

Genome Sequence of *Porphyromonas gingivalis* Strain AJW4

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***Porphyromonas gingivalis* is associated with oral and systemic diseases. Strain-specific *P. gingivalis* invasion phenotypes have been correlated with disease presentation in infected laboratory animals. Here, we present the genome sequence of AJW4, a minimally invasive strain, with a single contig of 2,372,492 bp and a G+C content of 48.27%.**

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Porphyromonas gingivalis is an anaerobic bacterium (1) found in the oral tissues of chronic periodontitis patients (2, 3) and selected nonoral tissues of systemic disease patients (4–6). Invasion of nonphagocytic host cells may be a mechanism by which *P. gingivalis* promotes oral and systemic disease (7). *In vitro* studies have shown variation among strain invasion phenotypes (8–10). Animals inoculated with highly invasive strains displayed oral (11) and vascular (12) disease, while invasion-deficient strains generated no disease. Presently, the genomic sequences and corresponding invasion phenotypes are available for only three highly invasive *P. gingivalis* strains, W83, ATCC 33277, and A7436 (13–15). AJW4 is the only widely available minimally invasive strain of *P. gingivalis* (7, 8). Isolated at the State University of New York-Buffalo in 1988 (16), AJW4 exhibited 1% average *P. gingivalis* invasion efficiency in an *in vitro* analysis (8). Microarray analyses comparing AJW4 and W83, an invasive strain, detected 120 putative genes in W83 that were heavily modified or absent in AJW4 (7), including sequences linked to invasion capacity (17). This study was undertaken to determine the complete genome sequence of AJW4 and expedite the identification of the genetic components required for successful invasion by *P. gingivalis*.

P. gingivalis strain AJW4 was obtained from B. G. Loos (State University of New York at Buffalo, NY) and grown as previously described (18). Genomic DNA was obtained using the Wizard genomic DNA (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 (Roche) (19). A total of 546,865 reads of 143,355,863 bases, with an average read length of 262 bp, were generated.

The GS-20 reads were assembled using Velvet version 0.7.63 (<https://www.ebi.ac.uk/~zerbino/velvet/>) (20) and Newbler version 2.3 (Roche) (19). Gaps between contigs were closed by edit-

ing in Consed (<http://www.phrap.org/consed/consed.html>) (21–23) and by PCR-augmented Sanger sequencing. The genome was annotated using the RAST (<http://metagenomics.anl.gov>) (24) and IMG-ER (<http://img.jgi.doe.gov/er>) (25) servers and then amended using the Gene Prediction Improvement Pipeline software (<https://geneprimp.jgi-psf.org>) (26).

The genome of *P. gingivalis* AJW4 has approximately 59-fold coverage and contains a single contig of 2,372,492 bp (G+C content, 48.27%). A total of 2,073 genes were annotated, which included 2,006 predicted coding sequences (CDSs), 53 tRNAs, 20 rRNAs, and 1 transfer-messenger RNA (tmRNA). There are 231 subsystems in the genome; also, 193 protein metabolism, 158 cofactors, vitamins, prosthetic groups, and pigments, 64 RNA metabolism, 87 DNA metabolism, 96 carbohydrate, and 19 membrane transport subsystem features were observed.

The annotated *P. gingivalis* AJW4 genome was compared to that of *P. gingivalis* strains W83, ATCC 33277, and TDC60 using RAST (24) and IMG-ER (25). All-to-all BLASTP comparisons of the predicted protein sequences showed that AJW4 possesses 154 strain-specific CDSs, of which 123 are annotated as hypothetical proteins. Notably, AJW4 and W83 contain similar quantities of DNA base pairs and predicted genes, suggesting that the widespread loss of genetic information is not a basis for the minimally invasive AJW4 phenotype.

The availability of the AJW4 genome aids explorations of the genomic elements necessary for the invasion of host cells by *P. gingivalis*, a key aspect of its association with oral and systemic pathologies.

Nucleotide sequence accession number. This genome sequencing project was deposited in GenBank under the accession no. [CP011996](https://www.ncbi.nlm.nih.gov/nuccore/CP011996). The version described is the first version.

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REFERENCES

- Mayrand D, Holt SC. 1988. Biology of asaccharolytic black-pigmented *Bacteroides* species. *Microbiol Res* 52:134–152.
- Griffen AL, Becker MR, Lyons SR, Moeschberger ML, Leys EJ. 1998. Prevalence of *Porphyromonas gingivalis* and periodontal health status. *J Clin Microbiol* 36:3239–3242.
- Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL, Jr. 1998. Microbial complexes in subgingival plaque. *J Clin Periodontol* 25: 134–144. <http://dx.doi.org/10.1111/j.1600-051X.1998.tb02419.x>.
- Katz J, Chegini N, Shiverick KT, Lamont RJ. 2009. Localization of *P. gingivalis* in preterm delivery placenta. *J Dent Res* 88:575–578. <http://dx.doi.org/10.1177/0022034509338032>.
- Totaro M, Cattani P, Ria F, Toluoso B, Gremese E, Fedele A, D'Onghia S, Marchetti S, 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. <http://dx.doi.org/10.1186/ar4243>.
- Louhelainen A, Aho J, Tuomisto S, Aittoniemi J, Vuento R, Karhunen PJ, Pessi T. 2014. Oral bacterial DNA findings in pericardial fluid. *J Oral Microbiol* 6:25835. <http://dx.doi.org/10.3402/jom.v6.25835>.
- Dolgilevich S, Rafferty B, Luchinskaya D, Kozarov E. 2011. Genomic comparison of invasive and rare non-invasive strains reveals *Porphyromonas gingivalis* genetic polymorphisms. *J Oral Microbiol* 3:5764.
- Dorn BR, Burks JN, Seifert KN, Progulske-Fox A. 2000. Invasion of endothelial and epithelial cells by strains of *Porphyromonas gingivalis*. *FEMS Microbiol Lett* 187:139–144. <http://dx.doi.org/10.1111/j.1574-6968.2000.tb09150.x>.
- Jandik KA, Bélanger M, Low SL, Dorn BR, Yang MCK, Progulske-Fox A. 2008. Invasive differences among *Porphyromonas gingivalis* strains from healthy and diseased periodontal sites. *J Periodontol Res* 43:524–530. <http://dx.doi.org/10.1111/j.1600-0765.2007.01064.x>.
- 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. <http://dx.doi.org/10.1371/journal.pone.0052606>.
- Malek R, Fisher JG, Caleca A, Stinson M, van Oss CJ, Lee JY, Cho MI, Genco RJ, Evans RT, Dyer DW. 1994. Inactivation of the *Porphyromonas gingivalis* *fimA* gene blocks periodontal damage in gnotobiotic rats. *J Bacteriol* 176:1052–1059.
- Deshpande RG, Khan MB, Genco CA. 1998. Invasion of aortic and heart endothelial cells by *Porphyromonas gingivalis*. *Infect Immun* 66: 5337–5343.
- 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. <http://dx.doi.org/10.1128/JB.185.18.5591-5601.2003>.
- 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. <http://dx.doi.org/10.1093/dnares/dsn013>.
- 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):300927–15. <http://dx.doi.org/10.1128/genomeA.00927-15>.
- Chen PB, Davern LB, Aguirre A. 1991. Experimental *Porphyromonas gingivalis* infection in nonimmune athymic BALB/c mice. *Infect Immun* 59:4706–4709.
- Nagano K, Murakami Y, Nishikawa K, Sakakibara J, Shimozato K, Yoshimura F. 2007. Characterization of RagA and RagB in *Porphyromonas gingivalis*: study using gene-deletion mutants. *J Med Microbiol* 56: 1536–1548. <http://dx.doi.org/10.1099/jmm.0.47289-0>.
- Bélanger M, Rodrigues P, Progulske-Fox A. 2007. Genetic manipulation of *Porphyromonas gingivalis*. *Curr Protoc Microbiol* Chapter 13: Unit13C.2.
- Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen Y, 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. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* 437:376–380. <http://dx.doi.org/10.1038/nature03959>.
- Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <http://dx.doi.org/10.1101/gr.074492.107>.
- Ewing B, Green P. 1998. Base-calling of automated sequencer traces using Phred. II. Error probabilities. *Genome Res* 8:186–194. <http://dx.doi.org/10.1101/gr.8.3.186>.
- 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. <http://dx.doi.org/10.1101/gr.8.3.175>.
- Gordon D, Abajian C, Green P. 1998. Consed: a graphical tool for sequence finishing. *Genome Res* 8:195–202. <http://dx.doi.org/10.1101/gr.8.3.195>.
- Meyer F, Paarmann D, D'Souza M, Olson R, Glass E, Kubal M, Paczian T, Rodriguez A, Stevens R, Wilke A, Wilkening J, Edwards R. 2008. The metagenomics RAST server—a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics* 9:386. <http://dx.doi.org/10.1186/1471-2105-9-386>.
- 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. <http://dx.doi.org/10.1093/nar/gkr1044>.
- 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. <http://dx.doi.org/10.1038/nmeth.1457>.