AMERICAN SOCIETY FOR MICROBIOLOGY
genomeAnnouncements™

PROKARYOTES

Genome Sequence of *Porphyromonas gingivalis* Strain A7A1-28

Gary Xie,[a] Ryan P. Chastain-Gross,[b,c]* Myriam Bélanger,[b,c]* Dibyendu Kumar,[d]* Joan A. Whitlock,[b,c]* Li Liu,[d]* William G. Farmerie,[d] Collin L. Zeng,[b] Hajnalka E. Daligault,[a] Cliff S. Han,[a] Thomas S. Brettin,[a]* Ann Progulske-Fox[b,c]

Bioenergy and Biome Sciences (B-11), Bioscience Division, Los Alamos National Laboratory, Los Alamos, New Mexico, USA[a]; Department of Oral Biology, University of Florida, Gainesville, Florida, USA[b]; Center for Molecular Microbiology, University of Florida, Gainesville, Florida, USA[c]; Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, Florida, USA[d]

ABSTRACT *Porphyromonas gingivalis* is an oral opportunistic pathogen. Sequenced *P. gingivalis* laboratory strains display limited diversity in antigens that modulate host responses. Here, we present the genome sequence of A7A1-28, a strain possessing atypical fimbrillin and capsule types, with a single contig of 2,249,024 bp and a G+C content of 48.58%.

Received 6 January 2017 **Accepted** 11 January 2017 **Published** 9 March 2017

Citation Xie G, Chastain-Gross RP, Bélanger M, Kumar D, Whitlock JA, Liu L, Farmerie WG, Zeng CL, Daligault HE, Han CS, Brettin TS, Progulske-Fox A. 2017. Genome sequence of *Porphyromonas gingivalis* strain A7A1-28. Genome Announc 5:e00021-17. https://doi.org/10.1128/genomeA.00021-17.

Address correspondence to Ryan P. Chastain-Gross, gross.ryanp@ufl.edu, or Ann Progulske-Fox, apfox@dental.ufl.edu.

* Present address: Ryan P. Chastain-Gross, Department of Urology, College of Medicine, University of Florida, Gainesville, Florida, USA; Myriam Bélanger, Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA; Dibyendu Kumar, Waksman Genomics Facility, Waksman Institute of Microbiology, Rutgers University, Piscataway, New Jersey, USA; Joan A. Whitlock, Department of Medicine, College of Medicine, University of Florida, Gainesville, Florida, USA; Li Liu, Department of Biomedical Informatics, Arizona State University, Scottsdale, Arizona, USA; Thomas S. Brettin, Computing, Environment and Life Sciences Directorate, Argonne National Laboratory, Lemont, Illinois, USA.

G.X. and R.P.C.-G. contributed equally to this work.

Porphyromonas gingivalis is an anaerobic bacterium (1) associated with periodontal disease (2–4) and multiple systemic diseases (5–7). *P. gingivalis* may manipulate host responses to orchestrate dysbiosis and disease (8, 9), potentially through extensive variation in fimbrillin genotypes (10–12) and capsule serotypes (13, 14). Notably, common *P. gingivalis* laboratory strains display limited fimbrillin and capsule diversity (11, 15–18), limiting laboratory modeling of periodontal disease. This has extended to genome sequencing: fimbrillin type I, capsule absent (15, 17, 19) (ATCC 33277 [20] and 381 [21]), and fimbrillin type IV, capsule K1 (15, 17, 22, 23) (W83 [24] and A7436 [25]). Fimbrillin and capsule information is unavailable for other sequenced laboratory strains of *P. gingivalis* (26–28). A7A1-28 is a widely available strain that exhibits fimbrillin type II and capsule K3 (15, 17, 19). Isolated in 1985 by Neiders and Chen at SUNY-Buffalo (Buffalo, NY, USA) from a type 2 diabetes patient (29, 30), A7A1-28 stimulates *in vitro* responses that dramatically differ from those elicited by W83 or ATCC 33277 (13, 14); however, these differences have not been evaluated at the genome level. This study was undertaken to determine the complete genome sequence of A7A1-28 and facilitate investigations of the variety of host responses elicited by strains of *P. gingivalis*.

P. gingivalis strain A7A1-28 was obtained from Kesavalu Lakshmyya (University of Florida) and grown as previously described (31). Genomic DNA was obtained using the Wizard gDNA purification kit (Promega) and processed to generate shotgun and 8-kb paired-end libraries, which were sequenced using the 454 Life Sciences GS-20 instrument (32) (Roche). A total of 468,259 reads of 235,999,749 bp, with an average read length of 504 bp, were generated.

The GS-20 reads were assembled using Velvet version 0.7.63 (https://www.ebi.ac.uk/~zerbino/velvet) (33) and Newbler version 2.3 (Roche) (32). Gaps between contigs were closed by editing in Consed (http://www.phrap.org/consed/consed.html) (34–36) and by PCR-augmented Sanger sequencing. The genome was annotated using the RAST (http://metagenomics.anl.gov) (37) and IMG-ER servers (http://img.jgi.doe.gov/er) (38) and then amended using Gene Prediction Improvement Pipeline software (39).

The genome of *P. gingivalis* A7A1-28 has approximately 94-fold coverage and contains a single contig of 2,249,024 bp (G+C content of 48.58%). A total of 1,982 genes were annotated, which included 1,915 predicted coding sequences (CDSs), 53 tRNAs, 12 rRNAs, and one tmRNA. There are 229 subsystems in the genome. Further, 191 protein metabolism, 128 cofactors, vitamins, prosthetic groups, and pigments, 65 RNA metabolism, 94 DNA metabolism, 99 carbohydrate, and 17 membrane transport subsystem features were observed.

The annotated *P. gingivalis* A7A1-28 genome was compared to *P. gingivalis* strains W83, ATCC 33277, and TDC60 using RAST (37) and IMG-ER (38). All-to-all BLASTP comparisons of predicted protein sequences showed that A7A1-28 possesses 119 strain-specific CDSs, of which 98 are annotated as hypothetical proteins. Further, A7A1-28 contains a variety of mobile genetic elements, including seven *Bacteroides* conjugative transposons absent from W83, ATCC 33277, and TDC60. Genome synteny analysis revealed that the gene order in A7A1-28 resembles that of A7436 and AJW4, suggesting that local mutations may generate unique phenotypes observed in A7A1-28.

The availability of the A7A1-28 genome aids investigators in efforts to decipher interactions between *P. gingivalis* and host tissue, which are critical to homeostasis in the subgingival microbiome.

Accession number(s). This genome sequencing project was deposited in GenBank under the accession number CP013131. The version described is the first version.

ACKNOWLEDGMENTS

This study was supported by a University of Florida College of Dentistry Multi-Investigator Pilot Program project grant (to A.P.F.), as well as National Institute for Dental and Craniofacial Research grant DE013545-07S1 (to A.P.F.), and contract Y1-DE-6006-02 (to Los Alamos National Laboratory). We thank the staff of the University of Florida Interdisciplinary Center for Biotechnology Research, especially Regina Shaw, for excellent technical assistance.

REFERENCES

1. Mayrand D, Holt SC. 1988. Biology of asaccharolytic black-pigmented *Bacteroides* species. Microbiol Rev 52:134–152.
2. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL, Jr. 1998. Microbial complexes in subgingival plaque. J Clin Periodontol 25:134–144. https://doi.org/10.1111/j.1600-051X.1998.tb02419.x.
3. da Silva-Boghossian CM, do Souto RM, Luiz RR, Colombo AP. 2011. Association of red complex, *A. actinomycetemcomitans* and non-oral bacteria with periodontal diseases. Arch Oral Biol 56:899–906. https://doi.org/10.1016/j.archoralbio.2011.02.009.
4. Colombo AP, Boches SK, Cotton SL, Goodson JM, Kent R, Haffajee AD, Socransky SS, Hasturk H, Van Dyke TE, Dewhirst F, Paster BJ. 2009. Comparisons of subgingival microbial profiles of refractory periodontitis, severe periodontitis, and periodontal health using the human oral microbe identification microarray. J Periodontol 80:1421–1432. https://doi.org/10.1902/jop.2009.090185.
5. Vanterpool SF, Been JV, Houben ML, Nikkels PG, De Krijger RR, Zimmermann LJ, Kramer BW, Progulske-Fox A, Reyes L. 2016. *Porphyromonas gingivalis* within placental villous mesenchyme and umbilical cord stroma is associated with adverse pregnancy outcome. PLoS One 11:e0146157. https://doi.org/10.1371/journal.pone.0146157.
6. Totaro MC, Cattani P, Ria F, Tolusso B, Gremese E, Fedele AL, D'Onghia S, Marchetti S, Di Sante G, Canestri S, Ferraccioli G. 2013. *Porphyromonas gingivalis* and the pathogenesis of rheumatoid arthritis: analysis of various compartments including the synovial tissue. Arthritis Res Ther 15:R66. https://doi.org/10.1186/ar4243.
7. Serra e Silva Filho W, Casarin RC, Nicolela EL, Jr, Passos HM, Sallum AW, Gonçalves RB. 2014. Microbial diversity similarities in periodontal pockets and atheromatous plaques of cardiovascular disease patients. PLoS One 9:e109761. https://doi.org/10.1371/journal.pone.0109761.
8. Hajishengallis G, Lamont RJ. 2014. Breaking bad: manipulation of the host response by *Porphyromonas gingivalis*. Eur J Immunol 44:328–338. https://doi.org/10.1002/eji.201344202.
9. Hajishengallis G. 2015. Periodontitis: from microbial immune subversion to systemic inflammation. Nat Rev Immunol 15:30–44. https://doi.org/10.1038/nri3785.
10. Nakagawa I, Inaba H, Yamamura T, Kato T, Kawai S, Ooshima T, Amano A. 2006. Invasion of epithelial cells and proteolysis of cellular focal adhesion components by distinct types of *Porphyromonas gingivalis* fimbriae. Infect Immun 74:3773–3782. https://doi.org/10.1128/IAI.01902-05.
11. Enersen M, Nakano K, Amano A. 2013. *Porphyromonas gingivalis* fimbriae. J Oral Microbiol 5:20265. https://doi.org/10.3402/jom.v5i0.20265.
12. Kato T, Kawai S, Nakano K, Inaba H, Kuboniwa M, Nakagawa I, Tsuda K, Omori H, Ooshima T, Yoshimori T, Amano A. 2007. Virulence of *Porphyromonas gingivalis* is altered by substitution of fimbria gene with different genotype. Cell Microbiol 9:753–765. https://doi.org/10.1111/j.1462-5822.2006.00825.x.
13. Vernal R, León R, Silva A, van Winkelhoff AJ, Garcia-Sanz JA, Sanz M. 2009. Differential cytokine expression by human dendritic cells in response to different *Porphyromonas gingivalis* capsular serotypes. J Clin Periodontol 36:823–829. https://doi.org/10.1111/j.1600-051X.2009.01462.x.
14. Vernal R, Diaz-Guerra E, Silva A, Sanz M, Garcia-Sanz JA. 2014. Distinct human T-lymphocyte responses triggered by *Porphyromonas gingivalis* capsular serotypes. J Clin Periodontol 41:19–30. https://doi.org/10.1111/jcpe.12176.
15. Aduse-Opoku J, Slaney JM, Hashim A, Gallagher A, Gallagher RP, Rangarajan M, Boutaga K, Laine ML, Van Winkelhoff AJ, Curtis MA. 2006. Identification and characterization of the capsular polysaccharide (K-antigen) locus of *Porphyromonas gingivalis*. Infect Immun 74:449–460. https://doi.org/10.1128/IAI.74.1.449-460.2006.
16. Laine ML, Appelmelk BJ, van Winkelhoff AJ. 1997. Prevalence and distribution of six capsular serotypes of *Porphyromonas gingivalis* in periodontitis patients. J Dent Res 76:1840–1844. https://doi.org/10.1177/00220345970760120601.
17. Laine ML, van Winkelhoff AJ. 1998. Virulence of six capsular serotypes of *Porphyromonas gingivalis* in a mouse model. Oral Microbiol Immunol 13:322–325. https://doi.org/10.1111/j.1399-302X.1998.tb00714.x.
18. Enersen M. 2011. *Porphyromonas gingivalis*: a clonal pathogen? Diversities in housekeeping genes and the major fimbriae gene. J Oral Microbiol 3:8487. https://doi.org/10.3402/jom.v3i0.8487.
19. Nakano K, Kuboniwa M, Nakagawa I, Yamamura T, Nomura R, Okahashi N, Ooshima T, Amano A. 2004. Comparison of inflammatory changes caused by *Porphyromonas gingivalis* with distinct *fimA* genotypes in a mouse abscess model. Oral Microbiol Immunol 19:205–209. https://doi.org/10.1111/j.0902-0055.2004.00133.x.

20. Naito M, Hirakawa H, Yamashita A, Ohara N, Shoji M, Yukitake H, Nakayama K, Toh H, Yoshimura F, Kuhara S, Hattori M, Hayashi T, Nakayama K. 2008. Determination of the genome sequence of *Porphyromonas gingivalis* strain ATCC 33277 and genomic comparison with strain W83 revealed extensive genome rearrangements in *P. gingivalis*. DNA Res 15:215–225. https://doi.org/10.1093/dnares/dsn013.
21. Chastain-Gross RP, Xie G, Bélanger M, Kumar D, Whitlock JA, Liu L, Raines SM, Farmerie WG, Daligault HE, Han CS, Brettin TS, Progulske-Fox A. 2017. Genome sequence of *Porphyromonas gingivalis* strain 381. Genome Announc 5(2):e01467-16. https://doi.org/10.1128/genomeA.01467-16.
22. Nagano K, Hasegawa Y, Abiko Y, Yoshida Y, Murakami Y, Yoshimura F. 2012. *Porphyromonas gingivalis* FimA fimbriae: fimbrial assembly by *fimA* alone in the *fim* gene cluster and differential antigenicity among *fimA* genotypes. PLoS One 7:e43722. https://doi.org/10.1371/journal.pone.0043722.
23. Rodrigues PH, Reyes L, Chadda AS, Bélanger M, Wallet SM, Akin D, Dunn W, Jr, Progulske-Fox A. 2012. *Porphyromonas gingivalis* strain specific interactions with human coronary artery endothelial cells: a comparative study. PLoS One 7:e52606. https://doi.org/10.1371/journal.pone.0052606.
24. Nelson KE, Fleischmann RD, DeBoy RT, Paulsen IT, Fouts DE, Eisen JA, Daugherty SC, Dodson RJ, Durkin AS, Gwinn M, Haft DH, Kolonay JF, Nelson WC, Mason T, Tallon L, Gray J, Granger D, Tettelin H, Dong H, Galvin JL, Duncan MJ, Dewhirst FE, Fraser CM. 2003. Complete genome sequence of the oral pathogenic bacterium *Porphyromonas gingivalis* strain W83. J Bacteriol 185:5591–5601. https://doi.org/10.1128/JB.185.18.5591-5601.2003.
25. Chastain-Gross RP, Xie G, Bélanger M, Kumar D, Whitlock JA, Liu L, Farmerie WG, Daligault HE, Han CS, Brettin TS, Progulske-Fox A. 2015. Genome sequence of *Porphyromonas gingivalis* Strain A7436. Genome Announc 3(5):e00927-15. https://doi.org/10.1128/genomeA.00927-15.
26. Xie G, Chastain-Gross RP, Bélanger M, Kumar D, Whitlock JA, Liu L, Farmerie WG, Daligault HE, Han CS, Brettin TS, Progulske-Fox A. 2015. Genome sequence of *Porphyromonas gingivalis* Strain AJW4. Genome Announc 3(6):e01304-15. https://doi.org/10.1128/genomeA.01304-15.
27. Watanabe T, Maruyama F, Nozawa T, Aoki A, Okano S, Shibata Y, Oshima K, Kurokawa K, Hattori M, Nakagawa I, Abiko Y. 2011. Complete genome sequence of the bacterium *Porphyromonas gingivalis* TDC60, which causes periodontal disease. J Bacteriol 193:4259–4260. https://doi.org/10.1128/JB.05269-11.
28. Siddiqui H, Yoder-Himes DR, Mizgalska D, Nguyen KA, Potempa J, Olsen I. 2014. Genome sequence of *Porphyromonas gingivalis* strain HG66 (DSM 28984). Genome Announc 2(5):e00947-14. https://doi.org/10.1128/genomeA.00947-14.
29. Nisengard R. 1987. Bacterial invasion in periodontal disease. J Periodontol 58:331–339. https://doi.org/10.1902/jop.1987.58.5.331.
30. Chen PB, Neiders ME, Millar SJ, Reynolds HS, Zambon JJ. 1987. Effect of immunization on experimental *Bacteroides gingivalis* infection in a murine model. Infect Immun 55:2534–2537.
31. Belanger M, Rodrigues P, Progulske-Fox A. 2007. Genetic manipulation of *Porphyromonas gingivalis*. Curr Protoc Microbiol 13:Unit13C.2. https://doi.org/10.1002/9780471729259.mc13c02s05.
32. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen Z, Dewell SB, Du L, Fierro JM, Gomes XV, Godwin BC, He W, Helgesen S, Ho CH, Irzyk GP, Jando SC, Alenquer ML, Jarvie TP, Jirage KB, Kim JB, Knight JR, Lanza JR, Leamon JH, Lefkowitz SM, Lei M, Li J, Lohman KL, Lu H, Makhijani VB, McDade KE, McKenna MP, Myers EW, Nickerson E, Nobile JR, Plant R, Puc BP, Ronan MT, Roth GT, Sarkis GJ, Simons JF, Simpson JW, Srinivasan M, Tartaro KR, Tomasz A, Vogt KA, Volkmer GA, Wang SH, Wang Y, Weiner MP, Yu P, Begley RF, Rothberg JM. 2005. Genome sequencing in microfabricated high-density picolitre reactors. Nature 437:376–380. https://doi.org/10.1038/nature03959.
33. Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome Res 18:821–829. https://doi.org/10.1101/gr.074492.107.
34. Ewing B, Green P. 1998. Base-calling of automated sequencer traces using Phred. II. Error probabilities. Genome Res 8:186–194. https://doi.org/10.1101/gr.8.3.186.
35. Ewing B, Hillier L, Wendl MC, Green P. 1998. Base-calling of automated sequencer traces using Phred. I. Accuracy assessment. Genome Res 8:175–185. https://doi.org/10.1101/gr.8.3.175.
36. Gordon D, Abajian C, Green P. 1998. Consed: a graphical tool for sequence finishing. Genome Res 8:195–202. https://doi.org/10.1101/gr.8.3.195.
37. Meyer F, Paarmann D, D'Souza M, Olson R, Glass EM, Kubal M, Paczian T, Rodriguez A, Stevens R, Wilke A, Wilkening J, Edwards RA. 2008. The metagenomics RAST server—a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics 9:386. https://doi.org/10.1186/1471-2105-9-386.
38. Markowitz VM, Chen IM, Palaniappan K, Chu K, Szeto E, Grechkin Y, Ratner A, Jacob B, Huang J, Williams P, Huntemann M, Anderson I, Mavromatis K, Ivanova NN, Kyrpides NC. 2012. IMG: the integrated microbial genomes database and comparative analysis system. Nucleic Acids Res 40:D115–D122. https://doi.org/10.1093/nar/gkr1044.
39. Pati A, Ivanova NN, Mikhailova N, Ovchinnikova G, Hooper SD, Lykidis A, Kyrpides NC. 2010. GenePRIMP: a gene prediction improvement pipeline for prokaryotic genomes. Nat Methods 7:455–457. https://doi.org/10.1038/nmeth.1457.