest stages. Lett. Appl. Microbiol. 44: 30–35.
Morales, H., Marin, S., Centelles, X., Ramos, A.J. and Sanchis, V. 2007a. Cold and ambient deck storage prior to processing as a critical control point for patulin accumulation. Int. J. Food Microbiol. 116: 260–265.
Morales, H., Sanchis, V., Rovira, A., Ramos, A.J. and Marín, S. 2007b. Patulin accumulation in apples during postharvest: Effects of controlled atmosphere storage and fungicide treatments. Food Control 18: 1443–1448.
Morales-Rodriguez, I., de Yanez-Morales, M.J., Silva-Rojas, H.V., Garcia-de-los-Santos, G. and Guzman-de-Pena, D.A. 2007. Biodiversity of *Fusarium* species in Mexico associated with ear rot in maize, and their

identification using a phylogenetic approach. Mycopathologia 163: 31–39.
Morantte, A.P., Palomar, M.K. and Lim, J.L. 1986. Fungal infection and its effect on stored sun-dried and kiln-dried copra. Ann. Trop. Res. 8: 32–41.
Mordue, J.E.M. 1971. *Colletotrichum capsici*. CMI Descriptions of Pathogenic Fungi and Bacteria: 317. Kew: Commonwealth Mycological Institute.
Moreau, C. 1980. Le *Penicillium roqueforti*, morphologie, physiologie, intéret en industrie fromagère, mycotoxines. Lait 60: 254–271.
Morello, L.G., Sartori, D., Martinez, A.L. de O., Vieira, M. L.C., Taniwaki, M.H. and Fungaro, M.H.P. 2007. Detection and quantification of *Aspergillus westerdijkiae* in coffee beans based on selective amplification of beta-tubulin gene by using real-time PCR. Int. J. Food Microbiol. 119: 270–276.
Moretti, A., Logrieco, A., Bottalico, A., Ritieni, A., Randazzo, G. and Corda, P. 1995. Beauvericin production by *Fusarium subglutinans* from different geographical areas. Mycol. Res. 99: 282–286.
Moretti, A., Belisario, A., Tafuri, A., Ritieni, A., Corazza, L. and Logrieco, A. 2002. Production of beauvericin by different races of *Fusarium oxysporum* f. sp. *melonis*, the Fusarium wilt agent of muskmelon. Eur. J. Plant Pathol. 108: 661–666.
Moretti, A, Mulè, G., Susca, A., González-Jaén, M.T. and Logrieco, A. 2004. Toxin profile, fertility and AFLP analysis of *Fusarium verticillioides* from banana fruits. Eur. J. Plant Pathol. 110: 601–609.
Morrissey, W.F., Davenport, B., Querol, A. and Dobson, A. D.W. 2004. The role of indigenous yeasts in traditional Irish cider fermentations. J. Appl. Microbiol. 97: 647–655.
Morton, F.J. and Smith, G. 1963. The genera *Scopulariopsis* Bainier, *Microascus* Zukal and *Doratomyces* Corda. Mycol. Papers 86: 1–96.
Mossel, D.A.A., Kleynen-Semmeling, A.M.C., Vincentie, H. M., Beerens, H. and Catsaras, M. 1970. Oxytetracycline-glucose-yeast extract agar for selective enumeration of moulds and yeasts in foods and clinical material. J. Appl. Bacteriol. 33: 454–457.
Moubasher, A.H., El-Naghy, M.A. and Abdel-Hafez, S.I. 1972. Studies on the fungus flora of three grains in Egypt. Mycopathol. Mycol. Appl. 47: 261–274.
Mounjouenpou, P., Gueule, D., Fontana-Tachon, A., Guyot, B., Tondje, P.R. and Guiraud, J.-P. 2008. Filamentous fungi producing ochratoxin A during cocoa processing in Cameroon. Int. J. Food Microbiol. 121: 234–241.
Mourad, K. and Nour-Eddine, K. 2006. Microbiological study of fermented Algerian green olives: isolation and identification of lactic acid bacteria and yeasts along with the effects of brine solutions obtained at the end of olive fermentation on *Lactobacillus plantarum* growth. Grasas Aceites 57: 292–300.
Mourichon, X. 1983. Contribution à l'étude des taches noires (fruitlet core rot) et leathery pocket de l'ananas causés par *Penicillium funiculosum* Thom en d'Côte d'Ivoire. Fruits 38: 601–609.
Mphande, F.A., Siame, B.A. and Taylor, J.E. 2004. Fungi, aflatoxins and cyclopiazonic acid associated with peanut retailing in Botswana. J. Food Prot. 67: 96–102.
Mrak, E.M. and Bonar, L. 1939. Film yeasts from pickle brines. Zentralbl. Bakteriol. Parasitenkd., Infektionskrankh. Hyg. Abt. II 100: 289–294.
Mrak, E.M., Vaughn, R.H., Miller, M.W. and Phaff, H.J. 1956. Yeasts occurring in brines during the fermentation and storage of green olives. Food Technol. Champaign 10: 416–419.
Mtisi, E. and McLaren, N.W. 2003. Diseases of sorghum and pearl millet in some Southern African countries. *In* Sorghum and Millets Diseases, ed. J.F. Leslie. Ames, IA: Iowa State University Press. pp. 427–430.
Mubatanhema, W., Moss, M.O., Frank, M.J. and Wilson, D. M. 1999. Prevalence of *Fusarium* species of the *Liseola* section on Zimbabwean corn and their ability to produce the mycotoxins zearalenone, moniliformin and fumonisin B1. Mycopathologia 148: 157–163.
Muhamad, L.J., Ito, H., Watanabe, H. and Tamura, N. 1986. Distribution of microorganisms in spices and their decontamination by gamma-irradiation. Agric. Biol. Chem. 50: 347–355.
Muhammad, S., Shehu, K. and Amusa, N.A. 2004. Survey of the market diseases and aflatoxin contamination of tomato (*Lycopersicon esculentum* Mill) fruits in Sokoto, northwestern Nigeria. Nutr. Food Sci. 34: 72–76.
Mühlencoert, E., Mayer, I., Zapf, M.W., Vogel, R.F. and Niessen, L. 2004. Production of ochratoxin A by *Aspergillus ochraceus*. Eur. J. Plant Pathol. 110: 651–659.
Mukta, D. and Bora, K.N. 1993. *Colletotrichum acutatum* – a new fruit rotting pathogen of guava (*Psidium guajava* L.) in storage. Indian J. Mycol. Plant Pathol. 23: 331.
Müller, H.M., Schröppel, E. and Thaler, M. 1981. Einfluss von Propionsäuredosierung und Kornfeuchte auf die Entwicklung und Mykotoxinbildung von Penicillien und Aspergillen in Körnermais. Landwirtsch. Forsch. 34: 23–34.
Müller, O. and Krawinkel, M. 2005. Malnutrition and health in developing countries. Can. Med. Assoc. J. 173: 279–286.
Müller, S.O., Eckert, I., Lutz, W.K., Stopper, H. 1996. Genotoxicity of the laxative drug components emodin, aloe-emodin and danthron in mammalian cells: Topoisomerase II mediated? Mutat. Res. 371: 165–173.
Munday, J.S., Thompson, D., Finch, S.C., Babu, J.V., Wilkins, A.L., di Menna, M.E. and Miles, C.O. 2008. Presumptive tremorgenic mycotoxicosis in a dog in New Zealand, after eating mouldy walnuts. N.Z. Vet. J. 56: 145–147.
Muniz, M. de F.S., Rocha, D.F., Silveira, N.S.S. and Menezes, M. 2003. Identification of fungi causal agents of postharvest diseases on commercialized fruits in Alagoas, Brazil. Summa Phytopathol. 29: 38–42.
Murdock, D.I. and Hatcher, W.S. 1978. A simple method to screen fruit juices and concentrates for heat-resistant mold. J. Food Prot. 41: 254–256.
Muriuki, G.K. and Siboe, G.M. 1995. Maize flour contaminated with toxigenic fungi and mycotoxins in Kenya. Afr. J. Health Sci. 2: 236–241.
Mushtaq, M., Faiza-Iftikhar and Sharfun-Nahar. 2007. Detection of yeast mycoflora from butter. Pak. J. Bot. 39: 887–896.

Mutti, P., Previdi, M.P., Quintavalla, S. and Spotti, E. 1988. Toxigenicity of mould strains isolated from salami as a function of culture medium. Ind. Conserve 63: 142–145.

Muys, G.T., van Gils, H.W. and de Vogel, P. 1966a. The determination and enumeration of the associative microflora of edible emulsions. Part I. Mayonnaise, salad dressings and tomato ketchup. Lab. Pract. 15: 648–652, 674.

Muys, G.T., van Gils, H.W. and de Vogel, P. 1966b. The determination and enumeration of the associative microflora of edible emulsions. Part II. The microbiological investigation of margarine. Lab. Pract. 15: 975–984.

Nair, N.G. 1985. Fungi associated with bunch rot of grapes in the Hunter Valley. Aust. J. Agric. Res. 36: 435–442.

Nair, N.G., Emmett, R.W. and Parker, F.E. 1987. Programming applications of dicarboximides to control bunch rot of grapes caused by *Botrytis cinerea*. Plant Pathol. 36: 175–179.

Nakai, V.K., Rocha, L. de O., Goncalez, E., Fonseca, H., Ortega, E.M.M. and Correa, B. 2008. Distribution of fungi and aflatoxins in a stored peanut variety. Food Chem. 106: 285–290.

Nalim, F.A. 2004. Studies on molecular phylogenetics of *Fusarium* species. Ph.D. thesis. Pennsylvania State University, University Park, PA.

Nandi, B. 1978. Glucosamine analysis of fungus-infected wheat as a method to determine the effect of antifungal compounds in grain preservation. Cereal Chem. 55: 121–126.

Narasimhan, B. and Muthumary, J.P. 2005. Mycobiota of sunflower seeds and samples collected from vegetable oil refinery located in Tamilnadu, India. Mycol. Prog. 4: 195–204.

Nash, S.M. and Snyder, W.C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology 52: 567–572.

Nassar, A.M. and Ismail, M.A. 1994. Psychrotrophic and mesophilic fungi isolated from imported frozen lean meat in Egypt. J. Food Saf. 14: 289–295.

Naude, T.W., O'Brien, O.M., McGregor, A.D.G., Roux, C. and Flaoyen, A. 2002. Tremorgenic neuromycotoxicosis in 2 dogs ascribed to the ingestion of penitrem A and possibly roquefortine in rice contaminated with *Penicillium crustosum*. J. S. Afr. Vet. Assoc. 73: 211–215.

Navi S.S., Bandyopadhyay R. and Hall A.J. 2002. Effects of crop season, storage conditions, cultivars and fungicide on postharvest mold fungi infecting sorghum grain. Int. Sorghum Millets Newslett. 43: 65–68.

Needham, R. and Magan, N. 2003. Detection and differentiation of toxigenic and non-toxigenic *Penicillium verrucosum* strains on bakery products using an electronic nose. Aspects Appl. Biol. 68: 217–222.

Neish, G.A., Farnsworth, E.R., Greenhalgh, R. and Young, J.C. 1983. Observations on the occurrence of *Fusarium* species and their toxins in corn in eastern Ontario. Can. J. Plant Pathol. 5: 11–16.

Nelson, P.E., Toussoun, T.A. and Cook, R.J., (eds). 1981. *Fusarium*: Diseases, Biology and Taxonomy. University Park, PA: Pennsylvania State University Press.

Nelson, P.E., Toussoun, T.A. and Marasas, W.F.O. 1983. *Fusarium* Species. An illustrated Manual for Identification. University Park, PA: Pennsylvania State University Press.

Nelson, P.E., Juba, J.H., Ross, P.F. and Rice, L.G. 1994. Fumonisin production by *Fusarium* species on solid substrates. J. AOAC Int. 77: 522–525.

Nesci, A., Barros, G., Castillo, C. and Etcheverry, M. 2006. Soil fungal population in preharvest maize ecosystem in different tillage practices in Argentina. Soil Tillage Res. 91: 143–149.

Neves, L., Pampulha, M.E. and Loureiro-Dias, M.C. 1994. Resistance of food spoilage yeasts to sorbic acid. Lett. Appl. Microbiol. 19: 8–11.

Newell, S.Y., Miller, J.D. and Fallon, R.D. 1987. Ergosterol content of salt-marsh fungi: effect of growth conditions and mycelial age. Mycologia 79: 688–695.

Newell, S.Y., Arsuffi, T.L. and Fallon, R.D. 1988. Fundamental procedures for determining ergosterol content of decaying plant material by liquid chromatography. Appl. Environ. Microbiol. 54: 1876–1879.

Ngabirano, H., Mugabe, B. and Kakuba, A. 2001. Mould and ochratoxin A (OTA) contamination in coffee samples from four districts in Uganda. 19th Int. Sci. Colloqu. Coffee, Trieste, Italy, 14–18 May 2001: 1–7.

Nicoletti, R., Lopez-Gresa, M.P., Manzo, E., Carella, A. and Ciavatta, M.L. 2007. Production and fungitoxic activity of Svh 642305, a secondary metabolite of *Penicillium canescens*. Mycopathologia 163: 295–301.

Nicoue, E.E., Emond, J.P., Vuillemard, J.C. and Nunes, M. C.D. 2004. Destruction of *Rhizopus stolonifer* and *Botrytis cinerea* by ozone/ions treatments. Phytoprotection 85: 81–87.

Nielsen, D.S., Honholt, S., Tano-Debrah, K. and Jespersen, L. 2005. Yeast populations associated with Ghanaian cocoa fermentations analysed using denaturing gradient gel electrophoresis (DGGE). Yeast (Chichester) 22: 271–284.

Nielsen, D.S., Teniola, O.D., Ban-Koffi, L., Owusu, M., Andersson, T.S. and Holzapfel, W.H. 2007. The microbiology of Ghanaian cocoa fermentations analysed using culture-dependent and culture-independent methods. Int. J. Food Microbiol. 114: 168–186.

Nielsen, K.F., Sumarah, M.W., Frisvad, J.C. and Miller, J. D. 2006. Production of metabolites from the *Penicillium roqueforti* complex. J. Agric. Food Chem. 54: 3756–3763.

Nielsen, P.V. 1992. Rapid detection of heat resistant fungi in fruit juices by an impedimetric method. *In* Modern Methods in Food Mycology, eds R.A. Samson, A.D. Hocking, J.I. Pitt and A.D. King. Amsterdam: Elsevier. pp. 311–319.

Nielsen, P.V., Beuchat, L.R. and Frisvad, J.C. 1988. Growth of and fumitremorgin production by *Neosartorya fischeri* as affected by temperature, light, and water activity. Appl. Environ. Microbiol. 54: 1504–1510.

Nielsen, P.V., Beuchat, L.R. and Frisvad, J.C. 1989. Influence of atmospheric oxygen-content on growth and fumitremorgin production by a heat-resistant mold, *Neosartorya fischeri*. J. Food Sci. 54: 679–682.

Niessen, L. 2007a. Current trends in molecular diagnosis of ochratoxin A producing fungi. *In*: Mycotechnology – Present Status and Future Prospects, M. Rai, ed. New Delhi: India: International Publishing House. pp. 84–105.

Niessen, L. 2007b. PCR -based diagnosis and quantification of mycotoxin producing fungi. Int. J. Food Microbiol. 119: 38–46.

Niessen, L., Donhauser, S., Weideneder, A., Geiger, E. and Vogel, H. 1991. Möglichkeiten einer verbesserten visuellen Beurteilung des mikrobiologischen Status von Malzen. Brauwelt 131: 1556–1560, 1562.

Niessen, L., Donhauser, S., Weideneder, A., Geiger, E. and Vogel, H. 1992. Mykologische Untersuchungen an Cerealien und Malzen im Zusammenhang mit dem Wildwerden (Gushing) des Bieres. Brauwelt 132: 702, 704–706, 709–712, 714.

Niessen, L., Schmidt, H. and Vogel, R.F. 2004. The use of tri5 gene sequences for PCR detection and taxonomy of trichothecene-producing species in the *Fusarium* section *Sporotrichiella*. Int. J. Food Microbiol. 95: 305–319.

Nigro, F., Schena, L., Ligorio, A., Pentimone, A., Ippolito, A. and Salerno, M.G. 2006. Control of table grape storage rots by pre-harvest applications of salts. Postharvest Biol. Technol. 42: 142–149.

Nilsson, K. and Bjurman, J. 1998. Chitin as an indicator of the biomass of two wood-decay fungi in relation to temperature, incubation time, and media composition. Can. J. Microbiol. 44: 575–581.

Nirenberg, H. 1976. Untersuchungen über die morphologische und biologische Differenzierung in Die *Fusarium* – Sektion *Liseola*. Mitt. Biol. Bundesanst. Land-Forstwirtsch., Berlin-Dahlem 169: 1–117.

Nirenberg. H. I. 1990. Recent advances in the taxonomy of *Fusarium*. Stud. Mycol. 32: 91–101.

Nirenberg, H.I., Schmitzelsherif, H. and Kling, C.I. 1995. Occurrence of Fusaria and some blackening moulds on durum wheat in Germany. 2. Incidence of some blackening moulds. Z. Pflanzenkrankh. Pflanzenschutz 102: 164–170.

Nisiotou, A.A., Spiropoulos, A.E. and Nychas, G.J.E. 2007. Yeast community structures and dynamics in healthy and *Botrytis*-affected grape must fermentations. Appl. Environ. Microbiol. 73: 6705–6713.

Nketsia-Tabiri, J., Adu-Gyamfi, A., Gbedemah, C.M and Sefa-Dedeh, S. 2003. Optimising processing conditions for irradiated cured fish. Int. Atomic Energy Agency Tech. Docs (IAEA-TECDOCs) No. 1337: 207–216.

Northolt, M.D., van Egmond, H.P. and Paulsch, W.E. 1978. Patulin production by some fungal species in relation to water activity and temperature. J. Food Prot. 41: 885–890.

Northolt, M.D., van Egmond, H.P. and Paulsch, W.E. 1979. Ochratoxin A production by some fungal species in relation to water activity and temperature. J. Food Prot. 42: 485–490.

Northolt, M.D., van Egmond, H.P., Soentoro, P. and Deijll, E. 1980. Fungal growth and the presence of sterigmatocystin in hard cheese. J. Assoc. Off. Anal. Chem. 63: 115–119.

Notermans, S. and Huevelman, C.J. 1985. Immunological detection of moulds in food by using the enzyme-linked immunosorbent assay (ELISA): preparation of antigens. Int. J. Food Microbiol. 2: 247–258.

Notermans, S. and Kamphuis, H.J. 1992. Detection of fungi in foods by latex agglutination: a collaborative study. *In* Modern Methods in Food Mycology, eds R.A. Samson, A.D. Hocking, J.I. Pitt and A.D. King. Amsterdam: Elsevier. pp. 205–212.

Notermans, S., Huevelman, C.J., Beumer, R.R. and Maas, R. 1986. Immunological detection of moulds in food: relation between antigen production and growth. Int. J. Food Microbiol. 3: 253–261.

Nottebrock, H., Scholer, H.J. and Wall, M. 1974. Taxonomy and identification of mucormycosis-causing fungi. I. Synonymity of *Absidia ramosa* with *A. corymbifera*. Sabouraudia 12: 64–74.

Nout, M.J.R. 2003. Traditional fermented products from Africa, Latin America and Asia. *In* Yeasts in Food, eds T. Boekhout and V. Robert. Cambridge, UK: Woodhead Publishing, pp. 451–473.

Nout, M.J.R. and Rombouts, F.M. 1990. Recent developments in tempe research. J. Appl. Bacteriol. 69: 609–633.

Nout, M.J.R., Bonants-van Laarhoven, T.G.M., de Jongh, P. and de Koster, P.G. 1987. Ergosterol content of *Rhizopus oligosporus* NRRL 5905 grown in liquid and solid substrates. Appl. Microbiol. Biotechnol. 26: 456–461.

Nunes, C., Usall, J., Teixido, N., Torres, R. and Viñas, I. 2002. Control of *Penicillium expansum* and *Botrytis cinerea* on apples and pears with the combination of *Candida sake* and *pantoea agglomerans*. J. Food Prot. 65: 178–184.

Nunes, C., Usall, J., Manso, T., Torres, R., Olmo, M. and García, J.M. 2007. Effect of high temperature treatments on growth of *Penicillium* spp. and their development on 'Valencia' oranges. Food Sci. Technol. Int. 13: 63–68.

Nuñez, F., Rodriguez, M.M., Córdoba, J.J., Bermudez, M.E. and Asensio, M.A. 1996. Yeast population during ripening of dry-cured Iberian ham. Int. J. Food Microbiol. 29: 271–280.

Nunez, M., Medina, M., Gaya, P. and Dias-Amado, C. 1981. Yeasts and moulds of Cabrales blue cheese. Lait 61: 62–79.

Nweke, F.U. and Enujeke, E.C. 2006. Fungi associated with spoilage of stored and marketed white yam tubers (*Dioscorea rotundata* Poir) in Umunede, Nigeria. Global J. Agric. Sci. 5: 11–12.

Nyvall, R.F., Percich, J.A. and Mirocha, C.J. 1999. Fusarium head blight of cultivated and natural wild rice (*Zizania palustris*) in Minnesota caused by *Fusarium graminearum* and associated *Fusarium* spp. Plant Dis. 83: 159–164.

Obadina, A.O., Oyewole, O.B. and Odubayo, M.O. 2007. Effect of storage on the safety and quality of *fufu* flour. J. Food Saf. 27: 148–156.

Obi, S.K.C. and Moneke, A.N. 1986. Pectinolytic and cellulolytic enzyme complex of fungi associated with soft rot of yams (*Dioscorea rotundata* Poir). Int. Biodeterior. 22: 295–299.

Obiri-Danso, K. 1994. Microbiological studies on corn dough fermentation. Cereal Chem. 71: 186–188.

O'Brien, M., O'Kiely, P., Forristal, P.D. and Fuller, H.T. 2007. Quantification and identification of fungal propagules in well-managed baled grass silage and in normal on-farm produced bales. Anim. Feed Sci. Technol. 132: 283–297.

Ockerman, H.W., Sánchez, F.J.C., Mariscal, M.A.O., Serrano, A.M.M., Muñoz M.C.T. and León-Crespo, F. 2001. Influence of temperature on proteolytic activity of indigenous Spanish molds in meat products. J. Muscle Foods 12: 263–273.

O'Donnell, K.L. 1979. Zygomycetes in culture. Athens, GA: University of Georgia.

O'Donnell, K., Cigelnik, E. and Casper, H.H. 1998. Molecular phylogenetic, morphological, and mycotoxin data support reidentification of the Quorn mycoprotein fungus as *Fusarium venenatum*. Fungal Genet. Biol. 23: 57–67.

O'Donnell, K., Lutzoni, F.M., Ward, T.J. and Benny, G.L. 2001. Evolutionary relationships among mucoralean fungi (Zygomycota): evidence of family polyphyly on a large scale. Mycologia 93: 286–296.

O'Donnell, K., Ward, T.J., Geiser, D.M., Kistler, H.C. and Aoki, T. 2004. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal Genet. Biol. 41: 600–623.

Odunfa, S.A. 1987. Microbial contaminants of carbonated soft drinks produced in Nigeria. Monatsschr. Brauwiss. 40: 220–222.

Ogawa, H., Yoshimura, A. and Sugiyama, J. 1997. Polyphyletic origins of species of the anamorphic genus *Geosmithia* and the relationships of the cleistothecial genera: evidence from 18S, 5S and 28S rDNA sequence analysis. Mycologia 89: 756–771.

Ogundana, S.K. 1972. The post-harvest decay of yam tubers and its preliminary control in Nigeria. *In* Biodeterioration of Materials. Vol. 2, eds A.H. Walters and E.H. Hueck-van der Plas. London: Applied Science Publishers. pp. 481–492.

Ogundana, S.K. and Dennis, C. 1981. Assessment of fungicides for the prevention of storage rot of yam tubers. Pestic. Sci. 12: 491–494.

Ogundana, S.K., Naqvi, S.H.Z. and Ekundayo, J.A. 1970. Fungi associated with soft rot of yams (*Dioscorea* spp.) in storage in Nigeria. Trans. Br. Mycol. Soc. 54: 445–451.

Ogundare, A.O. and Adetuyi, F.C. 2003. Studies on the microbial population of bread baked with wheat flour from south western Nigeria. J. Food Agric. Environ. 1: 83–87.

Ogundero, V.W. 1981. Degradation of Nigerian palm products by thermophilic fungi. Trans. Br. Mycol. Soc. 77: 267–271.

Ogundero, V.W. 1983. Thermophilic fungi and fermenting cocoa beans in Nigeria. Mycopathologia 82: 159–165.

Oh, J.Y., Sang, M.K., Ryoo, M.I., and Kim, K.D. 2008. Temporal changes of fungal and bacterial populations in rice under indoor storage conditions. . Plant Pathol. J. 24: 74–79.

Okagbue, R.N. 1990. Identification of yeasts and aerobic spore forming bacteria from cassava flour. Food Microbiol. 7: 27–32.

Okamoto, S., Hosoe, T., Itabashi, T., Nozawa, K., Okada, K., Takaki, G.M.C., Chikamori, M., Yaguchi, T., Fukushima, K., Miyaji, M. and Kawai, K. 2004. New decalin derivatives, eujavonic acids A and B, from *Eupenicillium javanicum*. J. Nat. Prod. 67: 1580–1583.

Okeke, B., Seigle-Murandi, F., Steiman, R., Benoit-Guyod, J.-L. and Kaouadji, M. 1993. Identification of mycotoxin-producing fungal strains: a step in the isolation of compounds active against rice fungal diseases. J. Agric. Food Chem. 41: 1731–1735.

Okigbo, R.N. 2003. Fungi associated with peels of postharvest yams (*Dioscorea* sp.) in storage. Global J. Pure Appl. Sci. 9: 19–23.

Okoli, C.A.N. and Erinle, I.D. 1989. Factors responsible for market losses of tomato fruits in the Zaria area of Nigeria. J. Hortic. Sci. 64: 69–71.

Okuda, T. 1994. Variation in colony characteristics of *Penicillium* strains resulting from minor variations in culture conditions. Mycologia 86: 259–262.

Okull, D.O., Demirci, A., Rosenberger, D. and LaBorde, L. F. 2006. Susceptibility of *Penicillium expansum* spores to sodium hypochlorite, electrolyzed oxidizing water, and chlorine dioxide solutions modified with non-ionic surfactants. J. Food Prot. 69: 1944–1948.

Oliveira, A.C., Peres, C.M., Pires, J.M.C., Pereira, C.S., Vitorino, S., Marques, J.J., Crespo, M.B.T. and San Romão, M.V. 2003. Cork stoppers industry: defining appropriate mould colonization. Microbiol. Res. 158: 117–124.

Oliveira, M., Brito, D., Catulo, L., Leitao, F., Gomes, L., Silva, S., Vilas-Boas, L., Peito, A., Fernandes, I., Gordo, F. and Peres, C. 2004. Biotechnology of olive fermentation of 'Galega' Portuguese variety. Grasas Aceites (Sevilla) 55: 219–226.

Olliver, M. and Rendle, T. 1934. A new problem in fruit preservation. Studies on *Byssochlamys fulva* and its effect on the tissues of processed fruit. J. Soc. Chem. Ind. Lond. 53: 166–172.

Olsen, M., Jonsson, N., Magan, N., Banks, J., Fanelli, C., Rizzo, A., Haikara, A., Dobson, A., Frisvad, J., Holmes, S., Olkku, J., Persson, S.J. and Borjesson, T. 2006. Prevention of ochratoxin A in cereals in Europe. *In* Advances in Food Mycology, eds A.D. Hocking, J.I. Pitt, R.A. Samson and U. Thrane. New York: Springer. pp. 317–342.

Olsen, M., Johnsson, P., Möller, T., Paladino, R. and Lindblad, M. 2008. *Aspergillus nomius*, an important aflatoxin producer in Brazil nuts? World Mycotoxin J. 1: 123–126.

Olsson, J., Börjesson, T., Lundstedt, T. and Schnürer, J. 2002. Detection and quantification of ochratoxin A and deoxynivalenol in barley grains by GC-MS and electronic nose. Int. J. Food Microbiol. 72: 203–214.

Olszak, M. 1994. Aetiology of sour cherry fungal diseases in Poland III. Pathogenicity of the isolated fungi. J. Fruit Ornamen. Plant Res. 2(4): 165–184.

Oluma, H.O.A. and Nwankiti, A.O. 2003. Seed-storage mycoflora of peanut cultivars grown in Nigerian savanna. Tropicultura 21: 79–85.

Olutiola, P.O. 1976. Some environmental and nutritional factors affecting growth and sporulation of *Aspergillus flavus*. Trans. Br. Mycol. Soc. 66: 131–136.

Omobuwajo, T.O. and Wilcox, B.F. 1989. Microbes associated with field spoilage of breadfruit (*Artocarpus communis*). Lebensm. Wiss. Technol. 22: 175–178.

O'Neill, K., Damoglou, A.P. and Patterson, M.F. 1991. Sensitivity of some common grain fungi to irradiation on grain and in phosphate-buffered saline. Lett. Appl. Microbiol. 12: 180–183.

Onifade, A.K. and Jeff-Agboola, Y.A. 2003. Effect of fungal infection on proximate nutrient composition of coconut (*Cocos nucifera* Linn) fruit. J. Food Agric. Environ. 1: 141–142.

Onishi, N. 1963. Osmophilic yeasts. Adv. Food Res. 12: 53–94.

Onwuzulu, O.C., Moleyar, V., Appiah, K.M., Aradhya, S. M. and Ranganna, S. 1995. Fungicidal control of *Fusarium* and *Alternaria* rots of fresh tomato fruit during storage under ambient conditions. Trop. Sci. 35: 49–57.

Onyike, N.B.N. and Nelson, P.E. 1992. *Fusarium* species associated with sorghum grain from Nigeria, Lesotho and Zimbabwe. Mycologia 84: 452–458.

Ormerod, J.G. 1967. The nutrition of the halophilic mold *Sporendonema epizoum*. Arch. Mikrobiol. 56: 31–39.

Orth, R. 1976. Wachstum und Toxinbildung von Patulin und Sterigmatocystin-bildung Schimmelpilzen unter kontrolieri Atmosphäre. Z. Lebensm. Unters. Forsch. 160: 359–366.

Osman, A.R., Mikhail, M.S., Aly, H.Y. and Soliman, N.K. 1988. Sorghum grain-borne fungi and their effect on grain viability under different storage conditions. Egypt. J. Phytopathol. 20: 47–61.

O'Toole, D.K. 1997. The role of microorganisms in soy sauce production. Adv. Appl. Microbiol. 45: 87–152.

Overy, D.P. and Frisvad, J.C. 2005. Mycotoxin production and Postharvest storage rot of ginger (*Zingiber officinale*) by *Penicillium brevicompactum*. J. Food Prot. 68:607–609.

Overy, D.P., Seifert, K.A., Savard, M.E. and Frisvad, J.C. 2003. Spoilage fungi and their mycotoxins in commercially marketed chestnuts. Int. J. Food Microbiol. 88: 69–77.

Overy, D.P., Karlshøj, K. and Due, M.J. 2005a. Low temperature growth and enzyme production in *Penicillium* ser. *Corymbifera* species, casual agents of blue mold storage rot in bulbs. J. Plant. Pathol. 87: 57–63.

Overy, D.P., Frisvad, J.C., Steinmeier, U. and Thrane, U. 2005b. Clarification of the agents causing blue mold storage rot upon various flower and vegetable bulbs: implications for mycotoxin contamination. Postharvest Biol. Technol. 35: 217–221.

Owens, J.D., Konirova, L. and Thomas, D.S. 1992. Causes of conductance change in yeast cultures. J. Appl. Bacteriol. 72: 32–38.

Oyeniran, J.O. 1980. The role of fungi in the deterioration of tropical stored products. Occasional Paper Ser., Niger. Stored Prod. Res. Inst. 2: 1–25.

Oyewole, O.B. 2001. Characteristics and significance of yeasts' involvement in cassava fermentation for 'fufu' production. Int. J. Food Microbiol. 65: 213–218.

Oyeyiola, G.P. 1991. Fermentation of millet to produce kamu a Nigerian starch-cake food. World J. Microbiol. Biotechnol. 7: 196–201.

Oyugi, E. and Buys, E.M. 2007. Microbiological quality of shredded Cheddar cheese packaged in modified atmospheres. Int. J. Dairy Technol. 60: 89–95.

Ozay, G., Aran, N. and Pala, M. 1995. Influence of harvesting and drying techniques on microflora and mycotoxin contamination of figs. Nahrung 39: 156–165.

Ozay, G., Seyhan, F., Pembeci, C., Saklar, S. and Yilmaz, A. 2008. Factors influencing fungal and aflatoxin levels in Turkish hazelnuts (*Corylus avellana* L.) during growth, harvest, drying and storage: a 3-year study. Food Addit. Contam. A 25: 209–218.

Pacheco, C.D. and De Massaguer, P.R. 2004. Biological validation of tomato pulp continuous heat process. J. Food Process Eng. 27: 449–463.

Pacin, A.M., González, H.H.L., Etcheverry, M., Resnik, S.L., Vivas, L. and Espin, S. 2002. Fungi associated with food and feed commodities from Ecuador. Mycopathologia 156: 87–92.

Palacios-Cabrera, H., Taniwaki, M.H., Hashimoto, J.M. and Castle de Menezes, H. 2005. Growth of *Aspergillus ochraceus*, *A. carbonarius* and *A. niger* on culture media at different water activities and temperatures. Braz. J. Microbiol. 36: 24–28.

Palou, E., López-Malo, A., Barbosa-Canovas, G.V., Welti-Chanes, J. and Swanson, B.G. 1997a. High hydrostatic pressure as a hurdle for *Zygosaccharomyces bailii* inactivation. J. Food Sci. 62: 855–857.

Palou, E., López-Malo, A. Barbosa-Canovas, G.V., Welti-Chanes, J. and Swanson, B.G. 1997b. Effect of water activity on high hydrostatic pressure inhibition of *Zygosaccharomyces bailii*. Lett. Appl. Microbiol. 24: 417–420.

Palou, L., Cristo, C.H., Smilanick, J.L., Adaskaveg, J.E., Zoffoli, J.P. 2002. Effects of continuous 0.3 ppm ozone exposure on decay development and physiological responses of peaches and table grapes in cold storage. Postharvest Biol. Technol. 24: 39–48.

Pan, J., Vicente, A.R., Martínez, G.A., Chaves, A.R. and Civello, P.M. 2004. Combined use of UV-C irradiation and heat treatment to improve postharvest life of strawberry fruit. J. Sci. Food Agric. 84: 1831–1838.

Panaccione, D.G. and Coyle, C.M. 2005. Abundant respirable ergot alkaloids from the common airborne fungus *Aspergillus fumigatus*. Appl. Environ. Microbiol. 71: 3106–3111.

Panagou, E.Z., Skandamis, P.N. and Nychas, G.-J.E. 2002. Heat resistance of *Monascus ruber* ascospores isolated from thermally processed green olives of the Conservolea variety. Int. J. Food Microbiol. 76: 11–18.

Panagou, E.Z., Skandamis, P.N. and Nychas, G.-J.E. 2003. Modelling the combined effect of temperature, pH and a_w on the growth rate of *Monascus ruber*, a heat-resistant fungus isolated from green table olives. J. Appl. Microbiol. 94: 146–156.

Panagou, E.Z., Skandamis, P.N. and Nychas, G.-J.E. 2005. Use of gradient plates to study combined effects of temperature, pH and NaCl concentration on growth of *Monascus ruber* van Tieghem, as an ascomycete fungus isolated from green table oles. Appl. Environ. Microbiol. 71: 392–399.

Panagou, E.Z., Kodogiannis, V. and Nychas, G.-J.E. 2007. Modelling fungal growth using radial basis function neural networks: the case of the ascomycetous fungus *Monascus ruber*. Int. J. Food Microbiol. 117: 276–286.

Panasenko, V.T. 1967. Ecology of microfungi. Bot. Rev. 33: 189–215.

Pandya, Y., Jewett Jr, F.F. and Hoover, D. 1995. Concurrent effects of high hydrostatic pressure, acidity and heat on the destruction and injury of yeasts. J. Food Prot. 58: 301–304.

Panon, G., Massiot, P. and Drilleau, J.F. 1995. Pectinolytic enzyme production by yeasts in cider fermentation. Sci. Aliments 15: 31–42.

Paolesse, R., Alimelli, A., Martinelli, E., Di Natale, C., D'Amico, A., D'Egidio, M.G., Aureli, G., Ricelli, A.

and Fanelli, C. 2006. Detection of fungal contamination of cereal grain samples by an electronic nose. Sens. Actuators B 119: 425–430.

Papa, F., Zambonelli, C. and Grazia, L. 1995. UV ray utilization for the control of moulding in salami. Ind. Aliment. (Pinerolo, Italy) 34: 17–19.

Papagianni, M., Ambrosiadis, I. and Filiousis, G. 2007. Mould growth on traditional Greek sausages and penicillin production by *Penicillium* isolates. Meat Sci. 76: 653–657.

Papouskova, K. and Sychrova, H. 2007. The co-action of osmotic and high temperature stresses results in a growth improvement of *Debaryomyces hansenii* cells. Int. J. Food Microbiol. 118: 1–7.

Pardo, E., Marín, S., Solsona, A., Sanchis, V. and Ramos, A. J. 2004a. Modelling of germination and growth of ochratoxigenic isolates of *Aspergillus ochraceus* as affected by water acvtivity and temperature on a barley-based medium. Food Microbiol. 21: 267–274.

Pardo, E., Marín, S., Sanchis, V. and Ramos, A.J. 2004b. Prediction of fungal growth and ochratoxin A production by *Aspergillus ochraceus* on irradiated barley grains as influenced by temperature and water activity. Int. J. Food Microbiol. 95: 79–88.

Pardo, E., Ramos, A.J., Sanchis, V. and Marín, S. 2005a. Modelling of effects of water activity and temperature on germination and growth of ochratoxigenic isolates of *Aspergillus ochraceus* on a green coffee-based medium. Int. J. Food Microbiol. 98: 1–9.

Pardo, E., Marín, S., Ramos, A.J. and Sanchis, V. 2005b. Mycelial growth and ochratoxin A production by isolates of *Aspergillus ochraceus* influenced by water activity and temperature on irradiated green coffee beans. J. Food Prot. 68: 133–138.

Pardo, E., Marín, S., Sanchis, V. and Ramos, A.J. 2005c. Impact of relative humidity and temperature on visible fungal growth and OTA production of ochratoxigenic *Aspergillus ochraceus* isolates on grapes. Food Microbiol. 22: 383–389.

Pardo, E., Marín, S., Ramos, A.J. and Sanchis, V. 2006a. Ecophysiology of ochratoxigenic *Aspergillus ochraceus* and *Penicillium verrucosum* isolates. Predictive models for fungal spoilage prevention. Food Addit. Contam. 23: 398–410.

Pardo, E., Sanchis, V., Ramos, A.J. and Marín, S. 2006b. Non-specificity of nutritional substrate for ochratoxin A production by isolates of *Aspergillus ochraceus*. Food Microbiol. 23: 351–358.

Pardo, E., Malet, M., Marín, S., Sanchis, V. and Ramos, A.J. 2006c. Effects of water activity and temperature on germination and growth profiles of ochratoxigenic *Penicillium verrucosum* isolates on barley meal extract agar. Int. J. Food Microbiol. 106: 25–31.

Pařenicová, L., Skouboe, P., Frisvad, J.C., Samson, R.A., Rossen, L., Ten Hoor-Suykerbuyk, M. and Visser, J. 2001. Combined molecular and biochemical approach identifies *Aspergillus japonicus* and *Aspergillus aculeatus* as two species. Appl. Environ. Microbiol. 67: 521–527.

Park, H.G. and Jong, S.C. 2003. Molecular characterization of *Monascus* strains based on the D1/D2 regions of LSU rRNA genes. Mycoscience 44: 25–32.

Park, H.G., Stamenova, E. and Jong, S.-C. 2004a. Phylogenetic relationships of *Monascus* species inferred from the ITS and the partial beta-tubulin gene. Botan. Bull. Acad. Sinica 45: 325–330.

Park, J. and Chu, F.S. 1993. Immunochemical analysis of trichothecenes produced by various Fusaria. Mycopathologia 121: 179–192.

Park, J.-O., Hargreaves, J.R., McConville, E.J., Stirling, G. R. and Ghisalberti, E.L. 2004b. Production of leucinostatins and nematicidal activity of Australian isolates of *Paecilomyces lilacinus* (Thom) Samson. Lett. Appl. Microbiol. 38: 271–276.

Park, J.W., Shon, D.H. and Kim, Y.B. 2003. Application of an enzyme-linked immunosorbent assay for detecting mold contamination in agricultural commodities and comparison with conventional assays. Food Agric. Immunol. 15: 159–166.

Park, J.W., Choi, S.Y., Hwang, H.J. and Kim, Y.B. 2005a. Fungal mycoflora and mycotoxins in Korean polished rice destined for humans. Int. J. Food Microbiol. 103: 305–314.

Park, K.-J. and Lee, Y.-W. 1990. National occurrence of *Fusarium* mycotoxins in Korean barley samples harvested in 1987 and 1989. Maikotokishin (Proc. Jpn. Assoc. Mycotoxicol.) 31: 37–41.

Park, S.-I., Stan, S.D., Daeschel, M.A. and Zhao, Y. 2005b. Antifungal coatings on fresh strawberries (*Fragaria* x *ananassa*) to control mold growth during cold storage. J. Food Sci. 70: M202–M207.

Parsi, Z. and Gorecki, T. 2006. Determination of ergosterol as an indicator of fungal biomass in various samples using non-discriminating flash pyrolysis. J. Chromatogr. A 1130: 145–150.

Partida-Martinez, L.P., Flores de Looβ, C., Ishida, K., Ishida, M., Roth, M., Buder, K. and Hertweck, C. 2007. Rhizonin, the first mycotoxin isolated from the Zygomycota, is not a fungal metabolite but is produced by bacterial endosymbionts. Appl. Environ. Microbiol. 73: 793–797.

Passoth, V., Fredlund, E., Druvefors, U.A. and Schnurer, J. 2006. Biotechnology, physiology and genetics of the yeast *Pichia anomala*. FEMS Yeast Res. 6: 3–13.

Paster, N., Lisker, N. and Chet, I. 1983. Ochratoxin A production by *Aspergillus ochraceus* Wilhelm grown under controlled atmospheres. Appl. Environ. Microbiol. 45: 1136–1139.

Paster, N., Pushinsky, A., Menasherov, M. and Chet, H. 1992. Inhibitory effect of *Aspergillus niger* on the growth of *Aspergillus ochraceus* and *Aspergillus flavus*, and on aflatoxin formation. J. Sci. Food Agric. 58: 589–591.

Paster, N., Huppert, D. and Barkai-Golan, R. 1995. Production of patulin by different strains of *Penicillium expansum* in pear and apple cultivars stored at different temperatures and modified atmospheres. Food Addit. Contam. 12: 51–58.

Patel, K.D. and Pathak, V.N. 1993. Incidence of guava fruit rots and losses due to *Rhizopus* and *Botryodiplodia* in Udaipur and Ahmedabad markets. Indian J. Mycol. Plant Pathol. 23: 273–277.

Patel, P.D. and Williams, A.P. 1985. A note on enumeration of food spoilage yeasts by measurement of adenosine triphosphate (ATP) after growth at various temperatures. J. Appl. Bacteriol. 59: 133–136.

Pateraki, M., Deekanea, A., Mitchell, D., Lydakis, D and Magan, N. 2007. Influence of sulphur dioxide, controlled atmospheres and water availability on in vitro germination, growth and ochratoxin A production by strains of *Aspergillus carbonarius* isolated from grapes. Postharvest Biol. Technol. 44: 141–149.

Paterson, R.R.M. 2006. Identification and quantification of mycotoxigenic fungi by PCR. Process Biochem. 41: 1467–1474.

Patiño, B., Mirete, S., Vazquez, C., Jiménez, M., Rodriguez, M.T. and Gonzalez-Jaén, M.T. 2006. Characterization of *Fusarium verticillioides* strains by PCR-RFLP analysis of the intergenic spacer region of the rDNA. J. Sci. Food Agric. 86: 429–435.

Patiño, B., Medina, Á, Doménech, M., González-Jaén, M.T., Jiménez, M. and Vázquez, C. 2007. Polymerase chain reaction (PCR) identification of *Penicillium brevicompactum*, a grape contaminant and mycophenolic acid producer. Food Addit. Contam. 24:165–172.

Patterson, M. and Damoglou, A.P. 1986. The effect of water activity and pH on the production of mycotoxins by fungi growing on a bread analogue. Lett. Appl. Microbiol. 3: 123–125.

Patterson, D.S.P., Roberts, B.A., Shreeve, B.J., MacDonald, S.M. and Hayes, A.W. 1979. Tremorgenic toxins produced by soil fungi. Appl. Environ. Microbiol. 37: 172–173.

Paula, C.R., Krebs, V.L.J., Auler, M.E., Ruiz, L.S., Matsumoto, F.E., Silva, E.H., Diniz, E.M.A. and Vaz, F.A.C. 2006. Nosocomial infection in newborns by *Pichia anomala* in a Brazilian intensive care unit. Med. Mycol. 44: 479–484.

Payne, G.A. 1992. Aflatoxin in maize. Crit. Rev. Plant Sci. 10: 423–440.

Pearson, B.M. and McKee, R.A. 1992. Rapid identification of *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii* and *Zygosaccharomyces rouxii*. Int. J. Food Microbiol. 16: 63–67.

Pecchini, G. 1997. Ergosterol, an index of quality for grated cheese. Ind. Aliment. 36: 1126–1130.

Pech, B., Lavoue, G., Parfait, A. and Belin, J.M. 1984. Rum fermentation: suitability of strains of *Schizosaccharomyces pombe*. Sci. Aliments 4: 67–72.

Peckham, J.C., Mitchell, F.E., Jones Jr., O.H. and Doupnik Jr., B. 1972. Atypical interstitial pneumonia in cattle fed moldy sweet potatoes. J. Am. Vet. Med. Assoc. 160: 169–172.

Pedras, M.S.C., Abrams, S.R. and Seguin-Swartz, G. 1988. Isolation of the first naturally occurring epimonothiodioxoperazine, a fungal toxin produced by *Phoma lingam*. Tetrahedron Lett. 29: 3471–3474.

Peever, T.L., Carpenter-Boggs, L., Timmer, L.W., Carris, L.M. and Bhatia, A. 2005. Citrus black rot is caused by phylogenetically distinct lineages of *Alternaria alternata*. Phytopathology 95: 512–518.

Pegg, K.G., Moffett, M.L. and Colbran, R.C. 1974. Diseases of ginger in Queensland. Queensl. Agric. J. 100: 611–618.

Peintner, U., Geiger, J. and Pöder, R. 2000. The mycobiota of Speck, a traditional Tyrolean smoked and cured ham. J. Food Prot. 63: 1399–1403.

Pelhate, J. 1968. Inventaire de la mycoflore des blés de conservation. Bull. Trimest. Soc. Mycol. Fr. 84: 127–143.

Peng, F-C., Cheng, C.-H., Lui, J.-C., Wu, M.-F., Lee, S.-H. and Pan, H.-J. 2004. Production of polyclonal antibodies against territrem B and detection of territrem B in the conidia of *Aspergillus terreus* 23-1 by immunoelectron microscopy. J. Agric. Food Chem. 52: 3360–3365.

Penman, D., Britton, G., Hardwick, K., Collin, H.A. and Isaac, S. 2000. Chitin as a measure of biomass of *Crinipellis perniciosa*, causal agent of witches' broom disease of *Theobroma cacao*. Mycol. Res. 104: 671–675.

Penrose, L.J., Nicholls, M.R. and Koffmann, W. 1984. Apple fruit rot caused by *Trichoderma harzianum*. Australas. Plant Pathol. 13: 46–47.

Pereira, C.S., Soares, G.A.M., Oliveira, A.C., Rosa, M.E., Pereira, H., Moreno, N. and San Romão, M.V. 2006. Effect of fungal colonization on mechanical performance of cork. Int. Biodet. Biodeg. 57: 244–250.

Perello, A.E. and Moreno, M.V. 2005. First report of *Phoma sorghina* (Sacc.) Boerema Dorenbosch & van Kest on wheat leaves (*Triticum aestivum* L.) in Argentina. Mycopathologia 159: 75–78.

Perkowski, J., Chelkowski, J. and Wakulinski, W. 1988. Deoxynivalenol and 3-acetyldeoxynivalenol and *Fusarium* species in winter triticale. Mycotoxin Res. 4: 97–101.

Persley, D.M. and Ploetz, R.C. 2003. Diseases of papaya. *In*: Diseases of tropical fruit crops, ed. R.C. Ploetz, Wallingford, UK: CABI Publishing. pp 373–412.

Peters, J.C., Lees, A.K., Cullen, D.W., Sullivan, L., Stroud, G.P. and Cunnington, A.C. 2008. Characterization of *Fusarium* spp. responsible for causing dry rot of potato in Great Britain. Plant Pathol. 57: 262–271.

Peters, R.D., Barasubiye, T. and Driscoll, J. 2007. Dry rot of rutabaga caused by *Fusarium avenaceum*. HortScience 42: 737–739.

Peterson, S.W. 2000. Phylogenetic relationships in *Aspergillus* based on rDNA sequence analysis. *In* Integration of Modern Taxonomic Methods for *Penicillium* and *Aspergillus* Classification, eds R.A. Samson and J.I. Pitt. London: Harwood Academic Publishers. pp. 323–355.

Peterson, S.W. 2004. Multilocus DNA sequence analysis shows that *Penicillium biourgeianum* is a distinct species closely related to *P. brevicompactum* and *P. olsonii*. Mycol. Res. 108: 434–440.

Peterson, S.W. 2006. Multilocus sequence analysis of *Penicillium* and *Eupenicillium* species. Rev. Iberoamer. Micol. 23: 134–138.

Peterson, S.W. 2008. Phylogenetic analysis of *Aspergillus* species using DNA sequences from four loci. Mycologia 100: 205–226.

Petters, H.I., Flannigan, B. and Austin, B. 1988. Quantitative and qualitative studies of the microflora of barley malt production. J. Appl. Bacteriol. 65: 279–297.

Pfohl-Leszkowicz, A. and Manderville, R.A. 2007. Ochratoxin A: an overview on toxicity and carcinogenicity in humans and animals. Mol. Nutr. Food Res. 51: 61–99.

Philipp, S. and Pedersen, P.D. 1988. Mould cultures for the food industry. A short review with special reference to the cheese and sausage production. Danish Dairy Food Ind. Worldwide 6: 8, 10–12.

Phillips, S., Widjaja, S., Wallbridge, A. and Cooke, R. 1988. Rice yellowing during post-harvest drying by aeration and during storage. J. Stored Prod. Res. 24: 173–181.

Phister, T.G. and Mills, D.A. 2003. Real-time PCR assay for detection and enumeration of *Dekkera bruxellensis* in wine. Appl. Environ. Microbiol. 69: 7430–7434.

Phister, T.G., Rawsthorne, H., Joseph, C.M.L. and Mills, D. A. 2007. Real-time PCR assay for detection and enumeration of *Hanseniaspora* species from wine and juice. Am. J. Enol. Viticult. 58: 229–233.

Pieckova, E., Valik, L. and Gorner, F. 2002. Moulds in yoghurts. Bull. Potravinarskeho Vyskumu 41: 291–301.

Pierson, C.F. 1966. Effect of temperature on the growth of *Rhizopus stolonifer* on peaches and agar. Phytopathology 56: 276–278.

Pietri, A., Bertuzzi, T., Pallaroni, L. and Piva, G. 2004. Occurrence of mycotoxins and ergosterol in maize harvested over 5 years in Northern Italy. Food Addit. Contam. 21: 479–487.

Pietri, A., Bertuzzi, T., Gualla, A. and Piva, G. 2006. Occurrence of ochratoxin A in raw ham muscles and in pork products from Northern Italy. Ital. J. Food Sci. 18: 99–106.

Pillai, T., Raut, B.T., Paulkar, P.K., Agarkar, G.D. and Thakare, A.R. 2003. Assessment of different methods for detection of mycoflora of sesamum. Ann. Plant Physiol. 17: 153–156.

Pina, C., Santos, C., Couto, J.A. and Hogg, T. 2004. Ethanol tolerance of five non-*Saccharomyces* wine yeasts in comparison with a strain of *Saccharomyces cerevisiae* – influence of different culture conditions. Food Microbiol. 21: 439–447.

Pinzari, F., Fanelli, C., Canhoto, O. and Magan, N. 2004. Electronic nose for the early detection of moulds in libraries and archives. Indoor Built Environ. 13: 387–395.

Piskorska-Pliszczynska, J. and Borkowska-Opacka, B. 1984. Natural occurrence of ochratoxin A and two ochratoxin-producing fungal strains in cheesecake. Bull. Vet. Inst. Pulawy 27: 95–98.

Pitt, J.I. 1963. Microbiological aspects of prune preservation. M.Sc. thesis. Kensington, NSW: University of New South Wales.

Pitt, J.I. 1966. Two new species of *Chrysosporium*. Trans. Br. Mycol. Soc. 49: 467–470.

Pitt, J.I. 1973. An appraisal of identification methods for *Penicillium* species: novel taxonomic criteria based on temperature and water relations. Mycologia 65: 1135–1157.

Pitt, J.I. 1974a. Resistance of some food spoilage yeasts to preservatives. Food Technol. Aust. 26: 238–241.

Pitt, J.I. 1974b. A synoptic key to the genus *Eupenicillium* and to sclerotigenic *Penicillium* species. Can. J. Bot. 52: 2231–2236.

Pitt, J.I. 1975. Xerophilic fungi and the spoilage of foods of plant origin. *In* Water Relations of Foods, ed. R.B. Duckworth. London: Academic Press. pp. 273–307.

Pitt, J.I. 1979a. *Geosmithia* gen. nov. for *Penicillium lavendulum* and related species. Can. J. Bot. 57: 2021–2030.

Pitt, J.I. 1979b. The Genus *Penicillium* and Its Teleomorphic States *Eupenicillium* and *Talaromyces*. London: Academic Press.

Pitt, J.I. 1979c. *Penicillium crustosum* and *P. simplicissimum*, the correct names for two common species producing tremorgenic mycotoxins. Mycologia 71: 1166–1177.

Pitt, J.I. 1981. Food spoilage and biodeterioration. *In* Biology of Conidial Fungal, Vol. 2, eds G.T. Cole and B. Kendrick. New York: Academic Press. pp. 111–142.

Pitt, J.I. 1984. The significance of potentially toxigenic fungi in foods. Food Technol. Aust. 36: 218–219.

Pitt, J.I. 1985. Nomenclatorial and taxonomic problems in the genus *Eurotium*. *In* Advances in *Penicillium* and *Aspergillus* Systematics, eds R.A. Samson and J.I. Pitt. pp. 383–396. New York: Plenum Press.

Pitt, J.I. 1986. Properties of the ideal fungal enumeration medium. *In* Methods for the Mycological Examination of Food, eds. A.D. King, J.I. Pitt, L.R. Beuchat and J.E. L. Corry. New York: Plenum Press. pp. 63–65.

Pitt, J.I. 1987. *Penicillium viridicatum*, *Penicillium verrucosum* and production of ochratoxin A. Appl. Environ. Microbiol. 53: 266–269.

Pitt, J.I. 1989a. Recent developments in the study of *Penicillium* and *Aspergillus* systematics. Soc. Appl. Bacteriol. Symp. Supp. 1989: 37S–45S.

Pitt, J.I. 1989b. Field studies on *Aspergillus flavus* and aflatoxins in Australian groundnuts. *In* Aflatoxins in groundnut: proceedings of the International Workshop, 6–9 October, l987, ICRISAT Center, India. Patancheru, India: ICRISAT. pp. 223–235.

Pitt, J.I. 1993a. A modified creatine sucrose medium for differentiation of species in *Penicillium* subgenus *Penicillium*. J. Appl. Bacteriol. 75: 559–563.

Pitt, J.I. 1993b. Corrections to species names in physiological studies on *Aspergillus flavus* and *Aspergillus parasiticus*. J. Food Prot. 56: 265–269.

Pitt, J.I. 1995. Phylogeny in the genus *Penicillium* – a morphologist's perspective. Can. J. Bot. 73(Suppl. 1): S768–S777.

Pitt, J.I. 2000. A Laboratory Guide to Common *Penicillium* Species, 3rd edn. Sydney, NSW: Food Science Australia, CSIRO.

Pitt, J.I. and Christian, J.H.B. 1968. Water relations of xerophilic fungi isolated from prunes. Appl. Microbiol. 16: 1853–1858.

Pitt, J.I. and Christian, J.H.B. 1970. Heat resistance of xerophilic fungi based on microscopical assessment of spore survival. Appl. Microbiol. 20: 682–686.

Pitt, J.I. and Hocking, A.D. 1977. Influence of solute and hydrogen ion concentration on the water relations of some xerophilic fungi. J. Gen. Microbiol. 101: 35–40.

Pitt, J.I. and Hocking, A.D. 1982. Food spoilage fungi. I. *Xeromyces bisporus* Fraser. CSIRO Food Res. Q. 42: 1–6.

Pitt, J.I. and Hocking, A.D. 1985. New species of fungi from Indonesian dried fish. Mycotaxon 22: 197–208.

Pitt, J.I. and Hocking, A.D. 1996. Current knowledge of fungi and mycotoxins associated with food commodities in Southeast Asia. *In* Mycotoxin contamination in grains, eds E. Highley and G.I. Johnson. Canberra, ACT: Australian Centre for International Agricultural Research. ACIAR Technical Reports 37: 5–10.

Pitt, J.I. and Hocking, A.D. 1997. *Fungi and Food Spoilage,* 2nd edn. London: Blackie Academic and Professional.

Pitt, J.I. and Hocking A.D. 2006. Mycotoxins in Australia: biocontrol of aflatoxin in peanuts. Mycopathologia 162: 233–243.

Pitt, J.I. and Leistner, L. 1991. Toxigenic *Penicillium* species. *In* Mycotoxins and Animal Foods, eds J.E. Smith and R. S. Henderson. Boca Raton, FL: CRC Press. pp. 91–99.

Pitt, J.I. and Miscamble, B.F. 1995. Water relations of *Aspergillus flavus* and closely related species. J. Food Prot. 58: 86–90.

Pitt, J.I. and Richardson, K.C. 1973. Spoilage by preservative-resistant yeasts. CSIRO Food Res. Q. 33: 80–85.

Pitt, J.I. and Samson, R.A. 1990a. Systematics of *Penicillium* and *Aspergillus* – past, present and future. *In* Modern Concepts in *Penicillium* and *Aspergillus* Classification, eds R.A. Samson and J.I. Pitt. New York: Plenum Press. pp. 3–13.

Pitt, J.I. and Samson, R.A. 1990b. Approaches to *Penicillium* and *Aspergillus* systematics. Stud. Mycol., Baarn 32: 77–90.

Pitt, J.I. and Samson, R.A. 1990c. Taxonomy of *Aspergillus* section *Restricta*. *In* Modern Concepts in *Penicillium* and *Aspergillus* Classification, eds R.A. Samson and J.I. Pitt. New York: Plenum Press. pp. 249–257.

Pitt, J.I. and Samson, R.A. 1993. Species names in current use in the *Trichocomaceae* (Fungi, Eurotiales). *In* Names in Current Use in the Families *Trichocomaceae*, *Cladoniaceae*, *Pinaceae*, and *Lemnaceae*, ed. W. Greuter. Königstein, Germany: Koeltz Scientific Books. Regnum Vegetabile 128: 13–57.

Pitt, J.I., Hocking, A.D. and Glenn, D.R. 1983. An improved medium for the detection of *Aspergillus flavus* and *A. parasiticus*. J. Appl. Bacteriol. 54: 109–114.

Pitt, J.I., Cruickshank, R.H. and Leistner, L. 1986. *Penicillium commune*, *P. camembertii*, the origin of white cheese moulds, and the production of cyclopiazonic acid. Food Microbiol. 3: 363–371.

Pitt, J.I., Klich, M.A., Shaffer, G.P., Cruickshank, R.H., Frisvad, J.C., Mullaney, E.J., Onions, A.H.S., Samson, R.A. and Williams, A.P. 1990. Differentiation of *Penicillium glabrum* from *Penicillium spinulosum* and other closely related species: an integrated taxonomic approach. Syst. Appl. Microbiol. 13: 304–309.

Pitt, J.I., Spotts, R.A., Holmes, R.J. and Cruickshank, R.H. 1991. *Penicillium solitum* revived, and its role as a pathogen of pomaceous fruit. Phytopathology 81: 1108–1112.

Pitt, J.I., Hocking, A.D., Bhudhasamai, K., Miscamble, B.F. , Wheeler, K.A. and Tanboon-Ek, P. 1993. The normal mycoflora of commodities from Thailand. 1. Nuts and oilseeds. Int. J. Food Microbiol. 20: 211–226.

Pitt, J.I., Hocking, A.D., Bhudhasamai, K., Miscamble, B.F. , Wheeler, K.A. and Tanboon-Ek, P. 1994. The normal mycoflora of commodities from Thailand. 2. Beans, rice, small grains and other commodities. Int. J. Food Microbiol. 23: 35–53.

Pitt, J.I., Hocking, A.D., Miscamble, B.F., Dharmaputra, O. S., Kuswanto, K.R., Rahayu, E.S., and Sardjono. 1998a. The mycoflora of food commodities from Indonesia. J. Food Mycol. 1: 41–60.

Pitt, J.I., Hocking, A.D., Jackson, K.L., Mullins, J.D. and Webley, D.J. 1998b. The occurrence of *Alternaria* species and related mycotoxins in international wheat. J. Food Mycol. 1**:** 103–113.

Pitt, J.I., Samson, R.A. and Frisvad, J.C. 2000. List of accepted species and their synonyms in the family *Trichocomaceae*. *In* Integration of Modern Taxonomic Methods for *Penicillium* and *Aspergillus* Classification, eds R.A. Samson and J.I Pitt. Amsterdam: Hardwood Academic Publishers. pp. 9–51.

Platt, H.W., Morrall, R.A.A. and Gruen, H.E. 1977. The effects of substrate, temperature and photoperiod on conidiation of *Pyrenophora tritici-repentis*. Can. J. Bot. 55: 254–259.

Plaza, P., Usall, J., Teixidó, N. and Viñas, I. 2003. Effect of water activity and temperature on germination and growth of *Penicillium digitatum*, *P. italicum* and *Geotrichum candidum*. J. Appl. Microbiol. 94: 549–554.

Plaza, P., Sanbruno, A., Usall, J., Lamarca, N., Torres, R., Pons, J. and Viñas, I. 2004. Integration of curing treatments with degreening to control the main postharvest diseases of clementine mandarins. Postharvest Biol. Technol. 34: 29–37.

Pohland, A.E. and Wood, G.E. 1987. Occurrence of mycotoxins in food. *In* Mycotoxins in Food, ed. P. Krogh. London: Academic Press. pp. 35–64.

Pohland, A.E., Nesheim, S. and Friedman, L. 1992. Ochratoxin A: a review. Pure Appl. Chem. 64: 1029–1046.

Pollastro, S., Dongiovanni, C., Angelini, R.M. de M., Abbatecola, A., Natale, P., Guido, M.A. de and Faretra, F. 2005. Grape rot and contamination of wine by ochratoxin A. Inf. Fitopatol. 55: 15–21.

Polonelli, L., Morace, G., Rosa, R., Castagnola, M. and Frisvad, J.C. 1987. Antigenic characterization of *Penicillium camemberti* and related common cheese contaminants. Appl. Environ. Microbiol. 53: 872–878.

Poncini, L. and Wimmer, F.L. 1986. Characterization of the yeasts (Blastomycetes) in some Fijian honeys. Acta Aliment. Polonica 12: 143–151.

Ponsone, L., Combina, M., Dalcero, A. and Chulze, S. 2007. Ochratoxin A and ochratoxigenic *Aspergillus* species in Argentinean wine grapes cultivated under organic and non-organic systems. Int. J. Food Microbiol. 114: 131–135.

Pose, G., Ludemann, V., Segura, J. and Fernandez Pinto, V. 2004. Mycotoxin production by *Alternaria* strains isolated from tomatoes affected by blackmold in Argentina. Mycotox. Res. 20: 80–86.

Powers, E.M. and Berkowitz, D. 1990. Efficacy of an oxygen scavenger to modify the atmosphere and prevent mold growth on meal, ready-to-eat pouched bread. J. Food Prot. 53: 767–771.

Pozzi, C.R., Braghini, R., Arcaro, J.R.P., Zorzete, P., Israel, A.L.M., Pozar, I.O., Denucci, S. and Correa, B. 2005. Mycoflora and occurrence of alternariol and alternariol monomethyl ether in Brazilian sunflower from sowing to harvest. J. Agric. Food Chem. 53: 5824–5828.

Prakichaiwattana, C.J., Fleet, G.H. and Heard, G.M. 2004. Application and evaluation of denaturing gradient gel electrophoresis to analyse the yeast ecology of wine grapes. FEMS Yeast Res. 4: 865–877.

Praphailong, W. and Fleet, G.H. 1997. The effect of pH, sodium chloride, sucrose, sorbate and benzoate on the growth of food spoilage yeasts. Food Microbiol. 14: 459–468.

Pratt, R.G., 2006. Enhancement of sporulation in species of *Bipolaris*, *Curvularia*, *Drechslera*, and *Exserohilum* by growth on cellulose-containing substrates. Mycopathologia 162: 133–140.

Prista, C., Loureiro-Dias, M.C., Montiel, V., García, R. and Ramos, J. 2005. Mechanisms underlying the halotolerant way of *Debaryomyces hansenii*. FEMS Yeast Res. 5: 693–701.

Proctor, R.H., Plattner, R.D., Brown, D.W., Seo, J.A. and Lee, Y.W. 2004. Discontinuous distribution of fumonisin biosynthetic genes in the *Gibberella fujikuroi* species complex. Mycol. Res. 108: 815–822.

Pronyk, C., Abramson, D., Muir, W.E. and White, N.D.G. 2006. Correlation of total ergosterol levels in stored canola with fungal deterioration. J. Stored Prod. Res. 42: 162–172.

Prusky, D., Gat, Z. and Burd, P. 1993. Effect of relative humidity during mango growth on the incidence of quiescent infections of *Alternaria alternata*. Plant Dis. 77: 249–252.

Prusky, D., McEvoy, J.L., Saftner, R., Conway, W.S. and Jones, R. 2004. Relationship between host acidification and virulence of *Penicillium* spp. on apple and citrus fruit. Phytopathology 94: 44–51.

Pryor, B.M. and Bigelow, D.M. 2003. Molecular characterization of *Embellisia* and *Nimbya* species and their relationship to *Alternaria, Ulocladium* and *Stemphylium*. Mycologia 95: 1141–1154.

Pryor, B.M. and Gilbertson, R.L. 2000. Molecular phylogenetic relationships amongst *Alternaria* species and related fungi based upon analysis of nuclear ITS and mt SSU rDNA sequences. Mycol. Res. 104: 1312–1321.

Puel, O., TaDrist, S., Delaforge, M., Oswald, I.P. and Lebrhi, A. 2007. The inability of *Byssochlamyss fulva* to produce patulin is related to absence of 6-methylsalisalic acid synthase and isoepoxydon dehydrogenase genes. Int. J. Food Microbiol. 115: 131–139.

Pulvirenti, A. and Giudici, P. 2003. Identification of yeasts by molecular techniques. Bulletin de l'OIV 76 (869/870): 635–649.

Pulvirenti, A., Solieri, L., Gullo, M., De Vero, L. and Giudici, P. 2004. Occurrence and dominance of yeast species in sourdough. Lett. Appl. Microbiol. 38: 113–117.

Punithalingam, E. 1976. *Botryodiplodia theobromae*. CMI Descriptions of Pathogenic Fungi and Bacteria. 519.

Purchase, I.F.H. 1971. The acute toxicity of the mycotoxin cyclopiazonic acid to rats. Toxicol. Appl. Pharmacol. 18: 114–123.

Put, H.M.C. 1974. The limitations of oxytetracycline as a selective agent in media for the enumeration of fungi in soil, feeds and foods in comparison with the selectivity obtained by globenicol (chloramphenicol). Arch. Lebensmittelhyg. 25: 73–83.

Put, H.M.C. and de Jong, J. 1982. The heat resistance of ascospores of four *Saccharomyces* spp. isolated from spoiled heat-processed soft drinks and fruit products. J. Appl. Bacteriol. 3: 235–243.

Put, H.M.C. and Kruiswijk, J.T. 1964. Disintegration and organoleptic deterioration of processed strawberries caused by the mould *Byssochlamys nivea*. J. Appl. Bacteriol. 27: 53–58.

Put, H.M.C., de Jong, J., Sand, F.E.M.J. and van Grinsven, A.M. 1976. Heat resistance studies on yeast spp. causing spoilage in soft drinks. J. Appl. Bacteriol. 40: 135–152.

Quarta, A., Mita, G., Haidukowski, M., Santino, A., Mulè, G. and Visconti, A. 2005. Assessment of trichothecene chemotypes of *Fusarium culmorum* occurring in Europe. Food Addit. Contam. 22: 309–315.

Quarta, A., Mita, G., Haidukowski, M., Logrieco, A., Mulè, G. and Visconti, A. 2006. Multiplex PCR assay for the identification of nivalenol, 3- and 15-acetyl-deoxynivalenol chemotypes in *Fusarium*. FEMS Microbiol. Lett. 259: 7–13.

Quimio, T.H., Pordesimo, A.N. and Quimio, A.J. 1975. Control of papaya fruit rots by postharvest dip in thiabendazole. Philipp. Agric. 59: 7–11.

Qin, G.Z., Tian, S. P., Xu, Y., Chan, Z. L. and Li, B.Q. 2006. Combination of antagonistic yeasts with two food additives for control of brown rot caused by *Monilinia fructicola* on sweet cherry fruit. J. Appl. Microbiol. 100: 508–515.

Quintana, M.C.D., Garcia, P.G. and Fernández, A.G. 2003. Characteristics of the growth of table olive yeasts at low temperature. Grasas Aceites 54: 264–271.

Quintana, M.C.D., Arroyo, F.N., Garcia, P.G. and Fernández, A.G. 2005. Growth in brine, at low temperature and different organic acids, of yeasts from table olives. Grasas Aceites 56: 9–15.

Quintavalla, S. and Spotti, E. 1993. Heat resistance of *Talaromyces flavus*, *Neosartorya fischeri* and *Byssochlamys nivea* isolated from fresh fruits. Microbiol. Aliments Nutr. 11: 335–341.

Rabie, C.J., van Rensburg, S.J., van der Watt, J.J. and Lübben, A. 1975. Onyalai – the possible involvement of a mycotoxin produced by *Phoma sorghina* in the aetiology. S. Afr. Med. J. 49: 1647–1650.

Rabie, C.J., Lübben, A., Schipper, M.A.A., van Heerden, F. R. and Fincham, J.E. 1985. Toxigenicity of *Rhizopus* species. Int. J. Food Microbiol. 1: 263–270.

Raj, M.H., Niranjana, S.R., Nayaka, S.C. and Shetty, H.S. 2007. Health status of farmers' saved paddy, sorghum, sunflower and cowpea seeds in Karnataka, India. World J. Agric. Sci. 3: 167–177.

Rajak, RC., Parwekar, S., Malviya, H. and Hasija, S.K. 1991. Keratin degradation by fungi isolated from the grounds of a gelatin factory in Jabalpur, India. Mycopathologia 114: 83–87.

Rajashekhara, E., Suresh, E.R. and Ethiraj, S. 1996. Influence of different heating media on thermal resistance of *Neosartorya fischeri* isolated from papaya fruit. J. Appl. Bacteriol. 81: 337–340.

Rajashekhara, E., Suresh, E.R. and Ethiraj, S. 2000. Modulation of thermal resistance of ascospores of *Neosartorya fischeri* by acidulants and preservatives in mango and grape juice. Food Microbiol. 17: 269–275.

Ramakrishna, N., Lacey, J. and Smith, J.E. 1993. Effect of water activity and temperature on the growth of fungi interacting on grain. Mycol. Res. 97: 1393–1402.

Ramakrishna, N., Lacey, J. and Smith, J.E. 1996. The effects of fungal competition on colonization of barley grain by *Fusarium sporotrichioides* on T-2 toxin formation. Food Addit. Contam. 13: 939–948.

Ramaswamy, K., Gunasekaran, N. and Rajendran, S. 2004. Microbiota of fumigated spice powders. Indian J. Microbiol. 44: 303–305.

Ramirez, M.L., Chulze, S. and Magan, N. 2006a. Temperature and water activity effects on growth and temporal

deoxynivalenol production by two Argentinean strains of *Fusarium graminearum* on irradiated wheat grain. Int. J. Food Microbiol. 106: 291–296.

Ramirez, M.L., Reynoso, M.M., Farnochi, M.C. and Chulze, S. 2006b. Vegetative compatibility and mycotoxin chemotypes among *Fusarium graminearum* (*Gibberella zeae*) isolates from wheat in Argentina. Eur. J. Plant Pathol. 115: 139–148.

Rand T.G., Giles, S., Flemming, J., Miller, J.D. and Puniani, E. 2005. Inflammatory and cytotoxic responses in mouse lungs exposed to purified toxins from building isolated *Penicillium brevicompactum* Dierckx and *P. chrysogenum* Thom. Toxicol. Sci. 87: 213–222.

Ranganna, B., Raghavan, G.S.V. and Kushalappa, A.C. 1998. Hot water dipping to enhance storability of potatoes. Postharvest Biol. Technol. 13: 215–223.

Rankine, B.C. 1964. Hydrogen sulphide production by yeasts. J. Sci. Food Agric. 15: 872–877.

Raper, K.B. 1957. Nomenclature in *Aspergillus* and *Penicillium*. Mycologia 49: 644–662.

Raper, K.B. and Fennell, D.I. 1965. The Genus *Aspergillus*. Baltimore, MD: Williams and Wilkins.

Raper, K.B. and Thom, C. 1949. A Manual of the Penicillia. Baltimore, MD: Williams and Wilkins.

Rapp, P. and Backhaus, S. 1992. Formation of extracellular lipases by filamentous fungi, yeasts, and bacteria. Enzyme Microb. Technol. 14: 938–943.

Raso, J., Calderon, M.L., Gongora, M., Barbosa-Canovas, G.V. and Swanson, B.G. 1998. Inactivation of *Zygosaccharomyces bailii* in fruit juices by heat, high hydrostatic pressure and pulsed electric fields. J. Food Sci. 63: 1042–1044.

Rastogi, S., Dogra, R.K.S., Khanna, S.K. and Das, M. 2006. Skin tumorigenic potential of aflatoxin B1 in mice. Food Chem. Toxicol. 44: 670–677.

Raters, M. and Matissek, R. 2005. Study on the distribution of mycotoxins in coca beans. Mycotoxin Res. 21: 182–186.

Raters, M. and Matissek, R. 2007. No OTA in fresh cocoa beans. Mycotoxin Res. 23: 85–87.

Rath, G. and Mohanty, G.N. 1985. *Aspergillus* rot of stored garlic and its control. Indian J. Mycol. Plant Pathol. 15: 323–324.

Rath, G.C. and Mohanty, G.N. 1986. *Fusarium* rot of stored garlic. Indian Phytopathol. 39: 614–615.

Ratomahenina, R., Chabalier, C. and Galzy, P. 1994. Concerning *Sporendonema casei* Desmazieres. Latte 19: 616–617.

Ravelomanana, R., Guiraud, J.P., Vincent, J.C. and Galzy, P. 1984. Study of the yeast flora of the traditional cocoa fermentation in Madagascar. Rev. Ferment. Ind. Aliment. 39: 103–106.

Ravichandran, V. and Sullia, S.B. 1983. Pathogenic fungi from sweet potato. Curr. Sci. 52: 1031.

Rawsthorne, H. and Phister, T.G. 2006. A real-time PCR assay for the enumeration and detection of *Zygosaccharomyces bailii* from wine and fruit juices. Int. J. Food Microbiol. 112: 1–7.

Ray, R.C. and Misra, R.S. 1995. Spoilage of sweet potato tubers in tropics. I. Microorganisms associated. Adv. Hortic. Sci. 9: 19–22.

Ray, R.C. and Ravi, V. 2005. Post harvest spoilage of sweet potato in tropics and control measures. Crit. Rev. Food Sci. Nutr. 45: 623–644.

Ray, R.C., Padmaja, G. and Balagopalan, C. 1990. Extracellular rhodanese production by *Rhizopus oryzae*. Zentralbl. Mikrobiol. 145: 259–268.

Ray, R.C., Chowdhury, S.R. and Balagopalan, C. 1994. Minimizing weight loss and microbial rotting of sweet potatoes (*Ipomoea batatas* L.) in storage under tropical ambient conditions. Adv. Hortic. Sci. 8: 159–163.

Ray-Schroeder, L.L. 1983. Effect of sorbate on growth, spore viability and mycotoxin production of selected *Penicillium* species. Diss. Abstr. Int. B 43: 3524.

Reboux, G., Piarroux, R., Mauny, F., Madroszyk, A., Millon, L., Bardonnet, K. and Dalphin, J.C. 2001. Role of molds in farmer's lung disease in Eastern France. Am. J. Resp. Crit. Care Med. 163: 1534–1539.

Recca, J. and Mrak, E.M. 1952. Yeasts occurring in citrus products. Food Technol. Champaign 6: 450–454.

Reddy, B.A., Saifulla, M., Mallesh, S.B. and Kumar, R.M. 2006. Seed mycoflora of pigeonpea (*Cajanus cajan* L. Mills.) and its management. Int. J. Agric. Sci. 2: 522–525.

Reddy, C.S. 2005. Alterations in protein kinase A signalling and cleft palate: a review. Hum. Exp. Toxicol. 24: 235–242.

Reichart, O. and Mohacsi-Farkas, C. 1994. Mathematical modelling of the combined effect of water activity, pH and redox potential on the heat destruction. Int. J. Food Microbiol. 24: 103–112.

Renouf, V., Claisse, O. and Lonvaud-Funel, A. 2007. Inventory and monitoring of wine microbial consortia. Appl. Microbiol. Biotechnol. 75: 149–164.

Restaino, L., Frampton, E.W., Hemphill, J.B. and Palnikar, P. 1995. Efficacy of ozonated water against various food-related microorganisms. Appl. Environ. Microbiol. 61: 3471–3475.

Restuccia, C., Giusino, F., Licciardello, F., Randazzo, C., Caggia, C. and Mutarore, G. 2006. Biological control of peach fungal pathogens by commercial products and indigenous yeasts. J. Food Prot. 69: 2465–2470.

Resurreccion, A.A. and Koehler, P.E. 1977. Toxicity of *Aspergillus amstelodami*. J. Food Sci. 42: 482–487.

Reyes, A.A. 1990. Pathogenicity, growth, and sporulation of *Mucor mucedo* and *Botrytis cinerea* in cold or CA storage. HortScience 25: 549–552.

Reyes M.E.Q., Rohrbach, K.G. and Paull, R.E. 2004. Microbial antagonists control postharvest black rot of pineapple fruit. Postharvest Biol. Technol. 33: 193–203.

Reynoso, M.M., Torres, A.M. and Chulze, S.N. 2004. Fusaproliferin, beauvericin and fumonisin production by different mating populations among the *Gibberella fujikuroi* complex isolated from maize. Mycol. Res. 108: 154–160.

Reynoso, M.M., Torres, A.M. and Chulze, S.N. 2006. Biological species in the *Gibberella fujikuroi* species complex isolated from maize kernels in Argentina. Plant Pathol. J. (Faisalabad) 5: 350–355.

Rheeder, J.P., Marasas, W.F.O., Thiel, P.G., Sydenham, E.W., Shephard, G.S. and van Schalkwyk, D.J. 1992. *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. Phytopathology 82: 352–357.

Rheeder, J.P., Sydenham, E.W., Marasas, W.F.O., Thiel, P.G., Shephard, G.S., Schlechter, M., Stockenstrom, S., Cronje, D.W. and Viljoen, J.H. 1995. Fungal infestation and mycotoxin contamination of South African commercial maize harvested in 1989 and 1990. S. Afr. J. Sci. 91: 127–131.

Riba, A., Mokrane, S., Mathieu, F., Lebrihi, A. and Sabaou, N. 2008. Mycoflora and ochratoxin A producing strains of *Aspergillus* in Algerian wheat. Int. J. Food Microbiol. 122: 85–92.

Ribeiro, N.C.A., Bezerra, J.L. and Lopez, A.S. 1986. Mycobiota of cacao fermentation in Bahia State, Brazil. Rev. Theobroma 16: 47–55.

Ribeiro Jr, P.M. and Dias, M.S.C. 2005. Diseases of passion fruit. Inf. Agropec. 26(228): 36–39.

Ribeiro, S.A.L., Cavalcanti, M.A.Q., Fernandes, M.J.S. and Lima, D.M.M. 2003. Filamentous fungi isolated from corn-derived products. Rev. Brasil. Botan. 26: 223–229.

Rice, S.L. 1980. Patulin production by *Byssochlamys* spp. in canned grape juice. J. Food Sci. 45: 485–488, 495.

Rice, S.L., Beuchat, L.R. and Worthington, R.E. 1977. Patulin production by *Byssochlamys* spp. in fruit juices. Appl. Environ. Microbiol. 34: 791–796.

Richard, J.L., Peden, W.M. and Williams, P.P. 1994. Gliotoxin inhibits transformation and its cytotoxic to turkey peripheral blood lymphocytes. Mycopathologia 126: 109–114.

Richardson, K.C. 1965. Incidence of *Byssochlamys fulva* in Queensland grown canned strawberries. Queensl. J. Agric. Anim. Sci. 22: 347–350.

Riddle, H.F.V., Channell, S., Blyth, W., Weir, D.M., Lloyd, M., Amos, W.M.G. and Grant, I.W.B. 1968. Allergic alveolitis in a maltworker. Thorax 23: 271–280.

Ride, J.P. and Drysdale, R.B. 1972. A rapid method for the chemical estimation of filamentous fungi in plant tissue. Physiol. Plant Pathol. 2: 7–15.

Rifai, M.A. 1969. A revision of the genus *Trichoderma*. Mycol. Papers 116: 1–56.

Rippon, L.E. 1980. Wastage of postharvest fruit and its control. CSIRO Food Res. Q. 40: 1–12.

Rizzo, I., Vedoya, G., Maurutto, S., Haidukowski, M. and Varsavsky, E. 2004. Assessment of toxigenic fungi on Argentinean medicinal herbs. Microbiol. Res. 159: 113–120.

Roberts, R.G. and Reymond, S.T. 1994. Chlorine dioxide for reduction of postharvest pathogen inoculum during handling of tree fruits. Appl. Environ. Microbiol. 60: 2864–2868.

Robertson, A. and Patel, N. 1989. Development of an enzyme immunoassay for the quantification of mould in tomato paste – an alternative to the Howard mould count. Food Agric. Immunol. 1: 73–81.

Robertson, A., Patel, N. and Sargeant, J.G. 1988. Immunofluorescence detection of mould – an aid to the Howard mould counting technique. Food Microbiol. 5: 33–42.

Robiglio, A.L. and Lopez, S.E. 1995. Mycotoxin production by *Alternaria alternata* strains isolated from red delicious apples in Argentina. Int. J. Food Microbiol. 24: 413–417.

Robine, E., Lacaze, I., Moularat, S., Ritoux, S. and Boissier, M. 2005. Characterisation of exposure to airborne fungi: measurement of ergosterol. J. Microbiol. Meth. 63: 185–192.

Rockland, L.B. and Beuchat, L.R., (eds). 1987. Water Activity: Theory and Applications to Food. New York: Marcel Dekker.

Rodríguez, M., Núñez, F., Córdora, J.J., Bermúdez, M.E. and Asensio, M.A. 1998. Evaluation of proteolytic activity of micro-organisms isolated from dry cured ham. J. Appl. Microbiol. 85: 905–912.

Rohde, S. and Rabenstein, F. 2005. Standardization of an indirect PTA-ELISA for detection of *Fusarium* spp. in infected grains. Mycotox. Res. 21: 100–104.

Rohm, H., Lechner, F. and Lehner, M. 1990. Microflora of Austrian natural-set yogurt. J. Food Prot. 53: 478–480.

Rohm, H., Eliskases-Lechner, F. and Bräuer, M. 1992. Diversity of yeasts in selected dairy products. J. Appl. Bacteriol. 72: 370–376.

Rohrbach, K.G. and Schmitt, D. 2003. Diseases of pineapple. *In* Diseases of tropical fruit crops, ed. R.C. Ploetz, Wallingford, UK: CABI Publishing. pp 443–464.

Rohrbach, K.G. and Taniguchi, G. 1984. Effects of temperature, moisture, and stage of inflorescence development on infection of pineapple by *Penicillium funiculosum* and *Fusarium moniliforme* var. *subglutinans*. Phytopathology 74: 995–1000.

Rojas, F.J., Jodral, M., Gosalvez, F. and Pozo, R. 1991. Mycoflora and toxigenic *Aspergillus flavus* in Spanish dry-cured ham. Int. J. Food Microbiol. 13: 249–256.

Roland, J.O. and Beuchat, L.R. 1984. Influence of temperature and water activity on growth and patulin production by *Byssochlamys nivea* in apple juice. Appl. Environ. Microbiol. 47: 205–207.

Roland, J.O., Beuchat, L.R., Worthington, R.E. and Hitchcock, H.L. 1984. Effects of sorbate, benzoate, sulfur dioxide and temperature on growth and patulin production by *Byssochlamys nivea* in grape juice. J. Food Prot. 47: 237–241.

Romano, P., Suzzi, G., Zironi, R. and Comi, G. 1993. Biometric study of acetoin production in *Hanseniaspora guilliermondii* and *Kloeckera apiculata*. Appl. Environ. Microbiol. 59: 1838–1841.

Rombouts, F.M. and Nout, M.R.J. 1995. Microbial fermentation in the production of plant foods. J. Appl. Bacteriol. Symp. Suppl. 79: 108S–117S.

Romero, S.M., Comerio, R.M., Larumbe, G., Ritieni, A., Vaamonde, G. and Fernandez Pinto, V. 2005. Toxigenic fungi isolated from dried vine fruits in Argentina. Int. J. Food Microbiol. 104: 43–49.

Romero, S.M., Patriarca, A., Fernandez Pinto, V. and Vaamonde, G. 2007. Effect of water activity and temperature on growth of ochratoxigenic strains of *Aspergillus carbonarius* isolated from Argentinean dried vine fruits. Int. J. Food Microbiol. 115: 140–143.

Roostita, R and Fleet, G.H. 1996. The occurrence and growth of yeasts in Camembert and Blue-veined cheeses. Int. J. Food Microbiol. 28: 393–404.

Rosa, C.A., Novak, F.R., de Almeida, J.A.G., Mendonca-Hagler, L.C. and Hagler, A.N. 1990. Yeasts from human milk collected in Rio de Janeiro, Brazil. Rev. Microbiol. 21: 361–363.

Ros-Chumillas, M., Egea-Cortines, M., Lopez-Gomez, A. and Weiss, J. 2007. Evaluation of a rapid DNA extraction method to detect yeast cells by PCR in orange juice. Food Control 18: 33–39.

Rosenberger, D.A., Engle, C.A. and Meyer, F.W. 2006. *Penicillium expansum* invades apples through stems

during controlled atmosphere storage. Plant Health Prog. (December): 1–8.

Rosi, I. 1993. Extracellular proteolytic activity in yeasts of enological interest. Ann. Microbiol. Enzymol. 43: 77–84.

Rosi, I., Vinella, M. and Domizio, P. 1994. Characterization of beta-glucosidase activity in yeasts of oenological origin. J. Appl. Bacteriol. 77: 519–527.

Ross, H.M., Harden, T.J., Nichol, A.W. and Deeth, H.C. 2000. Isolation and investigation of micro-organisms causing brown defects in mould-ripened cheeses. Aust. J. Dairy Technol. 55: 5–8.

Ross, P.F., Nelson, P.E., Richard, J.L., Osweiler, G.D., Rice, L.G., Plattner, R.D. and Wilson, T.M. 1990. Production of fumonisins by *Fusarium moniliforme* and *Fusarium proliferatum* isolates associated with equine leukoencephalomalacia and a pulmonary edema syndrome in swine. Appl. Environ. Microbiol. 56: 3225–3226.

Rossi, V., Terzi, V., Moggi, F., Morcia, C., Faccioli, P., Haidukowski, M. and Pascale, M. 2007. Assessment of *Fusarium* infection in wheat heads using a quantitative polymerase chain reaction (qPCR) assay. Food Addit. Contam. 24: 1121–1130.

Roussos, S., Zaouia, N., Salih, G., Tantaoui-Elaraki, A., Lamrani, K., Cheheb, M., Hassouni, H. Verhé, F., Perraud-Gaime, I., Augur, C. and Ismaili-Alaoui, M. 2006. Characterization of filamentous fungi isolated from Moroccan olive and olive cake: Toxinogenic potential of *Aspergillus* strains. Mol. Nutr. Food Res. 50: 500–506.

Roy, A.N., Sharma, R.B. and Gupta, K.C. 1977. Occurrence of three new rot diseases of stored garlic (*Allium sativum* L.). Curr. Sci. 46: 716–717.

Ruggia, A. and Galiero, G. 2005. The contamination of milk with aflatoxins. Bubalus Bubalis 11: 24–25.

Rundberget, T, Skaar, I., O'Brien, O. and Flåøyen, A. 2004. Penitrem and thomitrems formation by *Penicillium crustosum*. Mycopathologia 157: 349–357.

Ryall, A.L. and Lipton, W.J. 1979. Handling, Transportation and Storage of Fruits and Vegetables. Westport, CT: AVI Publishing Co.

Ryall, A.L. and Pentzer, W.T. 1982. Handling, Transportation and Storage of Fruits and Vegetables. Vol. 2. Fruits and Tree Nuts, 2nd edn. Westport, CT: AVI Publishing Co.

Sabaa-Srur, A.U.O., Brecht, J.K., Sargent, S.A. and Bartz, J. A. 1993. Recommended chlorine levels for treatment of float-tank water in tomato packinghouses. Acta Hortic. 343: 337–338.

Sabater-Vilar, M., Nijmeijer, S. and Fink-Gremmels, J. 2003a. Genotoxicity of five tremorgenic mycotoxins (fumitremogens B, paxiline, penitrem A, verruculogen and verrucosidin) produced by molds isolated from fermented meats. J. Food Prot. 66: 2123–2129.

Sabater-Vilar, M., Kuilman-Wahls, M.E.M. and Fink-Gremmels, J. 2003b. Inhibition of aflatoxin B1 mutagenicity by cyclopiazonic acid in the presence of human liver preparations. Toxicol. Lett. 143: 291–299.

Sabater-Vilar, M., Maas, R.F.M., De Bosschere, H., Ducatelle, R. and Fink-Gremmels, J. 2004. Patulin produced by *Aspergillus clavatus* isolated from feed containing malting residues associated with a lethal neurotoxicosis in cattle. Mycopathologia 158: 419–426.

Sage, L., Krivobok, S., Delbos, E., Seigle-Murandi, F. and Creppy, E.E. 2002. Fungal flora and ochratoxin A production in grapes and musts from France. J. Agric. Food Chem. 50: 1306–1311.

Sahgal, N., Needham, R., Cabañes, F.J. and Magan, N. 2007. Potential for detection and discrimination between mycotoxigenic and non-toxigenic spoilage moulds using volatile production patterns: a review. Food Addit. Contam. 24: 1161–1168.

Sahin, I. and Kalyoncuoglu, M.E. 1994. Erkenntnisse über die Schimmelpilze in der Mikroflora von Hasel-, Walnüssen und Sonnen-Blumenkernen. Chem. Mikrobiol. Technol. Lebensm. 16: 85–92.

Saito, M., Enomoto, M., Tatsuno, T. and Uraguchi, K. 1971a. Yellowed rice toxins. *In* Microbial Toxins: a Comprehensive Treatise. Vol. 6. Fungal Toxins, eds A. Ciegler, S. Kadis and S.J. Ajl. London: Academic Press. pp. 299–380.

Saito, M., Ohtsubo, K., Umeda, M., Enomoto, M., Kurata, H., Udagawa, S., Sakabe, F. and Ichinoe, M. 1971b. Screening tests using HeLa cells and mice for detection of mycotoxin-producing fungi isolated from foodstuffs. Jpn J. Exp. Med. 41: 1–20.

Saito, M., Rai, D.R. and Masuda, R. 2003. Isolation of *Penicillium hirsutum* from spoiled, packaged asparagus spears in Japan. J. Gen. Plant Pathol. 69: 304–306.

Sakai, A., Tanaka, H., Konishi, Y., Hanazawa, R., Ota, T., Nakahara, Y., Sekiguchi, S., Oshida, E., Takino, M., Ichinoe, M., Yoshikawa, K., Yoshizawa, T. and Takatori, K. 2005. Mycological examination of domestic unpolished rice and mycotoxin production by isolated *Penicillium islandicum*. J. Food Hyg. Soc. Jpn. 46: 205–212.

Salami, A.O. and Popoola, O.O. 2007. Thermal control of some post harvest rot pathogens of Irish potato (*Solanum tuberosum* L.). J. Agric. Sci. Belgrade. 52: 17–31.

Saleemullah, A.I., Khalil, I.A. and Shah, H. 2006. Aflatoxin contents of stored and artificially inoculated cereals and nuts. Food Chem. 98: 699–703.

Sales, A.C. and Yoshizawa, T. 2005a. Updated profile of aflatoxin and *Aspergillus* section *Flavi* contamination in rice and its byproducts from the Philippines. Food Addit. Contam. 22: 429–436.

Sales, A.C. and Yoshizawa, T. 2005b. Mold counts and *Aspergillus* section *Flavi* populations in rice and its byproducts from the Philippines. J. Food Prot. 68: 120–125.

Salomão, B.C.M., Slongo, A.P. and Aragão, G.M.F. 2007. Heat resistance of *Neosartorya fischeri* in various juices. LWT Food Sci. Technol. 40: 676–680.

Salomão, B.C.M., Massaguer, P.R. and Aragao, G.M.F. 2008. Isolation and selection of heat resistant molds in the production process of apple nectar. Ciencia Tecnol. Alim. 28: 116–121.

Salovaara, H. and Savolainen, J. 1984. Yeast type isolated from Finnish sour rye dough starters. Acta Aliment. Pol. 10: 241–245.

Samapundo, S., Devlieghere, F., de Meulenaer, B., Geeraerd, A.H., van Impe, J.F. and Debevere, J. M. 2005a. Predictive modelling of the individual and combined effect of water activity and temperature on the radial growth of *Fusarium verticilliodes* and *F. proliferatum* on corn. Int. J. Food Microbiol. 105: 35–52.

Samapundo, S., Devliehgere, F., de Meulenaer, B. and Debevere, J. 2005b. Effect of water activity and temperature on growth and the relationship between fumonisin production and the radial growth of *Fusarium verticillioides* and *Fusarium proliferatum* on corn. J. Food Prot. 68: 1054–1059.

Samapundo, S., Meulenaer, B. de, Atukwase, A., Debevere, J. and Devlieghere, F. 2007a. The influence of modified atmospheres and their interaction with water activity on the radial growth and fumonisin B1 production of *Fusarium verticillioides* and *F. proliferatum* on corn. Part I: The effect of initial headspace carbon dioxide concentration. Int. J. Food Microbiol. 114: 160–167.

Samapundo, S., Meulenaer, B. de, Atukwase, A., Debevere, J. and Devlieghere, F. 2007b. The influence of modified atmospheres and their interaction with water activity on the radial growth and fumonisin B1 production of *Fusarium verticillioides* and *F. proliferatum* on corn. Part II: The effect of initial headspace oxygen concentration. Int. J. Food Microbiol. 113: 339–345.

Samapundo, S., Devlieghere, F., Meulenaer, B. de, Lamboni, Y., Osei-Nimoh, D. and Debevere, J.M. 2007c. Interaction of water activity and bicarbonate salts in the inhibition of growth and mycotoxin production by *Fusarium* and *Aspergillus* species of importance to corn. Int. J. Food Microbiol. 116: 266–274.

Samelis, J. and Sofos, J.N. 2003. Yeasts in meat and meat products. *In* Yeasts in Food, eds T. Boekhout and V. Robert. Cambridge, UK: Woodhead Publishing. pp. 239–265.

Sams, C.E., Conway, W.S., Abbott, J.A., Lewis, R.J. and Ben-Shalom, N. 1993. Firmness and decay of apples following postharvest pressure infiltration of calcium and heat treatment. J. Am. Soc. Hortic. Sci. 118: 623–627.

Samson, R.A. 1974. *Paecilomyces* and some allied Hyphomycetes. Stud. Mycol., Baarn 6: 1–119.

Samson, R.A. 1979. A compilation of the Aspergilli described since 1965. Stud. Mycol., Baarn 18: 1–38.

Samson, R.A. and Frisvad, J.C. (Eds). 2004. *Penicillium* subgenus *Penicillium*: new taxonomic schemes, mycotoxins and other extrolites. Stud. Mycol. 49: 260.

Samson, R.A. and Gams, W. 1985. Typification of the species of *Aspergillus* and associated teleomorphs. *In* Advances in *Penicillium* and *Aspergillus* Systematics, eds R.A. Samson and J.I. Pitt. New York: Plenum Press. pp. 31–54.

Samson, R.A. and Hoekstra, E.S. 1994. Common fungi occurring in indoor environments. *In* Health Implications of Fungi in Indoor Environments, eds R.A. Samson, B. Flannigan, M.E. Flannigan, A.P. Verhoof, O.C.G. Adan and E.S. Hoekstra. Amsterdam: Elsevier. pp. 541–546.

Samson, R.A. and Pitt, J.I. 1985. Check list of common *Penicillium* species. *In* Advances in *Penicillium* and *Aspergillus* Systematics, eds R.A. Samson and J.I. Pitt. New York: Plenum Press. pp. 461–463.

Samson, R.A., Stolk, A.C. and Hadlok, R. 1976. Revision of the Subsection Fasciculata of *Penicillium* and some allied species. Stud. Mycol. Baarn 11: 1–47.

Samson, R.A., Eckardt, C. and Orth, R. 1977a. The taxonomy of *Penicillium* species from fermented cheeses. Antonie van Leeuwenhoek 43: 341–350.

Samson, R.A., Hadlok, R. and Stolk, A.C. 1977b. A taxonomic study of the *Penicillium chrysogenum* series. Antonie van Leeuwenhoek 43: 169–175.

Samson, R.A., Nielsen, P.V. and Frisvad, J.C. 1990. The genus *Neosartorya*: differentiation by scanning electron microscopy and mycotoxin profiles. *In* Modern Concepts in *Penicillium* and *Aspergillus* Classification, eds R.A. Samson and J.I. Pitt. New York: Plenum Press. pp. 455–467.

Samson, R.A., Hocking, A.D., Pitt, J.I. and King, A.D., eds. 1992. Modern Methods in Food Mycology. Amsterdam: Elsevier.

Samson, R.A., Flannigan, B., Flannigan, M., Verhoeff, A., Adan, O.C.G. and Hoekstra, E.S. 1994. Health Implication of Fungi in Indoor Environments. Air Quality Monographs, Vol. 2. Amsterdam: Elsevier.

Samson, R.A., Hoekstra, E.S., Frisvad, J.C. and Filtenborg, O., eds. 1995. Introduction to Foodborne Fungi, 4th edn. Baarn, Netherlands: Centraalbureau voor Schimmelcultures.

Samson, R.A., Hoekstra, E.S., and Frisvad, J.C. eds. 2004a. Introduction to Food- and Airborne Fungi, 7th edn. Utrecht, Netherlands: Centraalbureau voor Schimmelcultures.

Samson, R.A., Seifert, K.A., Kuijpers, A.F.A., Houbraken, J.A.M.P. and Frisvad, J.C. 2004b. Phylogenetic analysis of *Penicillium* subgenus *Penicillium* using partial β-tubulin sequences. Stud. Mycol. 49: 175–200.

Samuels, G.J. 2006. *Trichoderma:* systematics, the sexual state, and ecology. Phytopathology 96: 195–206.

Sand, F.E.M.J. and van Grinsven, A.M. 1976. Investigation of yeast strains isolated from Scandinavian soft drinks. Brauwissenschaft 29: 353–355.

Sanders, P.R., Sammons, B., Kaniewski, W., Haley, L., Layton, J., LaVallee, B.J., Delannay, X. and Tumer, N.E. 1992. Field resistance of transgenic tomatoes expressing the tobacco mosaic virus or tomato mosaic virus coat protein genes. Phytopathology 82: 683–690.

Sanderson, P.G. and Spotts, R.A. 1995. Postharvest decay of winter pear and apple fruit caused by species of *Penicillium*. Phytopathology 85: 103–110.

Sangalang, A.E., Burgess, L.W., Backhouse, D., Duff, J. and Wurst, M. 1995. Mycogeography of *Fusarium* species in soils from tropical, arid and Mediterranean regions of Australia. Mycol. Res. 99: 523–528.

Sanni, A.I. and Loenner, C. 1993. Identification of yeasts isolated from Nigerian traditional alcoholic beverages. Food Microbiol. 12: 517–523.

Santos, A. and Marquina, D. 2004. Killer toxin of *Pichia membranifaciens* and its possible use as a biocontrol agent against grey mould disease of grapevine. Microbiology (Reading) 150: 2527–2534.

Santos, A., Sánchez, A. and Marquina, D. 2004. Yeasts as biological agents to control *Botrytis cinerea*. Microbiol. Res. 159: 331–338.

Sanz, A., Martin, R., Mayoral, M.B., Hernandez, P.E., Gonzalez, I. and Garcia Lacarra, T. 2005. Development of a PCR-culture technique for rapid detection of yeast species in vacuum packed ham. Meat Sci. 71: 230–237.

Sarantinos, J., Hung, T.V., Black, R.G. and Brouwer, J.B. 1996. The effects of fungal infection on the chemical and functional properties of chickpeas (*Cicer arietinum*) and faba beans (*Vicia faba*). J. Sci. Food Agric. 70: 197–203.

Sartori, M.R., Taniwaki, M.H., Eiroa, M.N.U. and Pettinelli, A. 1991. Storage of maize with moderately high moisture content in an underground polyethylene silo. Colet. Inst. Tecnol. Aliment. (Campinas, Braz.) 21: 309–316.

Saudi, A.M. and Mansour, N.K. 1990. Fungal contamination of ready-to-eat-meals in airline catering. Fleischwirtschaft 70: 563–564.

Sauer, D.B., Storey, C.L. and Walker, D.E. 1984. Fungal populations in U.S. farm-stored grain and their relationship to moisture, storage time, regions and insect infestation. Phytopathology 74: 1050–1053.

Schaller, E., Bosset, J.O. and Escher, F. 1998. 'Electronic noses' and their application to food. Food Sci. Technol. 31: 305–316.

Schindler, A.F., Abadie, A.N., Gecan, J.S., Mislivec, P.B. and Brickey, P.M. 1974. Mycotoxins produced by fungi isolated from inshell pecans. J. Food Sci. 39: 213–214.

Schipper, M.A.A. 1975. On *Mucor mucedo*, *Mucor flavus* and related species. Stud. Mycol. Baarn 10: 1–33.

Schipper, M.A.A. 1976. On *Mucor circinelloides*, *Mucor racemosus* and related species. Stud. Mycol. Baarn 12: 1–40.

Schipper, M.A.A. 1978a. On certain species of *Mucor* with a key to all accepted species. Stud. Mycol. Baarn 17: 1–52.

Schipper, M.A.A. 1978b. On the genera *Rhizomucor* and *Parasitella*. Stud. Mycol. Baarn 17: 53–71.

Schipper, M.A.A. 1984. A revision of the genus *Rhizopus*. 1. The *Rh. stolonifer*-group and *Rh. oryzae*. Stud. Mycol. Baarn 25: 1–19.

Schipper, M.A.A. and Stalpers, J.A. 1984. A revision of the genus *Rhizopus*. 2. The *Rh. microsporus*-group. Stud. Mycol. Baarn 25: 20–34.

Schmidt, H., Adler, A., Holst-Jensen, A., Klemsdal, S.S., Logrieco, A., Mach, R.L., Nirenberg, H.I., Thrane, U., Torp, M., Vogel, R.F., Yli-Mattila, T. and Niessen, L. 2004. An integrated taxonomic study of *Fusarium langsethiae*, *Fusarium poae* and *Fusarium sporotrichioides* based on the use of composite datasets. Int. J. Food Microbiol. 95: 341–349.

Schmidt, J.L. and Lenoir, J. 1980. Study of the yeast flora composition of Camembert cheese. II. Lait 60: 272–282.

Schmidt, J.L., Graffard, C. and Lenoir, J. 1979. Biochemical aptitudes of yeasts isolated from Camembert cheese. I. Preliminary studies. Lait 59: 142–163.

Schmidt-Lorenz, W. and Gutschmidt, J. 1969. Mikrobielle und sensoriche Veranderungen gefrorener Brathahnchen und Poularden bei Lagerung im Temperaturbereich von –2.5°C bis -10°C. Fleischwirtschaft 49: 1033–1041.

Schneider, A., Horn, H. and Hammes, W.P. 2003. Zum Vorkommen osmophiler Hefen im Honig [The occurrence of osmophilic yeasts in honey]. Deutsche Lebensmittel-Rundschau 99: 310–319.

Schneider, R. 1954. Untersuchungen über Feuchtigkeitsanspruche parasitischer Pilze. Phytopathol. Z. 21: 63–78.

Schnürer, J. and Jonsson, A. 1992. Ergosterol levels and mould colony forming units in Swedish grains of food and feed grade. Acta Agric. Scand. Sect. B 42: 240–245.

Schnürer, J., Olsson, J. and Börjesson, T. 1999. Fungal volatiles as indicators of food and feeds spoilage. Fungal Genetics Biol. 27: 209–217.

Schoch, U., Lüthy, J. and Schlatter, C. 1984. Mykotoxine von *Penicillium roqueforti* und *P. camemberti* in Käse. II. Suche nach chemisch nicht identifizierten, biologisch aktiven Stoffen. Milchwissenschaft 39: 583–584.

Schol-Schwarz, M.B. 1959. The genus *Epicoccum*. Trans. Br. Mycol. Soc. 42: 149–173.

Schubert, K., Groenewald, J.Z., Braun, U., Dijksterhuis, J., Starink, M., Hill, C.F., Zalar, P., de Hoog, G.S. and Crous, P.W. 2007. Biodiversity in the *Cladosporium herbarum* complex (*Davidiellaceae, Capnodiales*), with standardisation of methods for *Cladosporium* taxonomy and diagnostics. Stud. Mycol. 58: 105–156.

Schwabe, M., Kamphuis, H., Trümner, U., Offenbächer, G. and Krämer, J. 1992. Comparison of the latex agglutination test and the ergosterol assay for the detection of moulds in foods and feedstuffs. Food Agric. Immunol. 4: 19–25.

Schwan, R.F. and Wheals, A.E. 2003. Mixed microbial fermentations of chocolate and coffee. *In* Yeasts in Food, eds T. Boekhout and V. Robert. Cambridge, UK: Woodhead Publishing, pp. 429–449.

Schweiggert, U., Mix, K., Schieber, A. and Carle, R. 2005. An innovative process for the production of spices through immediate thermal treatment of the plant material. Innov. Food Sci. Emerg. Technol. 6: 143–153.

Scott, D.B. 1965. Toxigenic fungi isolated from cereal and legume products. Mycopath. Mycol. Appl. 25: 213–222.

Scott, D.B. 1968. The Genus *Eupenicillium* Ludwig. Pretoria, South Africa: Council for Scientific and Industrial Research.

Scott, D.B. and Stolk, A.C. 1967. Studies on the genus *Eupenicillium* Ludwig. II. Perfect states of some Penicillia. Antonie van Leeuwenhoek 33: 297–314.

Scott, J.B. and Chakraborty, S. 2006. Multilocus sequence analysis of *Fusarium pseudograminearum* reveals a single phylogenetic species. Mycol. Res. 110: 1413–1425.

Scott, P.M. 1977. *Penicillium* mycotoxins. *In* Mycotoxic Fungi, Mycotoxins, Mycotoxicoses, an Encyclopedic Handbook. Vol. 1. Mycotoxigenic Fungi, eds T.D. Wyllie and L.G. Morehouse. New York: Marcel Dekker. pp. 283–356.

Scott, P.M. 1981. Toxins of *Penicillium* species used in cheese manufacture. J. Food Prot. 44: 702–710.

Scott, P.M., Abbas, H.K., Mirocha, C.J., Lawrence, G.A. and Weber, D. 1987. Formation of moniliformin by *Fusarium sporotrichioides* and *Fusarium culmorum*. Appl. Environ. Microbiol. 53: 196–197.

Scott, V.N. and Bernard, D.T. 1987. Heat resistance of *Talaromyces flavus* and *Neosartorya fischeri* isolated from commercial fruit juices. J. Food Prot. 50: 18–30.

Scott, W.J. 1957. Water relations of food spoilage microorganisms. Adv. Food Res. 7: 83–127.

Scudamore, K.A., Atkin, P.M. and Buckle, A.E. 1986. Natural occurrence of the naphthoquinone mycotoxins, xanthomegnin, viomellein and vioxanthin in cereals and animal feedstuffs. J. Stored Prod. Res. 22: 81–84.

Scudamore, K.A., Clarke, J.H. and Hetmanski, M.T. 1993. Isolation of *Penicillium* strains producing ochratoxin A, citrinin, xanthomegnin, viomellein and vioxanthin from stored cereal grains. Lett. Appl. Microbiol 17: 82–87.

Seefelder, W., Gossmann, M. and Humpf, H.U. 2002. Analysis of fumonisin B1 in *Fusarium proliferatum*-infected

asparagus spears and garlic bulbs from Germany by liquid chromatography-electrospray ionization mass spectrometry. J. Agric. Food Chem. 50: 2778–2781.

Seemüller, E. 1968. Untersuchungen über die morphologische und biologische differenzierung in der *Fusarium*-Sektion *Sporotrichiella*. Mitt. Biol. Bundesanst. Land-Forstwirtsch. Berlin-Dahlem 127: 1–93.

Seifert, K.A., Aoki, T., Baayen, R.P., Brayford, D., Burgess, L.W., Chulze, S., Gams, W., Geiser, D., de Gruyter, J., Leslie, J.F., Logrieco, A., Marasas, W.F.O., Nirenberg, H.I., O'Donnell, K., Rheeder, J., Samuels, G.J., Summerell, B.A., Thrane U. and Waalwijk, C. 2003. The name *Fusarium moniliforme* should no longer be used. Mycol. Res. 107: 643–644.

Seiler, D.A.L. 1980. Yeast spoilage of bakery products. *In* Biology and Activities of Yeasts, eds F.A. Skinner, S.M. Passmore and R.R. Davenport. London: Academic Press. pp. 141–152.

Seitz, L.M., Mohr, H.E., Burroughs, R. and Sauer, D.B. 1977. Ergosterol as an indicator of fungal invasion in grains. Cereal Chem. 54: 1207–1217.

Seitz, L.M., Sauer, D.B., Burroughs, R., Mohr, H.E. and Hubbard, J.D. 1979. Ergosterol as a measure of fungal growth. Phytopathology 69: 1202–1203.

Sekiyama, B.L., Ribeiro, A.B., Machinski, P.A. and Junior, M.M. 2005. Aflatoxins, ochratoxin A and zearaleone in maize-based food products. Braz. J. Microbiol. 36: 289–294.

Selmanoğlu, G. 2006. Evaluation of the reproductive toxicity of patulin in growing male rats. Food Chem. Toxicol. 44: 2019–2024.

Sempere, F. and Santamarina, M.P. 2006. Microscopic and macroscopic study of the interaction between *Alternaria alternata* (Fr.) Keissler and *Nigrospora oryzae* (Berk. & Broome) Petch. Ann. Microbiol. 56: 101–107.

Senser, F. 1979. Untersuchungen zum Aflatoxingehalt in Haselnüssen. Gordian 79: 117–123.

Sensidoni, A., Rondinini, G., Peressini, D., Maifreni, M. and Bortolomeazzi, R. 1994. Presence of an off-flavour associated with the use of sorbates in cheese and margarine. Ital. J. Food Sci. 6: 237–242.

Senyuva, H.Z. and Gilbert, J. 2008. Identification of fumonisin B2, HT-2 toxin, patulin, and zearalenone in dried figs by liquid chromatography-time-of-flight mass spectrometry and liquid chromatography-mass spectrometry. J. Food Prot. 71: 1500–1504.

Senyuva, H.Z., Gilbert, J., Ozcan, S. and Ulken, U. 2005. Survey for co-occurrence of ochratoxin A and aflatoxin B1 in dried figs in Turkey by using a single laboratory-validated alkaline extraction method for ochratoxin A. J. Food Prot. 68: 1512–1515.

Senyuva, H.Z., Gilbert, J. and Ulken, U. 2007. Aflatoxins in Turkish dried figs intended for export to the European Union. J. Food Prot. 70: 1029–1032.

Seo, J.A., Kim, J.C. and Lee, Y.W. 1996. Isolation and characterization of two new type C fumonisins produced by *Fusarium oxysporum*. J. Nat. Prod. 59: 1003–1005.

Sepitkova, J. and Jesenska, Z. 1986. Analysis of the mycoflora of malting barley and malt. Bulletin Potravinarskeho Vyskumu 25: 241–253.

Sepulveda, O.C. and Piontelli, L.E. 2005. *Aspergillus* population in maize and soja seed imported from Argentina: emphasis on section *Flavi*. Boletin Micol. 20: 41–55.

Serra, R. and Peterson, S.W. 2007. *Penicillium astrolabium* and *Penicillium neocrassum*, two new species isolated from grapes and their phylogenetic placement in the *P. olsonii* and *P. brevicompactum* clade. Mycologia 99: 78–87.

Serra, R., Braga, A. and Venancio A. 2005. Mycotoxin-producing and other fungi isolated from grapes for wine production, with particular emphasis on ochratoxin A. Res. Microbiol. 156: 515–521.

Serra, R., Lourenço, Alípio, P. and Venâncio, A. 2006. Influence of the region of origin on the mycobiota of grapes with emphasis on *Aspergillus* and *Penicillium* species. Mycol. Res. 110: 971–978.

Serra, R., Peterson, S. and Venancio, A. 2008. Multilocus sequence identification of *Penicillium* species in cork bark during plank preparation for the manufacture of stoppers. Res. Microbiol. 159: 178–186.

Sewram, V., Nieuwoudt, T.W., Marasas, W.F.O., Shephard, G.S. and Ritieni, A. 1999. Determination of the mycotoxin moniliformin in cultures of *Fusarium subglutinans* and in naturally contaminated maize by high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J. Chromatogr. A 848: 185–191.

Shabbir, S.M. and Rajasab, A.H. 2004. Diversity of *Fusarium* species on sorghum grain. Indian Phytopathol. 57: 450–453.

Shahnaz, D. and Ghaffar, A. 1991. Detection of the seed-borne mycoflora of sunflower. Pak. J. Bot. 23: 173–178.

Shantha, T., Rati, E.R. and Bhavani-Shankar, T.N. 1990. Behaviour of *Aspergillus flavus* in presence of *Aspergillus niger* during biosynthesis of aflatoxin B_1. Antonie van Leeuwenhoek 58: 121–127.

Sharma, P.D., Fisher, P.J. and Webster, J. 1977. Critique of the chitin assay technique for estimation of fungal biomass. Trans. Br. Mycol. Soc. 69: 479–483.

Sharma, R.B., Roy, A.N. and Singh, R.K. 2002. Fungal spores over fields of fruits and vegetables. Adv. Plant Sci. 15: 619–620.

Sharma, R.C., Kaul, J.L. and Sharma, R.L. 1989. Effect of post-inoculation fungicidal dips on post-harvest diseases of peaches. Int. J. Trop. Plant Dis. 7: 221–224.

Sharma, R.L. and Shukla, A. 2003. Effect of different packagings on soft rot (*Fusarium* spp.) of bell pepper in storage. J. Mycol. Plant Pathol. 33: 134–135.

Sharma, Y.P. and Sumbali, G. 1999a. Incidence of aflatoxin producing strains and aflatoxin contamination in dry fruit slices of quinces (*Cydonia oblonga* Mill.) from the Indian state of Jammu and Kashmir. Mycopathologia 148: 103–107.

Sharma, Y.P. and Sumbali, G. 1999b. Natural occurrence of ochratoxin A and toxigenic *Aspergillus ochraceus* strains in dry fruit slices of quinces from Jammu and Kashmir. Indian Phytopathol. 52: 148–150.

Sharman, M., Patey, A.L., Bloomfield, D.A. and Gilbert, J. 1991. Surveillance and control of aflatoxin contamination of dried figs and fig paste imported into the United Kingdom. Food Addit. Contam. 8: 299–304.

Sharpe, A.N. and Jackson, A.K. 1972. Stomaching: a new concept in bacteriological sample preparation. Appl. Microbiol. 24: 175–178.

Shearer, A.E.H., Mazzotta, A.S., Chuyate, R. and Gombas, D.E. 2002. Heat resistance of juice spoilage microorganisms. J. Food Prot. 65: 1271–1275.

Shephard, G.S., Thiel, P.G., Sydenham, E.W., Vleggaar, R. and Marasas, W.F.O. 1991. Reversed-phase high-performance liquid chromatography of tenuazonic acid and related tetramic acids. J. Chromatogr. 566: 195–205.

Shephard, G.S., Sewram V., Nieuwoudt, T.W., Marasas, W. F.O. and Ritieni, A. 1999. Production of the mycotoxins fusaproliferin and beauvericin by South African isolates in the *Fusarium* section *Liseola*. J. Agric. Food Chem. 47: 5111–5115.

Shephard, G.S., Marasas, W.F.O., Burger, H.M., Somdyala, N.I.M., Rheeder, J.P., Westhuizen, L. van der, Gatyeni, P. and van Schalkwyk, D.J. 2007. Exposure assessment for fumonisins in the former Transkei region of South Africa. Food Addit. Contam. 24: 621–629.

Shlosberg, A., Zadikov, I., Perl, S., Yakobson, B., Varod, Y., Elad, D., Rapoport, E. and Handji, V. 1991. *Aspergillus clavatus* as the probable cause of a lethal mass neurotoxicosis in sheep. Mycopathologia 114: 35–39.

Shoemaker, R.A. 1959. Nomenclature of *Drechslera* and *Bipolaris*, grass parasites segregated from '*Helminthosporium*'. Can. J. Bot. 37: 879–887.

Sholberg, P.L. 1990. A new postharvest rot of peaches in Canada caused by *Mucor piriformis*. Can. J. Plant Pathol. 12: 218–221.

Sholberg, P.L., Marchi, A. and Bechard, J. 1995. Biocontrol of postharvest diseases of apple using *Bacillus* spp. isolated from stored apples. Can. J. Microbiol. 41: 247–252.

Shotwell, O.L. 1977. Aflatoxin in corn. J. Am. Oil. Chem. Soc. 54: 216A–224A.

Shuangquan, B., Yu, B. and Miyamoto, T. 2006. Microflora in traditional strater cultures for fermented milk, hurunge, from Inner Mongolia, China. Anim. Sci. J. 77: 235–241.

Shukla, A. and Sharma, R.L. 2000. Incidence of soft rot of bell pepper in Himachal Pradesh. Mycol. Plant Pathol. 30: 107–109.

Suhr, K.I. and Nielsen, P.V. 2004. Effect of weak acid preservatives on growth of bakery product spoilage fungi at different water activities and pH values. Int. J. Food Microbiol. 95: 67–78.

Siemens, K. and Zawistowski, J. 1993. Occurrence of PR imine, a metabolite of *Penicillium roqueforti*, in blue cheese. J. Food Prot. 56: 317–319, 325.

Silva, C.F., Schwan, R.F., Dias, E.S. and Wheals, A.E. 2000. Microbial diversity during maturation and natural processing of coffee cherries of *Coffea arabica* in Braz. Int. J. Food Microbiol. 60: 251–260.

Silva, C.F., Batista, L.R. and Schwan, R.F. 2003. Incidence of *Aspergillus* produced by mycotoxins in berries and beans of coffee (*Coffea arabica* L.). Rev. Brasil. Armazenamenta 7: 30–36.

Simmonds, J.H. 1965. Papaw diseases. Queensl. Agric. J. 91: 666–667.

Simmons, E.G. 1967. Typification of *Alternaria*, *Stemphylium* and *Ulocladium*. Mycologia 59: 67–92.

Simmons, E.G. 1969. Perfect states of *Stemphylium*. Mycologia 61: 1–26.

Simmons, E.G. 1985. Perfect states of *Stemphylium*. II. Sydowia 38: 284–293.

Simmons, E.G. 1986. *Alternaria* themes and variations (22–26). Mycotaxon 25: 287–308.

Simmons, E.G. 2007. *Alternaria:* an Identification Manual. Utrecht, Netherlands: Centraalbureau voor Schimmelcultures.

Simoncini, N., Rotelli, D., Virgili, R. and Quintavalla, S. 2007. Dynamics and characterization of yeasts during ripening of typical Italian dry-cured ham. Food Microbiol. 24: 577–584.

Singh, A.K., Singh, R.N., Hussain, M.A. and Prasad, B.K. 2003. Effect of storage fungi of oil yielding seeds on germination and seedling diseases. J. Phytol. Res. 16: 163–168.

Singh, D. 2004. Effects of *Debaryomyces hansenii* and calcium salt on fruit rot (*Rhizopus macrosporus*) of peach. Ann. Plant Prot. 12: 310–313.

Singh, K., Singh, A. K., Singh, R. P. 2005. Detection of seed mycoflora of chick pea (*Cicer arietinum* L.). Ann. Plant Prot. Sci. 13: 167–171.

Singh, N.D., Sharma, A.K., Dwivedi, P., Patil, R.D. and Kumar, M. 2007. Citrinin and endosulfan induced teratogenic effects in Wistar rats. J. Appl. Toxicol. 27: 143–151.

Singh, P. and Ahmad, S.K. 1989. Prestorage aflatoxin contamination and *Aspergillus flavus* association with various growth stages of chickpea (*Cicer arietinum*). Ann. Agric. Sci. 34: 805–812.

Singh, P.K. and Shukla, A.N. 2005. Studies on mycoflora and natural occurrence of mycotoxins in *Prunus armeniaca* Linn. Indian J. For. 28: 397–399.

Singh, P.K. and Shukla, A.N. 2008. Survey of mycoflora counts, aflatoxin production biochemical changes in walnut kernels. J. Stored Prod. Res. 44: 169–172.

Singh, R.S. and Prashar, M. 1988. Two new post-harvest diseases of peach in India. J. Res. (Punjab Agric. Univ.) 25: 417–418.

Singh, S. 2005. Fungal chitin and its use for estimation of mycorrhizal infection. Mycorrhiza News 17(3): 2–9.

Sinha, A.K. and Ranjan, K.S. 1991. A report of mycotoxin contamination in Bhutanese cheese. J. Food Sci. Technol. India 28: 398–399.

Sinha, K.K., Bilgrami, K.S., Ranjan, K.S. and Prasad, T. 1988. Incidence of aflatoxins in mustard crop in Bihar. Indian Phytopathol. 41: 434–437.

Sinha, P., Sharma, R.P. and Roy, M.K. 1994. Management of storage rot in onion through gamma irradiation and chemicals. J. Food Sci. Technol. India 31: 341–343.

Sinha, R.N. and Wallace, H.A.H. 1977. Storage stability of farm-stored rapeseed and barley. Can. J. Plant Sci. 57: 351–365.

Sinha, R.N., Muir, W.E. and Sanderson, D.B. 1985. Quality assessment of stored wheat during drying with near-ambient temperature air. Can. J. Plant Sci. 65: 849–866.

Sinha, R.N., Tuma, D., Abramson, D. and Muir, W.E. 1988. Fungal volatiles associated with moldy grain in ventilated and nonventilated bin-stored wheat. Mycopathologia 101: 53–60.

Sinigaglia, M., Albenzio, M., Corbo, M.R. and Ciccarone, C. 2004. Comparison of mycoflora associated to Canestrato Pugliese cheese produced according to three protocols. Sci. Aliments 24: 159–172.

Sio, F. de, Laratta, B., Giovane, A., Quagliuolo, L., Castaldo, D. and Servillo, L. 2000. Analysis of free and

esterified ergosterol in tomato products. J. Agric. Food Chem. 48: 780–784.
Siriacha, P., Kawashima, K., Okazaki, H., Saito, M., Kawasugi, S. and Tonboon-Ek, P. 1988. *Aspergillus flavus* and aflatoxin contamination of maize ears and kernels in Thailand. Maikotokishin (Proc. Jpn. Assoc. Mycotoxicol.) 28: 23–28.
Sivanesan, A. 1987. Graminicolous species of *Bipolaris, Curvularia, Drechslera, Exserohilum* and their teleomorphs. Mycol. Papers 158: 1–261.
Skirdal, I.M. and Eklund, T. 1993. Microculture model studies on the effect of sorbic acid on *Penicillium chrysogenum*, *Cladosporium cladosporioides* and *Ulocladium atrum* at different pH levels. J. Appl. Bacteriol. 74: 191–195.
Skou, J.P. 1969. The effect of temperature on the growth and survival of *Aureobasidium pullulans* and of the radulasporic stage of *Guignardia fulvida* and *Sydowia polyspora*. Friesia 9: 226–236.
Skou, J.P. 1975. Two new species of *Ascosphaera* and notes on the conidial state of *Bettsia alvei*. Friesia 11: 62–74.
Skou, J.P. 1992. A series of xerophilic *Chrysosporium* species. Mycotaxon 43: 237–259.
Smilanick, J.L. and Denis-Arrue, R. 1992. Control of green mold of lemons with *Pseudomonas* species. Plant Dis. 76: 481–485.
Smilanick, J.L., Mansour, M.F., Mlikota Gabler, F. and Goodwine, W.R. 2006. The effectiveness of Pyrimethanil to inhibit germination of *Penicillium digitatum* and to control citrus green mold after harvest. Postharvest Biol. Technol. 42: 75–85.
Smith, B.J. and Gupton, C.L. 1993. Calcium applications before harvest affect the severity of anthracnose fruit rot of greenhouse-grown strawberries. Acta Hortic. 348: 477–482.
Smith, D. 1984. Maintenance of fungi. *In* Maintenance of Microorganisms. A Manual of Laboratory Methods, eds B.E. Kirsop and J.J. Snell. London: Academic Press. pp. 83–107.
Smith, D. and Onions, A.H.S. 1983. A comparison of some preservation techniques for fungi. Trans. Br. Mycol. Soc. 81: 535–540.
Smith, D. and Onions, A.H.S. 1994. The Preservation and Maintenance of Living Fungi. 2nd edn. Wallingford, UK: CAB International.
Smith, G. 1939. Some new species of mould fungi. Trans. Br. Mycol. Soc. 22: 252–256.
Smith, G. 1949. The effect of adding trace elements to Czapek-Dox medium. Trans. Br. Mycol. Soc. 32: 280–283.
Smith, J.P. 1994. Modified atmosphere packaging of bakery products. Tech. Bull., Am. Inst. Baking Res. Dept 16: 1–9.
Smith, K.P., Havey, M.J. and Handelman, J. 1993. Suppression of cottony leak of cucumber with *Bacillus cereus* strain UW85. Plant Dis. 77: 139–142.
Smith, M.Th. and Yarrow, D. 1995. Yeasts. *In* Introduction to Foodborne Fungi, 4th edn, eds R.A. Samson, E.S. van Hoekstra, J.C. Frisvad and O. Filtenborg. Baarn, Netherlands: Centraalbureau voor Schimmelcultures. pp. 222–234.
Smith, S.L. and Hill, S.T. 1982. Influence of temperature and water activity on germination and growth of *Aspergillus restrictus* and *A. versicolor*. Trans. Br. Mycol. Soc. 79: 558–560.
Smoot, J.J. and Segall, R.H. 1963. Hot water as a postharvest treatment of mango anthracnose. Plant Dis. Rep. 47: 739–742.
Snow, D. 1949. Germination of mould spores at controlled humidities. Ann. Appl. Biol. 36: 1–13.
Snowdon, A.L. 1990. A Colour Atlas of Post-harvest Diseases and Disorders of Fruits and Vegetables. 1. General Introduction and Fruits. London: Wolfe Scientific.
Snowdon, A.L. 1991. A Colour Atlas of Post-Harvest Diseases and Disorders of Fruits and Vegetables. 2. Vegetables. London: Wolfe Scientific.
Soldevilla, C., Vazquez, C., Patino, B., Jurado, M. and Gonzalez-Jaen, M.T. 2005. Toxicogenic fungi associated to wheats and barleys of Castilla and Leon. Boletin de Sanida Vegetal, Plagas 31: 519–529.
Solieri, L., Landi, S., De Vero, L. and Giudici, P. 2006. Molecular assessment of indigenous yeast population from traditional balsamic vinegar. J. Appl. Microbiol. 101: 63–71.
Solfrizzo, M., Strange, R.N., Sabia, C. and Visconti, A. 1994. Production of a toxin stemphol by *Stemphylium* species. Nat. Toxins 2: 14–18.
Somani, A.K. 2004. Potato tuber rots and associated incitants. Potato J. 31: 201–204.
Sonjak, S., Frisvad, J.C. and Gunde-Cimerman, N. 2005. Comparison of secondary metabolite production by *Penicillium crustosum* strains, isolated from Arctic and other various ecological niches. FEMS Microbiol. 53: 51–60.
Sonoda, R.M., Hayslip, N.C. and Stoffella, P.J. 1981. Tomato fruit rot infection cycle in a fresh market packing operation. Proc. Fla State Hortic. Soc. 94: 281–282.
Sørensen, B.B. and Jakobsen, M. 1997. The combined effects of temperature, pH and NaCl on growth of *Debaryomyces hansenii* analyzed by flow cytometry and predictive microbiology. Int. J. Food Microbiol. 34: 209–220.
Sørensen, J.L., Nielsen, K.F. and Thrane, U. 2007. Analysis of moniliformin in maize plants using hydrophilic interaction chromatography. J. Agric. Food Chem. 55: 9764–9768.
Sørensen, L.M., Jacobsen, T., Nielsen, P.V., Frisvad, J.C. and Koch, A.G. 2008. Mycobiota in the processing areas of two different meat products. Int. J. Food Microbiol. 124: 58–64.
Sorenson, W.G., Hesseltine, C.W. and Shotwell, O.L. 1966. Effect of temperature on production of aflatoxin on rice by *Aspergillus flavus*. Mycopathol. Mycol. Appl. 33: 49–55.
Souheil, H., Vey, A., Thuet, P. and Trilles, J.P. 1999. Pathogenic and toxic effects of *Fusarium oxysporum* (Schlecht.) on survival and osmoregulatory capacity of *Penaeus japonicus* (Bate). Aquaculture 178: 209–224.
Souza, E.L. de, Sousa, C.P. de, Lima E.de O. and Freire, K. R.L. 2003. Isolation and identification of filamentous fungi in cassava flour (*Manihot esculenta* Crantz) and maize meal (*Zea mays* L.) sold in markets in the city of Joao Pessao, PB. Higiene Aliment. 17: 34–39.
Sousa, M.J., Teixeira, J.A. and Mota, M. 1993. Must deacidification with an induced flocculant yeast strain of *Schizosaccharomyces pombe*. Appl. Microbiol. Biotech. 39: 189–193.
Sousa, M.J., Mota, M. and Leao, C. 1995. Effects of ethanol and acetic acid on the transport of malic acid and glucose in

the yeast *Schizosaccharomyces pombe*: implications in wine deacidification. FEMS Microbiol. Lett. 126: 197–202.

Sousa, M.J., Miranda, L., CorteReal, M. and Leao, C. 1996. Transport of acetic acid in *Zygosaccharomyces bailii*: Effects of ethanol and their implications on the resistance of the yeast to acidic environments. Appl. Environ. Microbiol. 62: 3152–3157.

Southern, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98: 503–517.

Southwell, R.J., Moore, K.J., Manning, W. and Hayman, P. T. 2003. An outbreak of Fusarium head blight of durum wheat on the Liverpool Plains in northern New South Wales in 1999. Aust. Plant Pathol. 32: 465–471.

Spangenberg, D.S. and Ingham, S.C. 2000. Comparison of methods for enumeration of yeasts and molds in shredded low-moisture, part-skim Mozzarella cheese. J. Food Prot. 63: 529–533.

Sparks, D. 2007. Pink mould of pecan kernels in the southeastern USA is associated with scab, above-normal temperatures, and humidity during nut maturation. J. Hort. Sci. Biotechnol. 82: 946–950.

Spicher, G. 1984a. Die Erreger der Schimmelbildung bei Backwaren. I. Die auf verpackten Schnittbroten aufretenden Schimmelpilze. Getreide Mehl Brot 38: 77–80.

Spicher, G. 1984b. Die Erreger der Schimmelbildung bei Backwaren. III. Einige Beobachtungen über die Biologie der Erreger de "Kreidekrankheit" des Brotes. Getreide Mehl Brot: 38: 178–182.

Spicher, G. 1985. Die Erreger der Schimmelbildung bei Backwaren. I. Weitere Untersuchungen über die auf verpackten Schnittbroten aufretenden Schimmelpilze. Dtsch. Lebensm. Rundsch. 81: 16–20.

Spicher, G. 1986a. Die Sauerteiggärung. Chem. Mikrobiol. Technol. Lebensm. 10: 65–77.

Spicher, G. 1986b. Neue Erkenntnisse über die Erreger der 'Kreidekrankheit' des Brotes und Möglichkeiten zur Wachstumsverhinderung. Brot Backwaren 34: 208–213.

Spicher, G. and Isfort, G. 1987. Die Erreger der Schimmelbildung bei Backwaren. IX. Die auf vorgebackenen Brötchen, Toast- und Weichbrötchen auftretenden Schimmelpilze. Dtsch. Lebensm. Rundsch. 83: 246–249.

Spicher, G. and Isfort, G. 1988. Die Erreger der Schimmelbildung bei Backwaren. X. *Monascus ruber*, ein nicht alltäglicher Schimmelerreger des Brotes. Getreide Mehl Brot 42: 176–181.

Spicher, G. and Mellenthin, B. 1983. Zur Frage der mikrobiologischen Qualität von Getreidevollkornerzeugnissen. III. Die bei Speisegetreide und Mehlen auftretenden Hefen. Dtsch. Lebensm. Rundsch. 79: 35–38.

Spicher, G., Schröder, R. and Schöllhammer, K. 1979. Die Mikroflora des Sauerteiges. VII. Untersuchungen über die Art der in 'Reinzuchtsauern' auftretenden Hefen. Z. Lebensm. Unters. Forsch. 169: 71–81.

Splittstoesser, D.F. 1987. Fruits and fruit products. *In* Food and Beverage Mycology, 2nd edn, ed. L.R. Beuchat. New York: Van Nostrand Reinhold. pp. 101–128.

Splittstoesser, D.F., Kuss, F.R., Harrison, W. and Prest, D. B. 1971. Incidence of heat-resistant molds in Eastern orchards and vineyards. Appl. Microbiol. 21: 335–337.

Splittstoesser, D.F., Leasor, S.B. and Swanson, K.M.J. 1986. Effect of food composition on the heat resistance of yeast ascospores. J. Food Sci. 51: 1265–1267.

Splittstoesser, D.F., Lammers, J.M., Downing, D.L. and Churey, J.J. 1989. Heat resistance of *Eurotium herbariorum*, a xerophilic mold. J. Food Sci. 54: 683–685.

Sponholz, W.R., Lacher, M. and Dittrich, H.H. 1986. Die Bildung von Alditolen durch die Hefen des Weines. Chem. Mikrobiol. Technol. Lebensm. 10: 19–24.

Spotti, E., Mutti, P. and Campanini, M. 1988. Microbiological study of the 'phenol defect' of ham during ripening. Ind. Conserve 63: 343–346.

Spotti, E., Mutti, P. and Campanini, M. 1989. Occurrence of moulds on hams during preripening and ripening, contamination of the environment and growth on the muscle portion of hams. Ind. Conserve 64: 110–113.

Spotti, E., Quintavalla, S. and Mutti, P. 1992. Contaminazione da spore fungine termoresistenti di frutta, pomodoro e loro derivati. Ind. Conserve 67: 421–425.

Spotti, E., Mutti, P. and Scalari, F. 1994. *P. nalgiovense, P. gladioli, P. candidum and A. candidus*: possibility of their use as starter cultures. Ind. Conserve 69: 237–241.

Spotti, E., Chiavaro, E., Pari, E. and Busolli, C. 2001. Growth of *Penicllium verrocosum* in model systems based on raw ripened meat products. Part II. Ochratoxin A determination and comparison between a rapid immunofluorometric method and traditional RP-HPLC technique. Ind. Conserve 76: 167–183.

Spotts, R.A. and Cervantes, L.A. 1989. Evaluation of disinfestant–flotation salt–surfactant combinations on decay fungi of pear in a model dump tank. Phytopathology 79: 121–126.

Spotts, R.A. and Cervantes, L.A. 1993. Use of filtration for removal of conidia of *Penicillium expansum* from water in pome fruit packinghouses. Plant Dis. 828–830.

Spotts, R.A., Seradi, M., Mielke, E.A., Bai, J.H., Chen, P.M., Hansen, J.D., Neven, L.G. and Sanderson, P.G. 2006. Effect of high-pressure hot water washing treatment on fruit quality, insects, and disease in apples and pears. Part II. Effect on postharvest decay of d'Anjou pear fruit. Postharvest Biol. Technol. 40: 216–220.

Srinivasulu, B., Aruna, K., Kumar, K.V.K. and Rao, D.V.R. 2003. Investigations on postharvest aflatoxin contamination of copra. Indian Coconut J. 34: 8–9.

Stankovic, S., Levic, J., Petrovic, T., Logrieco, A. and Moretti, A. 2007. Pathogenicity and mycotoxin production by *Fusarium proliferatum* isolated from onion and garlic in Serbia. Eur. J. Plant Pathol. 118: 165–172.

Steenkamp, E.T., Wingfield, B.D., Desjardins, A.E., Marasas, W.F.O. and Wingfield, M.J. 2002. Cryptic speciation in *Fusarium subglutinans*. Mycologia 94: 1032–1043.

Steinbuch, E. 1965. Preparation of sauerkraut. Ann. Rept. Sprenger Institute, Wageningen, Netherlands. 1965: 56–57.

Steinbuch, E. 1966. Manufacturing of sauerkraut. Ann. Rept. Sprenger Institute, Wageningen, Netherlands 1966: 47.

Steiner, W.E., Rieker, R.H. and Battaglia, R. 1988. Aflatoxin contamination in dried figs: distribution and association with fluorescence. J. Agric. Food Chem. 36: 88–91.

Stenwig, H. and Liven, E. 1988. Mycological examination of improperly stored grains. Acta Agric. Scand. 38: 199–205.

Steyaert, R.H. 1949. Contribution à l'étude monographique de *Pestalotia* et *Monochaetia* (*Truncatella* gen. nov. et

Pestalotiopsis gen. nov.). Bull. Jard. Bot. Etat. Brux. 19: 285–354.

Stolk, A.C. 1969. Four new species of *Penicillium*. Antonie van Leeuwenhoek 35: 261–274.

Stolk, A.C. and Dakin, J.C. 1966. *Moniliella*, a new genus of Moniliales. Antonie van Leeuwenhoek 32: 399–409.

Stolk, A.C. and Samson, R.A. 1972. The genus *Talaromyces*. Studies on *Talaromyces* and related genera. II. Stud. Mycol., Baarn 2: 1–65.

Stolk, A.C. and Samson, R.A. 1983. The Ascomycete genus *Eupenicillium* and related *Penicillium* anamorphs. Stud. Mycol. Baarn 23: 1–149.

Stolk, A.C. and Samson, R.A. 1985. A new taxonomic scheme for *Penicillium* anamorphs. *In* Advances in *Penicillium* and *Aspergillus* Systematics, eds R.A. Samson and J.I. Pitt. New York: Plenum Press. pp. 163–192.

Stolk, A.C. and Scott, D.B. 1967. Studies on the genus *Eupenicillium* Ludwig. I. Taxonomy and nomenclature of Penicillia in relation to their sclerotioid ascocarpic states. Persoonia 4: 391–405.

Stoloff, L. 1977. Aflatoxins – an overview. *In* Mycotoxins in Human and Animal Health, eds J.V. Rodricks, C.W. Hesseltine and M.A. Mehlman. Park Forest South, IL: Pathotox Publishers. pp. 7–28.

Stoloff, L. and Friedman, L. 1976. Information bearing on the evaluation of the hazard to man from aflatoxin ingestion. PAG Bull. 6: 21–23.

Stoloff, L. and Trucksess, M.W. 1981. Effect of boiling, frying and baking on recovery of aflatoxin from naturally contaminated corn grits and cornmeal. J. Assoc. Off. Anal. Chem. 64: 678–680.

Stotzky, G. and Goos, R.D. 1965. Effect of high CO_2 and low O_2 tensions on the soil microbiota. Can. J. Microbiol. 11: 853–868.

Stratford, M. and Anslow, P.A. 1998. Evidence that sorbic acid does not inhibit yeast as a classic 'weak acid' preservative. Lett. Appl. Microbiol. 27: 203–206.

Stratford, M. and James, S.A. 2003. Non-alcoholic beverages and yeasts. *In* Yeasts in Food, eds T. Boekhout and V. Robert. Cambridge, UK: Woodhead Publishing. pp. 309–345.

Stratford, M. and Lambert, R.J.W. 1999. Weak-acid preservatives: mechanisms of adaptation and resistance by yeasts. Food Aust. 51: 26–29.

Stratford, M. Plumridge, A. and Archer, D.B. 2007. Decarboxylation of sorbic acid by spoilage yeasts is associated with the PAD1 gene. Appl. Environ. Microbiol. 73: 6534–6542.

Strosnider, H., Azziz-Baumgartner, E., Banziger, M., Bhat, R.V., Breiman, R., Brune, M.N., DeCock, K., Dilley, A., Groopman, J., Hell, K., Henry, S.H., Jeffers, D., Jolly, C., Jolly, P., Kibata, G.N., Lewis, L., Liu, X.M., Luber, G., McCoy, L., Mensah, P., Miraglia, M., Misore, A., Njapau, H., Ong, C.N., Onsongo, M.T.K., Page, S.W., Park, D., Patel, M., Phillips, T., Pineiro, M., Pronczuk, J., Rogers, H.S., Rubin, C., Sabino, M., Schaafsma, A., Shephard, G., Stroka, J., Wild, C., Williams, J.T. and Wilson, D. 2006. Workgroup report: Public health strategies for reducing aflatoxin exposure in developing countries. Environ. Health. Perspect. 114: 1898–1903.

Stubbs, S., Hutson, R., James, S. and Collins, M.D. 1994. Differentiation of the spoilage yeast *Zygosaccharomyces bailii* from other *Zygosaccharomyces* species using 18S rDNA as target for a non-radioactive ligase detection reaction. Lett. Appl. Microbiol. 19: 268–272.

Studer-Rohr, I., Dietrich, D.R., Schlatter, J. and Schlatter, C. 1995. The occurrence of ochratoxin A in coffee. Food Chem. Toxicol. 33: 341–355.

Stynen, D., Meulmans, L., Goris, A., Braendlin, N., and Symons, N. 1992. Characteristics of a latex agglutination test based on monoclonal antibodies for the detection of fungal antigens in food. *In* Modern Methods in Food Mycology, eds R.A. Samson, A.D. Hocking, J.I. Pitt and A.D. King. Amsterdam: Elsevier. pp. 213–219.

Suarez-Lepe, J.A. 1991. New biotechnology of preparation of base wines for manufacture of naturally sparkling wines. Ind. Bevande 20: 263–265, 269.

Suárez-Quiroz, M., de Louise, B., Gonzalez-Rios, O., Barel, M., Guyot, B., Schorr-Galindo, S. and Guiraud, J.-P. 2005. The impact of roasting on the ochratoxin A content of coffee. Int. J. Food Sci. Technol. 40: 605–611.

Subbarao, K.V., Michailides, T.J. and Morgan, D.P. 1993 Effects of osmotic potential and temperature on growth of two pathogens of figs and a biocontrol agent. Phytopathology 83: 1454–1459.

Subden, R.E., Husnik, J.I., van Twest, R., van der Merwe, G. and van Vuuren, H.J.J. 2003. Autochthonous microbial population in a Niagara Peninsula icewine must. Food Res. Int. 36: 747–751.

Subramanian, C.V. 1971. Hyphomycetes: An Account of Indian Species Except Cercosporae. New Delhi: Indian Council of Agricultural Research.

Sud, D., Sharma, O.P. and Sharma, P.N. 2005. Seed mycoflora in kidney bean (*Phaseolus vulgaris* L.) in Himachal Pradesh. Seed Res. 33: 103–107.

Sugihara, T.F., Kline, L. and Miller, M.W. 1971. Microorganisms of the San Francisco sour dough bread process. I. Yeasts responsible for leavening action. Appl. Microbiol. 21: 456–458.

Sugita-Konishi, Y., Nakajima, M., Tabata, S., Ishikuro, E., Tanaka, T., Norizuki, H. Itoh, Y., Aoyama, K., Fujita, K., Kai, S. and Kumagai, S. 2006. Occurrence of aflatoxins, ochratoxin A, and fumonisins in retail foods in Japan. J. Food Prot. 69: 1365–1370.

Sugiura, Y., Watanabe, Y., Tanaka, T., Yamamoto, S. and Ueno, Y. 1990. Occurrence of *Gibberella zeae* strains that produce both nivalenol and deoxynivalenol. Appl. Environ. Microbiol. 56: 3047–3051.

Sugiura, Y., Barr, J.R., Barr, D.B., Brock, J.W., Elie, C.M., Ueno, Y., Patterson, D.G.Jr., Potter, M.E. and Reiss, E. 1999. Physiological characteristics and mycotoxins of human clinical isolates of *Fusarium* species. Mycol. Res. 103: 1462–1468.

Suhr, K.I. and Nielsen, P.V. 2004. Effect of weak acid preservatives on growth of bakery product spoilage fungi at different water activities and pH values. Int. J. Food Microbiol. 95: 67–78.

Suhr, K.I. and Nielsen, P.V. 2005. Inhibition of fungal growth on wheat and rye bread by modified atmosphere packaging and active packaging using volatile mustard essential oil. J. Food Sci. 70: M37–M44.

Suihko, M.-L. and Mäkinen, V. 1984. Tolerance of acetate, propionate and sorbate by *Saccharomyces cerevisiae* and *Torulopsis holmii*. Food Microbiol. 1: 105–110.

Sujaya, I.N., Antara, N.S., Sone, T., Tamura, Y., Aryanta, W.R., Yokota, A., Asano, K. and Tomita, F. 2004. Identification and characterization of yeasts in brem, a traditional Balinese rice wine. World J. Microbiol. Biotechnol. 20: 143–150.

Sumbali, G. and Sharma, Y.P. 1997. Mycoflora associated with cut dried fruits of quinces (*Cydonia oblonga* Mill.) from Jammu province. Microbial Biotechnol.: 151–155.

Sumi, Y., Nagura, H. and Miyakawa, M. 1990. Induction of pituitary tumors in germ-free rats exposed to *Aspergillus versicolor*. Cancer Res. 50: 400–402.

Sumi, Y., Nagura, H., Takeuchi, M. and Miyakawa, M. 1994. Granulomatous lesions in the lung induced by inhalation of mold spores. Virchows Arch. 424: 661–668.

Summerell, B.A., Salleh, B. and Leslie, J.F. 2003. A utilitarian approach to *Fusarium* identification. Plant Dis. 87: 117–128.

Sun, G., Zhang, M., Zhang, R., Ma, H.N. and Gleason, M.L. 2006. *Wallemia* – a genus newly recorded from China. Mycotaxon 95: 277–280.

Sunesen, L.O. and Stahnke, L.H. 2003. Mould starter cultures for dry sausages – selection, application and effects. Meat Sci. 65: 935–948.

Sunesson, A.L., Vaes, W.H.J., Nilsson, C.A., Blomquist, G. R., Andersson B. and Carlson, R. 1995. Identification of volatile metabolites from five fungal species cultivated on two media. Appl. Environ. Microbiol. 61: 2911–2918.

Suresh, E.R., Onkarayya, H. and Ethiraj, S. 1982. A note on the yeast flora associated with fermentation of mango. J. Appl. Bacteriol. 52: 1–4.

Suriyarachchi, V.R. and Fleet, G.H. 1981. Occurrence and growth of yeasts in yogurts. Appl. Environ. Microbiol. 42: 574–579.

Susamma, P. and Menon, M.R. 1981. Microorganisms associated with the deterioration of copra. Madras Agric. J. 68: 686–687.

Susamma, P., Menon, M.R. and Alice, A. 1981. Quantitative changes in the amino acids of copra due to fungal infection. J. Food Sci. Technol. India 18: 254–255.

Susuri, L. and Doda-Gashi, N. 2003. Alfafa seed microflora and some characteristics of obtained isolates. Fragmenta Phytomed. Herbol. 28: 81–88.

Sutton, B.C. 1980. The Coelomycetes: Fungi Imperfecti with Pycnidia, Acervuli and Stromata. Kew: Commonwealth Mycological Institute.

Suzuki, T., Kurisu, M., Hoshino, Y., Ichinoe, M., Nose, N., Tokumaru, Y. and Watanabe, A. 1980. Production of trichothecene mycotoxins of *Fusarium* species in wheat and barley harvested in Saitama prefecture. Shokuhin Eiseigaku Zasshi (J. Food Hyg. Soc. Jpn) 21: 43–49.

Suzuki, T., Kurisu, M. and Ichinoe, M. 1981. Trichothecenes-producing fungi of *Fusarium* species. Maikotokishin (Proc. Jpn. Assoc. Mycotoxicol.) 13: 34–36.

Suzzi, G., Schirone, M., Martuscelli, M., Gatti, M., Fornasari, M.E. and Neviani, E. 2003. Yeasts associated with Manteca. FEMS Yeast Res. 3: 159–166.

Swart, A.W. and Holz, G. 1994. Colonization of table grape bunches by *Alternaria alternata* and rot of cold-stored grapes. S. Afr. J. Enol. Vitic. 15: 19–25.

Sydenham, E.W., Thiel, P.G., Marasas, W.F.O., Shephard, G.S., van Schalkwyk, D.J. and Koch, K.R. 1990. Natural occurrence of some *Fusarium* mycotoxins in corn from low and high esophageal cancer prevalence areas of the Transkei, Southern Africa. J. Agric. Food Chem. 38: 1900–1903.

Sydenham, E.W., Marasas, W.F.O., Thiel, P.G., Shephard, G. S. and Nieuwenhuis, J.J. 1991. Production of mycotoxins by selected *Fusarium graminearum* and *F. crookwellense* isolates. Food Addit. Contam. 8: 31–41.

Szczerbanik, M., Jobling, J., Morris, S. and Holford, P. 2007. Essential oil vapours control some common postharvest fungal pathogens. Aust. J. Exp. Agric. 47: 103–109.

Szekely, J.G., Chelack, W.S., Delany, S., Marquardt, R.R. and Frohlich, A.A. 1991. Scanning electron microscope observations of growth and ochratoxin A production of *Aspergillus alutaceus* variety *alutaceus* (formerly *A. ochraceus*) on gamma-irradiated barley. Food Struct. 10: 295–302.

Tabuc, C., Bailly, J.D., Bailly, S., Querin, A. and Guerre, P. 2004. Toxigenic potential of fungal mycoflora isolated from dry cured meat products: preliminary study. Rev. Med. Vet. 155: 287–291.

Tafuri, A., Ferracane, R. and Ritieni, A. 2004. Ochratoxin A in Italian marketed cocoa products. Food Chem. 88: 487–494.

Takahashi, H., Yasaki, H., Nanayama, U., Manabe, M. and Matsuura, S. 1984. Distribution of sterigmatocystin and fungal mycelium in individual brown rice kernels naturally infected by *Aspergillus versicolor*. Cereal Chem. 61: 48–52.

Takahashi, H., Uematsu, S., Oizumi, T., Mori, E., Yanagihori, S. and Ichinoe, M. 1995. Pink mold rot of muskmelons and tomato fruits and trichothecin production by *Trichothecium roseum*. Maikotokishin (Proc. Jpn. Assoc. Mycotoxicol.) 41: 53–59.

Takatori, K., Watanabe, K., Udagawa, S. and Kurata, H. 1977. Studies on the contamination of fungi and mycotoxins in spices. I. Mycoflora of imported spices and inhibitory effects of the spices on the growth of some fungi. Maikotokishin (Proc. Jpn. Assoc. Mycotoxicol.) 1: 36–38.

Taligoola, H.K., Ismail, M.A. and Chebon, S.K. 2004. Mycobiota associated with rice grains marketed in Uganda. J. Biol. Sci. 4: 271–278.

Tam, J., Mankotia, M., Mably, M., Pantazopoulos, P., Neil, R.J., Calway, P. and Scott, P.M. 2006. Survey of breakfast and infant cereals for aflatoxins B1, B2, G1 and G2. Food Addit. Contam. 23: 693–699.

Tamang, J.P. and Sarkar, P.K. 1995. Microflora of murcha – an amylolytic fermentation starter. Microbios 81: 115–122.

Tammer, B., Lehmann, I., Nieber, K. and Altenburger, R. 2007. Combined effects of mycotoxin mixtures on human T cell function. Toxicol. Lett. 170: 124–133.

Tan, M.K. and Niessen, L.M. 2003. Analysis of rDNA ITS sequences to determine genetic relationships among, and provide a basis for simplified diagnosis of, *Fusarium* species causing crown rot and head blight of cereals. Mycol. Res. 107: 811–821.

Tan, M.K., Simpfendorfer, S., Backhouse, D. and Murray, G.M. 2004. Occurrence of Fusarium head blight (FHB) in southern NSW in 2000: identification of causal fungi and determination of putative chemotype of *Fusarium graminearum* isolates by PCR. Australas. Plant Pathol. 33: 385–392.

Tanaka, K., Kushiro, M and Manabe, M. 2006. A review of studies and measures to improve the mycotoxical safety of traditional Japanese mold-fermented foods. Mycotoxin Res. 22: 153–158.

Tanaka, M., Sumida, T. and Mitsui, R. 2000. Effect of culture conditions on production of cellulases and xylanases by *Eupenicillium javanicum* – Note. Seibuttsu-Kogaku Kaishi 78: 43–47.

Tandon, M.P., Bhargava, V. and Jamaluddin. 1975. Some new fruit rot diseases. Curr. Sci. 44: 708.

Tangni, E.K. and Pussemier, L. 2007. Ergosterol and mycotoxins in grain dusts from fourteen Belgian cereal storages: a preliminary screening survey. J. Sci. Food Agric. 87: 1263–1270.

Taniwaki, M.H. 1995. Growth and mycotoxin production by fungi under modified atmospheres. Ph.D. thesis. Kensington, NSW: University of New South Wales.

Taniwaki, M.H., Pitt, J.I., Urbano, G.R., Teixeira, A.A. and Leitão M.F.F. 1999. Fungi producing ochratoxin A in coffee. Proceedings of the 18th ASIC Coffee Conference, Helsinki, Finland. pp 239–247.

Taniwaki, M.H., Hocking, A.D., Pitt, J.I. and Fleet, G.H. 2001a. Growth of fungi and mycotoxin production on cheese under modified atmospheres. Int. J. Food Microbiol. 68: 125–133.

Taniwaki, M.H., Silva, N. da, Banhe, A.A. and Iamanaka, B. T. 2001b. Comparison of culture media, Simplate, and Petrifilm for enumeration of yeasts and molds in food. J. Food Prot. 64: 1592–1596.

Taniwaki, M.H., Pitt, J.I., Teixeira, A.A. and Iamanaka, B. T. 2003. The source of ochratoxin A in Brazilian coffee and its formation in relation to processing methods. Int. J. Food Microbiol. 82: 173–179.

Taniwaki, M.H., Teixeira, A.A., Teixeira, A.R.R., Meletti, M.A., Iamanaka, B.T., Carvalho, M.A., Pfenning, L.H., Almeida, A.R. and Pitt, J.I. 2005. The influence of fungi on the flavour of coffee beverages. ASIC 20th Int. Conf. Coffee Sci. Bangalore, India, 11–15 October 2004, pp. 317–321.

Taniwaki, M.H., Pitt, J.I., Hocking, A.D. and Fleet, G.H. 2006. Comparison of hyphal length, ergosterol, mycelium dry weight and colony diameter for quantifying growth of fungi from foods, *In*: Advances in Food Mycology, A.D. Hocking, J.I. Pitt, R.A. Samson and U. Thrane eds. New York: Springer. pp. 49–67.

Tariq, M., Dawar, S., Abid, M. and Shaukat, S.S. 2005. Seedborne mycoflora of soybean. Int. J. Biol. Biotech. 2: 711–713.

Teakle, D.S. 1965. *Fusarium* rhizome rot of ginger in Queensland. Queensl. J. Agric. Anim. Sci. 23: 263–272.

Teixeira, H., Machado, J. da C., Oride, D., Alves, M. C. and Noda, A. 2005. Water restriction technique: effect on *Acremonium strictum*, seeds protrusion and obtaining of maize seeds infected. Fitopatologia Bras. 30: 109–114.

Teixeira, M.A., Bettiol, W. and Cesnik, R. 2001. Pathogenicity of *Colletotrichum gloesporioides*, *Orthezia praelonga* pathogenic agent, to citrus leaves, flowers and fruits. Summa Phytopathol. 27: 352–357.

Tekauz, A., McCallum, B., Ames, N. and Fetch, J.M. 2004. Fusarium head blight of oat – current status in western Canada. Can. J. Plant Pathol. 26: 473–479.

Teoh, A.L., Heard, G. and Cox, J. 2004. Yeast ecology of Kombucha fermentation. Int. J. Food Microbiol. 95: 119–126.

Terao, K. 1983. Sterigmatocystin – a masked potent carcinogenic mycotoxin. J. Toxicol. Tox. Rev. 2: 77–110.

Terao, K., Ito E., Udagawa, S., Kawai, K. and Nozawa, K. 1988. Acute intoxication induced by emestrin, a new mycotoxin from *Emericella* species. Maikotokishin (Proc. Jpn. Assoc. Mycotoxicol.) 27: 31–32.

Terao, K., Ito, E., Kawai, K., Nozawa, K. and Udagawa, S. 1990. Experimental acute poisoning in mice induced by emestrin, a new mycotoxin isolated from *Emericella* species. Mycopathologia 112: 71–79.

Téren, J., Varga, J., Hamari, Z., Rinyu, E. and Kevei, F. 1996. Immunochemical detection of ochratoxin A in black *Aspergillus* strains. Mycopathologia 134: 171–176.

Téren, J., Palagyi, A and Varga, J. 1997. Isolation of ochratoxin producing Aspergilli from green coffee beans of different origin. Cereal Res. Commun. 25: 305–306.

Teuber, M. and Engel, G. 1983. Low risk of mycotoxin production in cheese. Microbiol. Aliments Nutr. 1: 193–197.

Tharappan, B. and Ahmad, R. 2006. Fungal colonization and biochemical changes in coffee beans undergoing monsooning. Food Chem. 94: 247–252.

Theron, D.J. and Holz, G. 1990. Effect of temperature on dry rot development of potato tubers inoculated with different *Fusarium* spp. Potato Res. 33: 109–117.

Thom, C. and Raper, K.B. 1941. The *Aspergillus glaucus* group. U.S. Dep. Agric. Misc. Pub. 426: 1–46.

Thomas, D.S. and Davenport, R.R. 1985. *Zygosaccharomyces bailii* – a profile of characteristics and spoilage activities. Food Microbiol. 2: 157–169.

Thrane, U. 1988. Screening for fusarin C production by European isolates of *Fusarium* species. Mycotoxin Res. 4: 2–10.

Thrane, U. 2001. Developments in the taxonomy of *Fusarium* species based on secondary metabolites. *In Fusarium*: Paul E. Nelson Memorial Symposium, eds B.A. Summerell, J. F. Leslie, D. Backhouse, W.L. Bryden and L.W. Burgess. St Paul, MN: APS Press. pp. 29–49.

Thrane, U., Adler, A., Clasen, P.-E., Galvano, F., Langseth, W., Lew, H., Logrieco, A., Nielsen, K.F. and Ritieni, A. 2004. Diversity in metabolite production by *Fusarium langsethiae*, *Fusarium poae*, and *Fusarium sporotrichioides*. Int. J. Food Microbiol. 95: 257–266.

Timmer, L.M., Brown, G.E. and Zitko, S.E. 1998. The role of *Colletotrichum* spp. in postharvest anthracnose of citrus and survival of *C. acutatum* on fruit. Plant Dis. 82: 415–418.

Tilbury, R.H. 1980. Xerotolerant yeasts at high sugar concentrations. *In* Microbial Growth and Survival in Extreme Environments, eds G.W. Gould and J.E.L. Corry. Tech. Ser., Soc. Appl. Bacteriol. 15: 103–128.

Tindale, C.R., Whitfield, F.B., Levingston, S.D. and Nguyen, T.H.L. 1989. Fungi isolated from packaging materials: their role in the production of 2,4,6-trichloroanisole. J. Sci. Food Agric. 49: 437–447.

Tjamos, S.E., Antoniou, P.P., Kazantzidou, A., Antonopoulos, D.F., Papageorgiou, I. and Tjamos, E.C. 2004. *Aspergillus niger* and *Aspergillus carbonarius* in

Corinth raisin and wine-producing vineyards in Greece: population composition, Ochratoxin A production and chemical control. J. Phytopathol. 152: 250–255.

Tognon, G., Campagnoli, A., Pinotti, L., Dell'Orto, V. and Cheli, F. 2005. Implementation of the electronic nose for the identification of mycotoxins in durum wheat (*Triticum durum*). Vet. Res. Commun. 29(Supp. 2): 391–393.

Toit, L.J. du, Inglis, D.A. and Pelter, G.Q. 2003. *Fusarium proliferatum* pathogenic on onion bulbs in Washington. Plant Dis. 87: 750.

Tolleson, W.H., Dooley, K.L., Sheldon, W.G., Thurman, J. D., Bucci, T.J. and Howard, P.C. 1996. The mycotoxin fumonisin induces apoptosis in cultured human cells and livers and kidneys of rats. Adv. Exp. Med. Biol. 392: 237–250.

Tonon, S.A., Marucci, R.S., Jerke, G. and Garcia, A. 1997. Mycoflora of paddy and milled rice produced in the region of Northeastern Argentina and Southern Paraguay. Int. J. Food Microbiol. 37: 231–235.

Torkar, K.G. and Vengust, A. 2008. The presence of yeasts, moulds and aflatoxin M-1 in raw milk and cheese in Slovenia. Food Control 19: 570–577.

Török, T. and King, A.D. 1991. Thermal inactivation kinetics of food-borne yeasts. J. Food Sci. 56: 6–9, 59.

Török, T., Rockhold, D. and King, A.D. 1993. Use of electrophoretic karyotyping and DNA-DNA hybridization in yeast identification. Int. J. Food Microbiol. 19: 63–80.

Torp, M. and Nirenberg, H.I. 2004. *Fusarium langsethiae* sp. nov. on cereals in Europe. Int. J. Food Microbiol. 95: 247–256.

Torres, A.M., Reynoso, M.M., Rojo, F.G., Ramirez, M.L. and Chulze, S.N. 2001. *Fusarium* species (section *Liseola*) and its mycotoxins in maize harvested in northern Argentina. Food Addit. Contam. 18: 836–843.

Torres, M., Viladrich, R., Sanchis, V. and Canela, R. 1992. Influence of age on ergosterol content in mycelium of *Aspergillus ochraceus*. Lett. Appl. Microbiol. 15: 20–22.

Torres, R., Nunes, C., García, J.M., Abadias, M., Viñas, I., Manso, T., Olmo, M. and Usall, J. 2007. Application of *Pantoea agglomerans* CPA-2 in combination with heated sodium bicarbonate solutions to control the major postharvest diseases affecting citrus fruit at several Mediterranean locations. Eur. J. Plant Pathol. 118: 73–83.

Tosun, N. and Delen, N. 1998. Minimising of contamination of aflatoxigenic fungi and subsequent aflatoxin development in fig orchards by fungicides. Acta Horticult. 480: 193–197.

Tothill, I.E., Harris, D. and Magan, N. 1992. The relationship between fungal growth and ergosterol content of wheat grain. Mycol. Res. 96: 965–970.

Tournas, V.H. 2005. Spoilage of vegetable crops by bacteria and fungi and related health hazards. Crit. Rev. Microbiol. 31: 33–44.

Tournas, V.H. and Katsoudas, E. 2005. Mould and yeast flora in fresh berries, grapes and citrus fruits. Int. J. Food Microbiol. 105: 11–17.

Tournas, V.H. and Stack, M.E. 2001. Production of alternariol and alternariol methyl ether by *Alternaria alternata* grown on fruits at various temperatures. J. Food Prot. 64: 528–532.

Townsend, J.F., Cox, J.K.B., Sprouse, R.F. and Lucas, F.V. 1971. Fungal flora of South Vietnamese fish and rice. J. Trop. Med. Hyg. 74: 98–100.

Tran-Dinh, N., Pitt, J.I. and Carter, D.A. 1999. Molecular genotype analysis of natural toxigenic and nontoxigenic isolates of *Aspergillus flavus* and *A. parasiticus*. Mycol. Res. 103: 1485–1490.

Tran-Dinh, N., Kennedy, I., Bui, T. and Carter, D. 2009. Survey of Vietnamese peanuts, corn and soil for the presence of *Aspergillus flavus* and *Aspergillus parasiticus* and their associated mycotoxin, aflatoxin. Mycopathologia (submitted).

Tresner, H.D. and Hayes, J.A. 1971. Sodium chloride tolerance of terrestrial fungi. Appl. Microbiol. 22: 210–213.

Trinci, A.P.J. 1994. Evolution of the Quorn myco-protein fungus, *Fusarium graminearum* A3/5. Microbiology 140: 2181–2188.

Tripathi, M.N., Shukla, D.N. and Dwivedi, B.K. 1999. Effect of Hydrogen-ion concentration on the growth and development of *Fusarium* spp. Bioved 10: 31–37.

Troller, J.A. and Christian, J.H.B. 1978. Water Activity and Food. New York: Academic Press.

Trucksess, M.W. and Scott, P.M. 2008. Mycotoxins in botanicals and dried fruits: A review. Food Addit. Contam. 25: 181–192.

Trung, T.S., Bailly, J.D., Querin, A., Le Bars, P. and Guerre, P. 2001. Fungal contamination of rice from south Vietnam, mycotoxinogenesis of selected strains and residues in rice. Rev. Med. Vet. 152: 555–560.

Tsai, G.J. and Yu, S.C. 1997. An enzyme-linked immunosorbent assay for the detection of *Aspergillus parasiticus* and *Aspergillus flavus*. J. Food Prot. 60: 978–984.

Tsai, G.J. and Yu, S.C. 1999. Detecting *Aspergillus parasiticus* in cereals by an enzyme-linked immunosorbent assay. Int. J. Food Microbiol. 50: 181–189.

Tseng, T.C. and Tu, J.C. 1997. Mycoflora and mycotoxins in adzuki and mung beans produced in Ontario, Canada. Microbios 90(363): 87–95.

Tseng, T.C., Tu, J.C. and Tzean, S.S. 1995. Mycoflora and mycotoxins in dry bean (*Phaseolus vulgaris*) produced in Taiwan and in Ontario, Canada. Bot. Bull. Acad. Sinica 36: 229–234.

Tseng, T.C., Tu, J.C. and Soo, L.C. 1996. Natural occurrence of mycotoxins in *Fusarium*-infected beans. Microbios 84(338): 21–28.

Tsubouchi, H., Yamamoto, K., Hisada, K. and Sakabe, Y. 1984. A survey of occurrence of mycotoxins and toxigenic fungi in imported green coffee beans. Maikotokishin (Proc. Jpn. Assoc. Mycotoxicol.) 19: 14–21.

Tsubouchi, H., Terada, H., Yamamoto, K., Hisada, K. and Sakabe, Y. 1985. Caffeine degradation and increased ochratoxin A production by toxigenic strains of *Aspergillus ochraceus* isolated from green coffee beans. Mycopathologia 90: 181–186.

Tsuruta, O. and Saito, M. 1980. Mycological damage of domestic brown rice during storage in warehouses under natural conditions. 3. Changes in mycoflora during storage. Trans. Mycol. Soc. Jpn 21: 121–125.

Tsuyoshi, N., Fudou, R., Yamanaka, S., Kozaki, M., Tamang, N, Thapa, S. and Tamang, J.P. 2005.

Identification of yeast strains isolated from marcha in Sikkim, a microbial starter for amylolytic fermentation. Int. J. Food Microbiol. 99: 135–146.

Tubaki, K. 1973. An undescribed halophilic species of *Scopulariopsis*. Trans. Mycol. Soc. Jpn 14: 367–369.

Tuite, J.F. and Christensen, C.M. 1957. Grain storage studies. 14. Moisture content of wheat seed in relation to invasion of the seed by species of the *Aspergillus glaucus* group, and effect of invasion upon germination of the seed. Phytopathology 47: 323–327.

Tuthill, D.E., Frisvad, J.C. and Christensen, M. 2001. Systematics of *Penicillium* based on rDNA sequences, morphology and secondary metabolites. Mycologia 93: 298–308.

Tzortzakis, N., Singleton, I. and Barnes, J. 2007. Deployment of low-level ozone-enrichment for the preservation of chilled fresh produce. Postharvest Biol. Technol. 43: 261–270.

Udagawa, S. 1959. Taxonomic studies of fungi on stored rice grains. III. *Penicillium* group (Penicillia and related genera). 2. Tokyo Nogyo Daigaku Nogaku Shuho (J. Agric. Sci. Tokyo) 5: 5–21.

Udagawa, S. 1988. Mycotoxicoses – the present problem and prevention of mycotoxins. Asian Med. J. 31: 599–604.

Udagawa, S. and Suzuki, S. 1994. *Talaromyces spectabilis*, a new species of food-borne ascomycetes. Mycotaxon 50: 81–88.

Udagawa, S. and Tsuruta, O. 1973. Isolation of an osmophilic *Penicillium* associated with cereals during long-term storage. Trans. Mycol. Soc. Jpn 14: 395–402.

Udagawa, S., Kobatake, M. and Kurata, H. 1977. Re-estimation of preservation effectiveness of potassium sorbate (food additive) in jams and marmalade. Eisei Shikensho Hokoku (Bull. Nat. Inst. Hyg. Sci., Tokyo) 95: 88–92.

Uduebo, A.E. 1974. Effect of high temperature on the growth, sporulation, and pigment production of *Botryodiplodia theobromae*. Can. J. Bot. 52: 2631–2634.

Ueno, Y. 1972. Temperature dependent production of citreoviridin, a neurotoxin of *Penicillium citreo-viride* Biourge. Jpn. J. Exp. Med. 42: 107–114.

Ueno, Y. and Ueno, I. 1972. Isolation and acute toxicity of citreoviridin, a neurotoxic mycotoxin of *Penicillium citreo-viride* Biourge. Jpn. J. Exp. Med. 42: 91–105.

Unterholzner, O., Aurich, M. and Platter, K. 1988. Geschmacks- und Geruchsfehler bei Rotweinen verursacht durch *Schizosaccharomyces pombe* L. Mitt. Klosterneuburg, Rebe und Wein, Obstbau und Früchteverwertung 38: 66–70.

Uraguchi, K. 1969. Mycotoxic origin of cardiac beriberi. J. Stored Prod. Res. 5: 227–236.

Uraguchi, K. 1971. Yellowed rice toxins. IV. Citreoviridin. *In* Microbial Toxins. Vol. 4. Fungal Toxins, eds A. Ciegler, S. Kadis and S.J. Ajl. London: Academic Press. pp. 367–380.

Uraguchi, K., Saito, M., Noguchi, Y., Takahashi, K., Enomoto, M., and Tatsuno, T. 1972. Chronic toxicity and carcinogenicity in mice of the purified mycotoxins, luteoskyrin and cyclochlorotine. Food Cosmet. Toxicol. 10: 193–207.

Urbano, G.R., Taniwaki, M.H., Leitao, M.F.D. and Vicentini, M.C. 2001. Occurrence of ochratoxin A-producing fungi in raw Brazilian coffee. J. Food Prot. 64: 1226–1230.

US Food and Drug Administration. 1992. Bacteriological Analytical Manual, 7th edn. Arlington, VA: AOAC International.

Usha, C.M., Patkar, K.L., Shetty, H.S., Kennedy, R. and Lacey, J. 1994. Mycoflora of developing sorghum grains with special reference to *Aspergillus flavus*. Trop. Sci. 34: 353–360.

Utkhede, R.S. and Mathur, S. 2004. Internal fruit rot caused by *Fusarium subglutinans* in greenhouse sweet peppers. Can. J. Plant Pathol. 26: 386–390.

Vaamonde, G., Scarmato, G. and Bonera, N. 1987. Zearalenone production by *Fusarium* species isolated from soybeans. Int. J. Food Microbiol. 4: 129–133.

Vaamonde, G., Patriarca, A., Fernández Pinto, V. Comerio, R. and Degrossi, C. 2003. Variability of aflatoxin and cyclopiazonic acid production by *Aspergillus* section *Flavi* from different substrates in Argentina. Int. J. Food Microbiol. 88: 79–84.

Vágvölgyi, C., Heinrich, H., Ács, K. and Papp, T. 2004. Genetic variability in the species *Rhizopus stolonifer,* assessed by random amplified polymorphic DNA analysis. Antonie van Leeuwenhoek 86: 181–188.

Valdez, J.G., Makuch, M.A., Ordovini, A.F., Masuelli, R.W., Overy, D.P. and Piccolo, R.J. 2006. First report of *Penicillium allii* as a field pathogen of garlic (*Allium sativum*). Plant Pathol. 55: 583.

Valera, H.R., Gomes, J., Lakshmi, S., Gururaja, R., Suryanarayan, S. and Kumar, D. 2005. Lovastatin production by solid state fermentation using *Aspergillus flavipes*. Enz. Microbial Technol. 37: 521–526.

Valero, A., Marín, S., Ramos, A.J. and Sanchis, V. 2005. Ochratoxin A-producing species in grapes and sun-dried grapes and their relation to ecophysiological factors. Lett. Appl. Microbiol. 41: 196–201.

Valero, A., Sanchis, V., Ramos, A.J. and Marín, S. 2007a. Studies on the interactions between grape-associated filamentous fungi on a synthetic medium. Int. J. Food Microbiol. 113: 271–276.

Valero, A., Begum, M., Leong, S.L., Hocking, A.D., Ramos, A.J., Sanchis, V. and Marín, S. 2007b. Effect of germicidal UVC light on fungi isolated from grapes and raisins. Lett. Appl. Microbiol. 45: 238–243.

Valletrisco, M. and Niola, I. 1983. Possible effect of fungal diseases on apple juice quality. Ind. Bevande 12: 457–462.

Van Beyma, F.H. 1933. Beschreibung einiger neuer Pilzarten aus dem Centraalbureau voor Schimmelcultures – Baarn (Holland). Zentralbl. Bakteriol. Parasitenkd. Infektionskrankh. Hyg. Abt. II, 88: 134–141.

Van der Horst, M., Samson, R.A. and Karman, H. 1992. Comparison of two commercial kits to detect moulds by latex agglutination. *In* Modern Methods in Food Mycology, eds R.A. Samson, A.D. Hocking, J.I. Pitt and A.D. King. Amsterdam: Elsevier. pp. 241–245.

Van der Merwe, K.J., Steyn, P.S. and Fourie, L. 1965. Ochratoxin A, a toxic metabolite produced by *Aspergillus ochraceus* Wilh. Nature (London) 205: 1112–1113.

Van der Riet, W.B. 1976. Studies on the mycoflora of biltong. S. Afr. Food Rev. 3: 105, 107, 109, 111.

Van der Riet, W.B. and Pinches, S.E. 1991. Control of *Byssochlamys fulva* in fruit juices by means of intermittent treatment with dimethyldicarbonate. Lebensm. Wiss. Technol. 24: 501–503.

Van der Riet, W.B. and van der Walt, W.H. 1985. Effect of ionizing radiation on ascospores of three strains of *Byssochlamys fulva* in apple juice. J. Food Prot. 48: 1016–1018.

Van der Riet, W.B., Botha, A. and Pinches, S.E. 1989. The effect of dimethyldicarbonate on vegetative growth and ascospores of *Byssochlamys fulva* suspended in apple juice and strawberry nectar. Int. J. Food Microbiol. 8: 95–102.

Van der Spuy, J.E., Matthee, F.N. and Crafford, D.J.A. 1975. The heat resistance of moulds *Penicillium vermiculatum* Dangeard and *Penicillium brefeldianum* Dodge in apple juice. Phytophylactica 7: 105–108.

Van der Vossen, J.M.B.M., Rahaoui, H., de Nus, M.W.C.M. and Hartog, B.J. 2003. PCR methods for tracing and detection of yeasts in the food chain. *In* Yeasts in Food, eds T. Boekhout and V. Robert. Cambridge, UK: Woodhead Publishing. pp.123–138.

Van der Walt, A.M., van der Linde, E., Alberts, M., Modjajdi, P., Jivan, S.D. and Bezuidenhout, C.C. 2006. Fumonisin-producing *Fusarium* strains and fumonisins in traditional African vegetables (morogo). S. Afr. J. Sci. 102: 151–155.

Van Dyk, K. 2004. Fungi associated with root and crown rot of wheat and barley in Tanzania. Afr. Plant Prot. 10: 117–124.

Van Eck, J.H., Prior, B.A. and Brandt, E.V. 1993. The water relations of growth and polyhydroxy alcohol production by ascomycetous yeasts. J. Gen. Microbiol. 139: 1047–1054.

Van Egmond, H.P. 1989. Aflatoxin M_1: occurrence, toxicity, regulation. *In* Mycotoxins in Dairy Products, ed. H.P. van Egmond. London: Elsevier Applied Science. pp. 11–55.

Van Oorschot, C.A.N. 1980. A revision of *Chrysosporium* and allied genera. Stud. Mycol., Baarn 20: 1–89.

Vanden Bossche, H., Engelen, M. and Rochette, F. 2003. Antifungal agents of use in animal health – chemical, biological and pharmacological aspects. J. Vet. Pharmacol. Ther. 26: 5–29.

Var, I., Kabak, B. and Gök, F. 2007. Survey of aflatoxin B1 in helva, a traditional Turkish food, by TLC. Food Control 18: 59–62.

Varga, J. 2006. Molecular typing of aspergilli: recent developments and outcomes. Med. Mycol. 44 (Suppl 1): S149–S161.

Varga, J. and Kozakiewicz, Z. 2006. Ochratoxin A in grapes and grape-derived products. Trends Food Sci. Technol. 17: 72–81.

Varga, J., Kevei, F., Vriesema, A., Debets, F, Kozakiewicz, Z. and Croft, J.H. 1994. Mitochondrial DNA restriction fragment length polymorphisms in field isolates of the *Aspergillus niger* aggregate. Can. J. Microbiol. 40: 612–621.

Varga, J., Rigó, K., Molnár, J., Tóth, B., Szencz, S., Téren, J. and Kozakiewicz. 2003. Mycotoxin production and evolutionary relationships among species of *Aspergillus* section *Clavati*. Antonie van Leeuwenhoek 83: 191–200.

Varga, M., Bartok, T. and Mesterhazy, A. 2006. Determination of ergosterol in *Fusarium*-infected wheat by liquid chromatography-atmospheric pressure photoionization mass spectrometry. J. Chromatogr. A 1103: 278–283.

Vaughn, R.H., Douglas, H.C. and Gilliland, J.R. 1943. Production of Spanish-type green olives. Bull. Calif. Agric. Exp. Stn. 678.

Velez-Rodriguez, L. and Rivera-Vargas L.L. 2007. Recent studies of fungal pathogens of onions in Puerto Rico. J. Agric. Univ. Puerto Rico. 91: 31–45.

Venkatasubbaiah, P., Dwarakanath, C.T. and Sreenivasa-Murthy, V. 1985. Involvement of yeast flora in idli batter fermentation. J. Food Sci. Technol. India 22: 88–90.

Venter, S.L. and Steyn, P.J. 1998. Correlation between fusaric acid production and virulence of isolates of *Fusarium oxysporum* that causes potato dry rot in South Africa. Potato Res. 41: 289–294.

Vergeade, J., Guiraud, J., Larpent, J.P. and Galzy, P. 1976. Yeasts of Saint-Nectaire cheeses. Lait 56: 275–285.

Veringa, H.A., van den Berg, G. and Daamen, C.B.G. 1989. Factors affecting the growth of *Aspergillus versicolor* and the production of sterigmatocystin on cheese. Neth. Milk Dairy J. 43: 311–326.

Vesonder, R.F., Lambert, R., Wicklow, D.T. and Biehl, M. L. 1988. *Eurotium* spp. and echinulin in feed refusal by swine. Appl. Environ. Microbiol. 54: 830–831.

Vesonder, R.F., Logrieco, A., Bottalico, A., Altomare, C. and Petersen, S.W. 1995. *Fusarium* species associated with banana fruit and their potential toxicity. Mycotoxin Res. 11: 93–98.

Viana, F.M.P., Freire, F.C.O., Barguil, B.M., Alves, R.E., Santos, A.A., Cardoso, J.E. and Vidal, J.C. 2002. Post-harvest basal rot of coconut fruits in the State of Ceara, Brazil. Fitopatol. Brasil. 27: 545.

Vieira-Dalode, G., Jespersen, L., Hounhouligan, J., Moller, P.L., Nago, C.M. and Jakobsen, M. 2007. Lactic acid bacteria and yeasts associated with gowe production from sorghum in Benin. J. Appl. Microbiol. 103: 342–349.

Vijayalakshmi, M. and Rao, A.S. 1993. Effect of seed-borne fungi on quality of sunflower (*Helianthus annus*) oil. Indian J. Agric. Sci. 63: 529–531.

Viljoen, B.C. and Greyling, T. 1995. Yeasts associated with Cheddar and Gouda making. Int. J. Food Microbiol. 28: 79–88.

Viljoen, B.C., Khoury, A.R. and Hattingh, A. 2003a. Seasonal diversity of yeasts associated with white-surface mould-ripened cheeses. Food Res. Int. 36: 275–283.

Viljoen, B.C., Lourens-Hattingh, A., Ikalafeng, B. and Peter, G. 2003b. Temperature abuse initiating yeast growth in yoghurt. Food Res. Int. 36: 193–197.

Villa-Carvajal, M., Querol, A. and Belloch, C. 2006. Identification of species in the genus *Pichia* by restriction of the internal transcribed spacers (ITS1 and ITS2) and the 5.8S ribosomal DNA gene: identification of *Pichia* species by 5.8S-ITS rDNA RFLP. Antonie van Leeuwenhoek 90: 171–181.

Vinaixa, M., Marin, S., Brezmes, J., Llobet, E., Vilanova, X., Correig, X., Ramos, A. and Sanchis, V. 2004. Early detection of fungal growth in bakery products by use of an electronic nose based on mass spectrometry. J. Agric. Food Chem. 52: 6068–6074.

Viñas, I., Morlans, I. and Sanchis, V. 1990. Potential for the development of tolerance by *Aspergillus amstelodami, A. repens* and *A. ruber* after repeated exposure to potassium sorbate. Zentralbl. Mikrobiol. 145: 187–193.

Viñas, I., Bonet, J. and Sanchis, V. 1992. Incidence and mycotoxin production by *Alternaria tenuis* in decayed apples. Lett. Appl. Microbiol. 14: 284–287.

Vincent, M.A. and Pitt, J.I. 1989. *Penicillium allii*, a new species from Egyptian garlic. Mycologia 81: 300–303.

Vindelov, J. and Arneborg, N. 2002. Effects of temperature, water activity, and syrup film composition on the growth of *Wallemia sebi*: development and assessment of a model predicting growth lags in syrup agar and crystalline sugar. Appl. Environ. Microbiol. 68: 1652–1657.

Visconti, A. and Doko, M.B. 1994. Survey of fumonisin production by *Fusarium* isolated from cereals in Europe. J. AOAC Int. 77: 546–550.

Visconti, A., Chelkowski, J., Solfrizzo, M. and Bottalico, A. 1990. Mycotoxins in corn ears naturally infected with *Fusarium graminearum* and *F. crookwellense*. Can. J. Plant Pathol. 12: 187–189.

Vismer, H.F., Sydenham, E.W., Schlechter, M., Brown, N.L. , Hocking, A.D., Rheeder, J.P. and Marasas, W.F.O. 1996. Patulin producing *Penicillium* species isolated from naturally infected apples in South Africa. S. Afr. J. Sci. 92: 530–534.

Viswanath, P., Kavitha, S., Farhana, A. and Appaiah, K.M. 2006. A survey of ochratoxin A in wheat and barley in India. J. Food Saf. 27: 111–123.

Voigt, K., Cigelnik, E. and O'Donnell, K. 1999. Phylogeny and PCR identification of clinically important Zygomycetes based on nuclear ribosomal-DNA sequence data. J. Clin. Microbiol. 37: 3957–3964.

Von Arx, J.A. 1957. Die Arten der Gattung *Colletotrichum*. Phytopathol. Z. 29: 413–468.

Von Arx, J.A. 1970. The Genera of Fungi Sporulating in Pure Culture. Lehre, Germany: J. Cramer.

Von Arx, J.A. 1977. Notes on *Dipodascus*, *Endomyces* and *Geotrichum* with the description of two new species. Antonie van Leeuwenhoek 43: 333–340.

Von Arx, J.A. 1981a. On *Monilia sitophila* and some families of Ascomycetes. Sydowia 34: 13–29.

Von Arx, J.A. 1981b. The Genera of Fungi Sporulating in Pure Culture, 3rd edn. Vaduz, Germany: J. Cramer.

Von Arx, J.A., Guarro, J. and Figueras, M.J. 1986. The Ascomycete Genus *Chaetomium*. Berlin: J. Cramer.

Von Schelhorn, M. 1950. Untersuchungen über den Verberb wasserarmer Lebensmittel durch osmophile Mikroorganismen. I. Verberb von Lebensmittel durch osmophile Hefen. Z. Lebensm. Unters. Forsch. 91: 117–124.

Vrany, J., Rasochova, M., Dobias, J. and Horackova, V. 1989. *Fusarium* on surface of tubers of selected potato varieties. Zentralbl. Mikrobiol. 144: 399–404.

Vytřasová, J., Přibáňová, P. and Marvanová, L. 2002. Occurrence of xerophilic fungi in bakery gingerbread production. Int. J. Food Microbiol. 72: 91–96.

Wade, N.L. and Gipps, P.G. 1971. Post-harvest control of brown rot and *Rhizopus* rot in peaches with benomyl and dichloran. Aust. J. Exp. Agric. Anim. Husb. 13: 600–603.

Wade, N.L. and Morris, S.C. 1982. Causes and control of cantaloupe post-harvest wastage in Australia. Plant Dis. 66: 549–552.

Wade, N.L., Kavanagh, E.E. and Sepiah, M. 1993. Effects of modified atmosphere storage on banana postharvest diseases and the control of bunch main-stalk rot. Postharvest Biol. Technol. 3: 143–154.

Wade, W.N., Vasdinnyei, R., Deak, T. and Beuchat, L.R. 2003. Proteolytic yeasts isolated from raw, ripe tomatoes and metabolic association of *Geotrichum candidum* with *Salmonella*. Int. J. Food Microbiol. 86: 101–111.

Waghray, S., Reddy, C.S. and Reddy, A.P.K. 1988. Seed mycoflora and aflatoxin contamination in rice. Indian Phytopathol. 41: 492–494.

Waje, C.K., Dizon, E.I., Serrano, E.P. and Mabesa, R.C. 2005. Mycological stability of minimally-processed young coconut (*Cocos nucifera* L.) as influenced by different chemical and physical treatments. Philipp. Agric. Sci. 88: 84–94.

Walcott, R.R., Gitaitis, R.D. and Langston, D.B. 2004. Detection of *Botrytis aclada* in onion seed using magnetic capture hybridization and the polymerase chain reaction. Seed Sci. Technol. 32: 425–438.

Walker, H.W. 1977. Spoilage of food by yeasts. Food Technol., Champaign 23(2): 57–61, 65.

Walker, H.W. and Ayres, J.C. 1970. Yeasts as spoilage organisms. *In* The Yeasts. Vol. 3, eds A.H. Rose and J. S. Harrison. London: Academic Press. pp. 500–527.

Wallace, H.A.H., Sinha, R.N. and Mills, J.T. 1976. Fungi associated with small wheat bulks during prolonged storage in Manitoba. Can. J. Bot. 54: 1332–1343.

Wallbridge, A. 1981. Fungi associated with crown-rot disease of boxed bananas from the Windward Islands during a two-year survey. Trans. Br. Mycol. Soc. 77: 567–577.

Wan, K., Yousef, A.E., Schwartz, S.J. and Wang, H.H. 2006. Rapid, specific, and sensitive detection of spoilage molds in orange juice using a real-time Taqman PCR assay. J. Food Prot. 69: 385–390.

Wang, E., Norred, W.P., Bacon, C.W., Riley, R.T. and Merrill, A.H. 1991. Inhibition of sphingolipid biosynthesis by fumonisins. J. Biol. Chem. 266: 14486–14490.

Wang, J.-S. and Groopman, J.D. 1999. DNA damage by mycotoxins. Mutation Res. 424: 167–181.

Wang, L. and Zhuang, W.Y. 2007. Phylogenetic analyses of penicillia based on partial calmodulin gene sequences. Biosystems 88: 113–126.

Wang, Y. and Guo, L.-D. 2004. Morphological and molecular identification of an endophytic fungus *Epicoccum nigrum*. Mycosystema 23: 474–479.

Wang, Y.-Z., Ju, X.-L. and Zhou, Y.-G. 2005. The variability of citrinin production in *Monascus* type cultures. Food Microbiol. 22: 145–148.

Warnock, D.W. 1971. Assay of fungal mycelium in grains of barley, including the use of the fluorescent antibody technique for individual species. J. Gen. Microbiol. 67: 197–205.

Warth, A.D. 1977. Mechanism of resistance of *Saccharomyces bailii* to benzoic, sorbic and other weak acids used as food preservatives. J. Appl. Bacteriol. 43: 215–230.

Warth, A.D. 1985. Resistance of yeast species to benzoic and sorbic acids and to sulfur dioxide. J. Food Prot. 48: 564–569.

Warth, A.D. 1986. Effects of nutrients and pH on the resistance of *Zygosaccharomyces bailii* to benzoic acid. Int. J. Food Microbiol. 3: 263–271.

Warth, A.D. 1988. Effect of benzoic acid on growth yield of yeasts differing in their resistance to preservatives. Appl. Environ. Microbiol. 54: 2091–2095.

Warth, A.D. 1989a. Transport of benzoic and propanoic acids by *Zygosaccharomyces bailii*. J. Gen. Microbiol. 135: 1383–1390.

Warth, A.D. 1989b. Relationships among cell size, membrane permeability, and preservative resistance in yeast species. Appl. Environ. Microbiol. 55: 2995–2999.

Warth, A.D. 1989c. Relationships between the resistance of yeasts to acetic, propanoic and benzoic acids and to methyl paraben and pH. Int. J. Food Microbiol. 8: 343–349.

Warth, A.D. 1991. Mechanism of action of benzoic acid on *Zygosaccharomyces bailii*: effects on glycolytic metabolite levels, energy production, and intracellular pH. Appl. Environ. Microbiol. 57: 3410–3414.

Watkins, K.L., Fazekas, G. and Palmer, M.V. 1990. Patulin in Australian apple juice. Food Aust. 42: 438–439.

Watson, D.H. 1984. An assessment of food contamination by toxic products of *Alternaria*. J. Food Prot. 47: 485–488.

Watson-Craik, I.A., Aidoo, K.E. and Anderson, J.G. 1989. Induction of conductance and capacitance changes by food-borne fungi. Food Microbiol. 6: 231–244.

Watson-Craik, I.A., Aidoo, K.E. and Anderson, J.G. 1990. Development and evaluation of a medium for the monitoring of food-borne moulds by capacitance changes. Food Microbiol. 7: 129–145.

Webb, T.A. and Mundt, J.O. 1978. Molds on vegetables at the time of harvest. Appl. Environ. Microbiol. 35: 655–658.

Weber, Z., Kostecki, M., Bargen, S. von, Gossmann, M., Waskiewicz, A., Bocianowski, J., Knaflewski, M., Buttner, C. and Golinski, P. 2006. *Fusarium* species colonizing spears and forming mycotoxins in field samples of asparagus from Germany and Poland. J. Phytopathol. 154: 209–216.

Webley, D.J., Jackson, K.L., Mullins, J.D., Hocking, A.D. and Pitt, J.I. 1997. *Alternaria* toxins in weather damaged wheat and sorghum in the 1995–1996 Australian harvest. Aust. J. Agric. Res. 48: 1249–1255.

Weete, J.D. 1974. Fungal Lipid Biochemistry: Distribution and Metabolism. New York: Plenum Press.

Weidenbörner, M. and Kunz, B. 1994. Untersuchungen zur Mykoflora von Getreideflocken. Getreide Mehl Brot. 48: 114–116.

Weidenbörner, M., Wieczorek, C., Appel, S. and Kunz, B. 2000. Whole wheat and white wheat flour – the mycobiota and potential mycotoxins. Food Microbiol. 17: 103–107.

Wells, J.M. 1980. Toxigenic fungi isolated from late-season pecans. J. Food Saf. 2: 213–220.

Wells, J.M. and Payne, J.A. 1975. Toxigenic *Aspergillus* and *Penicillium* isolates from weevil-damaged chestnuts. Appl. Microbiol. 30: 536–540.

Wells, J.M. and Payne, J.A. 1976. Toxigenic species of *Penicillium*, *Fusarium* and *Aspergillus* from weevil-damaged pecans. Can. J. Microbiol. 22: 281–285.

Wells, J.M. and Uota, M. 1970. Germination and growth of five fungi in low-oxygen and high-carbon dioxide atmospheres. Phytopathology 60: 50–53.

Wells, J.M., Cole, R.J. and Kirksey, J.W. 1975. Emodin, a toxic metabolite of *Aspergillus wentii* isolated from weevil-damaged chestnuts. Appl. Microbiol. 30: 26–28.

Wells, J.M., Cole, R.J., Cutler, H.C. and Spalding, D.H. 1981. *Curvularia lunata*, a new source of cytochalasin B. Appl. Environ. Microbiol. 41: 967–971.

Westall, S. and Filtenborg, O. 1998. Spoilage yeasts of decorated soft cheese packed in modified atmosphere. Food Microbiol. 15: 243–249.

Wheeler, J.L., Harrison, M.A. and Koehler, P.E. 1987. Presence and stability of patulin in pasteurized apple cider. J. Food Sci. 52: 479–480.

Wheeler, K.A. and Hocking, A.D. 1988. Water relations of *Paecilomyces variotii, Eurotium amstelodami, Aspergillus candidus* and *Aspergillus sydowii*, xerophilic fungi isolated from Indonesian dried fish. Int. J. Food Microbiol. 7: 73–78.

Wheeler, K.A. and Hocking, A.D. 1993. Interactions among xerophilic fungi associated with dried salted fish. J. Appl. Bacteriol. 74: 164–169.

Wheeler, K.A., Hocking, A.D., Pitt, J.I. and Anggawati, A. 1986. Fungi associated with Indonesian dried fish. Food Microbiol. 3: 351–357.

Wheeler, K.A., Hocking, A.D. and Pitt, J.I. 1988a. Effects of temperature and water activity on germination and growth of *Wallemia sebi*. Trans. Br. Mycol. Soc. 90: 365–368.

Wheeler, K.A., Hocking, A.D. and Pitt, J.I. 1988b. Water relations of some *Aspergillus* species isolated from dried fish. Trans. Br. Mycol. Soc. 91: 631–638.

Wheeler, K.A., Hocking, A.D. and Pitt, J.I. 1988c. Influence of temperature on the water relations of *Polypaecilum pisce* and *Basipetospora halophila*, two halophilic xerophiles. J. Gen. Microbiol. 134: 2255–2260.

Wheeler, K.A., Hurdman, B.F. and Pitt, J.I. 1991. Influence of pH on the growth of some toxigenic species of *Aspergillus*, *Penicillium* and *Fusarium*. Int. J. Food Microbiol. 12: 141–150.

Whipps, J.M. and Lewis, D.H. 1980. Methodology of a chitin assay. Trans. Br. Mycol. Soc. 74: 416–418.

White, M.M., James, T.Y., O'Donnell, K., Cafaro, M.J., Tanabe, Y. and Sugiyama, J. 2006. Phylogeny of the Zygomycota based on nuclear ribosomal sequence data. Mycologia 98: 872–884.

Whitfield, F.B., Nguyen, T.H.L. and Tindale, C.R. 1991. Effect of relative humidity and incubation time on the O-methylation of chlorophenols in fibreboard by *Paecilomyces variotii*. J. Sci. Food Agric. 55: 19–26.

Wichmann, G., Herbarth, O. and Lehmann, I. 2002. The mycotoxins citrinin, gliotoxin, and patulin affect interferon-gamma rather than interleukin-4 production in human blood cells. Environ. Toxicol. 17: 211–218.

Wicklow, D.T. 1983. Taxonomic features and ecological significance of sclerotia. *In* Aflatoxin and *Aspergillus flavus* in Corn, eds U.L. Diener, R.L. Asquith and J.W. Dickens. Auburn, AL: Alabama Agricultural Experiment Station. pp. 6–12.

Wicklow, D.T. 1995. The mycology of stored grain: an ecological perspective. *In* Stored Grain Ecosystems, eds D.S. Jayas, N.D.G. White and W.E. Muir. New York: Marcel Dekker. pp. 197–249.

Wicklow, D.T. and Cole, R.J. 1984. Citreoviridin in standing corn infested by *Eupenicillium ochrosalmoneum*. Mycologia 76: 959–961.

Wicklow, D.T. and Shotwell, O.L. 1983. Intrafungal distribution of aflatoxins among conidia and sclerotia of *Aspergillus flavus* and *Aspergillus parasiticus*. Can. J. Microbiol. 29: 1–5.

Wicklow, D.T., Horn, B.W. and Shotwell, O.L. 1987. Aflatoxin formation in pre-harvest maize ears coinoculated with *Aspergillus flavus* and *Aspergillus niger*. Mycologia 79: 679–682.

Wicklow, D.T., Stubblefield, R.D., Horn, B.W. and Shotwell, O.L. 1988. Citreoviridin levels in *Eupenicillium ochrosalmoneum*-infested maize kernels at harvest. Appl. Environ. Microbiol. 54: 1096–1098.

Wicklow, D.T., Weaver, D.K. and Throne, J.E. 1998. Fungal colonists of maize grain conditioned at constant temperatures and humidities. J. Stored Prod. Res. 34: 355–361.

Wicklow, D.T, Dowd, P.F., Alfatafta, A.A. and Gloer, J.B. 1996. Ochratoxin A: an antiinsectan metabolite from the sclerotia of *Aspergillus carbonarius* NRRL 369. Can. J. Microbiol. 42: 1100–1103.

Wicks, T. and Blumbieris, M. 1981. *Phytophthora* fruit rot of Delicious apples in South Australia. Australas. Plant Pathol. 10: 23–24.

Wiebe, M.G. 2004. Quorn™ myco-protein – overview of a successful fungal product. Mycologist 18: 17–20.

Wijeratnam, R.S.W., Hewajulige, I.G.H. and Abeyratne, N. 2005. Postharvest hot water treatment for the control of *Thielaviopsis* black rot of pineapple. Postharvest Biol. Technol. 36: 323–327.

Wild, B.L. 1983. Double resistance by citrus green mould *Penicillium digitatum* to the fungicides guazatine and benomyl. Ann. Appl. Biol. 103: 237–241.

Wiley, B.J. and Fennell, D.I. 1973. Ascocarps of *Aspergillus stromatoides, A. niveus*, and *A. flavipes*. Mycologia 65: 752–760.

Wiley, B.J. and Simmons, E.G. 1973. New species and a new genus of Plectomycetes with *Aspergillus* states. Mycologia 65: 934–938.

Wilkins, C.K. and Scholl, S. 1989. Volatile metabolites of some barley storage molds. Int. J. Food Microbiol. 8: 11–17.

Will, F., Hasselbeck, G. and Dietrich, H. 1992. Charakterisierung einer gelartigen Truebung aus Himbeersaft. Fluess. Obst 59: 352–353.

Williams, C.C., Cameron, E.J. and Williams, O.B. 1941. A facultatively anaerobic mold of unusual heat resistance. Food Res. 6: 69–73.

Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M. and Aggarwal, D. 2004. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. Am. J. Clin. Nutr. 80: 1106–1122.

Williams, K.C., Blaney, B.J., Dodman, R.L. and Palmer, C.L. 1992. Assessment for animal feed of maize kernels naturally-infected predominantly with *Fusarium moniliforme* and *Diplodia maydis*. Aust. J. Agric. Sci. 43: 773–782.

Williams, W.P., Windham, G.L., Buckley, P.M. and Daves, C.A. 2006. Aflatoxin accumulation in corn hybrids infested at different growth stages with southwestern corn borer (Lepidoptera: Crambidae). J. Agric. Urban Entomol. 23: 97–103.

Willingham, S.L., Cooke, A.W., Coates, L.M. and Pegg, K. G. 2000. Pepper spot: A new preharvest *Colletotrichum* disease of avocado cv. Hass. Aust. Plant Pathol. 29: 151.

Wilson, B.J., Yang, D.T.C. and Boyd, M.R. 1970. Toxicity of mould-damaged sweet potatoes (*Ipomoea batatas*). Nature (London) 227: 521–522.

Wilson, C.L. and Chalutz, E. 1989. Postharvest biological control of *Penicillium* rots with antagonistic yeasts and bacteria. Sci. Horticult. 40: 105–112.

Wilson, J.P. 2002. Fungi associated with the stalk rot complex of pearl millet. Plant Dis. 86: 833–839.

Wilson, J.P., Hanna, W.W., Wilson, D.M., Beaver, R.W. and Casper, H.H. 1993. Fungal and mycotoxin contamination of pearl millet grain in response to environmental conditions in Georgia. Plant Dis. 77: 121–124.

Wilson, J.P., Jurjevic, Z., Hannah, W., Wilson, D.M., Potter, T.L. and Coy, A.E. 2006. Host-specific variation in infection by toxigenic fungi and contamination by mycotoxins in pearl millet and corn. Mycopathologia 161: 101–107.

Wilson, T., Rabie, C.J., Fincham, J.E., Steyn, P.S. and Schipper, M.A.A. 1984. Toxicity of rhizonin A, isolated from *Rhizopus microsporus*, in laboratory animals. Food Chem. Toxicol. 22: 275–281.

Windham, G.L. and Williams, W.P. 2007. Systemic infection of stalks and ears of corn hybrids by *Aspergillus parasiticus*. Mycopathologia 164: 249–254.

Wing, N., Bryden, W.L., Lauren, D.R. and Burgess, L.W. 1993. Toxigenicity of *Fusarium* species and subspecies in section *Gibbosum* from different regions of Australia. Mycol. Res. 97: 1441–1446.

Wogan, G.N. 1992. Aflatoxins as risk factors for hepatocellular carcinoma in humans. Cancer Res. (Suppl) 52: 211s–2118s.

Wojcik-Stopczynska, B. 2006. Microbiological characteristic of cacao beans, products of its processing and of the processing environment. Rozprawy – Akademia Rolnicza w Szczecinie 238: 113 (in Polish).

Wollenweber, H.W. and Reinking, O.A. 1935. Die Fusarien, ihre Beschreibung, Schadwirkung und Kekampfung. Berlin: Paul Parey.

Wolter, H., Laing, E. and Viljoen, B.C. 2000. Isolation and identification of yeasts associated with intermediate moisture meats. Food Technol. Biotechnol. 38: 69–75.

Wood, G.M., Mann, P.J., Lewis, D.F., Reid, W.J. and Moss, M.O. 1990. Studies on a toxic metabolite from the mould *Wallemia*. Food Addit. Contam. 7: 69–77.

Woodward, K.N. 2005. Veterinary pharmacovigilance. Part 6. Predictability of adverse reactions in animals from laboratory toxicology studies. J. Vet. Pharmacol. Ther. 28: 213–231.

Wu, P.C., Su, H.J.J. and Ho, H.M. 2000. A comparison of sampling media for environmental viable fungi collected in a hospital environment. Environ. Res. 82: 253–257.

Wu, W., Cook, M.E., Chu, F.S., Buttles, T., Hunger, J. and Sutherland, P. 1997. Case study of bovine dermatitis caused by oat straw infected with *Fusarium sporotrichioides*. Vet. Rec. 140: 399–400.

Wyatt, M.K., Parish, M.E., Widmer, W.W. and Kimbrough, J. 1995. Characterization of mould growth in orange juice. Food Microbiol. 12: 347–355.

Wyatt, R.D. and Hamilton, P.B. 1972. The effect of rubratoxin in broiler chickens. Poult. Sci. 51: 1383–1387.

Xu, G., Chen, Y., Yu, H., Cameleyre, X. and Blanc, P.J. 2003. HPLC fluorescence method for determination of citrinin in *Monascus* cultures. Arch. Lebensmittelhyg. 54: 82–84.

Yaguchi, T., Udagawa, S. and Nishimura, K. 2005. *Geosmithia argillacea* is the anamorph of *Talaromyces*

eburneus as a heat resistant fungus. Cryptogam. Mycol. 26: 133–141.

Yamada, Y., Matsuda, M. and Mikata, K. 1996. The phylogeny of species of the genus *Saccharomycopsis* Schiönning (Saccharomycetaceae) based on the partial sequences of 18S and 26S ribosomal RNAs. Biosci. Biotech. Biochem. 60: 1303–1307.

Yamani, M.I. and Abu-Jaber, M.M. 1994. Yeast flora of labaneh produced by in-bag straining of cow milk set yogurt. J. Dairy Sci. 77: 3558–3564.

Yamazaki, M., Horie, Y, Suzuki, S., Fujimoto, H. and Maebayashi, Y. 1971. Toxic fungal metabolites isolated from home-made miso. Shokuhin Eiseigaku Zesshi (J. Food Hyg. Soc. Jpn) 12: 370–375.

Yamazaki, M., Fujimoto, H. and Miyaki, K. 1972. Metabolites of some strains of *Penicillium* isolated from foods. Yakugaku Zasshi (J. Pharmaceut. Soc. Jpn) 92: 101–104.

Yang, W., Kim, W-S., Fang, A. and Demain, A.L. 2003. Carbon and nitrogen source nutrition of fumagillin biosynthesis by *Aspergillus fumigatus*. Curr. Microbiol. 46: 275–279.

Yarrow, D. 1984. Genus 22. *Saccharomyces* Meyen ex Reess. *In* The Yeasts: A Taxonomic Study, 3rd edn, ed. N.J.W. Kreger-van Rij. Amsterdam: Elsevier. pp. 379–395.

Yates, A.R., Seaman, A. and Woodbine, M. 1967. Growth of *Byssochlamys nivea* in various carbon dioxide atmospheres. Can. J. Microbiol. 13: 1120–1123.

Yli-Mattila, T., Paavanen-Huhtala, S., Parikka, P., Konstantinova, P. and Gagkaeva, T.Y. 2004. Molecular and morphological diversity of *Fusarium* species in Finland and northwestern Russia. Eur J. Plant Pathol. 110: 573–585.

Yoder, J.A., Benoit, J.B. and Zettler, L.W. 2003. Effects of salt and temperature on the growth rate of a tick-associated fungus, *Scopulariopsis brevicaulis* Bainier (Deuteromycota). Int. J. Acarol. 29: 265–269.

Yong, R.K. and Cousin, M.A. 2001. Detection of moulds producing aflatoxins in maize and peanuts by an immunoassay. Int. J. Food Microbiol. 65: 27–38.

Yoshida, M., Tanigawa, M., Maeda, H. and Ifuku, Y. 1996. Rapid detection of microbial contaminants in fruit juice by enzyme-linked immunosorbent assay. J. Food Hyg. Soc. Jpn 37: 179–186.

Yoshizawa, T., Matsuura, Y., Tsuchiya, Y., Morooka, N., Kitani, K., Ichinoe, M. and Kurata, H. 1979. On the toxigenic Fusaria invading barley and wheat in southern Japan. Shokuhin Eiseigaku Zasshi (J. Food Hyg. Soc. Jpn) 20: 21–26.

Yoshizawa, T., Yamashita, A. and Luo, Y. 1994. Fumonisin occurrence in corn from high- and low-risk areas for human esophageal cancer in China. Appl. Environ. Microbiol. 60: 1626–1629.

Yoshizawa, T., Yamashita, A. and Chokethaworn, N. 1996. Occurrence of fumonisins and aflatoxins in corn from Thailand. Food Addit. Contam. 13: 163–168.

Youssef, M.S., Abo-Dahab, N.F. and Abou-Seidah, A.A. 2000. Mycobiota and mycotoxin contamination of dried raisins in Egypt. Afr. J. Mycol. Biotechnol. 8: 69–86.

Yu, T., Chen, J., Chen, R., Huang, B., Liu, D. and Zheng, X. 2007. Biocontrol of blue and gray mold diseases of pear fruit by integration of antagonistic yeast with salicylic acid. Int. J. Food Microbiol. 116: 339–345.

Yuan, G.-F., Liu, C.-S. and Chen, C.-C. 1995. Differentiation of *Aspergillus parasiticus* from *Aspergillus sojae* by random amplification of polymorphic DNA. Appl. Environ. Microbiol. 61: 2384–2387.

Yun, H., Lim, S., Chung, J., Jo, C., Park, J., Kwon, J. and Kim, D. 2006. Isolation and characterization of *Penicillium crustosum*, a patulin producing fungus, from apples. Food Sci. Biotech. 15: 896–901.

Zaake, S. 1979. Nachweis und Bedeutung geträkeschädlicher Hefen. Monatsschr. Brau. 32: 250–356.

Zadra, C., Cardinali, G., Corte, L., Fatichenti, F. and Marucchini, C. 2006. Biodegradation of the fungicide iprodione by *Zygosaccharomyces rouxii* strain DBVPG 6399. J. Agric. Food Chem. 54: 4734–4739.

Zalar, P., de Hoog, G.S., Schroers, H.J., Frank, J.M. and Gunde-Cimerman, N. 2005. Taxonomy and phylogeny of the xerophilic genus *Wallemia* (Wallemiomycetes and Wallemiales, *cl. et ord. nov.*). Antonie van Leeuwenhoek 87: 311–328.

Zalar, P., de Hoog, G.S., Schroers, H.-J., Crous, P.W., Groenewald. J.Z. and Gunde-Cimerman, N. 2007. Phylogeny and ecology of the ubiquitous saprobe *Cladosporium sphaerospermum*, with descriptions of seven new species from hypersaline environments. Stud. Mycol. 58: 157–183.

Zardetto, S. 2005. Effect of modified atmosphere packaging at abuse temperature on the growth of *Penicillium aurantiogriseum* isolated from fresh filled pasta. Food Microbiol. 22: 367–371.

Zare, R. and Ershad, D. 1997. *Fusarium* species isolated from cereals in Gorgan area. Iran. J. Plant Pathol. 33: 1–14 (Pe), 1–4 (En).

Zein, G.N., Moussa, A.M., Abou-Zeid, M.M., Gomaa, E.A. and Nofel, A. 1983. Studies on Kareish cheese in the local markets of Monoufia. I. Yeast content. Egypt. J. Dairy Sci. 11: 317–319.

Zhang, H.Y., Zheng, X.D. and Yu, T. 2007a. Biological control of postharvest diseases of peach with *Cryptococcus laurentii*. Food Control 18: 287–291.

Zhang, J.B., Li, H.P., Dang, F.J., Qu, B., Xu, Y.B., Zhao, C. S. and Liao, Y.C. 2007b. Determination of the trichothecene mycotoxin chemotypes and associated geographical distribution and phylogenetic species of the *Fusarium graminearum* clade from China. Mycol. Res. 111: 967–975.

Zhao, X.R., Lin, Q. and Brookes, P.C. 2005. Does soil ergosterol concentration provide a reliable estimate of soil fungal biomass? Soil Biol. Biochem. 37: 311–317.

Zheng, R.Y. and Chen, G.Q. 2001. A monograph of *Cunninghamella*. Mycotaxon 80: 1–75.

Zheng, R.-Z., Chen, G.-Q., Huang, H. and Liu, X.-Y. 2007a. A monograph of *Rhizopus*. Sydowia 59: 273–372.

Zheng, X., Yu, T., Chen, R., Huang, B. and Wu, V.C-H. 2007b. Inhibiting *Penicillium expansum* infection of pear fruit by *Cryptococcus laurentii* and cytokinin. Postharvest Biol. Technol. 45: 221–227.

Zill, G., Engelharst, G. and Wallnöfer, P.R. 1988. Determination of ergosterol as a measure of fungal growth using Si 60 HPLC. Z. Lebensm. Unters. Forsch. 187: 246–249.

Zipkes, M.R., Gilchrist, J.E. and Peeler, J.T. 1981. Comparison of yeast and mould counts by spiral, pour and streak plate methods. J. Assoc. Off. Anal. Chem. 64: 1465–1469.

Zitter, T.A. and Wien, H.C. 1984. Outbreak of *Alternaria alternata* causing fruit rot of tomatoes in upstate New York. Plant Dis. Reptr. 68: 628.

Zohri, A.A. and Saber, S.M. 1993. Filamentous fungi and mycotoxins detected in coconut. Zentralbl. Mikrobiol. 148: 325–332.

Zohri, A.A., Abdel-Sater, M.A. and Ismail, M.A. 1995. Incidence of aflatoxins and mould flora in corn snacks. J. Food Sci. Technol. India 32: 289–294.

Zur, G., Shimoni, E., Hallerman, E. and Kashi, Y. 2002. Detection of *Alternaria* fungal contamination in cereal grains by a polymerase chain reaction-based assay. J. Food Prot. 65: 1433–1440.

Index

Note: Bold face denotes fungal species description