



Complete Genome Sequence of *Streptococcus oralis* 34

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ABSTRACT *Streptococcus oralis* is an early colonizer and one of the most abundant species found in the human oral cavity. We report the complete genome sequence of *S. oralis* 34 (1,920,884 bp; GC content, 41.3%), commonly used in many oral microbiology studies exploring bacterial attachment and interaction(s) within mixed-species model systems.

Streptococcus oralis is a Gram-positive, nonmotile, alpha-hemolytic bacterium and one of the most abundant commensal bacteria in the human oral cavity, considered to be an early colonizer of dental plaque (1, 2). *S. oralis* belongs to the Mitis group of streptococci (3), which contains the major human pathogens *Streptococcus pneumoniae* and *Streptococcus mitis*. Although *S. oralis* is associated with oral health, *S. oralis* can gain access to the bloodstream and cause subacute infective endocarditis (IE) as an opportunistic pathogen (4–6). In 2016, *S. oralis* was split into three different subspecies, *S. oralis* subsp. *oralis*, *S. oralis* subsp. *tigurinus*, and *S. oralis* subsp. *dentisani* (7). However, due to genetic recombination and horizontal gene transfer via natural transformation between members of the Mitis group, classification of Mitis group species is often difficult (8).

S. oralis isolate 34 was originally obtained from R. J. Gibbons at the Forsyth Dental Center in Boston, MA, and at that time was called *Streptococcus sanguis* 34 (9). The isolate was used frequently in studies of oral bacterial adherence, both to surfaces and to other oral bacteria (10–12). Today, *S. oralis* 34 is still commonly used in oral microbiology research due to its robust biofilm formation properties (13, 14) and is commonly used within mixed-species models (15–17). However, a complete genome sequence for this organism was lacking.

For whole-genome sequencing, *S. oralis* 34 was resuscitated from a –80°C glycerol stock by streaking onto a brain heart infusion (BHI) agar plate and grown within an incubator at 37°C, 5% CO₂, for 48 h. Then, 5 ml of BHI broth was inoculated with the strain and grown overnight (37°C, 5% CO₂). The following morning, genomic DNA was purified using the DNeasy PowerLyzer microbial kit (Qiagen; catalog no. 12255-50), and the concentration was determined using a Qubit Flex fluorometer (Thermo Fisher Scientific; catalog no. Q33327) and the Qubit double-stranded DNA (dsDNA) broad-range (BR) assay kit (Thermo Fisher Scientific; catalog no. Q32850). The Microbial Genome Sequencing Center (MiGS; Pittsburgh, PA) performed combined short- and long-read sequencing (Small Nanopore Combo sequencing package; Illumina and Oxford Nanopore Technologies [ONT], respectively) and *de novo* assembly. Default parameters were used except where otherwise noted. Short reads were obtained using the Illumina Nextera kit and NextSeq 550 platform (18). For ONT sequencing, libraries were prepared using the kit SQK-LSK109 to the manufacturer's specifications (no DNA size selection/shearing), sequencing was performed on a MinION R9 flow cell, and base calling was performed using Guppy v4.2.2 (GPU mode) (19). Illumina paired-end reads (2 × 151 bp) and ONT long reads were provided as fastq files (Illumina, 1,701,038 reads, 468,658,143 bases, 244× coverage; ONT, 634,222 reads, 317,505,913 bases, 165× coverage). Quality control and adapter trimming were performed using bcl2fastq v2.20.0.445 (20) and Porechop v0.2.3_seqan.2.1.1 (21) for Illumina and ONT sequencing, respectively. Hybrid assembly with the Illumina and ONT reads was performed using Unicycler v0.4.8 (22). The

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assembly statistics were recorded using QUAST v5.0.2 (23). The assembly annotation was performed using Prokka v1.14.5 (24).

The *S. oralis* 34 sequence was deposited at GenBank as one circular contig (1,920,884 bp; GC content, 41.3%). GenBank annotated the genome sequence using the Prokaryotic Genome Annotation Pipeline (PGAP) v5.2 (25).

Data availability. The *S. oralis* 34 sequence is available in GenBank under accession no. CP079724, BioProject accession no. PRJNA746546, and BioSample accession no. SAMN20209571. The raw sequence reads are accessible under Sequence Read Archive accession no. SRX11573463 (Illumina) and SRX11573464 (MinION).

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