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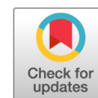
GENOME SEQUENCES



Complete Genome Sequence of *Aggregatibacter actinomycetemcomitans* Strain CU1000N

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ABSTRACT Here, we report the complete genome sequence of *Aggregatibacter actinomycetemcomitans* strain CU1000N. 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 that is implicated in the development of localized aggressive periodontitis (LAP) (1, 2). *A. actinomycetemcomitans* strain CU1000 was isolated in 1992 in New York City from the first-molar site of a 13-year-old, medically healthy, African American female patient with classic symptoms of LAP (3). CU1000N is a spontaneous nalidixic acid-resistant (DNA gyrase point mutant) strain of CU1000 (4). Despite the lack of a complete genome sequence, CU1000N, a “rough” strain, is commonly used as a model for pathogenesis and basic bacteriology studies (3–15). Rough strains of *A. actinomycetemcomitans* display the classic star-shaped colony morphology observed in clinical isolates from LAP patients; therefore, they are more appropriate for the study of *A. actinomycetemcomitans* biology than are those displaying a “smooth” colony phenotype (16, 17).

The CU1000N isolate was kept as a frozen stock at −80°C and was two passages from the original stock of the spontaneous mutant. It was streaked for isolation from −80°C to an entire Trypticase soy broth agar plate supplemented with glucose (8 g/L), sodium bicarbonate (10% [wt/vol]), and yeast extract (6 g/L) and was grown for 2 days under microaerophilic conditions (10% carbon dioxide). The rough phenotype was confirmed microscopically from the single colonies on the plate. Cells were scraped from the agar plate from the primary streak, resuspended in 2 mL of AE buffer (Qiagen), centrifuged (16,000 × *g* at 4°C for 2 min), homogenized, and resuspended in 100 μL of AE buffer. The genomic DNA was purified by phenol-chloroform-ethanol precipitation (18). DNA integrity was verified by agarose gel electrophoresis, purity was evaluated spectrophotometrically, and the concentration was determined fluorometrically.

Libraries were created for sequencing using the multiplex microbial SMRTbell library preparation protocol for the PacBio Sequel system. The SMRTbell template preparation kit 1.0-SPv3 and a SMRTbell barcoded adapter 96-well plate were used. SMRTbell libraries were pooled, size selected using BluePippin with a 5,000-bp cutoff value, and sequenced on a PacBio Sequel system. The polymerase reads were demultiplexed and broken into subreads (PacBio single-molecule real-time [SMRT] Link v7.0.1). The sequencing produced 47,445 polymerase reads and 47,057 postfiltered subreads (N_{50}, 9,973 nucleotides [nt]). Filtering as an intermediate step in assembling the reads and *de novo* assembly of the subreads were performed using Falcon (PacBio SMRT Link

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v7.0.1), which yielded a single contig of 2,337,866 nt (19). The resulting contig was circularized and permuted to *dnaA* using Circlator v1.5.5 (20). Errors were corrected using Arrow (PacBio SMRT Link v7.0.1). The final assembly was 2,331,529 nt and had an average coverage of 703×. It had a GC content of 44.2%, consistent with other completed *A. actinomycetemcomitans* genomes. The species of the final assembly was verified using taxator-tk v1.2 with the nonredundant-microbial_20140513 database (refpack from http://research.bifo.helmholtz-hzi.de/software) (21).

Annotation was performed by NCBI using the Prokaryotic Genome Annotation Pipeline (PGAP) v5.2 (22). The chromosome contains 2,268 genes, including 2,191 coding sequences, 19 rRNAs, 54 tRNAs including selenocysteine, 3 noncoding RNAs, and 1 predicted CRISPR.

Data availability. This whole-genome project has been deposited in DDBJ/ENA/GenBank under the accession number CP076449. The raw reads have been deposited in the NCBI SRA under the BioProject accession number PRJNA735719. The version described in this paper is the first version.

REFERENCES

1. Slots J, Ting M. 1999. *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in human periodontal disease: occurrence and treatment. Periodontol 2000 20:82–121. https://doi.org/10.1111/j.1600-0757.1999.tb00159.x.
2. Slots J, Reynolds HS, Genco RJ. 1980. *Actinobacillus actinomycetemcomitans* in human periodontal disease: a cross-sectional microbiological investigation. Infect Immun 29:1013–1020. https://doi.org/10.1128/iai.29.3.1013-1020.1980.
3. Fine DH, Furgang D, Schreiner HC, Goncharoff P, Charlesworth J, Ghazwan G, Fitzgerald-Bocarsly P, Figurski DH. 1999. Phenotypic variation in *Actinobacillus actinomycetemcomitans* during laboratory growth: implications for virulence. Microbiology 145:1335–1347. https://doi.org/10.1099/13500872-145-6-1335.
4. Kachlany SC, Planet PJ, Bhattacharjee MK, Kollia E, DeSalle R, Fine DH, Figurski DH. 2000. Nonspecific adherence by *Actinobacillus actinomycetemcomitans* requires genes widespread in bacteria and archaea. J Bacteriol 182:6169–6176. https://doi.org/10.1128/JB.182.21.6169-6176.2000.
5. Fine DH, Furgang D, Kaplan J, Charlesworth J, Figurski DH. 1999. Tenacious adhesion of *Actinobacillus actinomycetemcomitans* strain CU1000 to salivary-coated hydroxyapatite. Arch Oral Biol 44:1063–1076. https://doi.org/10.1016/S0003-9969(99)00089-8.
6. Kaplan JB, Perry MB, MacLean LL, Furgang D, Wilson ME, Fine DH. 2001. Structural and genetic analyses of O polysaccharide from *Actinobacillus actinomycetemcomitans* serotype f. Infect Immun 69:5375–5384. https://doi.org/10.1128/IAI.69.9.5375-5384.2001.
7. Kaplan JB, Ragunath C, Ramasubbu N, Fine DH. 2003. Detachment of *Actinobacillus actinomycetemcomitans* biofilm cells by an endogenous β-hexosaminidase activity. J Bacteriol 185:4693–4698. https://doi.org/10.1128/JB.185.16.4693-4698.2003.
8. Fine DH, Furgang D, Barnett ML. 2001. Comparative antimicrobial activities of antiseptic mouthrinses against isogenic planktonic and biofilm forms of *Actinobacillus actinomycetemcomitans*. J Clin Periodontol 28:697–700. https://doi.org/10.1034/j.1600-051x.2001.028007697.x.
9. Kachlany SC, Planet PJ, Desalle R, Fine DH, Figurski DH, Kaplan JB. 2001. *flp-1*, the first representative of a new pilin gene subfamily, is required for non-specific adherence of *Actinobacillus actinomycetemcomitans*. Mol Microbiol 40:542–554. https://doi.org/10.1046/j.1365-2958.2001.02422.x.
10. Kachlany SC, Fine DH, Figurski DH. 2000. Secretion of RTX leukotoxin by *Actinobacillus actinomycetemcomitans*. Infect Immun 68:6094–6100. https://doi.org/10.1128/IAI.68.11.6094-6100.2000.
11. Kram KE, Hovel-Miner GA, Tomich M, Figurski DH. 2008. Transcriptional regulation of the *tad* locus in *Aggregatibacter actinomycetemcomitans*: a termination cascade. J Bacteriol 190:3859–3868. https://doi.org/10.1128/JB.00128-08.
12. Kaplan JB, Meyenhofer MF, Fine DH. 2003. Biofilm growth and detachment of *Actinobacillus actinomycetemcomitans*. J Bacteriol 185:1399–1404. https://doi.org/10.1128/JB.185.4.1399-1404.2003.
13. Ihalin R, Zhong D, Karched M, Chen C, Asikainen S. 2018. Phosphorylcholine is located in *Aggregatibacter actinomycetemcomitans* fimbrial protein Flp 1. Med Microbiol Immunol 207:329–338. https://doi.org/10.1007/s00430-018-0554-1.
14. Izano EA, Wang H, Ragunath C, Ramasubbu N, Kaplan JB. 2007. Detachment and killing of *Aggregatibacter actinomycetemcomitans* biofilms by dispersin B and SDS. J Dent Res 86:618–622. https://doi.org/10.1177/154405910708600707.
15. Schreiner H, Markowitz K, Miryalkar M, Moore D, Diehl S, Fine DH. 2011. *Aggregatibacter actinomycetemcomitans*-induced bone loss and antibody response in three rat strains. J Periodontol 82:142–150. https://doi.org/10.1902/jop.2010.100250.
16. Preus HR, Sunday GJ, Haraszthy VI, Zambon JJ. 1992. Rapid identification of *Actinobacillus actinomycetemcomitans* based on analysis of 23S ribosomal RNA. Oral Microbiol Immunol 7:372–375. https://doi.org/10.1111/j.1399-302x.1992.tb00639.x.
17. Slots J. 1982. Selective medium for isolation of *Actinobacillus actinomycetemcomitans*. J Clin Microbiol 15:606–609. https://doi.org/10.1128/jcm.15.4.606-609.1982.
18. Green MR, Sambrook J. 2017. Isolation of high-molecular-weight DNA using organic solvents. Cold Spring Harb Protoc 2017:pdb.prot093450. https://doi.org/10.1101/pdb.prot093450.
19. Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 10:563–569. https://doi.org/10.1038/nmeth.2474.
20. Hunt M, Silva ND, Otto TD, Parkhill J, Keane JA, Harris SR. 2015. Circlator: automated circularization of genome assemblies using long sequencing reads. Genome Biol 16:294. https://doi.org/10.1186/s13059-015-0849-0.
21. Droge J, Gregor I, McHardy AC. 2015. Taxator-tk: precise taxonomic assignment of metagenomes by fast approximation of evolutionary neighborhoods. Bioinformatics 31:817–824. https://doi.org/10.1093/bioinformatics/btu745.
22. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.