Viridans streptococci (also called oral streptococci) constitute part of the normal flora of the human oral cavity, and the respiratory, genital and alimentary tracts. They can be associated with human diseases, such as dental caries (Streptococcus sobrinus, Streptococcus mutans), endocarditis (Streptococcus salivarius, S. mutans, Streptococcus sanguinis, Streptococcus sinensis), meningitis (S. salivarius), septicemia (Streptococcus pyogenes, S. salivarius), pneumonia (Streptococcus pneumoniae) or abscesses (Streptococcus intermedius, S. salivarius) (Dyson et al., 1999; Gauduchon et al., 2003; Woo et al., 2002). In a recent review of the genus, viridans streptococci were divided into five major groups: the mutants, salivarius, anginusos, sanguinis and mitis groups (Facklam, 2002). DNA sequence analyses have greatly contributed to improvements in the taxonomy of the streptococci. One of the most useful tools applied to the revision of the classification system for the genus Streptococcus is 16S rRNA gene sequencing (Bentley et al., 1991). However, discrimination to species level is sometimes difficult. Recently, other DNA targets have been proposed: the D-alanine:D-alanine ligase gene (Garnier et al., 1997), the transfer DNA spacers (De Ghelder et al., 1999), the groESL gene (Teng et al., 2002), the 16S–23S rRNA gene spacer region (Chen et al., 2004), the rpoB gene (Drancourt et al., 2004) and the sodA gene (Poyart et al., 1998). At present, identification of streptococci to species level is most often achieved by sequencing a fragment of the gene encoding the manganese-dependent superoxide dismutase (sodA) and a fragment of the gene encoding the beta subunit of RNA polymerase (rpoB).

Here we describe a novel species belonging to the Streptococcus sanguinis group, isolated from a human blood sample.

**Isolation and characterization**

A 52-year-old man was admitted to the intensive care unit of the Hôpital de la Timone, Marseille, in June 2004 with a bullet wound to the head. He presented with cerebral oedema, which required surgical intervention. Two days later, he developed acute respiratory failure, which required that he was intubated. After treatment with cloxacillin, the clinical status of the patient gradually improved and he became apyretic. Ten days later fever and pneumonia were noted. A blood sample for culture was taken. Two bacteria were cultivated from one of three blood samples: one isolate showed Gram-positive cocci arranged in chains (this was identified as Streptococcus intermedius). Strain 4401825 was negative. API 20Strep strips (bioMérieux) were used to characterize this isolate biochemically, but owing to its doubtful identification, partial sequencing of the 16S rRNA, rpoB and sodA genes was also performed. Antimicrobial susceptibility was determined according to the NCCLS. Strain 4401825 was susceptible to amoxicillin, erythromycin, doxycycline, rifampicin, gentamicin (at 500 μg) and vancomycin. The patient was treated with ceftriaxone. After 7 weeks hospitalization the patient’s symptoms gradually
improved and he left the intensive care unit for further rehabilitation.

Following growth on sheep-blood agar (bioMérieux) for 48 h at 37 °C in a 5% CO₂-enriched atmosphere, surface colonies of strain 4401825T were circular, white to greyish, shiny, convex, non-haemolytic and approximately 1–2 mm in diameter. Growth of the isolate occurred in the presence of air, 5% CO₂ and microaerophilic and anaerobic atmospheres, the latter two being created using a GENbag microaer and GENbag anaer (both bioMérieux), respectively. Bacterial growth was tested at different temperatures (25, 30, 37, 45 and 50 °C). Growth was observed at 25–45 °C on sheep-blood agar, with optimal growth at 37 °C. In liquid trypticase soy broth (TSB; Becton Dickinson), growth was not observed at 45 °C. The size and ultrastructure of cells were determined by electron microscopy. Cells were grown in liquid TSB medium for 24 h and stained with 1% (w/v) phosphotungstic acid. The samples were examined on a Morgagni 268D (Philips) electron microscope at an operating voltage of 60 kV. The cells were 0.3–0.7 μm in diameter.

Catalase activity was determined by using the ID colour catalase test kit (bioMérieux) and was found to be negative. The ability to grow in the presence of high NaCl concentrations [2, 5, 7 and 10% (w/v)] was tested in liquid TSB medium. The strain grew only in the presence of 2% NaCl. Lancefield grouping was tested with the Streptex kit (BioRad); reaction with G-group antiserum was detected. The commercially available API 20Strep and API Rapid ID32 Strep test strips (bioMérieux) were used to characterize the biochemical properties of strain 4401825T according to the manufacturer’s instructions and incubation at 37 °C. Diagnostic traits are given in Table 1 and in the species description below. The phenotypic characteristics were compared with those of S. gordonii, S. sanguinis and S. ferus. Strain 4401825T was included in the S. sanguinis group on the basis of several characteristic traits: absence of acetoin production from sodium pyruvate, absence of acid production from mannitol and presence of arginine dihydrolase enzymic activity. However, it was distinguished from other recognized species of the S. sanguinis group based on several other biochemical characteristics.

DNA was extracted using the FastDNA kit (BIO 101) as described by the manufacturer. PCR amplification of 16S rRNA, rpoB and sodA genes was performed using the

### Table 1. Traits useful for differentiating Streptococcus massiliensis sp. nov. from other streptococcal viridans species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
<td>Acetoin production</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of hippurate</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td>Enzyme activities:</td>
<td></td>
<td></td>
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<tr>
<td>Arginine dihydrodase</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>β-Glucosidase (aesculin)</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>β-Galactosidase</td>
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<td>+</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>−</td>
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<td>−</td>
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<td>−</td>
<td>+</td>
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<tr>
<td>Methyl β-D-glucopyranoside</td>
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<td>+</td>
<td>−</td>
<td>−</td>
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<td>Sequence similarity with strain 4401825T (%):</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>16S rRNA</td>
<td>(100)</td>
<td>95.0</td>
<td>95.5</td>
<td>95.2</td>
<td>95.4</td>
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<tr>
<td>rpoB</td>
<td>(100)</td>
<td>85.7</td>
<td>86.0</td>
<td>85.9</td>
<td>82.4</td>
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<tr>
<td>sodA</td>
<td>(100)</td>
<td>84.6</td>
<td>83.5</td>
<td>81.4</td>
<td>74.5</td>
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</table>
universal primer pair fD1 and rp2 (Weisburg et al., 1991),
the primers StreptoF and StreptoR (Drancourt et al., 2004)
and the primer pair d1 and d2 (Poyart et al., 1998),
respectively. PCR products were purified using MultiScreen
PCR (Millipore) and sequencing reactions were carried out
using a DNA sequencing kit (BigDye Terminator Cycle
Sequencing version 2.0 ready reactions; Applied Biosystems)
as described by the manufacturer. Sequencing products
were purified and electrophoresis was performed with an
ABI 3100 Genetic Analyzer (Applied Biosystems). Gene
sequences were aligned by using the multisequence align-
ment program CLUSTAL X (version 1.8). Distance matrices
determined following the assumptions described by Kimura
(1980) were used to elaborate a dendrogram using the
neighbour-joining method (Saitou & Nei, 1987). A boot-
strap analysis was performed to investigate the stability of
the trees obtained. Bootstrap values were obtained for a
consensus tree based on 100 randomly generated trees. The
data were also examined by using parsimony analysis.
Phylogenetic relationships with closely related species
were determined by using MEGA version 2.1 (Kumar et al., 2001).
The data were also examined using maximum-parsimony
(DNAML in PHYLIP 3.6) (Felsenstein, 1989). The percentage
similarity between 16S rRNA, rpoB and sodA gene sequences
were determined using NALIGN in the PC/GENE software
package (IntelliGenetics).

A 1470 nt 16S rRNA gene fragment of strain 4401825T was
sequenced. Sequence similarity between strain 4401825T
and S. ferus was 95.4%; lower sequence similarity values
were found with all other recognized species of the genus
Streptococcus. Phylogenetic analyses inferred from 16S rRNA
gene sequence comparisons using the neighbour-joining
(Fig. 1), parsimony and maximum-likelihood methods
showed that the new isolate grouped with S. ferus, which
was originally described as a ‘mutans-like’ Streptococcus
on the basis of phenotypic criteria (Coykendall, 1977;
Facklam, 2002) but later was found to be distantly related
to all then described streptococci (Whatmore & Whiley,
2002). The phylogenetic position of the new isolate was
not concordant with its phenotypic characterization. In
order to clarify the phylogenetic position of strain
4401825T, sequencing of sodA and rpoB gene fragments
was undertaken. Phylogenetic analyses inferred from sodA
and rpoB gene sequence comparisons by the neighbour-
joining (Fig. 2), parsimony and maximum-likelihood
methods showed that strain 4401825T clustered within the
S. sanguinis group. Phylogenetic trees inferred after con-
catenation of the two sequences showed higher bootstrap
values. Based on the data presented, we consider 4401825T
to be the type strain of a novel Streptococcus species, for
which the name Streptococcus massiliensis sp. nov. is
proposed.

**Fig. 1.** Phylogenetic tree of bacteria belonging to the genus Streptococcus inferred from comparison of 16S rRNA gene
sequences (1470 nt). Nucleotide accession numbers for the sequences used to construct this dendrogram are given in
parentheses. Enterococcus faecalis was used as the outgroup. Bar, 0.02 nucleotide changes per nucleotide position.
Description of *Streptococcus massiliensis* sp. nov.

*Streptococcus massiliensis* (mas.si.li.en sis. L. masc. adj. massiliensis of Massilia, the ancient Greek and Roman name for Marseille, France, where the type strain was isolated).

Gram-positive, non-motile, non-spore-forming cocci, arranged in pairs or short chains that are 0.3–0.7 μm in diameter. Surface colonies on sheep-blood agar are circular, white to greyish, shiny, convex, non-haemolytic and 1–2 mm in diameter after 48 h at 37°C in CO2-enriched atmosphere. Growth also occurs in aerobic, microaerophilic and anaerobic atmospheres. Optimum growth temperature is 37°C but growth is observed at 25–37°C. Growth occurs in the presence of 2% (w/v) NaCl. The type strain carries Lancefield G group antigen and is resistant to optochin. Catalase-negative. Acetoin production is negative and hydrolysis of hippurate is positive. Enzyme activity of arginine dihydrolase, leucine aminopeptidase, alanyl-phenylalanyl-proline arylamidase, glycyl-tryptophan arylamidase and alkaline phosphatase is detected. No activity is detected for β-glucosidase, β-galactosidase, β-glucuronidase, α-galactosidase, urease, pyrrolidonyl arylamidase, *N*-acetyl-β-glucosaminidase or β-mannosidase. Maltose is fermented. The following sugars are not fermented: arabinose, mannitol, sorbitol, lactose, trehalose, inulin, raffinose, glycogen, sucrose, pullulan, tagatose, methyl β-D-glucopyranoside, melibiose, ribose, starch and melezitose.

The type strain, 4401825T (= CIP 108498T = CCUG 49690T), was isolated from a human blood sample.

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References


Streptococcus massiliensis sp. nov.


