Staphylococcus pasteuri sp. nov., Isolated from Human, Animal, and Food Specimens

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A new novobiocin-susceptible species of the genus *Staphylococcus*, *Staphylococcus pasteuri*, is described on the basis of the results of a study of seven strains isolated from human, animal, and food specimens. DNA relatedness experiments (S1 nuclease method) showed that these strains form a homogeneous genomic species related at DNA homology levels of 2 to 13% to 27 type strains representing known *Staphylococcus* species. The use of a method based on rRNA gene restriction site polymorphism provides clear-cut distinction between this new species and *Staphylococcus warneri*, which is the most similar species phenotypically. The type strain of the new species is strain BM9357 (= ATCC 51129).

Taxonomy of the genus *Staphylococcus* was shaken by two important methodological steps: an extensive phenotypic investigation of human strains which led to the delimitation of 10 species in 1975 (22, 32) and DNA relatedness studies which have currently circumscribed 29 species (15, 17, 19, 23, 31, 36) and four named subspecies (3, 9, 20, 25).

We have recently developed a method based on rRNA gene restriction site polymorphism for identifying *Staphylococcus* species and subspecies (4, 7, 8, 12). The use of this method enabled the screening of seven staphylococcal strains, isolated from various sources (human, animal, and food specimens), that were phenotypically similar to *Staphylococcus warneri* but genotypically more closely related to each other than to any of the 271 strains validly classified as belonging to 31 staphylococcal taxa (7, 8). In the present study, DNA-DNA hybridization experiments grouped these seven strains into a new species, *Staphylococcus pasteuri*, described in this paper.

MATERIALS AND METHODS

Bacterial strains. Seven epidemiologically unrelated isolates were recovered from human clinical specimens (BM9357^T [T = type strain] from vomit; BM9363 from urine; BM10425, BM10426, and BM10427 from hemocultures), mixed vegetables (BM9359), and goat's milk (BM10507). A loopful of each sample was streaked onto a nutrient sheep blood agar plate, and the plates were incubated for 24 h at 37°C. The medium used for propagation of all isolates was brain heart infusion (Difco Laboratories, Detroit, Mich.). All cultures were stored as frozen suspensions at –20°C in brain heart infusion containing 30% (vol/vol) glycerol (Prolabo, Paris, France). The type strains of 27 *Staphylococcus* species (Table 1) were purchased from the following institutions: American Type Culture Collection, Rockville, Md., National Collection of Type Cultures, London, England, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany, and Czechoslovak Collection of Microorganisms, J. E. Purkyně University, Brno, Czechoslovakia. The three *Staphylococcus hominis* strains and the nine *S. warneri* strains (Table 2), collected by the French National Refer-

ence Center for Staphylococci (Institut Pasteur, Paris, France), were previously identified by our method based on rRNA gene restriction site polymorphism (4, 8).

Phenotypic characterization. To observe daily morphology and pigmentation of colonies, bacterial strains were grown on P agar (24) and P agar containing 1.7% (vol/vol) skim milk at 35°C for 3 days and at 25°C for an additional 2 days (21, 23). Cell morphology was observed with Gram-stained smears. Growth at various temperatures and salt tolerance were evaluated after incubation for 48 h (24). Anaerobic growth was tested by using a semisolid Brewer thioglycolate medium (Difco) (13). The configuration of lactic acid produced from glucose under anaerobic conditions (33) was analyzed enzymatically with a commercial kit (Boehringer Mannheim GmbH, Mannheim, Germany). Catalase activity was tested by inoculating a small amount of a plate culture into a drop of 3% H₂O₂. Free coagulase and clumping factor were detected by using the Staphylocoagulase test with rabbit plasma (Diagnostics Pasteur, Marnes-la-Coquette, France) and the Staphyslide test (BioMérieux, Marcy l'Etoile, France), respectively. The following characteristics were determined as previously described: fibrinolysin activity (5) tested with rabbit plasma, oxidase activity (14), DNase activity (26), β-glucosidase activity (37), caseinolytic activity (10), and nitrate reduction and acid production aerobically from various carbohydrates (24). Hemolysis was tested on tryptic soy agar (Difco) containing 5% (vol/vol) washed sheep erythrocytes (Diagnostics Pasteur), and the hemolysins produced were characterized by their synergy with β-toxin, using the CAMP disk procedure (18). Gelatin hydrolysis and egg yolk reaction were detected in tryptic soy agar supplemented with 0.5% (wt/vol) gelatin (Merck, Darmstadt, Germany) and 5% (vol/vol) egg yolk emulsion, respectively (35). Esculin hydrolysis was detected by using an ID32 Staph gallery (API BioMérieux, La Balme les Grottes, Montalieu-Vercieu, France), as were the activities of the following enzymes: β-galactosidase, β-glucuronidase, pyrolidonyl arylamidase, arginine arylamidase, and N-acetylglucosaminidase. Determination of acetoin production in Clark and Lubs' liquid medium (Diagnostics Pasteur), detection of urease activity on Christensen urea agar (Diagnostics Pasteur), and detection of arginine dihydrolase and ornithine decarboxylase activities in Moeller's liquid media (Diagnos-

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TABLE 1. DNA relatedness between *S. pasteurii* and 27 *Staphylococcus* species

Source of unlabeled DNA ^a	% Reassociation at 60°C with labeled DNA from BM9357 ^T
<i>S. pasteurii</i> BM9357 ^T	100
<i>S. pasteurii</i> BM9359	93
<i>S. pasteurii</i> BM9363	98
<i>S. pasteurii</i> BM10507	94
<i>S. arlettae</i> DSM 20672 ^T	3
<i>S. aureus</i> ATCC 12600 ^T	6
<i>S. auricularis</i> ATCC 33753 ^T	6
<i>S. capitis</i> ATCC 27840 ^T	6
<i>S. caprae</i> CCM 3573 ^T	13
<i>S. carnosus</i> DSM 20501 ^T	2
<i>S. caseolyticus</i> ATCC 13548 ^T	5
<i>S. chromogenes</i> NCTC 10530 ^T	5
<i>S. cohnii</i> ATCC 29974 ^T	8
<i>S. delphini</i> ATCC 49171 ^T	5
<i>S. epidermidis</i> ATCC 14990 ^T	4
<i>S. equorum</i> DSM 20674 ^T	6
<i>S. felis</i> ATCC 49168 ^T	2
<i>S. gallinarum</i> CCM 3572 ^T	7
<i>S. haemolyticus</i> ATCC 29970 ^T	6
<i>S. hominis</i> ATCC 27844 ^T	7
<i>S. hyicus</i> ATCC 11249 ^T	3
<i>S. intermedius</i> ATCC 29663 ^T	5
<i>S. kloosii</i> DSM 20676 ^T	4
<i>S. lentus</i> ATCC 29070 ^T	2
<i>S. lugdunensis</i> ATCC 43809 ^T	6
<i>S. saprophyticus</i> ATCC 15305 ^T	11
<i>S. schleiferi</i> ATCC 43808 ^T	2
<i>S. simulans</i> ATCC 27848 ^T	5
<i>S. sciuri</i> ATCC 29062 ^T	2
<i>S. warneri</i> ATCC 27836 ^T	11
<i>S. xylosus</i> ATCC 29971 ^T	8

^a The strains whose designations begin with BM are from the collection of the French National Reference Center for Staphylococci (Institut Pasteur). DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany; ATCC, American Type Culture Collection, Rockville, Md.; CCM, Czechoslovak Collection of Microorganisms, J. E. Purkyne University, Brno, Czechoslovakia; NCTC, National Collection of Type Cultures, London, England.

tics Pasteur) were performed according to the manufacturer's instructions.

Susceptibility to various antimicrobial agents was determined on Mueller-Hinton agar by a standard diffusion technique (1) with disks containing penicillin G, oxacillin, cefalotin, spectinomycin, streptomycin, kanamycin, neomycin, tobramycin, gentamicin, chloramphenicol, tetracycline, minocycline, erythromycin, oleandomycin, spiramycin, lincomycin, sulfonamides, trimethoprim, pefloxacin, fusidic acid, rifampin, fosfomycin, vancomycin, nitrofurantoin, novobiocin, and vibriostatic agent 0/129 (all obtained from Diagnostics Pasteur), as well as bacitracin (Eurobio, Les Ulis, France). Disks were prepared with pristinamycin I (40 µg; Rhône-Poulenc-Rorer, Vitry-sur-Seine, France), pristinamycin II (20 µg; Rhône-Poulenc-Rorer), cadmium acetate (0.2 µmol; Merck), sodium arseniate (2 µmol; Merck), mercuric nitrate (0.2 µmol; Merck), ethidium bromide (200 µg; Sigma Chemical Co., St. Louis, Mo.), acriflavine (200 µg; Sigma), cetyltrimethylammonium bromide (10 µg; International Biotechnologies, Inc., New Haven, Conn.), propamidine isothionate (200 µg; May and Baker, London, England), chlorhexidine digluconate (400 µg; Coopération Pharmaceutique Française, Melun, France), lysostaphin (100 U; Sig-

ma), and desferal (1 mg; Ciba-Geigy Ltd., Lane Cove, Australia).

Isolation of cell walls. Bacteria from 500 ml of a culture grown in brain heart infusion to an optical density of 0.7 at 578 nm were harvested by centrifugation at 12,000 × g for 10 min. After suspension in 1 M NaCl, the cells were disintegrated with glass beads (diameter, 0.1 mm; Braun, Melsungen, Germany) in a cooled cell grinder (Dyno Mill, Bachofen, Switzerland), keeping the temperature below 4°C. After the beads were separated from the suspension with a sintered glass filter, the suspension was mixed with 250 ml of 1% sodium dodecyl sulfate (SDS) and heated for 30 min at 60°C to remove membranes and noncovalently bound protein. After centrifugation at 12,000 × g for 10 min and washing with distilled water (four times), covalently bound protein was removed by incubation with 0.2 mg of trypsin (Sigma) per ml in 0.15 M Tris-HCl buffer (pH 7) for 24 h. After extensive washing with buffer and distilled water, the resulting cell walls were lyophilized.

Amino acid analysis of cell walls. Samples were prepared by hydrolyzing isolated cell walls with 4 M HCl at 100°C for 15 h. Amino acid analyses were performed with a model LC5001 analyzer (Biotronik, Maintal, Germany) and sodium citrate buffer. Amino acids and amino sugars were detected with the ninhydrin reagent.

Isolation of teichoic acids. Bacteria from 30 ml of an overnight culture were harvested by centrifugation (4,000 × g, 10 min), washed with NaCl (0.9%), and lyophilized. The lyophilized cells were suspended in 3 ml of 70% (wt/vol) aqueous phenol, centrifuged, and then heated for 30 min at 68°C. The sediment was washed with 30 ml of distilled water and lyophilized. Lyophilized cells (20 mg) were treated with 40% hydrofluoric acid (16 h at 4°C) and centrifuged, and the supernatant was dried in vacuo. The material was dissolved in 2 M HCl, hydrolyzed (3 h at 100°C), and again dried in vacuo. Then several reagents were added in the following order: 300 µl of dimethyl sulfoxide, 300 µl of cyclohexane, 100 µl of trimethylchlorosilane, and 200 µl of hexamethylene disilazane. After 30 min at room temperature, 5 µl of the upper phase was sampled for analysis.

Gas chromatography analysis of teichoic acids. We used a model 5890A gas chromatograph (Hewlett-Packard, Böblingen, Germany) equipped with an HP-5 capillary column (0.2 µm by 0.2 mm by 25 m), a flame ionization detector, and a model 5898A computer workstation for data registration and evaluation. Operating conditions were as follows: injection port temperature, 250°C; detector temperature, 300°C; carrier gas pressure, 62 kPa (nitrogen); oven temperature programmed to increase from 100°C (for 1 min) to 300°C (for 10 min) with increments of 5°C/min. Peaks were identified by comparing the retention times with retention times of standard samples.

Isolation of DNA. Large-scale preparation of cellular DNA was carried out by using 200 ml of a culture in PY broth (1% tryptone, 0.5% yeast extract, 0.1% glucose, 0.5% NaCl, 0.1% Na₂HPO₄; pH 7.2). At late logarithmic growth phase, penicillin was added at a final concentration of 100 µg ml⁻¹, and incubation was continued for 1 h. Cells were harvested by centrifugation, washed with 0.15 M EDTA (pH 8), and resuspended in 5 ml of TE buffer (10 mM Tris base [pH 8], 1 mM EDTA). They were then treated with lysozyme (40 U ml⁻¹; Sigma) and lysostaphin (15 U ml⁻¹; Sigma) at 37°C until visible lysis occurred. Lysis was completed by adding 1% (wt/vol) SDS and proteinase K (50 µg ml⁻¹; Merck) and leaving the cells for 30 min at 37°C. Sodium chloride and cetyltrimethylammonium bromide were added at final con-

TABLE 2. Characteristics differentiating the 21 strains analyzed phenotypically

Characteristic	<i>S. pasteurii</i> strains							<i>S. warneri</i> strains										<i>S. hominis</i> strains			
	BM9357 ^T	BM9359	BM9363	BM10507	BM10425	BM10426	BM10427	ATCC 27836 ^T	BM9372	BM9382	BM9408	BM9414	BM9418	76066	75594	CH108	CH30	ATCC 27844 ^T	SM67	75611	78354
Colony diam of >5 mm	+	-	+	-	-	-	-	+	-	-	+	+	+	-	+	+	-	-	-	-	-
Pigmentation (carotenoid)	(+)	(+)	(+)	+	+	-	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+
Anaerobic growth (thioglycolate)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	w	w	w	w
Production of:																					
D-Lactate	+	+	+	+	+	+	+	+	+	+	+	ND	ND	ND	ND	+	+	+	ND	ND	+
L-Lactate	+	+	+	+	+	+	+	+	+	+	+	ND	ND	ND	ND	+	+	-	ND	ND	-
β-Glucosidase	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	-	+	+
β-Glucuronidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	w	-	-
Egg yolk reaction	+	+	+	+	+	+	+	-	-	+	+	-	+	+	+	-	+	-	-	-	-
Synergistic hemolysis	+	+	+	+	+	+	+	NH	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin hydrolysis	+	+	-	+	+	+	+	-	+	+	+	+	-	+	-	+	+	-	-	-	-
Nitrate reduction	-	+	+	-	+	+	+	-	+	-	+	-	+	-	+	-	-	+	-	+	+
Acetoin production	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-
Arginine dihydrolase	+	+	-	-	+	+	w	+	-	+	+	-	+	+	-	+	w	w	-	-	-
Casein hydrolysis	+	-	-	-	-	w	-	-	-	w	w	+	-	+	-	+	+	+	+	+	+
N-Acetylglucosaminidase	-	-	w	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Acid produced (aerobically) from:																					
D-Trehalose	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
D-Mannose	-	-	-	-	-	-	-	w	-	-	-	-	-	-	-	-	+	-	-	-	-
D-Ribose	-	-	-	-	-	-	-	+	(+)	-	(+)	(+)	(+)	(+)	(+)	(+)	-	-	-	-	-
α-Lactose	(+)	-	-	+	-	-	-	-	-	(+)	(+)	(+)	-	(w)	w	-	+	+	+	+	+
Maltose	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Mannitol	+	+	-	+	+	+	+	+	+	+	-	-	-	+	-	+	+	-	-	-	-
D-Melezitose	(w)	-	+	+	w	-	(w)	(w)	-	-	-	-	(w)	-	(w)	-	-	(w)	(w)	(w)	(w)
D-Turanose	-	-	-	(w)	w	-	w	-	-	-	-	-	-	-	(w)	-	-	+	+	+	+
Resistance to lysostaphin (100 U)	+	+	+	+	+	+	+	-	+	-	-	-	+	-	-	-	-	+	-	+	+
Resistance to desferal (1 mg)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-

+ , positive reaction; -, negative reaction; w, weakly positive reaction. Parentheses indicate a delayed reaction. ND, not determined; NH, nonhemolytic.

centrations of 1 M and 1% (wt/vol), respectively, and the lysate was incubated for 10 min at 65°C prior to sequential extraction with equal volumes of phenol-chloroform-isoamyl alcohol (25/24/1, by volume) and chloroform-isoamyl alcohol (24/1, by volume) (2). Isopropanol (0.6 volume) was added to the last aqueous layer. Precipitated DNA was recovered by centrifugation, washed in 70% (vol/vol) ethanol, dried, resuspended in TE buffer, and treated with RNase (3 U ml⁻¹; Boehringer) for 30 min at 37°C. The purity and concentration of DNA were assayed spectrophotometrically.

rRNA gene restriction site polymorphism. Cellular DNAs isolated from the staphylococcal strains were cleaved by *EcoRI* (Amersham International plc, Amersham, England) and probed under stringent conditions with ³²P-labeled plasmid pBA2 (4). The sizes of the hybridizing bands were calculated by using the Raoul I DNA ladder (Appligène, Illkirch, France) as a molecular weight standard. The hybridization patterns (HP) obtained were compared with those previously described (7, 8). The similarity between any two HP was assessed by calculating the Dice coefficient (11).

DNA-DNA hybridization. The S1 nuclease method (6) was used for DNA-DNA hybridization according to a procedure (the S1 nuclease-trichloroacetic acid procedure) detailed elsewhere (16). Native cellular DNA was labeled in vitro by

nick translation with tritium-labeled deoxynucleotides (Amersham).

DNA base composition. The guanosine-plus-cytosine (G+C) content was determined from DNA melting temperatures by the equation of Owen et al. (29). *Escherichia coli* K-12 DNA was included as a standard.

RESULTS AND DISCUSSION

Morphological, physiological, and biochemical characteristics. Microscopic examination of the seven *S. pasteurii* strains revealed gram-positive spherical cells, 0.5 to 1.5 μm in diameter, occurring in pairs, tetrads, and irregular clusters. All strains produced catalase and were able to grow aerobically and anaerobically when incubated for 24 h in thioglycolate medium. They were resistant to bacitracin and vibriostatic agent 0/129 but susceptible to nitrofurantoin. These properties are shared by the strains belonging to the genus *Staphylococcus*.

When the *S. pasteurii* strains were tested by the micro-method ID32 Staph System, the results indicated that four of them (BM9357^T, BM9359, BM10425, and BM10426) could be classified as *S. warneri*, whereas the remaining three

strains (BM9363, BM10427, and BM10507) could not be identified.

All the phenotypic tests listed in Materials and Methods and used to characterize the seven *S. pasteurii* strains were also carried out for 10 *S. warneri* strains and for 4 *S. hominis* strains, including the type strains of these two phenotypically related species (21, 23, 37). The 21 staphylococcal strains analyzed had numerous properties in common including growth on agar at 15 to 45°C and on agar supplemented with 5 to 15% (wt/vol) sodium chloride, production of catalase and urease, acid production (aerobically) from D-glucose, glycerol, β -D-fructose, and sucrose, inability to hydrolyze esculin, and no production of free coagulase, clumping factor, fibrinolysin, oxidase, DNase, β -galactosidase, alkaline phosphatase, pyrrolydonyl arylamidase, arginine arylamidase, or ornithine decarboxylase or acid from L-arabinose, D-cellobiose, α -D-melibiose, D-raffinose, xylose, or D-xylose. The characteristics differentiating these 21 strains are reported in Table 2. Susceptibility to desferal appeared to be the best test to discriminate the 4 *S. hominis* strains from the 17 other strains belonging to *S. pasteurii* and *S. warneri*; the use of this test to distinguish *S. hominis* from *S. warneri* has been reported by Lindsay and Riley (27). Yellow pigmentation of the colonies and resistance to lysostaphin on Mueller-Hinton agar are the traits that most clearly distinguished *S. pasteurii* from *S. warneri* strains. Indeed, six of the seven *S. pasteurii* strains produced yellow pigment (better visualized on P agar supplemented with milk), whereas only 1 of the 10 *S. warneri* strains did; the 7 *S. pasteurii* strains were all resistant to lysostaphin, whereas only 2 of the 10 *S. warneri* strains exhibited such resistance.

Cell wall analysis of *S. pasteurii*. All seven strains exhibited the same L-Lys-Gly₄-Ser peptidoglycan type. Since the glycine/serine ratio is known to be sensitive to the composition of the culture medium, cell walls from two strains (BM9357^T and BM10507) were also analyzed after growth in 2.5% Bacto Peptone (Difco) supplemented with 0.5% NaCl and 0.5% glycine (30). However, amino acid analysis revealed no significant change in the glycine/serine ratio under these conditions. The L-Lys-Gly₄-Ser type was also consistent with the observed lysostaphin resistance of the seven *S. pasteurii* strains, since the existence of a pentaglycine interpeptide chain is a prerequisite for efficient action of this endopeptidase (28, 38).

Teichoic acid analysis by gas chromatography revealed the existence of the glycerol type, but not of the ribitol type, of teichoic acids in all seven *S. pasteurii* strains. *N*-acetylglucosamine was detected, by both amino acid analysis and gas chromatography, in strain BM10507, the only strain isolated from a goat. No *N*-acetylglucosamine or any other amino sugar was found in any of the other six strains. *N*-acetylglucosamine can occur among staphylococci in three structurally different types of teichoic acid (34): (i) branching from the poly(polyolphosphate) chain, (ii) forming part of the main chain of a poly(glycerolphosphateglycosylphosphate) teichoic acid, and (iii) forming the main chain of a poly(glycosylphosphate) teichoic acid. The last two types, known to occur in *Staphylococcus hyicus* and *Staphylococcus sciuri* and in *Staphylococcus auricularis* and *Staphylococcus caseolyticus*, respectively (34), are very improbable for strain BM10507 because this strain has glycerol in its teichoic acid and because the amount of *N*-acetylglucosamine detected was fairly low (approximately 2 mol per mol of muramic acid). Thus, *N*-acetylglucosamine

FIG. 1. *Eco*RI restriction patterns of *S. pasteurii* and *S. warneri* probed with ³²P-labeled plasmid pBA2. Lane 1, Raoul I DNA ladder (Appligène); lane 2, *S. warneri* ATCC 27836^T (HP E22); lane 3, *S. pasteurii* BM9357^T (HP E850); lane 4, *S. pasteurii* BM10425 (HP E851); lane 5, *S. pasteurii* BM10426 (HP E852).

appears to be a possible side chain substituent of the polyglycerolphosphate teichoic acid in *S. pasteurii*.

HP obtained with the probe pBA2 (ribosomal DNA). Three distinct HP were observed for the *Eco*RI-digested cellular DNAs of the seven *S. pasteurii* strains (HP E850 for BM9357^T; HP E851 for BM9359, BM10425, BM10427, and BM10507; and HP E852 for BM9363 and BM10426). As shown in Fig. 1 (lanes 3 to 5), the HP had a core of eight common hybridizing bands which are species specific, yielding an easy distinction from the single *Eco*RI HP, HP E22 (lane 2), detected for the 13 *S. warneri* strains currently analyzed (4, 7, 8). Each of the three HP detected for the *S. pasteurii* strains was compared with each of the 81 *Eco*RI HP detected for the 271 strains validly classified as belonging to 31 staphylococcal taxa (7, 8). The highest intertaxon percentage of similarity (66.6%) was lower than the values calculated for the seven *S. pasteurii* strains among themselves (84.2 to 100%). These results suggested that the strains belonging to *S. pasteurii* were homogeneously clus-

tered and easily distinguishable from all other staphylococcal taxa currently described. The variety of patterns observed for *S. pasteurii* strains could be used to type these strains.

Percentages of DNA-DNA homology. The DNA relatedness results obtained with labeled reference DNA from *S. pasteurii* BM9357^T are shown in Table 1. The four *S. pasteurii* strains chosen for this part of the study had been recovered from distinct sources (human, animal, and food specimens) and had three distinct HP. As seen in Table 1, these four strains formed a tight genomic species since strain BM9357^T was related at levels of more than 93% to the three other *S. pasteurii* strains and at levels of no more than 13% to the 27 other staphylococcal species tested, including *S. warneri* (11%).

DNA base composition of *S. pasteurii*. The average base composition of each of the two *S. pasteurii* strains tested, BM9357^T and BM9363, was 35 mol% G+C. This value falls within the extreme values observed in the genus *Staphylococcus* (30 to 41 mol% G+C) (17, 21).

Description of *Staphylococcus pasteurii* sp. nov. *Staphylococcus pasteurii* (pas' teur. i. L. gen. n. *pasteurii* of Pasteur, honoring the French microbiologist Louis Pasteur for his contribution in 1878 to the recognition of staphylococci as pathogenic agents and also referring to the research institute, Institut Pasteur, Paris, France, where the new species was characterized). The description of *S. pasteurii* is based on a total of seven strains isolated from different sources. The cells are nonsporulating, nonmotile, gram-positive cocci, 0.5 to 1.5 µm in diameter, occurring singly, in pairs, in tetrads, or in clusters. These organisms grow on P agar at 15 to 45°C. After incubation on P agar at 35°C for 3 days and at 25°C for an additional 2 days, colony diameter varies from 4 to 7 mm. The colonies are smooth, glistening, and raised with slightly elevated centers and regular edges. The yellow pigment produced by six of the seven strains is better visualized when P agar is supplemented with skim milk. Growth is evident throughout a tube of thioglycolate medium and on P agar supplemented with 5 to 15% (wt/vol) sodium chloride. All strains produce both D- and L-lactate isomers from glucose under anaerobic conditions. They exhibit catalase, urease, β-glucuronidase, and β-glucosidase activities. They clear egg yolk medium and give synergistic hemolysis with β-toxin. They produce acid aerobically from D-glucose, glycerol, β-D-fructose, sucrose, and D-trehalose. In contrast, negative results are obtained for the following tests: free coagulase, clumping factor, fibrinolysin, oxidase, DNase, β-galactosidase, alkaline phosphatase, pyrrolidonyl arylamidase, arginine arylamidase, ornithine decarboxylase, and esculin hydrolysis. No acid is produced aerobically from L-arabinose, D-cellobiose, α-D-melibiose, D-raffinose, xylitol, D-xylose, D-mannose, or D-ribose.

Agar disk diffusion tests show that the seven strains are resistant to lysostaphin, vibriostatic agent 0/129, and bacitracin, whereas they are susceptible to oxacillin, cefalotin, spectinomycin, streptomycin, kanamycin, neomycin, tobramycin, gentamicin, minocycline, pristinamycin II, sulfonamides, trimethoprim, pefloxacin, fusidic acid, rifampin, fosfomycin, vancomycin, nitrofurantoin, novobiocin, cadmium acetate, mercuric nitrate, cetyltrimethylammonium bromide, propamidine isothionate, chlorhexidine digluconate, and desferal (Tables 2 and 3).

Description of the type strain. Strain BM9357^T (= ATCC 51129^T) was isolated from a vomit sample. In addition to all the characteristics shared by the seven *S. pasteurii* strains, the type strain has the following properties: gelatin and

TABLE 3. Resistance and susceptibility of *S. pasteurii* strains to various antimicrobial agents

Antibiotic (load per disk)	BM9357 ^T	BM9359	BM9363	BM10507	BM10425	BM10426	BM10427
Penicillin G (6 µg)	R ^a	S	S	S	R	S	R
Oxacillin (5 µg)	S	S	S	S	S	S	S
Cefalotin (30 µg)	S	S	S	S	S	S	S
Spectinomycin (100 µg), streptomycin (10 U), kanamycin (30 U), neomycin (30 U), tobramycin (10 µg), and gentamicin (15 µg)	S	S	S	S	S	S	S
Chloramphenicol (30 µg)	S	S	S	R	S	S	S
Tetracycline (30 U)	S	S	S	R	R	S	S
Minocycline (30 U)	S	S	S	S	S	S	S
Erythromycin (15 U)	S	S	S	R _c	R _c	S	R _c
Oleandomycin (15 U)	S	S	S	R _c	R _c	S	R _c
Spiramycin (100 µg)	S	S	S	R _c	R _c	S	R _c
Lincomycin (15 µg)	S	S	S	R _c	R _c	S	R _c
Pristinamycin I (40 µg)	S	S	S	R _c	R _c	S	R _c
Pristinamycin II (20 µg)	S	S	S	S	S	S	S
Sulfonamides (200 µg), trimethoprim (5 µg), pefloxacin (5 µg), fusidic acid (10 µg), rifampin (30 µg), fosfomycin (50 µg), vancomycin (30 µg), nitrofurantoin (300 µg), and novobiocin (5 µg)	S	S	S	S	S	S	S
Bacitracin (0.4 U)	R _c	R _c	R _c	R _c	R _c	R _c	R _c
Vibriostatic agent 0/129 (0.5 mg)	R _c	R _c	R _c	R _c	R _c	R _c	R
Cadmium acetate (0.2 µmol)	S	S	S	S	S	S	S
Sodium arseniate (2 µmol)	R	R	R	R	R	S	R
Mercuric nitrate (0.2 µmol)	S	S	S	S	S	S	S
Ethidium bromide (200 µg)	S	S	S	S	R	S	R
Acriflavine (200 µg)	S	S	S	S	R	S	R
CTAB (10 µg) ^b	S	S	S	S	S	S	S
Propamidine isothionate (200 µg)	S	S	S	S	S	S	S
Chlorhexidine digluconate (400 µg)	S	S	S	S	S	S	S

^a R, resistant; S, susceptible; R_c, resistant with growth up to the edge of the disk.

^b CTAB, cetyltrimethylammonium bromide.

casein hydrolysis; acetoin production; arginine dihydrolase activity; acid production from α-lactose, maltose, and D-mannitol; inability to reduce nitrates, to hydrolyze N-acetylglucosamine, or to produce acid from D-melezitose or D-turanose; and resistance to penicillin G by penicillinase production, to erythromycin and oleandomycin (inducible MLS phenotype), and to sodium arseniate. The G+C content of the DNA is 37 mol%.

Differentiation of *S. pasteurii* from the other staphylococcal taxa. The new species *S. pasteurii* can be phenotypically distinguished from all of the other novobiocin-susceptible staphylococcal taxa except *S. warneri* by the characteristics listed in Table 4. Although the production of yellow pigment and the resistance to lysostaphin appeared to be the best conventional tests to distinguish *S. pasteurii* from *S. warneri* (Table 2), phenotypic discrimination between these two species remains difficult since the presence or absence of these characteristics is not shared by more than 90% of the strains belonging to each taxon (Tables 2 and 4). Therefore, rRNA gene restriction site polymorphism by using plasmid pBA2 as a probe (4, 7, 8, 12) is currently the most appropri-

TABLE 4. Simplified phenotypic scheme for the identification of novobiocin-susceptible staphylococci^a

Characteristic	<i>S. aureus</i>	<i>S. aureus</i> subsp. <i>anaerobius</i>	<i>S. auricularis</i>	<i>S. capitis</i>	<i>S. capitis</i> subsp. <i>ureolyticus</i>	<i>S. caprae</i>	<i>S. carnosus</i>	<i>S. caseolyticus</i>	<i>S. chromogenes</i>	<i>S. delphini</i>	<i>S. epidermidis</i>	<i>S. felis</i>	<i>S. haemolyticus</i>	<i>S. hominis</i>	<i>S. hyicus</i>	<i>S. intermedius</i>	<i>S. lugdunensis</i>	<i>S. muscae</i>	<i>S. saccharolyticus</i>	<i>S. schleiferi</i>	<i>S. schleiferi</i> subsp. <i>coagulans</i>	<i>S. simulans</i>	<i>S. warneri</i>	<i>S. pasteurii</i>
Pigmentation (carotenoid)	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Free coagulase (rabbit plasma)	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clumping factor	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Heat-stable nuclease	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ornithine decarboxylase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkaline phosphatase	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acetoin production	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urease	w	ND	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
β -Glucosidase	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid produced (aerobically) from:																								
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Mannitol	+	ND	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-Mannose	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-Trehalose	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-Ribose	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-Xylose	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Desferal resistance (1 mg/disk)	+	ND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

^a Data from references 17, 19, 20, 21, 23, and 27 and this study (data for *S. hominis*, *S. warneri*, and *S. pasteurii*).^b +, 90% or more of the strains are positive; -, 90% or more of the strains are negative; w, 90% or more of the strains are weakly positive; v, 11 to 89% of the strains are positive; ND, not determined. Parentheses indicate a delayed reaction.

ate method to differentiate *S. pasteurii* from all of the staphylococcal taxa described previously, including *S. warneri*.

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REFERENCES

- Acar, J. F., and F. W. Goldstein. 1986. Disk susceptibility test, p. 27-63. In V. Lorian (ed.), *Antibiotics in laboratory medicine*. The Williams & Wilkins Co., Baltimore.
- Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.). 1987. *Current protocols in molecular biology*. John Wiley & Sons, Inc., New York.
- Bannerman, T. L., and W. E. Kloos. 1991. *Staphylococcus capitis* subsp. *ureolyticus* subsp. nov. from human skin. *Int. J. Syst. Bacteriol.* **41**:144-147.
- Chesneau, O., S. Aubert, A. Morvan, J. L. Guesdon, and N. El Solh. 1992. Usefulness of the ID32 Staph System and a method based on rRNA gene restriction site polymorphism analysis for species and subspecies identification of staphylococcal clinical isolates. *J. Clin. Microbiol.* **30**:2346-2352.
- Christie, R., and H. Wilson. 1941. A test of staphylococcal fibrinolysis. *Aust. J. Exp. Biol. Med. Sci.* **19**:329-332.
- Crosa, J. M., D. J. Brenner, and S. Falkow. 1973. Use of a single-strand-specific nuclease for analysis of bacterial and plasmid deoxyribonucleic acid homo- and heteroduplexes. *J. Bacteriol.* **115**:904-911.
- De Buyser, M. L., A. Morvan, S. Aubert, F. Dilasser, and N. El Solh. 1992. Evaluation of a ribosomal RNA gene probe for the identification of species and subspecies within the genus *Staphylococcus*. *J. Gen. Microbiol.* **135**:889-899.
- De Buyser, M. L., A. Morvan, F. Grimont, and N. El Solh. 1989. Characterization of *Staphylococcus* species by ribosomal RNA gene restriction patterns. *J. Gen. Microbiol.* **135**:989-999.
- De La Fuente, R., G. Suarez, and K. H. Schleifer. 1985. *Staphylococcus aureus* subsp. *anaerobius* subsp. nov., the causal agent of abscess disease of sheep. *Int. J. Syst. Bacteriol.* **35**:99-102.
- Devriese, L. A. 1979. Identification of clumping factor negative staphylococci isolated from cows' udders. *Res. Vet. Sci.* **27**:313-320.
- Dice, L. R. 1945. Measures of the amount of ecologic association between species. *Ecology* **26**:297-302.
- El Solh, N., M. L. De Buyser, A. Morvan, F. Grimont, S. Walcher-Salesse, S. Aubert, C. Monzon-Moreno, O. Chesneau, and J. Allignet. 1990. Use of *Bacillus subtilis* 16S rRNA genes as a probe to identify species, subspecies, and types in the genus *Staphylococcus*, p. 585-593. In R. P. Novick (ed.), *Molecular biology of the staphylococci*. VCH Publishers, Inc., New York.
- Evans, J. B., and W. E. Kloos. 1972. Use of shake cultures in a semisolid thioglycolate medium for differentiating staphylococci from micrococci. *Appl. Microbiol.* **23**:326-331.
- Faller, A., and K. H. Schleifer. 1981. Modified oxidase and benzidine tests for separation of staphylococci. *J. Clin. Microbiol.* **13**:1031-1035.
- Freney, J., Y. Brun, M. Bes, H. Meugnier, F. Grimont, P. A. D. Grimont, C. Nervi, and J. Fleurette. 1988. *Staphylococcus lugdunensis* sp. nov. and *Staphylococcus schleiferi* sp. nov., two species from human clinical specimens. *Int. J. Syst. Bacteriol.* **38**:168-172.
- Grimont, P. A. D., M. Y. Popoff, F. Grimont, C. Coynault, and M. Lemelin. 1980. Reproducibility and correlation study of three deoxyribonucleic acid hybridization procedures. *Curr. Microbiol.* **4**:325-330.
- Hajek, V., W. Ludwig, K. H. Schleifer, N. Springer, W. Zitzelsberger, R. M. Kroppenstedt, and M. Kocur. 1992. *Staphylococcus muscae*, a new species isolated from flies. *Int. J. Syst. Bacteriol.* **42**:97-101.
- Hebert, G. A., and G. A. Hancock. 1985. Synergistic hemolysis exhibited by species of staphylococci. *J. Clin. Microbiol.* **22**:409-415.
- Igimi, S., S. Kawamura, E. Takahashi, and T. Mitsuoka. 1989. *Staphylococcus felis*, a new species from clinical specimens from cats. *Int. J. Syst. Bacteriol.* **39**:373-377.
- Igimi, S., E. Takahashi, and T. Mitsuoka. 1990. *Staphylococcus schleiferi* subsp. *coagulans* subsp. nov., isolated from the external auditory meatus of dogs with external ear otitis. *Int. J. Syst. Bacteriol.* **40**:409-411.
- Kloos, W. E., and D. W. Lambe, Jr. 1991. *Staphylococcus*, p. 222-237. In A. Balows, W. J. Hausler, Jr., K. L. Hermann, H. D. Isenberg, and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C.
- Kloos, W. E., and K. H. Schleifer. 1975. Isolation and characterization of staphylococci from human skin. II. Descriptions of four new species: *Staphylococcus warneri*, *Staphylococcus capitis*, *Staphylococcus hominis*, and *Staphylococcus simulans*. *Int. J. Syst. Bacteriol.* **25**:62-79.
- Kloos, W. E., and K. H. Schleifer. 1986. Genus IV. *Staphylococcus* Rosenbach 1884, 18^{AL}, (Nom. Cons. Opin. 17 Jud. Comm. 1958. 153), p. 1013-1035. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 2. The Williams & Wilkins Co., Baltimore.
- Kloos, W. E., T. G. Tornabene, and K. H. Schleifer. 1974. Isolation and characterization of micrococci from human skin, including two new species: *Micrococcus lylae* and *Micrococcus kristinae*. *Int. J. Syst. Bacteriol.* **24**:79-101.
- Kloos, W. E., and J. F. Wolfshohl. 1991. *Staphylococcus cohnii* subspecies: *Staphylococcus cohnii* subsp. *cohnii* subsp. nov. and *Staphylococcus cohnii* subsp. *urealyticum* subsp. nov. *Int. J. Syst. Bacteriol.* **41**:284-289.
- Lachica, R. V. F., P. D. Hoeprich, and C. Genigeorgis. 1972. Metachromatic agar-diffusion microslide technique for detecting staphylococcal nuclease in foods. *Appl. Microbiol.* **23**:168-169.
- Lindsay, J. A., and T. V. Riley. 1991. Susceptibility to desferrioxamine: a new test for the identification of *Staphylococcus epidermidis*. *J. Med. Microbiol.* **35**:45-48.
- Maidhof, H., B. Reinicke, P. Blümel, B. Berger-Bächi, and H. Labischinski. 1991. *femA*, which encodes a factor essential for expression of methicillin resistance, affects glycine content of peptidoglycan in methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains. *J. Bacteriol.* **173**:3507-3513.
- Owen, R. J., L. R. Hill, and S. P. Lapage. 1969. Determination of DNA base compositions from melting profiles in dilute buffers. *Biopolymers* **7**:503-516.
- Schleifer, K. H., W. P. Hammes, and O. Kandler. 1976. Effects of endogenous and exogenous factors on the primary structure of bacterial peptidoglycan. *Adv. Microb. Physiol.* **13**:245-292.
- Schleifer, K. H., R. Kilpper-Bälz, and L. A. Devriese. 1984. *Staphylococcus arlettae* sp. nov., *S. equorum* sp. nov. and *S. kloosii* sp. nov.: three new coagulase-negative, novobiocin-resistant species from animals. *Syst. Appl. Microbiol.* **5**:501-509.
- Schleifer, K. H., and W. E. Kloos. 1975. Isolation and characterization of staphylococci from human skin. I. Amended descriptions of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* and descriptions of three new species: *Staphylococcus cohnii*, *Staphylococcus haemolyticus*, and *Staphylococcus xylosus*. *Int. J. Syst. Bacteriol.* **25**:50-61.
- Schleifer, K. H., and M. Kocur. 1973. Classification of staphylococci based on chemical and biochemical properties. *Arch. Mikrobiol.* **93**:65-85.
- Schleifer, K. H., and R. M. Kroppenstedt. 1990. Chemical and molecular classification of staphylococci. *J. Appl. Bacteriol.*

- Symp. Suppl. **19**:9S–24S.
35. **Smith, R. F., and C. L. Bettge.** 1972. Comparative characteristics of human and porcine staphylococci and their differentiation in burn xenografting procedures. *Appl. Microbiol.* **24**:929–932.
36. **Varaldo, P. E., R. Kilpper-Bälz, F. Biavasco, G. Satta, and K. H. Schleifer.** 1988. *Staphylococcus delphini* sp. nov., a coagulase-positive species isolated from dolphins. *Int. J. Syst. Bacteriol.* **38**:436–439.
37. **White, D. G., R. J. Harmon, and B. E. Langlois.** 1990. Fluorogenic assay for differentiating *Staphylococcus warneri* and *Staphylococcus hominis* strains of bovine origin. *J. Clin. Microbiol.* **28**:602.
38. **Zygmunt, W. A., H. P. Browder, and P. A. Tavormina.** 1967. Lytic action of lysostaphin on susceptible and resistant strains of *Staphylococcus aureus*. *Can. J. Microbiol.* **13**:845–853.