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Streptococcus shenyangsis sp. nov., a New Species Isolated from the Oropharynx of a Healthy Child from Shenyang China

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Introduction

In the human body, there are approximately 100 trillion microbes, which are approximately ten times the number of human cells [1]. With the development of the Human Microbiome Program [2] and the continuous development of high-throughput sequencing technology [3], the understanding of human microbial flora has increased greatly. The oral cavity is the initial part of the human digestive tract and is connected to the respiratory tract. It is an important human microecological system. Streptococcus is ubiquitous in the mouth and is the dominant genus in the saliva and soft tissues of the mouth [4–6]. Here, we describe *Streptococcus shenyangsis* sp. nov. strain D19^T, which was isolated from the oropharynx of a healthy child.

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Materials and Methods

Specimen Collection and Isolation

A throat swab specimen-containing strain D19^T was collected from the oropharynx of a 6-year-old healthy child in Shenyang, China. Our study was approved by the Ethics Committee of Shenyang Medical College, and written informed consent was obtained from the parents of the participant. Samples were stored at − 80 ℃ before isolation and culture. The sample was placed in 1 ml of physiological saline, shaken using a vortex mixer, and diluted to 10^{-3}. A total of 0.1 ml was uniformly applied to BHI agar medium containing 5% defibrinated sheep blood (Solarbio). After incubating at 37 ℃ for 24 h, the gray-white colonies with alpha hemolysis were picked from the medium and purified by the continuous streaking technique.

16S rRNA and Conserved Sequence Analysis

The nucleic acids of the strain were extracted for gene amplification and cloning of 16S rRNA using the Wizard

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16S rRNA and Conserved Sequence Analysis

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D19^T were selected based on BLAST alignment, and the phylogenetic tree constructed with the 16S rRNA results. The following strains were selected: *Streptococcus oralis* subsp. *dentisani* 77477 (CAUK00000000.1) [24], *Streptococcus mitis* ATCC 49456 (MUYN00000000.1), *Streptococcus pneumoniae* ATCC 3340 (LN831051.), *Streptococcus pseudopneumoniae* ATCC BAA-960 (AICS00000000.1) [25], and *Streptococcus oralis* ATCC 3503 (LR134336.1). The selected strains were all type strains of Streptococcus species. Meanwhile, the complete genome sequence was retrieved from the FTP of NCBI (National Center for Biotechnology Information).

Results

Conserved Sequence Analysis

The 16S rRNA gene sequence of the novel strain was obtained by sequencing (1433 bp), uploaded to the GenBank database (MN061029) and compared with the sequence in GenBank by the NCBI server using BLAST. As a representative result, compared with strain D19^T, the type strains of Streptococcus with 16S rRNA similarities greater than 97% were *Streptococcus oralis* subsp. *dentisani* strain 7747 (99.23%), *Streptococcus pseudopneumoniae* strain 108 (98.88%), *Streptococcus mitis* strain ATCC 49456 (98.81%), *Streptococcus pneumoniae* strain ATCC 33400 (98.65%), *Streptococcus infantis* ATCC 700779 (98.32%), *Streptococcus oralis* subsp. *tigurinus* AZ_3a (98.11%), *Streptococcus rubneri* strain LMG 27207 (97.97%), *Streptococcus australis* strain AI-1 (97.62%), and *Streptococcus sanguinis* strain JCM 5708 (97.34%). The neighbor-joining and maximum likelihood methods and Kimura's two-parameter model were used to construct a tree inferred by comparison of the 16S rRNA gene sequences of *Streptococcus shenyangsis* D19^T and other members of the genus Streptococcus (Supplementary Fig. 1, Supplementary Fig. 2), and we constructed a neighbor-joining phylogenetic tree of the *Streptococcus mitis* group using 16S rRNA (Fig. 1). The results showed that *Streptococcus oralis* subsp. *dentisani* DSM 27088 ^{T}, *Streptococcus mitis* ATCC 49456 ^{T}, *Streptococcus pneumoniae* ATCC 33400 ^{T}, *Streptococcus pseudopneumoniae* ATCC BAA-960 T, and *Streptococcus oralis* ATCC 3503 ^{T} are most closely related to strain D19^T.

Phenotypic and Biochemical Characterization

Strain D19^T was shown to be Gram positive by classic Gram staining [26], nonmotile by a motility assay on soft agar plates [27] and a facultative anaerobic bacterium by growing cultures under different gas environments; the bacterium could grow at temperatures between 22 ℃ and 37 ℃, and the optimal culture temperature was 37 ℃. Strain D19^T was grown in 2.5% salt BHI agar medium containing 5% defibrinated sheep blood. The major cellular fatty acids of strain D19^T were C16:0 (35.1%), C14:0 (17.96%), and C18:1 w9c (10.24%). All described fatty acids are listed in Table 1. As determined with the API 50CH, API ZYM, and Rapid ID32 STREP kits, cells were able to produce acid

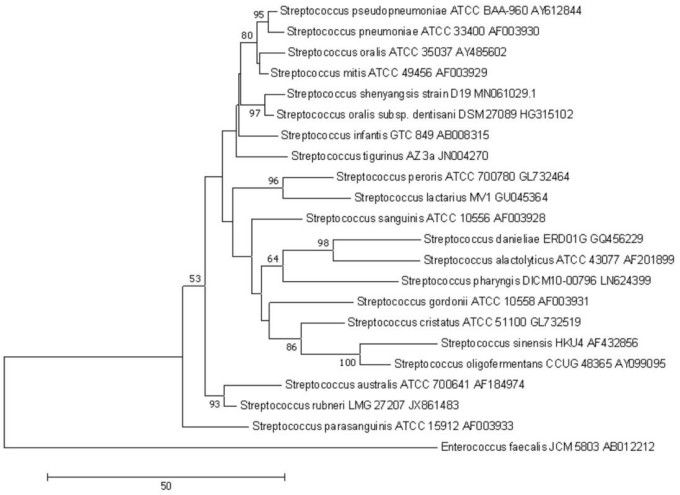

Fig. 1 Neighbor-joining phylogenetic tree inferred from comparison of 16S rRNA gene sequences of the *Streptococcus* D19^T and the closely related members of the main streptococcal mitis group. The 16S rRNA gene sequence of *Enterococcus faecalis* ATCC 19433 ^{T} was used as an outgroup. Bar, 0.01 Substitutions per site. Bootstrap values ≥ 50 are indicated

Table 1 Cellular fatty acid composition (%)

Fatty acids	Percent (%)
16:0	35.1
14:0	17.96
18:1 w9c	10.24
18:0	10.09
12:0	6.57
Sum in feature 5 (18:0 ante/18:2 w6,9c)	6.34
Sum in feature 8 (18:1 w7c)	5.35
Sum in feature 3 (16:1 w7c/16:1 w6c)	3.61
16:1 w9c	1.77
16:1 w5c	0.93
20:4 w6,9,12,15c	0.86
17:0	0.82
15:0	0.63

from lactose, sucrose, maltose, D-galactose, D-glucose, D-fructose, D-mannose, pullulan, N-acetylglucosamine, and 5-ketogluconate. Enzyme activities were detected for esterase lipase (c8), lipase (c14), cystin-arylamidase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, alkaline phosphatase, and APPA. The biochemical comparison results with adjacent strains are shown in Table 2. *Streptococcus shenyangsis* D19^T was sensitive to 10 different antibiotics (penicillin, ampicillin, cefepime, cefotaxime, ceftriaxone, linezolid, clindamycin, chloramphenicol, vancomycin, and erythromycin). Electron micrographs of strain D19^T are shown in Fig. 2.

Genome Properties

The genome of strain D19^T is 2,023,003 bp long with a G+C content of 39.93 mol% (Fig. 3). It is made up of 24 scaffolds composed of 27 contigs. Of the 1966 predicted genes, 1916 were protein-coding genes and 50 were RNAs (three genes were 5S rRNA, one gene was 16S rRNA, one gene was 23S rRNA, one gene was sRNA, and 44 genes were tRNA). A total of 1889 genes (96.08%) were assigned a putative function by NR BLAST. The remaining 263 genes (13.38%) were annotated as hypothetical proteins.

Genome Comparison

The genome of strain D19^T (VFSH00000000) was compared to the genomes of related members of Streptococcus. The genome size of strain D19^T (2.02 Mb) was greater

Table 2 Biochemical characteristics that differentiate the novel strains from 1. Str. D19^T; 2. S. *oralis* subsp.*dentisani* ATCC DSM 27088 ^{T}; 3. S. *mitis* 49465 ^{T} [28]; 4, S. *oralis* ATCC35037^T [28, 29]; 5, S. *pneumoniae* ATCC 33400 ^{T} [28]; 6, S. *pseudopneumoniae* ATCC BAA-960 T [25]

	1	2	3	4	5	6
Hemolysis	α	α	α	α	α	α
Acetoin production(Voges-proskauer test)	−	−	−	−	−	−
Hydrolysis of						
Arginine	−	−	v	−	+	−
Aesculin	−	−	−	v	v	−
Hippurate	−	−	−	−	−	−
Starch	−	−	v	+	−	−
Production of						
α-D-Galactosidase	v	v	+	−	+	−
β-D-Galactosidase	+	−	−	+	+	+
β-D-Glucosidase	v	−	−	−	−	v
Acid from						
Lactose	+	+	+	+	+	+
D-Mannitol	v	+	−	+	v	v
Melibiose	−	−	v	v	−	−
Raffinose	−	v	v	v	+	−
D-Ribose	−	−	v	v	−	−
D-Sorbitol	−		−	−	−	−
Sucrose	+	v	+	+	+	v
D-Tagatose	−		−	−	−	−
Trehalose	−	−	v	v	v	−

+positive, − negative

V variable

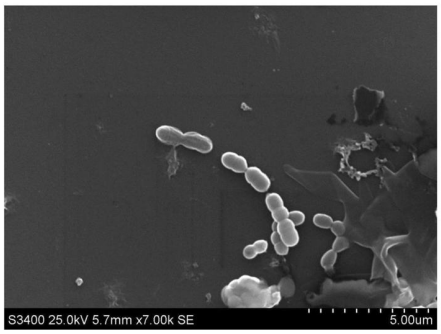

Fig. 2 Electron microscopy of Streptococcus *shenyangsis* sp.nov. strain D19^T, using a Hitachi S3400 electron microscope (FEI) operated at 25 kV. The scale bar represents 5 µm

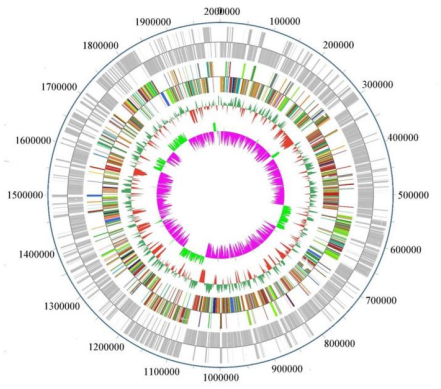

Fig. 3 Graphical circular map of the genome. The outermost circle is the position coordinates of the genomic sequence, from the outside to the inside, respectively, the coding gene (the positive circle is the positive chain, the negative chain is the inner chain), the COG function annotation (the positive chain by the outer circle, the negative chain by the inner circle), the genome GC content (inward red part indicates that the GC content in this area is lower than the whole genome average GC content, the outward green part is opposite), genomic GC skew value (inward pink part indicates that the area G content is lower than C) Content, the outward light green part is opposite)

than those of *Streptococcus oralis* subsp. *dentisani*, *Streptococcus mitis* ATCC 49456, and *Streptococcus oralis* ATCC 3503, but less than those of *Streptococcus pneumoniae* ATCC 33400 and *Streptococcus pseudopneumoniae* ATCC BAA-960. The G + C content of strain D19^T was equal to that of *Streptococcus pseudopneumoniae* ATCC BAA-960, higher than this of *Streptococcus pneumoniae* ATCC 33400 and lower than those of other streptococci compared. The number of protein-coding genes (1889) and the total number of genes (1966) of strain D19^T were lower than those of *Streptococcus pneumoniae* ATCC 33400 and *Streptococcus pseudopneumoniae* ATCC BAA-960 but higher than those of *Streptococcus oralis* subsp. *dentisani, Streptococcus mitis* ATCC 49456 and *Streptococcus oralis* ATCC 3503 (Table 3). In all comparative genomes, the distribution of genes in COG categories is presented in Fig. 4. The DDH values of strain D19^T compared to the related members of Streptococcus ranged from 31 to 52%, both of which are less than 70% (Table 4). The ANI values of strain D19^T compared to the related members of Streptococcus ranged from 86 to 92% (Table 4). By comparing strain D19^T with adjacent strains, it was found that there were differences in the genome length, GC content, and number of coding genes. The values of strain D19^T and adjacent strains in the dDDH and ANI comparisons were also much smaller than the critical values of 70 and 95%. This indicates that the genome of strain D19^T was not identical to those of the five adjacent strains.

Description of *Streptococcus shenyangsis* sp. nov. Strain D19^T

Streptococcus shenyangsis (shen.yang'sis L. gen. neutr. n. shenyang, pertaining to, or originating from Shenyang, the capital of Liaoning Province, China, where the sample was sampled.)

Streptococcus shenyangsis sp. nov. strain D19^T was cultured on nutrient agar medium containing 5% defibrinated sheep blood for 24 h, and it produced needle-sized, gray-white colonies. The optimum culture temperature was 37 ℃. The strain was Gram positive and nonmotile. The biochemical characteristics of *Streptococcus shenyangsis* D19^T and adjacent strains are shown in Table 1. *Streptococcus shenyangsis* D19^T was sensitive to 10 different antibiotics: penicillin, ampicillin, cefepime, cefotaxime, ceftriaxone, linezolid, clindamycin, chloramphenicol, vancomycin, and erythromycin. The major fatty acid was C16:0. The 16S rRNA and genome sequences are deposited in NCBI, and the accession numbers are MN061029.1 and VFSH00000000. The genome of strain D19^T was 2,023,003 bp long with a G + C content of 39.93 mol%. The type strain D19^T (GDMCC 1.1644 = JCM 33583) was isolated from the oropharynx of a healthy child in Shenyang, China.

Table 3 Genome comparison of closely related species: streptococcus D19^T

S. no	Name of organisms	Size (Mb)	G+C (mol%)	Protein-coding genes	Total genes
1	D19^T	2,023,003	39.9	1889	1966
2	*Streptococcus oralis* subsp.*dentisani* DSM 27088	1,868,539	41.1	1726	1850
3	*Streptococcus mitis* ATCC 49456	1,847,880	40.4	1750	1831
4	*Streptococcus pneumoniae* ATCC 33400	2,110,970	39.7	2064	2330
5	*Streptococcus pseudopneumoniae* ATCC BAA-960	2,085,750	39.9	2113	2150
6	*Streptococcus oralis* ATCC 3503	1,931,550	41.5	1785	1912

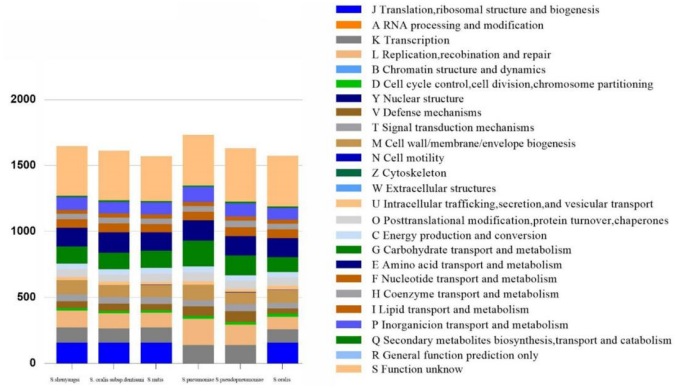

Fig. 4 Distribution of predicted genes of *streptococcus* D19^T and other related member streptococcus species into COG

Table 4 Pairwise comparison of *Streptococcus* D19^T with other related member of streptococcus using GGDC, formula 2 (DDH estimates based on identities/HSP length) and the ANI values

	DDH	ANI
Streptococcus oralis subsp.*dentisani*	31% [28.6–33.5%]	85.98%
Streptococcus mitis	51.70% [49–54.3%]	92.83%
Streptococcus pneumoniae	48.40% [45.8–51.1%]	91.69%
Streptococcus pseudopneumoniaes	47.90% [45.3–50.5%]	92.16%
Streptococcus oralis	31.50% [29.1–34.1%]	85.98%

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00284-021-02500-1.

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Declarations

Conflict of Interest The authors declare that there are no conflicts of interest.

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