Complete Genome Sequence of *Aggregatibacter actinomycetemcomitans* Strain IDH781

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We report here the complete genomic sequence and methylome of *Aggregatibacter actinomycetemcomitans* strain IDH781. This rough strain is used extensively as a model organism to characterize localized aggressive periodontitis pathogenesis, the basic biology and oral cavity colonization of *A. actinomycetemcomitans*, and its interactions with other members of the oral microbiome.

Aggregatibacter actinomycetemcomitans is a Gram-negative nonmotile facultative anaerobe of the oral microbiota implicated in the development of localized aggressive periodontitis (LAP) (1, 2). Despite the lack of a complete genomic sequence, IDH781, a rough strain, is commonly used as a model for pathogenesis and basic bacteriology studies (3–11). Rough strains of *A. actinomycetemcomitans* display the classic star-shaped colony morphology observed in clinical isolates from LAP patients, so they are more appropriate for the study of *A. actinomycetemcomitans* biology than those displaying a smooth colony phenotype (12, 13).

IDH781 was grown under anaerobic conditions (10% hydrogen, 10% carbon dioxide, and 80% nitrogen) on brain heart infusion agar supplemented with glucose, sodium bicarbonate, and yeast extract. The rough phenotype was confirmed microscopically. Cells were scraped from agar plates and lysed in 625 μg/ml proteinase K, 1.25 mg/ml lysozyme, and 2% sodium dodecyl sulfate. Genomic DNA was purified by phenol-chloroform/isoamyl extraction (14). DNA integrity was verified by agarose gel electrophoresis, purity was evaluated spectrophotometrically, and concentration was determined fluorometrically.

Sequencing libraries were constructed using the Pacific Biosciences 20-kb template preparation protocol and Sage Science's BluePippin size-selection system with a 5-kb fragment size cutoff. Pacific Biosciences single-molecule real-time (SMRT) sequencing was performed on an RSII instrument using a single SMRT cell with P6-C4 chemistry and a 3-h movie, producing 7,601 polymerase reads (N_{50}, 11,624 nucleotides [nt]) and 153,117 postfiltered subreads (N_{50}, 6,741 nt). *De novo* assembly with the HGAP assembler (version 2.3) yielded a single contig supported by a mean coverage of 283-fold (15).

The genome was circularized by permutation to start at the *dnaA* gene and remove terminal duplications using Circlator

(version 1.0.2), followed by resequencing using RS_Modification_and_Motif_Analysis (version 2.3) to correct errors at the original contig break and to detect DNA methylation motifs based on significant interpulse duration signals (15, 16). Two well-supported m6A motifs were detected, G**A**TC and AGG**A**G (bold indicates the methylated residue), with >98% of motifs in the genome detected. Seven other unique nonpalindromic modifications were detected at low frequency; these consisted of 2 putative m6A motifs (34% and 30%), 1 putative m4C motif (11%), and 4 unknown base modifications (20%, 15%, 8%, and 4%). The significance and importance of these low-frequency interpulse duration signals remain unknown.

The final assembly was 2,291,252 bp, with a G+C content of 44.3%, consistent with other completed *A. actinomycetemcomitans* genomes. To verify strain identity and detect possible horizontal gene transfer events, genomic intervals were taxonomically assigned using Taxator-tk (version 1.3.1e) with the nonredundant-microbial_20140513 database (refpack from http://research.bifo.helmholtz-hzi.de/software) (17). All classified regions (39.8% of the genome) were assigned to the species *A. actinomycetemcomitans*.

Annotation was performed by NCBI using the Prokaryotic Genome Automated Annotation Pipeline (PGAAP, best-placed reference protein; GeneMarkS+; version 3.3) (18). The chromosome contains 2,206 genes, with 2,129 coding sequences, 19 rRNAs, and 54 tRNAs for all 20 amino acids plus selenocysteine, 4 noncoding RNAs (ncRNAs), and 3 predicted clustered regularly interspaced short palindromic repeats (CRISPRs).

Accession number(s). The complete genome sequence of *A. actinomycetemcomitans* strain IDH781 has been deposited in GenBank under the accession number CP016553. The version described here is the first version.

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