

# Complete Genome Sequence of *Aggregatibacter actinomycetemcomitans* Strain IDH781

Anthony C. May,<sup>a</sup> Rachel L. Ehrlich,<sup>b,c</sup> Sergey Balashov,<sup>b,c</sup> Garth D. Ehrlich,<sup>b,c</sup> Mayilvahanan Shanmugam,<sup>a</sup> Daniel H. Fine,<sup>a</sup> Narayanan Ramasubbu,<sup>a</sup>  Joshua Chang Mell,<sup>b,c</sup>  Carla Cugini<sup>a</sup>

Department of Oral Biology, Rutgers School of Dental Medicine, Newark, New Jersey, USA<sup>a</sup>; Department of Microbiology and Immunology, Center for Genomic Sciences (CGS), Institute for Molecular Medicine & Infectious Disease, Drexel University College of Medicine, Philadelphia, Pennsylvania, USA<sup>b</sup>; Genomics Core Facility, Clinical and Translational Research Institute (CTRI), Drexel University College of Medicine, Philadelphia, Pennsylvania, USA<sup>c</sup>

**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.**

Received 20 September 2016 Accepted 22 September 2016 Published 10 November 2016

**Citation** May AC, Ehrlich RL, Balashov S, Ehrlich GD, Shanmugam M, Fine DH, Ramasubbu N, Mell JC, Cugini C. 2016. Complete genome sequence of *Aggregatibacter actinomycetemcomitans* strain IDH781. *Genome Announc* 4(6):e01285-16. doi:10.1128/genomeA.01285-16.

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Address correspondence to Carla Cugini, cc1337@sdm.rutgers.edu.

*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, GATC and AGGAG (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 (repack 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.

## FUNDING INFORMATION

This work, including the efforts of Mayilvahanan Shanmugam and Narayanan Ramasubbu, was funded by HHS | National Institutes of Health (NIH) (DE022544). This work, including the efforts of Rachel L. Ehrlich, Sergey Balashov, Garth D. Ehrlich, and Joshua Chang Mell, was funded by HHS | National Institutes of Health (NIH) (DC02148). This work, including the efforts of Daniel H. Fine, was funded by HHS | National Institutes of Health (NIH) (DE021172 and DE017968). This work, including the efforts of Anthony C. May and Carla Cugini, was funded by New Jersey Health Foundation (NJHF) (PC31-15).

G.D.E., J.C.M., R.L.E., and S.B. were supported by funding from the Drexel Clinical and Translational Research Institute (CTRL).

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