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Genome Sequence of the Bacteriocin-Producing Oral Probiotic *Streptococcus salivarius* Strain M18

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***Streptococcus salivarius* is a Gram-positive bacterial commensal and pioneer colonizer of the human oral cavity. Many strains produce ribosomally synthesized proteinaceous antibiotics (bacteriocins), and some strains have been developed for use as oral probiotics. Here, we present the draft genome sequence of the bacteriocin-producing oral probiotic *S. salivarius* strain M18.**

The Gram-positive bacterium *Streptococcus salivarius* is a pioneer colonizer of the human oral cavity, and large populations persist at this site for the host's lifetime (12). *S. salivarius* is the prototype species of the *S. salivarius* group, which includes the important dairy species *Streptococcus thermophilus* (6). Many *S. salivarius* strains produce ribosomally synthesized proteinaceous antibiotics (bacteriocins; reviewed in reference 14), typically encoded by megaplasmid-borne loci (10). As *S. salivarius* is generally associated with good oral health, several bacteriocinogenic strains with proven safety records have been developed as oral probiotics (2–4, 8, 12).

S. salivarius M18 (formerly strain Mia) is a megaplasmid-carrying oral probiotic exhibiting broad-spectrum inhibitory activity against several streptococcal pathogens, notably the caries-causing *Streptococcus mutans* (10). In order to provide a genetic basis for factors enhancing its probiotic candidature, e.g., bacteriocin repertoire and colonization-related genes, and also to establish whether the strain is free of virulence factors and antibiotic resistance determinants, the *S. salivarius* M18 genome was sequenced by a whole-genome shotgun strategy using a Roche GS-FLX pyrosequencer (7). Approximately 42.9 million base pairs (~18-fold coverage) was assembled by Roche GS *de novo* assembler (versions 1.1.03.24 and 2.3) into ~150 contigs. All putative chromosomal contigs were ordered relative to the megaplasmid-free *S. salivarius* CCHSS3 genome sequence (GenBank accession number FR873481). Gap closures were achieved by direct Sanger-based sequencing of PCR amplicons generated with specific primers designed for contig termini.

The high-quality draft *S. salivarius* M18 chromosome sequence currently comprises five supercontigs (2,142,944 bp; GC content of 39.6%). The remaining genomic gaps contain multiple copies of large (>6-kb) genes encoding putative highly repetitive serine-rich proteins homologous to the *Streptococcus gordonii* Hsa adhesin (9). The latter, which are conspicuously absent in *S. thermophilus*, may aid *S. salivarius* in colonizing oral surfaces. Automated annotation carried out by the rapid annotations using subsystems technology (RAST) (1) and NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) servers revealed 1,975 protein-coding sequences (CDSs), six rRNA operons, and 68 tRNA genes. A variety of insertion sequences were identified, with IS*Sag8* and IS*1193* being the most common. In addition, the chromosome contains a locus (*slm*) specifying the production of a new anti-*S. mutans* lantibiotic bacteriocin designated salivaricin M.

The *S. salivarius* M18 megaplasmid, pSsal-M18, is 183,037 bp long (GC content of 34.8%) and is the first completely sequenced streptococcal megaplasmid. Of 172 CDSs annotated by PGAAP, 16 belong to the known bacteriocin-encoding loci for salivaricins A2 (11), 9 (13), and MPS (5, 14). Interestingly, there is a region of several open reading frames (ORFs) (flanked by insertion sequences) nearly identical to chromosomal genes found in *S. salivarius* strains CCHSS3 and 57.I (GenBank accession number CP002888), indicating gene exchange between the chromosome and megaplasmid. The *S. salivarius* M18 genome sequence will not only be useful for comparative genomics but is essential for the development of a functional genomics platform facilitating molecular evolution and ecological studies.

Nucleotide sequence accession numbers. This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under accession number AGBV00000000. The version described in this paper is the first version, AGBV01000000.

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