**Streptococcus oricebi** sp. nov., isolated from the oral cavity of tufted capuchin

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A Gram-stain-positive, catalase-negative, coccus-shaped organism was isolated from the oral cavity of tufted capuchin (Cebus apella). Comparative 16S rRNA gene sequence analysis suggested classification of the organism within the genus *Streptococcus*. Strain M8T was related most closely to *Streptococcus oralis* ATCC 35037T (96.17 % similarity) followed by *Streptococcus massiliensis* CCUG 49690T (95.90 %) based on the 16S rRNA gene. Strain M8T was related most closely to *S. massiliensis* CCUG 49690T (86.58 %) based on the RNA polymerase β subunit-encoding gene (*rpoB*), and to *Streptococcus tigurinus* AZ_3aT (81.26 %) followed by *S. massiliensis* CCUG 49690T (80.45 %) based on the 60 kDa heat-shock protein gene (*groEL*). The phylogenetic trees of 16S rRNA, *rpoB* and *groEL* gene sequences showed that strain M8T was most closely related to *S. massiliensis*. Based on phenotypic characterization as well as 16S rRNA gene and housekeeping gene (*rpoB* and *groEL*) sequence data, a novel taxon, *Streptococcus oricebi* sp. nov. (type strain M8T = JCM 30719T = DSM 100101T), is proposed.

A large number of streptococcal species colonize the oral cavities of animals and humans. Novel streptococci from the oral cavity of various animals have previously been reported (Takada & Hirasawa, 2007, 2008; Takada et al., 2010, 2013; Shinozaki-Kuwahara et al., 2011, 2014; Okamoto et al., 2013; Saito et al., 2014). Some oral streptococci are known to form characteristic colonies on Mitis Salivarius (MS) agar, demonstrating the synthesis of exopolysaccharides from sucrose (Hamada et al., 1979; Coykendall & Gustafson, 1985; Kilian et al., 1989). In the present study, we examined various monkey oral isolates grown on MS agar, with a focus on strains showing a rough colony morphology. We used 16S rRNA gene sequencing to characterize one of the organisms isolated from tufted capuchin (*Cebus apella*) that did not correspond phenotypically to currently recognized *Streptococcus* species. On the basis of phenotypic and phylogenetic results, we here propose another novel species of the genus *Streptococcus*.

A strain (M8T) with rough colony morphology obtained from the oral cavity of a captive tufted capuchin (Nasu World Monkey Park, Tochigi, Japan) was isolated on MS agar (Difco). Bacterial samples were collected using the cotton swabs method as described by Takada et al. (2006). This strain formed small, raised colonies with an irregular margin on MS agar at 37 °C. The strain produced z-haemolysis on brain-heart infusion (Difco) agar supplemented with 5 % sheep blood at 37 °C in a candle extinction jar. Lancefield grouping was tested with the streptococcal grouping kit (Oxoid); a reaction with A-group antiserum was detected. A biochemical analysis was conducted using the Rapid ID 32 Strep, API 50 CH and API ZYM systems (bioMérieux) according to the manufacturer’s instructions. The main differences in the characteristics of strain M8T and other related species are shown in Table 1.

DNA was extracted from bacterial culture using the Promega Genome kit according to the manufacturer’s instructions. The G+C contents of the DNA preparations were determined using an HPLC method (Kaneko et al., 1986) with some modifications. Briefly, 10 μg of denatured DNA was hydrolysed with P1 nuclease [100 μg ml⁻¹ in 40 mM acetate buffer containing 2 mM ZnSO₄ (pH 5.3); Yamasa Shoyu] for 1 h at 50 °C. Alkaline phosphatase (0.8 U; Takara Shuzo) was then added, and the mixture was incubated at 37 °C for 1 h. The nucleosides were separated and quantified with the ACQUITY UPLC system (Waters) and a LaChrom C18-AQ column (Hitachi High-Tech Fielding) by using an equal amount of a nucleoside mixture (Yamasa Shoyu) as a standard. The nucleosides were eluted with a solvent containing 20 mM NH₄H₂PO₄ and acetonitrile (20 : 1, v/v) (Waters). The value obtained for strain M8T was 42.4 ± 0.22 mol% G+C (mean ± SD; range, 42.1–42.5 mol%). To assess the phylogenetic affinity of the novel isolate, its 16S rRNA gene was amplified by PCR using the primer set 27f...
sequence (1504 bp) of strain M8T showed similarities of EzTaxon-e server (Kim
rRNA gene sequence similarities were performed using the
phylogenetic neighbours and calculation of pairwise 16S
against the DDBJ database. The identification of the closest
of the novel isolate were identified by performing searches
jected to a comparative analysis. The closest known relatives
v1.1 Cycle Sequencing kit (Life Technologies) and then sub-
PRISM 3130 Genetic Analyzer using a Big Dye Terminator
1992). Amplicons were directly sequenced with an ABI
gene sequences, which were described previously (Hiraishi,
additional primers for the determination of 16S rRNA
AAAGGAGGTGATCCAGCC-3
(5
D-sorbitol, glycogen, melibiose, melezitose, D-arabitol, cyclodextrin, glycerol, erythritol, D-arabinose, D-xylose, L-xylose, D-adonitol, methyl
D-mannose and
D-mannose. All strains were able to hydrolyse
urea.

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(5’-AGAGTTTGATCCTGGCTCAG-3’) and 1525r (5’-
AAGGAGGTGATCCAGGC-3’) (Lane, 1991), and
additional primers for the determination of 16S rRNA
gene sequences, which were described previously (Hiraishi,
1992). Amplicons were directly sequenced with an ABI
PRISM 3130 Genetic Analyzer using a Big Dye Terminator
v1.1 Cycle Sequencing kit (Life Technologies) and then
subjected to a comparative analysis. The closest known relatives
of the novel isolate were identified by performing searches
against the DDBJ database. The identification of the closest
phylogenetic neighbours and calculation of pairwise 16S
rRNA gene sequence similarities were performed using the
EzTaxon-e server (Kim et al., 2012). The 16S rRNA gene
sequence (1504 bp) of strain M8T showed similarities of
96.17, 95.90 and 95.87 % to those of Streptococcus oralis
ATCC 35037T, Streptococcus massiliensis CCUG 49690T
and Streptococcus porcorum 682-03T, respectively, and these
values were less than 97 %, the threshold value for defining
different species (Stackebrandt & Goebel, 1994). The CLUS-
TAL W program (Thompson et al., 1994) was used to perform
a multiple alignment before reconstruction of the phyloge-
netic tree. Sequence similarity values were calculated auto-
matically by the MEGA 5.2 software. Nucleotide substitution
rates (Ks values) were calculated (Kimura, 1980) and the
trees were reconstructed by the neighbour-joining (Saitou
& Nei, 1987) and maximum-parsimony methods using
MEGA 5.2 software (Tamura et al., 2011). The results of the
phylogenetic analysis inferred from 16S rRNA gene sequence
comparisons using the neighbour-joining method (Fig. 1)
indicated that the new isolate was included in the anginosus
group and clustered with S. massiliensis, whereas the maxi-
mum-parsimony method showed S. massiliensis was
excluded from this group (Fig. S1, available in the online Supplementary Material). The segments of the genomic DNA of strain M8T encoding the groEL and rpoB genes were amplified by PCR and sequenced. Partial sequences of the groEL and rpoB genes were amplified using the primers described by Glazunova et al. (2009). The groEL and rpoB gene sequences used for phylogenetic analyses were obtained from the DDBJ database. Strain M8T was related most closely Streptococcus tigurinus AZ_3aT (81.26 % similarity) followed by S. massiliensis CCUG 49690T (80.45 %) based on the groEL (719 bp) gene sequence. Strain M8T was related most closely to S. massiliensis CCUG 49690T (86.58 % similarity) based on the rpoB (687 bp) gene sequence. Phylogenetic trees reconstructed by the neighbour-joining method with partial sequences of the groEL (Fig. 2) and rpoB (Fig. 3) genes confirmed the phylogenetic placement of representative strains within the genus Streptococcus. The phylogenetic tree of rpoB and groEL gene sequences showed that strain M8T was clustered with S. massiliensis. A phylogenetic analysis based on groEL and rpoB gene comparisons included strain M8T in the non-groupable streptococci (such as Streptococcus suis and Streptococcus acidominimus) and mutans group, respectively. Streptococci have been divided into six major groups (the anginosus, bovis, mitis, mutans, pyogenes and salivarius groups) based on their 16S rRNA gene sequences (Kawamura et al., 1995). The mitis group currently includes S. massiliensis based on a housekeeping gene sequencing analysis reported

Fig. 1. Phylogenetic tree reconstructed from the 16S rRNA gene sequence of members of the genus Streptococcus by the neighbour-joining method. Numbers on the tree indicate bootstrap values calculated for 1000 subsets for branch points >50 %. Bar, 0.005 substitutions per site.

Fig. 2. Neighbour-joining phylogenetic tree based on groEL gene sequences of strain M8T and related species. Numbers on the tree indicate bootstrap values calculated for 1000 subsets for branch points >50 %. Bar, 0.02 substitutions per site.
by Glazunova et al. (2006). However, based on its 16S rRNA gene sequence, *S. massiliensis* has been classified here within the mutans group. The mitis and anginosus groups were previously reported to belong to the same group based on a partial recN (the recombination and repair protein encoding gene) gene sequence analysis (Glazunova et al., 2010). Taken together, these findings indicated that strain M8<sup>T</sup> and *S. massiliensis* were not groupable based on the existing gene sequence analysis.

On the basis of phenotypic and phylogenetic criteria, strain M8<sup>T</sup> represents a novel species of the genus *Streptococcus*, for which the name *Streptococcus oricebi* sp. nov. is proposed.

**Description of *Streptococcus oricebi* sp. nov.**

*Streptococcus oricebi* (o.ri.ce'bi. L. n. os oris mouth; N.L. gen. n. oricebi of the mouth of *Cebus*).

Cells are Gram-stain-positive, non-spore-forming cocci, have a diameter of 0.8–1.0 μm, and occur in pairs or chains. Colonies are white, α-haemolytic and have a diameter of 0.6–0.9 mm after being incubated on blood agar at 37 °C for 48 h. On MS agar, colonies are small, dark blue, and have a crinkled appearance. Cells are facultatively anaerobic, catalase-negative and non-motile. Cells react with Lancefield group A antisera. A test for acid production shows a positive reaction with D-glucose, D-fructose, D-mannose, N-acetylglucosamine, salicin, maltose, lactose, sucrose, trehalose, raffinose and methyl β-D-glucopyranoside, but not with glycerol, erythritol, D-arabinose, D-ribose, D-xylene, L-xylene, D-adonitol, methyl β-D-xylpyranoside, D-galactose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, arbutin, cellobiose, melibiose, inulin, melezitose, starch, glycogen, xyitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabinol, L-arabinol, glucose, 2-keto-glucuronate, 5-keto-glucuronate, pullulan or cyclodextrin. Aesculin is hydrolysed, whereas hippuric acid and urea are not. The Voges–Proskauer test is positive. The test for enzyme activities shows a positive reaction with β-glucosidase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, arginine dihydrolase and alanine-phenylalanine-proline arylamidase, but not with alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase, trepsin, x-chymotrypsin, x-galactosidase, β-galactosidase, β-glucuronidase, x-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, x-fucosidase, pyrog glutamic acid arylamidase, glycyl-tryptophan arylamidase, β-mannosidase or urease.

The type strain, M8<sup>T</sup> (=JCM 30719<sup>T</sup>=DSM 100101<sup>T</sup>), was isolated from the oral cavity of tufted capuchin. The DNA G+C content of the type strain is 42.4 mol%.

![Fig. 3. Neighbour-joining phylogenetic tree based on rpoB gene sequences of strain M8<sup>T</sup> and related species. Numbers on the tree indicate bootstrap values calculated for 1000 subsets for branch points &gt;50 %. Bar, 0.02 substitutions per site.](image-url)
Acknowledgements

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References


