Staphylococcus succinus sp. nov., isolated from Dominican amber

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Two bacterial isolates, designated AMG-D1^T and AMG-D2, were recovered from 25–35-million-year-old Dominican amber. AMG-D1^T and AMG-D2 biochemically most closely resemble *Staphylococcus xylosus*; they differ physiologically from other staphylococci. Fatty acid analysis and comparisons with extensive databases were unable to show relatedness to any specific taxon. Moreover, AMG-D1^T and AMG-D2 contain tuberculostearic acid and *meso*-diaminopimelic acid, characteristic of the G+C-rich coryneform bacteria, as opposed to L-lysine characteristic of staphylococci. AMG-D1^T and AMG-D2 have a G+C ratio of 35 mol%. Phylogenetic analysis with the 16S rRNA gene indicated that AMG-D1^T and AMG-D2 were most closely related to *Staphylococcus equorum*, *S. xylosus*, *Staphylococcus saprophyticus* and other novobiocin-resistant staphylococci. Stringent DNA–DNA hybridization studies with AMG-D1^T revealed similarities of 38% with *S. equorum*, 23% with *S. xylosus* and 6% with *S. saprophyticus*. The results indicate that AMG-D1^T and AMG-D2 represent a novel species, which was named *Staphylococcus succinus* sp. nov. The type strain of the new species is AMG-D1 (ATCC 700337).

INTRODUCTION

Amber, a polymeric glass formed over time from the resins of conifers and some flowering plants, provides an excellent preservative matrix for biological specimens. Cano & Borucki reported the recovery of a viable *Bacillus sphaericus*-like isolate from the gut of a stingless bee found in Dominican amber estimated to be 25–40 million years old (3, 17). Observations made during the recovery of this spore-former were sufficiently compelling to attempt the recovery of non-spore-forming bacteria from inclusions in amber obtained from the same Dominican source.

Members of the genus *Staphylococcus* are ubiquitous and diverse. Currently, 29 recognized species isolated from soil, plants, animals and humans have been described (4, 14, 24, 26, 31). Subjected to extreme environmental conditions, such staphylococci are unusually durable. Viable organisms were recovered from cultures exposed to low temperatures (110–310 K), salt and other desiccating agents (32, 37). After exposure to a barrage of protons corresponding to about 250 years in solar space, *Staphylococcus aureus* showed very high survival rates (15). Additionally, Potts (25) has reported the recovery of viable Gram-positive, non-spore-forming bacilli and cocci from Pliocene permafrost sands and other air-dried environments.

Here we describe two *Staphylococcus*-like strains, AMG-D1^T and AMG-D2, which were isolated from plant and soil inclusions in Dominican amber. Using a polyphasic approach, including biochemical, physiological, morphological and molecular data (6), we grouped these two strains into a new species, *Staphylococcus succinus*.

METHODS

Amber decontamination. A piece of Dominican amber, measuring approximately 3 × 7 × 2 cm and containing small inclusions with the appearance of plant and soil debris, was examined for the presence of cracks or fissures, then rinsed

Abbreviations: DAP, *meso*-diaminopimelic acid; FAME, fatty acid methyl ester.

The GenBank accession numbers for the sequences reported in this paper are AF004219 (AMG-D1^T) and AF004220 (AMG-D2).

three times in sterile water for injection (McGaw) to remove surface particulates. The amber was soaked in 2% glutaraldehyde contained in a sterile medium bottle (Nalge) at 40 °C for 48 h. Incubation of the amber soaked in glutaraldehyde was carried out in a Branson 2200 Ultrasonic Cleaner (Branson Ultrasonic) filled with sterile, filtered, deionized water. Periodically, 30 min intervals of ultrasound were used to dislodge any microbubbles or debris that may have been trapped in surface flaws. After 48 h, the glutaraldehyde was replaced with filtered, 10% acidified bleach and incubation was continued at 25 °C for 24 h with periodic sonication. A final soak in triple-filtered 70% ethanol for 24 h at 25 °C was followed by transferring the flamed amber to a fresh medium bottle for confirmation of the decontamination process.

Media. All sterility evaluation and recovery cultures utilized either 100 ml vials of commercially prepared Trypticase Soy Broth (BBL) supplemented with 0·1% Tween 80 (Sigma) and 0·05% $MnSO_4$ (STSB) or commercially prepared (BBL) thioglycollate broth (TGB). Prior to use, all media were incubated for 7 d each at 25 and 37 °C and samples from each lot were evaluated for growth promotion using standard United States Pharmacopeia procedures (35).

Trypticase Soy Agar plates (TSA) (Remel) were used for subcultures and to determine colony morphology.

Sample preparation. Prior to cracking the amber, the effectiveness of the decontamination procedure was evaluated. The amber was immersed in STSB and incubated at room temperature for 14 d and 37 °C for 7 d. The STSB was then removed, Gram-stained and subcultured in a variety of media and conditions.

The culture flask, still containing the amber, was immersed 3–5 cm into a liquid nitrogen bath for 15 min. The frozen sample was immediately transferred to a sterile mortar and ground to a powder. The pulverized amber was suspended in a small volume of STSB and equal aliquots were inoculated into flasks containing either TGB or STSB. Paired cultures were incubated with periodic agitation at either 25 or 37 °C and observed for growth.

All procedures, except the 37 °C incubations, were performed in a Sterilgard Class II safety hood (Baker) by gowned, hooded, masked and gloved personnel. The hood surfaces and air were monitored for contamination during all procedures using commercially prepared Rodac and 12·7 cm settling plates (PML Microbiologicals).

Transmission electron microscopy. Isolated colonies from 24–48 h cultures growing on TSA or Soil Extract Agar were picked and gently resuspended in 2% glutaraldehyde, pH 7·0, for 24 h. The fixed bacteria were then washed in 0·1 M cacodylate buffer. Post-fixation was carried out with 1% OsO_4 in 0·1 M cacodylate buffer for 24 h, then the samples were dehydrated in an alcohol series, stained en bloc with 3% uranyl acetate, and mounted in epoxy resin. After the resin hardened, 1 μm sections were cut and microscopic examination was performed.

Biochemical and antibiogram determinations. Biochemical and antibiotic profiles were established following the manufacturer's recommended procedure (2) for MicroScan Pos Combo Type 6 panels (Baxter MicroScan). Briefly, four to five 24 h colonies from TSA plates were suspended in MicroScan Inoculum Water to a 0·5 McFarland density equivalent. Suspension (100 μl) was transferred to Pluronic-D water, which was used to rehydrate and inoculate the panels. Individual wells were interpreted for growth or biochemical reactions after 24 h incubation at 35 °C.

Cell wall analysis. Cell wall diamino acid composition was determined via TLC. The method of Komagata & Suzuki (16) was followed with the exception that Kodak silica gel TLC plates were used with a solvent of n-butanol/acetic acid/H_2O (80:20:20, by vol.). L-Lysine and a standard mixture of diaminopimelic acid (DAP) isomers were used to confirm the respective R_F values.

Fatty acid methyl ester (FAME) analysis. Twenty-four-hour cultures of D1 and D2 isolates grown in Trypticase Soy Broth Agar at 28 °C were used for the FAME analysis. Fatty acids were extracted, transmethylated with methanolic HCl and the FAMEs were analysed by gas chromatography. The FAME analysis was performed by Microbial ID in triplicate.

Mol % G+C content of DNA. The DNA extraction protocol was based on previously described procedures (11, 33). A bacterial pellet (0·05–0·1 g wet wt) was suspended in 2 ml suspension buffer (10 mM Tris/HCl, pH 8·0; 1 mM Na-EDTA; 0·35 M sucrose) and 2·5 mg lysozyme crystals were added. The lysozyme treatment was carried out at 37 °C for a minimum of 4 h (Gram-positive organisms) or 10 min (Gram-negative organisms). The cells were lysed during incubation for 1 h at 37 °C following the addition of 3 ml lysis buffer (100 mM Tris/HCl, pH 8·0; 0·3 M NaCl; 20 mM EDTA; 2%, w/v, SDS; 2%, v/v, 2-mercaptoethanol) and 10 μl proteinase K (100 μg ml^{-1}) (Sigma). An equal volume of phenol/chloroform/isoamyl alcohol (25:24:1, by vol.) was added to the lysate, and the aqueous phase was recovered and added to 5 ml chloroform/isoamyl alcohol (24:1, v/v). Following a second separation, the aqueous phase was transferred to a 50 ml centrifuge tube and 10 ml ice-cold 2-propanol was added. The DNA precipitated after incubation at −20 °C for 30 min and was pelleted by centrifugation at 26 500 *g* for 30 min at 0 °C. The 2-propanol was removed and the DNA was washed in 70% ice-cold ethanol and repelleted as before. Finally, the dried DNA was resuspended in Tris-EDTA buffer, pH 7·6.

The concentration of the DNA was estimated as described by Sambrook *et al.* (29). The purity was evaluated by determination of the A_{260}/A_{280} ratio (28). The enzymic digestion of the DNA was performed in triplicate as described by Gehrke *et al.* (10).

The nucleosides were separated using a modular 1100 series HPLC system (Hewlett Packard), a 12·5 cm ODS Hypersil C-18 column (Hewlett Packard) and an isocratic solvent system. The mobile phase was 8% methanol, 0·05 M KH_2PO_4, pH 4·4, flowing at 1·0 ml min^{-1}. To avoid having to account for modified cytosines, the deoxyguanosine/thymine nucleoside concentrations were measured by the A_{254} using a diode array detector. Their ratio was calculated and used to determine the mol% G+C content (20).

DNA relatedness, DNA sequencing and phylogenetic analysis. DNA hybridizations were performed as described by Rosselló-Mora *et al.* (27). Isolates AMG-D1^T and AMG-D2 were characterized via complete 16S rDNA sequencing using the technique of Dodge *et al.* (7). The flanking primers 5f and 1540r were used in PCR reactions to generate 1540 bp amplicons. One-hundred microlitre PCR reactions were carried out with kit-supplied buffer (PE Applied Biosystems), 350 μM total dNTPs (mix containing dATP, dCTP, dGTP, dTTP, dUTP), 1·5 mM $MgCl_2$, 20 pmol each primer, 2·5 units AmpliTaq and approximately 1 ng purified template DNA in a 5·0 μl volume. The thermal cycler

(Perkin-Elmer Gene-Amp PCR System 9600) was programmed for an initial 2 min denaturation at 94 °C, followed by 30 cycles of 94 °C denaturation (30 s), 55 °C annealing (30 s) and 72 °C extension (45 s) sequence, and ending with a 10 min 72 °C extension.

The 16S rRNA PCR products were purified to remove excess dNTPs using Microcon-100 concentrators (Amicon). The purified products were then used in cycle sequencing reactions using four-colour fluorescently labelled ddNTPs (FS Dye-Doe Terminator Cycle Sequencing kit; Applied Biosystems). Twelve overlapping forward and reverse primers, spanning the entire 16S rRNA gene in both directions, were used to maximize sequence data accuracy. Prior to electrophoresis, cycle sequencing products were purified of excess fluorescent ddNTPs, dNTPs and sequencing primers using Centri-sep spin columns (Princeton Separations). The purified samples were loaded onto 5% Long Ranger gels (FMC Bioproducts) and the electrophoresis was carried out for 7 h. Electropherograms were created from the gel scans using Data Collection version 1.2.1 (PE Applied Biosystems) and Sequence Analysis version 2.1.0 (PE/ABI). Sequence electropherogram files were analysed using Factura software version 1.2.0r6 (PE/ABI). Consensus sequences were aligned with the aid of CLUSTAL 5.0 and the alignment was verified manually. Phylogenetic analyses of the aligned sequences were carried out by the maximum likelihood algorithm using PHYLIP 3.2 (9).

New sequence was added to an alignment of about 5300 homologous bacterial 16S rRNA primary structures by using the aligning tool of the ARB program package (34). Similarity and distance matrices were calculated with the program ARB-PHYL of the same package. Phylogenetic trees were constructed using subsets of data that included representative sequences of staphylococci (19). These distance matrices, maximum likelihood and parsimony methods were from the programs PHYLIP (8), ARB and fastDNAml (19).

RESULTS AND DISCUSSION

Bacterial recovery

A visual and microscopic examination found the amber piece to be free of cracks and fissures. All sterility controls were negative for microbial growth. Five days post-inoculation of STSB with the crushed amber, a small white fleck, resembling precipitated protein, appeared floating on the surface of STSB medium incubated at 25 °C. Allowed to increase in size over 6 d, the 5 mm fleck was removed and streaked on TSA. After 48 h at 25 °C, multiple colonies of two types appeared: a white, entire, convex, opaque colony (AMG-D1^T) and a grey, lobated, convex colony with a rubbery consistency (AMG-D2). Gram stains of both colony types revealed Gram-positive cocci in a 'pilot wheel'-like arrangement. Both isolates were treated as different strains, and this discrimination has been supported, as shown below, by FAME and DNA–DNA pairing results.

Transmission electron photomicrographs (Fig. 1) revealed the presence of spherical cells with typical Gram-positive cell wall morphology. However, dividing cells exhibited unusual one, two and three internal cross-wall structures (Fig. 1).

Biochemical and antimicrobial sensitivity patterns

Similar biochemical patterns were observed for AMG-D1^T, AMG-D2 and *Staphylococcus xylosus*. Salient distinguishing characteristics include their ability to grow in the presence of 40% bile and the inability of both isolates to reduce nitrate to nitrite. Other biochemical characteristics useful in discriminating these strains from other novobiocin-resistant species include their ability to hydrolyse indoxyl phosphate and the fermentation of lactose, mannose and raffinose. These results are summarized in Table 1.

Both AMG-D1^T and AMG-D2 were sensitive to amikacin, amoxycillin/K clavulanate, ampicillin, cefazolin, cefotaxime, ceftriaxone, cefuroxime, cephalothin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, imipenem, nitrofurantoin, norfloxacin, oxacillin, penicillin, rifampin, streptomycin, sulfamethoxazole, tetracycline, ticarcillin/K clavulanate, trimethoprim/sulfamethoxazole and vancomycin, but were resistant to novobiocin.

Cell wall analysis

The TLC procedure readily discriminates L-lysine from DAP. Each plate included control lysates from *Staphylococcus epidermidis* and *Bacillus subtilis*, L-lysine and DAP isomer standards, and the sample preparations. The R_F values from three trials are presented in Table 2. These data indicate that AMG-D1^T and AMG-D2 cell walls contain DAP but not L-lysine. In contrast, *Staphylococcus* spp. cell walls characteristically contain only L-lysine (30).

FAME analysis

Comparison of AMG-D1^T and AMG-D2 FAME results to extensive databases using Sherlock software (MIDI) was unable to detect relatedness to any specific taxon. The presence of tuberculostearic acid (TBSA 10Me18:0), characteristic of coryneform bacteria, has not been previously described for *Staphylococcus* spp. (14, 22). However, both AMG-D1^T and AMG-D2 contain straight-chain saturated C20 fatty acid, which is typical of *Staphylococcus* species (Table 3).

Branched-chain fatty acids were the predominant fatty acids identified in both AMG-D1^T (90·29%) and AMG-D2 (88·51%). While not common, branched-chain fatty acids are the principal fatty acids of some *Bacillus* (12, 13) and *Staphylococcus* (22) strains. These results are summarized in Table 3.

Phylogenetic analysis

The 16S rRNA nucleotide sequences of AMG-D1^T and AMG-D2 were compared to homologous sequences of *Staphylococcus* spp. and other bacteria listed in the GenBank and RDP databases (19). BLAST searches indicate a close relationship of both AMG-

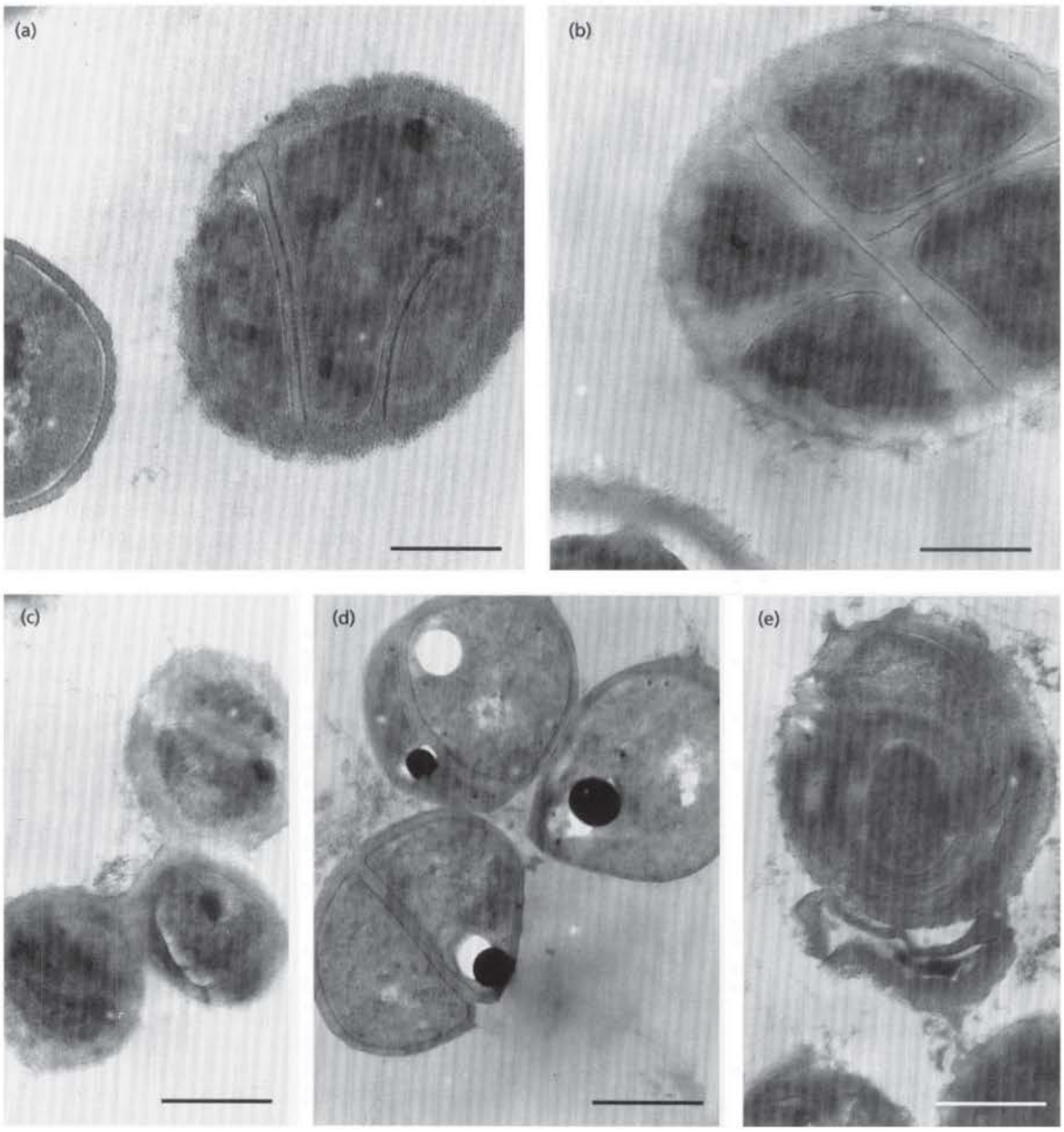

Fig. 1. Transmission electron photomicrographs of AMG-D1^T and AMG-D2. (a) AMG-D1^T growing on TSA for 48 h at 30 °C showing its characteristic form of cell division. Bar, 238 nm. (b) AMG-D2 growing on TSA for 48 h at 30 °C showing its characteristic form of cell division. Bar, 234 nm. (c) AMG-D1^T growing on TSA for 48 h at 30 °C, rosette formation. Bar, 219 nm. (d) AMG-D2 growing on TSA for 48 h at 32 °C showing (polyphosphate) inclusions. Bar, 278 nm. (e) AMG-D1^T growing on TSA for 72 h at 40 °C. Bar, 171 nm.

D1^T and AMG-D2 with members of the genus *Staphylococcus*. The calculated percentage 16S rRNA similarity between AMG-D1^T and AMG-D2 was 100 %. The values between these strains and *S. xylosus*, *Staphylococcus saprophyticus*, *Staphylococcus equorum*, *Staphylococcus cohnii* and *S. aureus* were 99·35, 99·13, 98·90, 98·84 and 97·45 %, respectively.

A 16S-rRNA-based tree reflecting the phylogenetic relationship of the strain AMG-D1^T and a selection of available type strains of the genus *Staphylococcus* is illustrated in Fig. 2. The tree was constructed based on the results of a distance matrix analysis of complete 16S rRNA sequences (19). It was evaluated by maximum likelihood and parsimony analyses. AMG-D1^T

Table 1. Biochemical characteristics of AMG-D1^T, AMG-D2 and other *Staphylococcus* spp.

Reactions are interpreted as the following: +, more than 90% of the strains are positive; −, more than 90% of the strains are negative; +/−, between 25 and 75% of the strains are positive; w, weak positive reaction. Values shown in this table are compilations of MicroScan results. PGR, *p*-nitrophenyl β-D-glucuronide; PGT, *p*-nitrophenyl β-D-galactopyranoside.

Biochemical assay	AMG-D1^T	AMG-D2	*S. xylosus*	*S. equorum*	*S. saprophyticus*
Growth in:					
Crystal violet	−	−	−	−	−
0·05 μg bacitracin ml^{-1}	+	+	+	+	+
40% bile	+	+	−	+/−	−
1·6 μg novobiocin ml^{-1}	+	+	+	+	+
Optochin	+	+	+	+	+
Reduction of nitrate	−	−	+	+	−
Production of glycosidase (PGR)	+	+	+	−	−
Production of glycosidase (PGT)	+	+	+	+/−	+/−
Hydrolysis of indoxyl phosphate	+	+	+/−	−	−
Voges–Proskauer test	−	−	+/−	−	+
Phosphatase	+	+	+	+/−	−
Production of pyrrolidonase	−	−	+/−	−	−
Dehydrolysation of arginine	−	−	−	−	−
Production of urease	+	+	+	+/−	+
Production of catalase	+	+	+	+	+
Production of oxidase	−	−	−	−	−
Fermentation of:					
Raffinose	−	w	−	−	−
Lactose	+	+	+/−	−	+/−
Mannose	+	+	+/−	−	−
Trehalose	+	+	+	−	−

Table 2. R_F values from the TLC of cell wall components of AMG-D1^T, AMG-D2, *B. subtilis* and *S. epidermidis*

Sample	R_F value		
	Trial 1	Trial 2	Trial 3
L-Lysine	0·23	0·23	0·23
DAP isomers	0·20	0·20	0·20
AMG-D1^T	0·20	0·20	0·20
AMG-D2	0·20	0·20	0·20
B. subtilis (ATCC 6051)	0·20	0·19	0·19
S. epidermidis (ATCC 14990)	0·23	0·24	0·24

and AMG-D2 were found to be most closely related to novobiocin-resistant *Staphylococcus* spp., and in particular to *S. equorum*, *S. xylosus* and *S. saprophyticus*. This relationship was also seen in biochemical studies.

Mol% G+C and DNA relatedness

Results are summarized in Table 4. The G+C content of both AMG-D1^T and AMG-D2 was 35 mol%, in accordance with the overall content of the genus (1, 14). To confirm that our protocol for mol% G+C determination was accurate, we analysed the mol% G+C from *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Salmonella dublin*, *Bacillus thuringiensis* and *S. epidermidis*. Our values were, in mol%, 50, 66, 61, 51, 37 and 35, respectively. Published values are 50–51, 67, 61–63, 50–53, 34–40 and 30–37%, for *C. freundii* (28), *P. aeruginosa* (23), *P. putida* (23), *Salmonella dublin* (18), *B. thuringiensis* (5) and *S. epidermidis* (14), respectively. These results are consistent with G+C contents of *Staphylococcus* (14).

The results of DNA–DNA similarity studies are also summarized in Table 4. DNA–DNA hybridization results indicated that the two *S. succinus* strains were tightly related (95% similarity) but conspicuously different (38–6%) from the phylogenetically most related species, *S. equorum*, *S. xylosus* and *S. saprophyticus*.

Attending to the recommendations of the systematics committee, these strains appeared sufficiently distant, both phylogenetically and genomically (DNA–DNA hybridization), from the rest of the staphylococci to be classified as a new species (21, 36). Based on these results, it appears that AMG-D1^T and AMG-D2 comprise two strains of a distinct species in the genus *Staphylococcus*. We propose they be classified as

Table 3. Fatty acid composition of AMG-D1^T and AMG-D2

Mean percentage fatty acid profiles for the described *Staphylococcus* spp. were provided by Microbial Identification.

Fatty acid	AMG-D1^T	AMG-D2	*S. equorum*	*S. xylosus*	*S. cohnii*	*S. saprophyticus*
11:0 iso	0·61	0·36	0·00	0·00	0·00	0·00
11:0 anteiso	0·23	0·33	0·00	0·00	0·00	0·00
12:0 iso	0·52	0·32	0·00	0·00	0·00	0·00
12:0	0·50	0·63	0·00	0·00	0·00	0·00
13:0 iso	28·38	17·39	1·22	1·44	0·00	0·55
13:0 anteiso	12·73	15·00	0·00	0·00	0·00	0·00
14:0 iso	1·39	1·06	1·10	2·40	2·48	1·88
14:0	1·50	2·31	0·76	1·13	0·50	1·10
15:0 iso	18·24	15·47	13·13	23·73	15·01	21·19
15:0 anteiso	18·12	24·74	60·15	39·24	43·28	41·55
16:0 iso	0·97	0·69	0·83	0·97	3·14	1·94
16:1ω11*c*	0·21	0·26	0·00	0·00	0·00	0·00
16:0	1·31	2·18	2·86	3·44	3·21	3·57
iso 17:1ω10*c*	1·53	0·97	0·00	0·00	0·00	0·00
17:0 iso	6·02	5·30	0·00	8·69	5·32	6·56
17:0 anteiso	2·29	3·28	6·00	4·48	10·18	4·66
18:1ω9*c*	0·00	0·00	1·83	0·00	0·00	0·00
18:0 iso	0·00	0·00	0·00	0·00	0·62	0·00
18:0	1·65	1·77	2·72	5·66	6·05	5·52
TBSA 10Me18:0	1·45	1·01	0·00	0·00	0·00	0·00
19:0 iso	0·79	1·29	1·41	2·75	1·83	1·92
19:0 anteiso	0·52	0·46	0·00	0·00	0·86	0·00
20:1ω9*c*	1·07	1·85	0·00	0·00	0·00	0·00
20:0	0·49	1·04	2·58	5·47	6·65	9·12

Table 4. DNA–DNA hybridization studies among AMG-D1^T, AMG-D2, *S. equorum* and *S. xylosus*

Isolate identification	Mean G+C content (mol %)	Similarity (%)	
		AMG-D1^T*	*S. equorum** (ATCC 43958)
AMG-D1^T	35	100	16
AMG-D2	35	95	22
S. equorum (ATCC 43958)	34	38	100
S. xylosus (ATCC 29971)	34	23	28
S. saprophyticus (ATCC 15305)	32	6	6

* Labelled DNA was used with these taxa in DNA–DNA similarity studies.

Staphylococcus succinus sp. nov. A description of the species follows.

Description of *Staphylococcus succinus* sp. nov.

Staphylococcus succinus (suc.cin.us. L. gen. n. *succinum* of amber).

Cells are Gram-positive, spherical (diameter 0·6–1·9 μm) and form characteristic rosettes with one central cell surrounded by two to five peripheral cells. Colonies after 2 d in TSA at 25 °C are raised with an elevated centre (umbonate), glossy, opaque white, rough and crenated, measuring 4–6 mm. AMG-D2 colonies, under the same conditions, are similarly sized, raised, glossy, opaque grey-white, smooth and entire. Growth occurs between 25 and 40 °C, but not at 42 °C. Optimal growth is obtained at 28 °C. The strains grow more slowly at 40 °C. They do not grow in the anaerobic portion of thioglycollate shake medium. Biochemical test results and the differential characteristics from other novobiocin-resistant staphylococci are shown in Table 1. The strains are PGR-positive, indoxyl-phosphate-positive, Voges–Proskauer-neg-

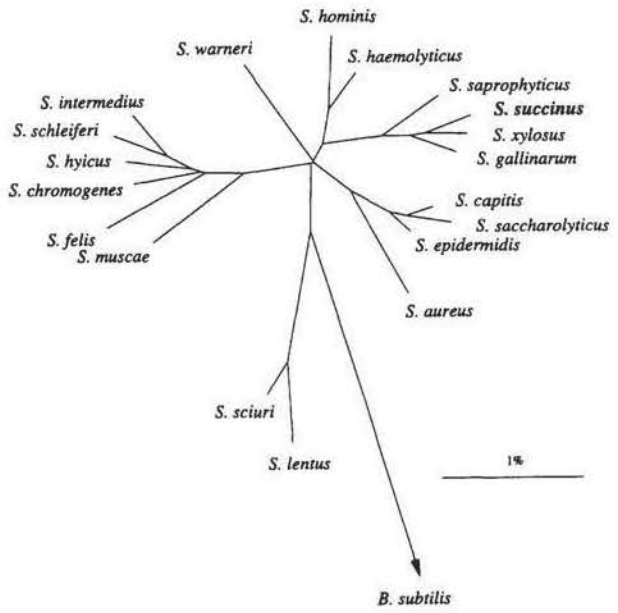

Fig. 2. Phylogenetic tree of AMG-D1^T and other *Staphylococcus* spp. based on complete 16S rRNA gene sequences. The bar indicates 1% of estimated sequence divergence. Topology of the major branches as well as the phylogenetic position of AMG-D1^T did not differ in all treeing approaches. Multifurcation indicates a tree topology which could not be significantly resolved based on the available data set.

ative, phosphatase-positive and ferment lactose, mannose and trehalose but are unable to reduce nitrate to nitrite. *S. succinus* can be differentiated from other novobiocin-resistant strains of *Staphylococcus* spp. using these analyses. Both strains contain peptidoglycan type DAP. The G+C content of the strains was tested and found to be 35 mol%. The type strain is resistant to novobiocin and sensitive to all other antibiotics tested. Principal membrane fatty acids are the saturated, odd-numbered, branched-chain fatty acids C13 iso, C13 anteiso, C15 iso, C15 anteiso, C17 iso and C17 anteiso. In addition, the cell membranes contain 10MeC18 (tuberculostearic acid). Strain AMG-D1 is the type strain (= ATCC 700337). The description of the strain corresponds to the description of the species.

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