Staphylococcus muscae, a New Species Isolated from Flies

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A new coagulase-negative species of the genus *Staphylococcus*, *Staphylococcus muscae*, is described on the basis of the results of a study of four strains that were isolated from flies. 16S rRNA sequences of the type strains of *S. muscae*, *Staphylococcus schleiferi*, and *Staphylococcus sciuri* were determined and used, together with the corresponding sequences of *Staphylococcus aureus* and *Staphylococcus epidermidis*, for a comparative analysis. The new species is characterized taxonomically; this species is differentiated from the other novobiocin-susceptible staphylococci by its physiological and biochemical activities, cell wall composition, and levels of genetic relatedness. The type strain of this species is strain MB4 (= CCM 4175).

A total of 28 species are currently recognized in the genus *Staphylococcus*; 19 species are listed in *Bergey's Manual of Systematic Bacteriology* (14), two species (*Staphylococcus chromogenes* and *Staphylococcus lentus*) have been elevated from the subspecies level (8, 19), and 7 species have been described since 1984 (*Staphylococcus arlettae*, *Staphylococcus delphini*, *Staphylococcus equorum*, *Staphylococcus felis*, *Staphylococcus kloosii*, *Staphylococcus lugdunensis*, and *Staphylococcus schleiferi*) (5, 11, 21, 26). Most of the new species were isolated from animals; the exceptions were *S. lugdunensis* and *S. schleiferi*, which were isolated from humans. The descriptions of all 28 *Staphylococcus* species were based on studies of strains that were isolated from mammals, birds, and foodstuffs of animal origin.

In this paper, we describe a new coagulase-negative species of staphylococci that was obtained from the body surfaces of flies. These organisms were found in about 7% of the flies caught in certain cow sheds but not on flies caught in human dwellings, stables, or piggeries (7). For this reason, this bacterium should be regarded as a transient rather than a resident on flies.

In this report we present phenetic and molecular taxonomic data for a new species, *Staphylococcus muscae*.

MATERIALS AND METHODS

Bacterial strains. Four strains were isolated from flies trapped in cow sheds; strains MB4^T, (T = type strain), MB21, and MB50 were isolated from *Stomoxys calcitrans*, and strain MB30 was isolated from *Musca domestica* (7). The medium used for isolation was blood agar base no. 2 (Oxoid) supplemented with 5% defibrinated ovine blood. Nutrient agar (Oxoid) containing glucose (2%, wt/vol) and P agar (15) were used to propagate all of the isolates. Inocula for tests were prepared from 1-day cultures that were incubated at 37°C. All strains were stored as frozen suspensions in glycerol broth at –25°C (10) and also in a freeze-dried state.

Methods. The procedures used for determining morphological, physiological, and biochemical characteristics have been described elsewhere (6). The type strains of the 14 novobiocin-susceptible *Staphylococcus* species (see Table 2)

were obtained from the Deutsche Sammlung von Mikroorganismen (DSM), Braunschweig, Germany, the American Type Culture Collection (ATCC), Rockville, Md., and the Czechoslovak Collection of Microorganisms (CCM), Brno, Czechoslovakia.

Phage typing was carried out by using the method of Blair and Williams (1). We used 24 phages (25) for human *Staphylococcus aureus* strains, 12 phages (24) for bovine *S. aureus* strains, 4 experimental phages (2) for *Staphylococcus intermedius* strains, and 4 phages (9) for *Staphylococcus hyicus* strains.

We used the methods described by Schleifer and Kandler (20) and Schleifer (18) for cell wall preparation and determination. Cellular fatty acids were determined by gas chromatography, using the method of Miller (17). DNA base composition was determined by thermal denaturation with a model 2600 spectrophotometer (Gilford Instruments Laboratories, Inc., Oberlin, Ohio) (3). DNA from *Escherichia coli* K-12 was used as a standard (13). Radioactive labeling of DNA and the DNA-DNA hybridization experiments were carried out by using the filter method as described previously (12). 16S rRNA genes (16S rDNA) were amplified in vitro by using the polymerase chain reaction as described previously by Ludwig et al. (16). The amplified 16S rDNA fragment was cloned in vector pBluescript (Stratagene, La Jolla, Calif.) and *E. coli* JM83. The cloned DNA was sequenced by using the chain termination method in combination with site-specific primers (16). Sequences of 16S rRNA genes were aligned, similarities between sequence pairs were determined, and an unrooted phylogenetic tree was constructed by using the method of Fitch and Margoliash (4) as described by Schleifer et al. (23).

RESULTS AND DISCUSSION

The four strains which we investigated were gram-positive, catalase-positive cocci that occurred in irregular clumps, were facultatively anaerobic, were susceptible to furadantin, and were resistant to bacitracin. The DNA base compositions of the four strains ranged from 40 to 41 mol% guanine plus cytosine (strains MB4^T and MB21, 40 mol%; strains MB30 and MB50, 41 mol%); these values are somewhat higher than the values obtained for the previously described staphylococci (30 to 39 mol%). The fatty acid compositions of three *S. muscae* strains are summarized in

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FIG. 1. Nucleotide sequences of 16S rRNAs of *S. muscae* (Smus), *S. sciuri* (Ssci), and *S. schleiferi* (Ssch). Bases which were not determined are indicated by n.

TABLE 1. Fatty acid compositions of *S. muscae* strains

Strain	Fatty acids composition (%) ^a										
	iso-C ₁₁	iso-C ₁₃	C _{14:0} ^b	iso-C ₁₅	anteiso-C ₁₅	C _{16:0}	iso-C ₁₇	anteiso-C ₁₇	C _{18:0}	iso-C ₁₉	C _{20:0}
MB4 ^T	tr ^c	1.1	3.7	40.9	1.0	14.8	5.8	1.9	10.7	1.0	10.1
MB21	0.4	1.8	7.8	40.6	9.7	18.3	4.3	1.5	9.4	0.6	5.5
MB30	0.3	1.9	7.9	34.6	9.7	23.6	2.7	1.1	10.9	0.4	6.3

^a Data are expressed as percentages of the total fatty acid methyl esters.^b 14:0, 14 carbons in straight-chain saturated (tetradecanoic) acid.^c Trace (less than 0.25%).

Table 1. In particular, the presence of long-chain saturated unbranched fatty acid C_{20:0} (*n*-eicosanoic acid) is typical of staphylococci (22). DNA-DNA hybridization studies showed that the four strains are genetically closely related to one another but not to any of the other staphylococcal species (Table 2). 16S rRNA gene fragments from strain MB4^T and the type strains of *S. schleiferi* and *Staphylococcus sciuri* were amplified, cloned, and sequenced; the sequences are aligned in Fig. 1. A comparison of these sequences and those of *S. aureus* and *Staphylococcus epidermidis* (15a) indicated that strain MB4^T is certainly a member of the genus *Staphylococcus* and is slightly more closely related to *S. schleiferi* than to the other staphylococci whose 16S rRNA sequences are known (Fig. 2). The cell walls contained a glycine-rich peptidoglycan, which is typical of staphylococci. Strains MB4^T and MB21 contained peptidoglycan type Lys-Gly₅₋₆, and strain MB30 contained peptidoglycan type Lys-Gly₄, Ser. Moreover, the phosphate contents of the cell walls (450 to 900 nmol of phosphate per mg of cell wall) and the occurrence of glycerol in cell wall hydrolysates indicated that a cell wall teichoic acid was present in all four strains (3a).

Description of *Staphylococcus muscae* sp. nov. The description of the new species *Staphylococcus muscae* (*mus'*cae, L. gen. n. *musca*, of a fly) was based on the test results obtained with strains MB4^T, MB21, MB30, and MB50.

All of the strains which we studied are small gram-positive cocci that range from 0.4 to 1.1 µm in diameter and are arranged predominantly in irregular clumps, occasionally in pairs and singly. Spherical or slightly ovoid cells are nonmotile and nonsporeforming.

Colonies on P agar (after 5 days at 37°C) are only slightly convex with gently raised centers, circular, entire, smooth,

faintly glistening, butyrous, opaque, grayish white, and 5 to 6 mm in diameter.

These organisms grow well in the presence of NaCl at concentrations up to 10%; no growth occurs at an NaCl concentration of 15%. They grow moderately at 25°C; no growth occurs at 10 and 45°C.

Facultative anaerobes; better growth occurs under aerobic conditions. Anaerobic growth in semisolid thioglycolate medium is evident after 24 to 48 h.

All of the strains produce catalase, phosphatase (strain MB50 weakly), and heat-labile nuclease and are positive in benzidine tests and weakly positive in methyl red tests. These organisms exhibit clear hemolysis on ovine blood agar medium (strains MB21 and MB30 weakly). All of the strains produce lecithinase, split Tween 20, Tween 40, and Tween 80, and reduce nitrates. On crystal violet agar, three of them produce white (positive, E type) colonies, while strain MB30 produces blue (negative, D type) colonies.

None of the strains produces oxidase, coagulase, clumping factor, fibrinolysin, thermostable nuclease, tellurite reductase, gelatinase, protease, urease, arginine dihydrolase, alpha- or beta-hemolysins, ornithine decarboxylase, acetyl-methylcarbinol, or beta-galactosidase. The strains do not hydrolyze starch or esculin.

The strains produce acid aerobically from glucose (without gas), fructose, sucrose, trehalose, turanose, xylose, and glycerol. No acid is produced from adonitol, arabinose, arbutin, cellobiose, dulcitol, fucose, galactose, inositol, inulin, lactose, maltose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sorbose, or tagatose. All of the strains except strain MB30 have the ability to produce acid anaerobically from glucose weakly.

All of the strains are resistant to lysozyme (MIC, >1,000 µg/ml) and susceptible to novobiocin (MIC, 0.06 to 0.1 µg/ml), penicillin (MIC, 0.01 to 0.06 µg/ml), oxacillin (MIC, 0.2 µg/ml), ampicillin (MIC, 0.06 to 1.0 µg/ml), cephaloridine

TABLE 2. Levels of DNA-DNA relatedness between *S. muscae* strains^a

Source of unlabeled DNA	% of homology with labeled DNA from strain:			
	MB4 ^T	MB21	MB30	MB50
<i>S. muscae</i> MB4 ^T	100 ^b	100	85	90
<i>S. muscae</i> MB21	100	100	90	ND
<i>S. muscae</i> MB30	95	95	100	ND

^a The values were determined under optimal hybridization conditions (25°C below the melting temperature of the DNA). We also determined the levels of relatedness between each of the *S. muscae* strains and the following other *Staphylococcus* strains: *S. arlettae* DSM 20672^T, *S. aureus* ATCC 12260^T, *S. auricularis* DSM 20609^T, *S. caprae* CCM 3573^T, *S. carnosus* DSM 20501^T, *S. cohnii* DSM 20260^T, *S. equorum* DSM 20674^T, *S. gallinarum* DSM 20610^T, *S. hyicus* ATCC 11249^T, *S. intermedius* CCM 5739^T, *S. kloosii* DSM 20676^T, *S. lentus* ATCC 29070^T, *S. lugdunensis* DSM 4804^T, and *S. schleiferi* DSM 4807^T. The values which we obtained were all less than 15%.

^b Percent relatedness. ND, not determined.

FIG. 2. Phylogenetic relationships of *S. muscae*. The tree was constructed by using distance values (K_{nuc}) (23). Bar = 0.05 K_{nuc} . B., *Bacillus*; C., *Clostridium*.

TABLE 3. Characteristics that differentiate *S. muscae* from other novobiocin-susceptible *Staphylococcus* species

Characteristic	<i>S. muscae</i>	<i>S. auricularis</i>	<i>S. capitis</i>	<i>S. caprae</i>	<i>S. carnosus</i>	<i>S. caseolyticus</i>	<i>S. chromogenes</i>	<i>S. epidermidis</i>	<i>S. felis</i>	<i>S. haemolyticus</i>	<i>S. hominis</i>	<i>S. lugdunensis</i>	<i>S. schleiferi</i>	<i>S. simulans</i>	<i>S. warneri</i>
Pigment	— ^a	—	—	—	—	v	+	—	—	v	v	v	—	—	v
Oxidase	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—
Alkaline phosphatase	+	—	—	+	+	—	+	+	+	—	—	—	+	w	—
Urease	—	—	—	+	—	ND	v	+	+	—	+	v	—	+	+
Hemolysis	w	—	v	+	—	—	—	v	v	+	v	w	w	v	v
Ornithine	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—
Clumping factor	—	—	—	—	—	ND	—	—	—	—	—	+	+	—	—
Heat-stable nuclease	—	—	—	—	v	ND	—	—	—	—	—	—	+	—	—
Acetoin	—	v	v	+	+	—	—	+	—	+	v	+	+	—	+
Acid produced from:															
Lactose	—	—	—	+	—	+	+	v	+	v	v	v	—	+	v
Maltose	—	v	—	v	—	+	v	+	—	+	+	+	—	—	v
Mannitol	—	—	+	—	+	—	v	—	v	v	—	—	—	+	v
Mannose	—	—	+	+	+	—	+	v	+	—	—	+	+	v	—
Ribose	—	—	—	—	+	+	+	v	v	v	—	—	—	v	v
Saccharose	+	v	v	—	—	v	+	+	v	+	+	+	—	+	+
Trehalose	+	+	—	+	v	+	+	—	+	+	+	+	v	v	+
Turanose	+	v	—	—	—	—	v	v	ND	v	v	w	—	—	—
Xylose	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—

^a +, positive; w, weak; v, variable; —, negative; ND, not determined.

(MIC, 0.1 to 0.2 µg/ml), erythromycin (MIC, 0.06 µg/ml), lincomycin (MIC, 0.2 to 0.5 µg/ml), clindamycin (MIC, 0.1 µg/ml), chloramphenicol (MIC, 2.0 µg/ml), tetracycline (MIC, 0.1 to 0.2 µg/ml), gentamicin (MIC, 0.2 µg/ml), and vancomycin (MIC, 1.0 µg/ml). All four strains are resistant to bacitracin (10 U per disc) and susceptible to furadantin (100 µg per disc) when the disc diffusion method is used.

The strains are resistant to all of the phages which we used at 100× routine test dilution. The dominant cellular fatty acids are iso-C_{15:0} (13 methyltetradecanoic) and C_{16:0} (hexadecanoic) acids (Table 1).

The peptidoglycan type of strains MB4^T and MB21 is Lys-Gly₅₋₆, and the peptidoglycan type of strain MB50 is Lys-Gly, Ser. None of the strains have protein A.

The average guanine-plus-cytosine content of the DNA is 40 to 41 mol%.

Strain MB4 (= CCM 4175) is the type strain of *S. muscae*. The characteristics of this strain are the same as those given above for the species and in Table 1.

The phenotypic properties that are useful for differentiating *S. muscae* from the other coagulase-negative staphylococcal species are shown in Table 3. *S. muscae* can be distinguished from the other species on the basis of its susceptibility to novobiocin, positive test for alkaline phosphatase, negative tests for oxidase, acetoin, pigment, and urease, carbohydrate reaction pattern, peptidoglycan type, and comparatively high DNA base composition.

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