Staphylococcus schleiferi subsp. coagulans subsp. nov., Isolated from the External Auditory Meatus of Dogs with External Ear Otitis

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A new subspecies, Staphylococcus schleiferi subsp. coagulans, was isolated from the external auditory meatus of dogs suffering from external ear otitis and is described on the basis of studies of 21 strains. Phenotypic studies showed that these strains are more closely related to Staphylococcus intermedius than to other staphylococci, but DNA hybridization studies indicated that they are closely related to Staphylococcus schleiferi N850274T. On the basis of biochemical distinctiveness (positive test tube coagulase test and different carbohydrate reactions) and the etiological importance (frequent isolation from otitis specimens from dogs) of these strains, we propose to classify them as a subspecies of S. schleiferi. The strains of this new subspecies are coagulase-positive but clumping factor-negative. A simple scheme for the differentiation of S. schleiferi subsp. coagulans from the other coagulase-positive staphylococci is presented.

The type strain is GA211 (=JCM 7470).

MATERIALS AND METHODS

Bacterial strains. Twenty-one strains (GA11, GA64, GA89, GA126, GA124, GA199, GA211, GA222, GA227, GA238, GA247, GA288, GA337, GA347, GA390, GA400, GA412, GA416, GA456, GA483, and GA499) were isolated mainly from canine otitis, whereas S. schleiferi was isolated from the external auditory meatus of dogs suffering from external ear otitis. The isolation medium was Trypticase soy agar (BBL Microbiolog)-dogs diagnosed as suffering from external ear otitis. Though strains from this species were obtained as previously described (11), no coagulase in tube tests (9). Despite a relatively high level of DNA homology, phenotypic characteristics of S. schleiferi do not match those of our strains.

RESULTS AND DISCUSSION

Except for a few carbohydrate reactions, the 21 strains are phenotypically homogeneous. All produce coagulase and heat-stable nuclease. The most distinctive characteristic is lack of acid production from trehalose.

DNA-DNA hybridization studies among coagulase-positive species of the genus Staphylococcus are listed in Table 1. These results indicate that all of seven strains tested are closely related, whereas the levels of DNA homology of these strains with other coagulase-positive staphylococci were rather low (5 to 27%). DNA-DNA hybridization of S. schleiferi N850274T DNA with labeled DNA from these strains revealed high levels of homology and indicated that these strains are closely related to S. schleiferi at the species level. However, we propose to classify them as a subspecies of S. schleiferi because the phenotypic characteristics do not match the description of S. schleiferi (9). If we consider the test tube coagulase test, clumping factor, colony diameter, and acid production from sucrose, D-mannitol, D-trehalose, and D-ribose as variable characteristics of S. schleiferi, we could hardly differentiate S. schleiferi from the other staphylococci. The test tube coagulase test is the most important and significant test for identification of staphylococcal strains isolated from clinical specimens. Our 21 strains produce coagulase, but S. schleiferi does not. Our strains were isolated mainly from canine otitis, whereas S. schleiferi is isolated from human clinical specimens. For epidemiolog-
ical purposes, it is desirable to distinguish between our strains and \textit{S. schleiferi}. Therefore, we propose the following.

\textit{Staphylococcus schleiferi} subsp. \textit{coagulans} subsp. nov. \textit{coagulans} (co.a'gu.lans. L. adj. \textit{coagulans} curdling, coagulating). The following description of \textit{coagulans} is based on a total of 21 strains, unless noted otherwise.

Cells are gram-positive cocci, 0.8 to 1.2 \(\mu\)m in diameter, occurring singly, in pairs, and, predominantly, in irregular clusters. Nonmotile. Nonsporforming. Colonies on horse blood agar after 24 h at 37°C are circular, entire, 1.5 to 2.0 mm in diameter, slightly convex, and opaque with a smooth glistening surface. Not pigmented. Facultatively anaerobic. Growth occurs in both the aerobic and anaerobic portions of semisolid thioglycolate medium.

All strains produce free coagulase (test tube coagulase test with rabbit plasma) but fail to produce fixed coagulase (clumping factor with human plasma). All strains produce \(\beta\)-hemolysin (hemolysis on sheep blood agar and hot-cold reaction), but \(\alpha\)-hemolysin and \(\delta\)-hemolysin are not detected. All strains reduce nitrate and produce heat-stable nuclease, acetoin, phosphatase, arginine dihydrolase, and urease. All strains are hyaluronidase and oxidase negative. All strains are susceptible to 1.6 \(\mu\)g of novobiocin per ml.

All strains produce acid from glucose, mannose, fructose, galactose, and glycerol. Acid is usually produced from ribose (95%) (negative for strain GA288) and lactose (86%) (negative for strains GA390, GA400, and GA416). Some strains produce acid from mannitol (48%) (strains GA11, GA22, GA227, GA238, GA247, GA288, GA412, GA456, GA483, and GA499) and sucrose (24%) (GA64, GA89, GA337, GA347 and GA400). No acid is produced from arabinose, xylose, rhamnose, maltose, cellobiose, trehalose, raffinose, melibiose, melezitose, glycerogen, sorbitol, erythritol, inositol, adonitol, dulcitol, or xylitol.

The G+C content of DNA, as determined on the basis of

\[\begin{array}{|c|c|c|c|c|}
\hline
\text{Source of unlabeled DNA} & \text{GA211} & \text{GA11} & \text{GA64} & \text{GA288} \\
\hline
\text{S. schleiferi subsp. coagulans GA211} & 100 & 94 & 88 & 85 \\
\text{S. schleiferi subsp. coagulans GA11} & 92 & 100 & 88 & 90 \\
\text{S. schleiferi subsp. coagulans GA64} & 76 & 72 & 100 & 85 \\
\text{S. schleiferi subsp. coagulans GA238} & 96 & 96 & 111 & 92 \\
\text{S. schleiferi subsp. coagulans GA288} & 98 & 67 & 108 & 100 \\
\text{S. schleiferi subsp. coagulans GA390} & 101 & 81 & 105 & 95 \\
\text{S. schleiferi subsp. coagulans GA400} & 76 & 90 & 95 & 89 \\
\text{S. schleiferi subsp. schleifer N850274\textsuperscript{a}} & 62 & 65 & 70 & 73 \\
\text{S. aureus subsp. aureus CCM 885\textsuperscript{b}} & 10 & 12 & 5 & 11 \\
\text{S. aureus subsp. anaerobius MVF-7\textsuperscript{b}} & 7 & 9 & 6 & 8 \\
\text{S. intermedius JCM 2422\textsuperscript{a}} & 25 & 22 & 27 & 21 \\
\text{S. hyicus JCM 2423\textsuperscript{a}} & 15 & 15 & 10 & 11 \\
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\end{array}\]

\(\text{a Level of relatedness after reassociation for 24 h at 58°C. Labeled DNA of strain GA211 shows 16\% relatedness with DNA of } S.\text{ felis ATCC 49168}\text{ and 13\% relatedness with DNA of } S.\text{ carnosus DSM 20501.}\)

\[\begin{array}{|c|c|c|c|c|c|c|c|}
\hline
\text{Characteristic} & \text{S. schleiferi \textit{subsp. coagulans}} & \text{S. \textit{schleiferi subsp. schleifer}} & \text{S. \textit{aureus subsp. aureus}} & \text{S. \textit{aureus subsp. anaerobius}} & \text{S. \textit{intermedius}} & \text{S. \textit{hyicus}} & \text{S. \textit{delphini}} \\
\hline
\text{Aerobic growth} & + & + & + & -/w & + & + & + \\
\text{Colony diameter \(>5\) mm\textsuperscript{c}} & + & - & - & - & - & - & - \\
\text{Pigment} & - & - & - & - & - & - & - \\
\text{Rabbit plasma coagulated} & + & - & + & + & + & - & - \\
\text{Clumping factor} & - & + & + & + & + & + & + \\
\text{Heat-stable nuclease} & + & + & + & + & + & + & + \\
\text{Hemolysins} & + & + & + & - & + & - & - \\
\text{Acetoin production} & + & + & + & + & + & + & + \\
\text{Hyaluronidase production} & - & ND & + & + & - & ND & - \\
\text{Acid produced from:} & & & & & & & \\
\text{Sucrose} & d & - & + & + & + & + & + \\
\text{Maltose} & - & - & + & - & - & - & - \\
\text{Galactose} & + & ND & - & + & + & + & + \\
\text{D-Mannitol} & d & + & - & d & - & + & + \\
\text{D-Trehalose} & - & + & - & + & + & + & + \\
\text{D-Ribose} & + & - & - & + & + & + & + \\
\hline
\end{array}\]

\(\text{a Data are from references 5-7, 9, 10, 12, and 17.}\)

\(\text{b +, More than 90\% of the strains are positive; } -, \text{more than 90\% of the strains are negative; } d, 11 \text{ to 89\% of the strains are positive; } w, \text{weak reaction; } -/w, \text{negative or weak reaction; ND, result not determined.}\)

\(\text{c Colony diameter is determined after incubation on Pagur (13) at 34 to 35°C for 3 days and at room temperature for an additional 2 days. Under these conditions colonies of } S.\text{ schleifer subsp. coagulans are 5.0 to 8.0 mm in diameter.}\)
the melting temperature in three strains (strains GA211, GA11, and GA64), ranges from 35 to 37 mol%.

The characteristics of the type strain, GA211 (=JCM 7470), are the same as those described above. The guanine-plus-cytosine content of its DNA is 35 mol%. The type strain was isolated from canine external ear otitis.

Selected characteristics that are useful in the identification of S. schleiferi subsp. coagulans and in distinguishing this taxon from other coagulase-positive Staphylococcus species are listed in Table 2. S. schleiferi subsp. coagulans is easily differentiated from coagulase-negative S. schleiferi subsp. schleiferi on the basis of its test tube coagulase test reaction and from the other coagulase-negative species on the basis of its production of coagulase, heat-stable nuclease, and β-hemolysin. S. schleiferi subsp. coagulans can be distinguished from other coagulase-positive species on the basis of its acetoin production, β-hemolysin production, negative hyaluronidase activity, and lack of acid production from trehalose or maltose.

LITERATURE CITED