Genome Sequence of *Porphyromonas gingivalis* Strain 381

Ryan P. Chastain-Gross,a,b,k Gary Xie,d Myriam Bélanger,a,b,k Dibyendu Kumar,c,e
Joan A. Whitlock,a,b,k Li Liu,∗ Sarah M. Raines,a William G. Farmerie,c
Hajnalka E. Daligault,d Cliff S. Han,d Thomas S. Brettin,d,k Ann Progulske-Foxa,b

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

ABSTRACT

*Porphyromonas gingivalis* is associated with both oral and systemic diseases. Strain-specific *P. gingivalis* invasion phenotypes do not reliably predict disease presentation during *in vivo* studies. Here, we present the genome sequence of 381, a common laboratory strain, with a single contig of 2,378,872 bp and a G+C content of 48.36%.

*Porphyromonas gingivalis* is an anaerobic bacterium (1) that is linked with periodontal disease (2–4) and multiple systemic diseases (5–7). In previous studies (8–11), *P. gingivalis* strains have shown a variety of pathogenic phenotypes *in vitro* and *in vivo*, but underlying genetic mechanisms are poorly defined. Presently, genomic sequences of well-known *P. gingivalis* laboratory strains W83, ATCC 33277, A7436, AJW4, and HG66 have been published (12–16), and W50 is available in the GenBank database (https://www.ncbi.nlm.nih.gov/assembly/483728). Isolated by Anne Tanner at the Forsyth Institute in Boston, Massachusetts, USA (17), 381 is a nonencapsulated strain of *P. gingivalis*. Notably, strains 381 and ATCC 33277 share similar capsule and fimbriae characteristics (18, 19), and both exhibit high invasion efficiencies during *in vitro* infection of human coronary artery endothelial cells (HCAECs) (8, 11). However, 381 induces autophagy and accesses alternative intracellular trafficking pathways, enabling persistence within HCAECs, while ATCC 33277 enters a lysosomal pathway and does not persist (8). In contrast, both strains induce similar inflammatory responses in a mouse abscess infection model (9, 20). This study was undertaken to determine the complete genome sequence of 381 and enable greater understanding of variations in host intracellular trafficking and host inflammatory responses among *P. gingivalis* strains.

*P. gingivalis* strain 381 was obtained from F. Macrina (Virginia Commonwealth University) and grown as previously described (21). Genomic DNA was obtained using the Wizard gDNA purification kit (Promega) and processed to generate shotgun and 3-kb paired-end libraries, which were sequenced using the 454 Life Sciences GS-20 instrument (22) (Roche). 806,578 reads of 123,742,668 bp, with an average read length of 153 bp, were generated.

GS-20 reads were assembled using Velvet version 0.7.63 (https://www.ebi.ac.uk/~zerbino/velvet) (23) and Newbler version 2.3 (Roche) (22). Gaps between contigs were closed by editing in Consed (http://www.phrap.org/consed/consed.html) (24–26) and by PCR-augmented Sanger sequencing. The genome was annotated using the RAST (http://metagenomics.anl.gov) (27) and IMG-ER servers (http://img.jgi.doe.gov/er) (28) and then amended using Gene Prediction Improvement Pipeline software (29).

The genome of *P. gingivalis* 381 has approximately 49-fold coverage and contains a single contig of 2,378,872 bp (G+C content of 48.36%). A total of 2,054 genes were annotated, which included 1,986 predicted coding sequences (CDSs), 53 tRNAs, 12...
rRNAs, and one tmRNA. There are 231 subsystems in the genome. Subsystem features observed included: protein metabolism (197), cofactors, vitamins, prosthetic groups and pigments (157), RNA metabolism (64), DNA metabolism (91), carbohydrates (96) and membrane transport (17).

The annotated \textit{P. gingivalis} 381 genome was compared to \textit{P. gingivalis} strains W83, ATCC 33277, and TDC60 using RAST (27) and IMG-ER (28). All-to-all BLASTp comparisons of predicted protein sequences showed that 381 possesses 64 strain-specific CDSs, all annotated as hypothetical proteins. Of note, 381 is a close relative of ATCC 33277 based on genome clustering analysis, and the gene order is nearly identical between 381 and ATCC 33277, except three minor differences due to inversion, duplication, or deletion of transposable elements.

The availability of the 381 genome enables exploration of how genomic differences among \textit{P. gingivalis} strains offer widely different \textit{in vitro} phenotypes, but may not confer competitive advantage in an animal model of infection.

**Accession number(s).** This genome sequencing project was deposited in GenBank under accession number CP012889. 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. We declare no conflict of interest.

**REFERENCES**

1. Mayrand D, Holt SC. 1988. Biology of asaccharolytic black-pigmented \textit{Bacteroides} species. Microbiol Rev 52:134–152.
2. Socransky SS, Haftajee 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, \textit{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, Haftajee AD, Socransky SS, Hasturk H, Van Dyke TE, Dewhirst F, Pastor BJ. 2009. Comparisons of subgingival microbial profiles of refractory periodontitis, severe periodontitis, and periodontal health using the human oral microbiome identification microarray. J Periodontol 80:1421–1432. [https://doi.org/10.1902/jop.2009.090018]
5. Vanterpool SF, Been JV, Houben ML, Nikkels PG, De Krijger RR, Zimmermann LJ, Kramer BW, Progulske-Fox A, Zimmermann RR, Zimmermann RJ. 2013. \textit{Porphyromonas gingivalis} within placental villous mesenchyme and umbilical cord stroma is associated with adverse pregnancy outcome. PLoS One 11: e014657. [https://doi.org/10.1371/journal.pone.014657]
6. Totaro MC, Cattani P, Ria F, Tolusso B, Gremese E, Fedele AL, D’Onghia EB, Diana PA, D’Onghia A. 2011. \textit{Porphyromonas gingivalis} and the pathogenesis of rheumatoid arthritis: analysis of various compartments including the synovial tissue. Arthritis Res Ther 13:R66. [https://doi.org/10.1186/ar4243]
7. Serra e Silva Filho W, Casarin RC, Nicolela EL, Jr, Passos HM, Sallum AW, Sallum AMC. 2012. \textit{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]
8. Laine ML, van Winkelhoff AJ. 1998. Virulence of six capsular serotypes of \textit{Porphyromonas gingivalis} in a mouse model. Oral Microbiol Immunol 13:322–325. [https://doi.org/10.1111/j.1399-302X.1998.tb00714.x]
9. Grenier D, Mayrand D. 1987. Selected characteristics of pathogenic and nonpathogenic strains of \textit{Bacteroides gingivalis}. J Clin Microbiol 25: 738–740.
10. Dom BR, Burks JN, Selfert KN, Progulske-Fox A. 2000. Invasion of endothelial and epithelial cells by strains of \textit{Porphyromonas gingivalis}. FEMS Microbiol Lett 187:139–144. [https://doi.org/10.1111/j.1574-6968.2000.tb09150.x]
11. Nelson KE, Fleischmann RD, DeBoy RT, Paulsen IT, Fouts DE, Eisen JA, Daugherty SC, Dodson RJ, Durkin AS, Gwinn M, Haft DH, Kelonay 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 \textit{Porphyromonas gingivalis} strain W83. J Bacteriol 185:591–5601. [https://doi.org/10.1128/ JB.185.18.591-5601.2003]
12. Naito M, Hirakawa H, Yamashita A, Ohara N, Shoji Y, Yukitake H, Nakayama K, Toh H, Yoshimura F, Kuhara S, Hattori M, Hayashi T, Nakayama K. 2008. Determination of the genome sequence of \textit{Porphyromonas gingivalis} strain ATCC 33277 and genomic comparison with \textit{W83} revealed extensive genome rearrangements in \textit{P. gingivalis}. DNA Res 15:215–225. [https://doi.org/10.1093/dnares/dsn013]
13. 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 \textit{Porphyromonas gingivalis} strain A7436. Genome Announc 3(5):e00927-15. [https://doi.org/10.1128/genomeA.00927-15]
14. 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 \textit{Porphyromonas gingivalis} strain AJW4. Genome Announc 3(6):e01304-15. [https://doi.org/10.1128/genomeA.01304-15]
15. Siddiqui H, Yoder-Himes DR, Mligalska D, Nguyen KA, Potempa J, Olsen I. 2014. Genome sequence of \textit{Porphyromonas gingivalis} strain HG66 (DSM 28984). Genome Announc 2(5):e00947-14. [https://doi.org/10.1128/genomeA.00947-14]
16. Tanner AC, Haffer C, Brathall GT, Visconti RA, Socransky SS. 1979. A study of the bacteria associated with advancing periodontitis in man. J Clin Periodontol 6:278–307. [https://doi.org/10.1111/j.1600-051X.1979.tb01931.x]
17. Chastain-Gross RP, Xie G, Bélanger M, Ria F, Tolusso B, Gremese E, Fedele AL, D’Onghia EB, Diana PA, D’Onghia A. 2011. \textit{Porphyromonas gingivalis} and the pathogenesis of rheumatoid arthritis: analysis of various compartments including the synovial tissue. Arthritis Res Ther 13:R66. [https://doi.org/10.1186/ar4243]
18. Siddiqui H, Yoder-Himes DR, Mligalska D, Nguyen KA, Potempa J, Olsen I. 2014. Genome sequence of \textit{Porphyromonas gingivalis} strain HG66 (DSM 28984). Genome Announc 2(5):e00947-14. [https://doi.org/10.1128/genomeA.00947-14]
19. Tannor AC, Haffer C, Brathall GT, Visconti RA, Socransky SS. 1979. A study of the bacteria associated with advancing periodontitis in man. J Clin Periodontol 6:278–307. [https://doi.org/10.1111/j.1600-051X.1979.tb01931.x]
20. Aduse-Opoku I, Slaney JM, Hashim A, Gallagher A, Gallagher RP, Ran-
garajan 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.

19. 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.

20. Ebersole JL, Kesavalu L, Schneider SL, Machen RL, Holt SC. 1995. Comparative virulence of periodontopathogens in a mouse abscess model. Oral Dis 1:115–128. https://doi.org/10.1111/j.1601-0825.1995.tb00174.x.

21. Belanger M, Rodrigues P, Progulske-Fox A. 2007. Genetic manipulation of Porphyromonas gingivalis. Curr Protoc Microbiol 13:13C.2. https://doi.org/10.1002/9780471729259.mc13c02s05.

22. 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, Ho CH, Irzyk GP, Jando SC, Alenquer ML, Jarvie TP, Jirage KB, Kim JB, Knight JR, Lanza JR, Leamon JH, Lefkowitz SM, Lu H, Makhijani VB, McDade KE, McKenna MP, Myers EW, Nobile JR, Plant R. 2005. Genome sequencing in microfabricated high-density picolitre reactors. Nature 437:376–380. https://doi.org/10.1038/nature03959.

23. 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.

24. 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.

25. 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.

26. 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.

27. 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.

28. 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.

29. 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.