

## *Streptococcus oligofermentans* sp. nov., a novel oral isolate from caries-free humans

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Five streptococcal strains were isolated from dental plaque and saliva of caries-free humans. The cells were Gram-positive, non-spore-forming, non-motile cocci that were arranged in short chains. The strains were catalase-negative, facultatively anaerobic and produced lactic acid exclusively from glucose fermentation. Biochemical analysis that used both conventional methods and the commercial API 20 Strep system showed that the five strains fermented only a few kinds of sugar. The mean DNA G + C content of the five novel strains was  $39.5 \pm 0.8$  mol%. Phylogenetic analysis based on 16S rDNA sequence homology indicated that the new isolates represented a novel member of the mitis group of the genus *Streptococcus*, related most closely to the recently described species *Streptococcus sinensis*. DNA–DNA relatedness between novel strain LMG 21535<sup>T</sup> and type strains of phylogenetically related species of oral streptococci was 7.1–16.4%. Therefore a novel *Streptococcus* species, *Streptococcus oligofermentans* sp. nov., is proposed. The type strain is LMG 21535<sup>T</sup> = AS 1.3089<sup>T</sup>.

Oral streptococci comprise part of the normal microbial flora of the oral cavity and upper respiratory tract of humans (Hardie & Marsh, 1978). However, they are also involved (usually as opportunistic pathogens) in a number of human diseases, such as dental caries (Fitzgerald *et al.*, 1960; Krasse, 1966) and bacterial endocarditis (Dyson *et al.*, 1999). On the basis of 16S rDNA homology, oral streptococci have been classified into five phylogenetic groups (Kawamura *et al.*, 1995). Among them, the mitis group has been considered to be difficult to identify by biochemical methods because of the lack of reliable traits (Ezaki *et al.*, 1988), so genetic characterizations are necessary. Recently, four new streptococcal species that belong to the mitis group have been proposed, based mainly on genetic and phylogenetic analyses (Kawamura *et al.*, 1998; Willcox *et al.*, 2001; Woo *et al.*, 2002).

During a survey of oral acid-producing bacteria of nasopharyngeal carcinoma patients, we isolated five strains of oral streptococci from dental plaques and saliva. Compared with described oral streptococcal species, these strains fermented only a few kinds of sugars. Phylogenetic analysis

based on 16S rDNA sequence homology and DNA–DNA relatedness indicated that the new isolates were closely related to the mitis group, but were a distinctive member of this group.

*Streptococcus sanguinis* ATCC 10556<sup>T</sup> and *Streptococcus mitis* NCTC 12261<sup>T</sup> were purchased from the China Microbiological Culture Collection Center (CMCC) and *Streptococcus sinensis* HKU4<sup>T</sup> and *Streptococcus gordonii* ATCC 10558<sup>T</sup> were kindly provided by Professor P. Woo of the University of Hong Kong and the China General Microbiological Culture Collection Center (CGMCC), respectively. In our laboratory, novel strains were isolated and purified on brain-heart infusion (BHI; Oxoid) agar plates supplemented with 5% defibrinated sheep blood, and cultivated at 37 °C under an atmosphere of N<sub>2</sub>/CO<sub>2</sub> (95/5%). End products of glucose fermentation in tryptone/peptone/yeast extract/glucose (TPYG) medium (Scardovi, 1986) were detected by GC (GC-14B; Shimadzu). Biochemical traits were determined by using both conventional methods (Hardie, 1986) and the API 20 Strep system (bioMérieux). All tests were performed in duplicate.

Genomic DNA was extracted and purified by using a previously described modification of the method of Marmur (1961) (Dong *et al.*, 2000). DNA G + C content was determined by thermal denaturation (Marmur & Doty, 1962). DNA–DNA relatedness was determined at 66.3 °C on the basis of the DNA–DNA liquid reassociation rate (De Ley *et al.*, 1970) by using a 752 spectrophotometer (Shanghai

Published online ahead of print on 20 December 2002 as DOI 10.1099/ijs.0.02493-0.

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of *Streptococcus oligofermentans* LMG 21535<sup>T</sup> is AY099095.

Tables showing the biochemical characteristics of *Streptococcus oligofermentans* and levels of DNA–DNA relatedness with closely related species are available as supplementary data in IJSEM Online.

Third Analytical Company, China) with a thermal controller. 16S rDNA was amplified by PCR with genomic DNA as the template and sequenced with an ABI PRISM 377XL DNA sequencer (Applied Biosystems). The most closely related sequences were retrieved from GenBank and aligned with those of the novel strains; similarity analysis was performed by using the DNAMAN program (version 4.0; Lynnon Biosoft). A phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987), implemented in the DNAMAN program. The stability of the clustering of the tree was evaluated by bootstrap analysis of 1000 datasets.

The novel strains produced lactic acid as the exclusive end-product of glucose fermentation. Their mean generation time was  $2.17 \pm 0.26$  h. The five strains utilized only a few sugars, such as sucrose, glucose, mannose and maltose; detailed data are available in IJSEM Online. Furthermore, they were different from most other oral streptococci in some biochemical characteristics (Table 1), such as not utilizing lactose or hydrolysing arginine; however, hippurate is hydrolysed.

The complete 16S rDNA sequence of novel strain LMG 21535<sup>T</sup> was compared with those of the type strains of 19 related oral streptococcal species and *Bacillus subtilis* NCFB 1769<sup>T</sup>. On the basis of 16S rDNA sequence similarity in a 1332 bp consensus fragment, a phylogenetic tree rooted with *B. subtilis* was constructed (Fig. 1). Strain LMG 21535<sup>T</sup> clustered in the mitis group with highest similarity to

*S. sinensis* HKU4<sup>T</sup> (97.7%), a recently described oral streptococcus (Woo *et al.*, 2002a, b). Similarity levels between strain LMG 21535<sup>T</sup> and other members of the mitis group ranged from 94.2 to 95.8 %, suggesting that this strain could represent a novel member of the mitis group, closely related to *S. sinensis*.

As strain LMG 21535<sup>T</sup> was phylogenetically related to the streptococci in the mitis group, DNA–DNA relatedness between them was analysed. DNA–DNA reassociation rates were 72–100 % among the five novel strains, indicating that they were a homogeneous genetic group. However, DNA–DNA relatedness between strain LMG 21535<sup>T</sup> and four streptococcal species in the mitis group was only 7.1–16.4 %, much lower than the DNA homology threshold for a species (70 %). Values of DNA–DNA relatedness between strain LMG 21535<sup>T</sup> and closely related species in the mitis group are available as supplementary data in IJSEM Online. The mean DNA G+C content of the five novel strains was  $39.46 \pm 0.79$  mol% (39.89 mol% for the type strain, LMG 21535<sup>T</sup>).

Although our strains exhibited high 16S rDNA similarity with *S. sinensis* (97.7 %), DNA–DNA relatedness between them was only 15 %. The difference in biochemical characteristics was also obvious (Table 1). By combining phenotypic, genotypic and phylogenetic characteristics, it is evident that the novel strains belong to a different species from *S. sinensis* and other oral streptococci, for which the name *Streptococcus oligofermentans* sp. nov. is proposed.

**Table 1.** Biochemical characteristics that differentiate *S. oligofermentans* sp. nov. from related *Streptococcus* species in the mitis group

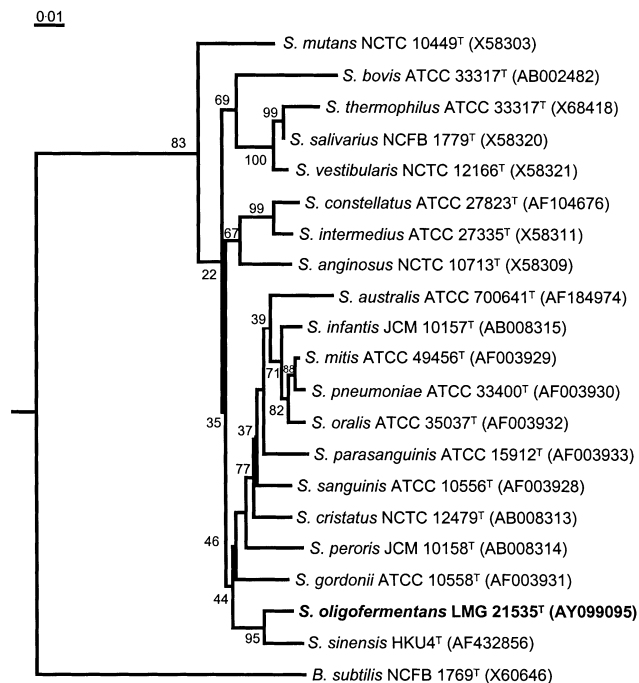
Species: 1, *S. oligofermentans*; 2, *S. sinensis*; 3, *S. sanguinis*; 4, *S. gordonii*; 5, *S. mitis*; 6, *Streptococcus peroris*; 7, *Streptococcus infantis*; 8, *Streptococcus australis*; 9, *Streptococcus oralis*; 10, *Streptococcus parasanguinis*; 11, *Streptococcus pneumoniae*; 12, *Streptococcus cristatus*. +, >90 % of strains positive; –, <10 % of strains positive; D+, 50–89 % of strains positive; D–, 11–49 % of strains positive; NT, not tested.

Character	1	2*	3†	4†	5†	6†	7†	8‡	9†	10†	11†	12†
Hydrolysis of:												
Arginine	–	+	+	+	–	–	–	+	–	+	D–	+
Hippurate	+	–	–	–	–	–	–	–	–	–	–	NT
Acid produced from:												
Lactose	D–	+	+	+	+	+	+	+	+	+	+	+
Raffinose	D–	–	D+	–	D–	–	–	–	D+	D+	+	D–
Trehalose	D–	+	+	+	–	–	–	–	D–	D–	+	D+
Melibiose	–	–	D+	–	D–	–	–	–	D+	D+	–	D–
Production of:												
α-Galactosidase	D–	–	D–	+	D+	–	–	–	D+	D+	+	D–
β-Galactosidase	–	–	+	+	D+	–	+	–	D–	D–	–	D–
Alkaline phosphatase	D–	–	D–	+	D+	+	–	+	D+	D+	–	–

\*Data from Woo *et al.* (2002a).

†Data from Kawamura *et al.* (1998).

‡Data from Willcox *et al.* (2001).



**Fig. 1.** Dendrogram based on 16S rDNA sequence homology in a consensus 1332 bp fragment, indicating the phylogenetic relationships of *S. oligofermentans* LMG 21535<sup>T</sup> and 19 other *Streptococcus* species. The tree was rooted with *B. subtilis* NCFB 1769<sup>T</sup> and constructed by using the neighbour-joining method with bootstrap values calculated from 1000 trees. Numbers at branch points represent bootstrap support (%); GenBank accession numbers of 16S rDNA sequences are given in parentheses. Bar, 1 % sequence divergence.

### Description of *Streptococcus oligofermentans* sp. nov.

*Streptococcus oligofermentans* (o.li.go.fer.men'tans. Gr. adj. *oligos* little, scanty; L. part. adj. *fermentans* fermenting; N.L. part. adj. *oligofermentans* fermenting few compounds).

Gram-positive, non-motile, non-spore-forming cocci, arranged in short chains, about 0.7 µm in diameter after 24 h growth in BHI medium at 37 °C. Catalase-negative. Colonies on BHI blood agar are even, locally rough, dark yellow with α-haemolysis and approximately 0.5–1.0 mm in diameter after 24 h cultivation. Facultatively anaerobic. Optimum temperature for growth is 37 °C; temperature range for growth is 25–41 °C. Optimum pH is 7.0; pH range for growth is 5.30–8.95. Sucrose, D-glucose, mannose and maltose are fermented; mannitol, salicin, sorbitol, arabinose, inulin, melibiose, cellobiose, arbutin, amygdalin, ribose, starch and glycogen are not fermented. Fermentation of lactose, trehalose and raffinose is variable. Hippurate is hydrolysed. Arginine and aesculin are not hydrolysed. Voges–Proskauer test is negative. DNA G+C content is 39.46 ± 0.79 mol% (39.89 mol% for the type strain).

The type strain is deposited in both the China General Microbiological Culture Collection Center (CGMCC; Beijing) and in BGGM/LMG (Gent, Belgium) under accession numbers AS 1.3089<sup>T</sup> and LMG 21535<sup>T</sup>, respectively. Strains were isolated from the dental plaque and saliva of humans.

### Acknowledgements

This study was supported by the grants of the '863' program from the Chinese Ministry of Sciences and Technology and the Chinese Academy of Sciences.

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