genomeA Journals.ASM.org

CrossMark

Complete Genome Sequence of *Pelosinus fermentans* JBW45, a Member of a Remarkably Competitive Group of *Negativicutes* in the *Firmicutes* Phylum

Kara B. De León,[a] Sagar M. Utturkar,[b] Laura B. Camilleri,[c,d] Dwayne A. Elias,[e] Adam P. Arkin,[f,g] Matthew W. Fields,[c,d] Steven D. Brown,[b,e] Judy D. Wall[a]

Division of Biochemistry, University of Missouri, Columbia, Missouri, USA[a]; Graduate School of Genome Science and Technology, University of Tennessee, Knoxville, Tennessee, USA[b]; Department of Microbiology and Immunology, Montana State University, Bozeman, Montana, USA[c]; Center for Biofilm Engineering, Montana State University, Bozeman, Montana, USA[d]; Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA[e]; Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA[f]; Department of Bioengineering, University of California, Berkeley, California, USA[g]

K.B.D.L. and S.M.U. contributed equally to this work.

The genome of *Pelosinus fermentans* JBW45, isolated from a chromium-contaminated site in Hanford, Washington, USA, has been completed with PacBio sequencing. Nine copies of the rRNA gene operon and multiple transposase genes with identical sequences resulted in breaks in the original draft genome and may suggest genomic instability of JBW45.

Received 7 August 2015 **Accepted** 11 August 2015 **Published** 24 September 2015

Citation De León KB, Utturkar SM, Camilleri LB, Elias DA, Arkin AP, Fields MW, Brown SD, Wall JD. 2015. Complete genome sequence of *Pelosinus fermentans* JBW45, a member of a remarkably competitive group of *Negativicutes* in the *Firmicutes* phylum. Genome Announc 3(5):e01090-15. doi:10.1128/genomeA.01090-15.

Copyright © 2015 De León et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to Judy D. Wall, wallj@missouri.edu.

Pelosinus fermentans JBW45 was isolated from groundwater stimulated for hexavalent-chromium reduction by injection of a polylactate compound (1). *Pelosinus* spp. are found in sites contaminated by heavy metals, explosives, and chlorinated solvents, at low or below-detection levels, but become dominant following nutrient addition *in vitro* (2–14) or *in situ* (1, 15, 16). A strict anaerobe, JBW45 likely resides in sediment-associated, anaerobic microsites (6). Although *Pelosinus* strains have been seen to form spores (17, 18), spore formation has not been documented for JBW45.

Previous genome sequencing of JBW45 with Illumina technology resulted in 98 contigs (1). Draft genome sequences for three other *Pelosinus* spp. from Hanford (A11, B4, and HCF1) and the type strain R7 from Russian kaolin clays are similar to each other but show little synteny with JBW45 or the completed genome of *Pelosinus* sp. UFO1, isolated from Oak Ridge, Tennessee, USA (19–21).

The complete genome sequence of JBW45 was determined with a Pacific Biosciences (PacBio, Menlo Park, California, USA) RSII instrument with P4-C2 chemistry as described previously (22). Two single-molecule real-time (SMRT) cells yielded 1,345,758,432 bases in 202,124 reads with a mean and maximum read length of 6,656 and 35,018 bases, respectively. SMRT analysis version 2.2 and HGAP version 3.0 (PacBio) were used for sequence assembly, which was improved with Pilon (23) and annotated as described previously (1). A single contig with 32-kb overlapping ends was generated, differing only by the presence of a putative transposase gene (JBW_01610). PCR across this region showed the transposase present in 4 of 14 JBW45 colonies, suggesting that the transposase may be actively moving and possibly contributing to evolutionary plasticity (24). Six identical copies (49.6% G+C content) were found in the completed JBW45 genome. This transposase was not found in the completed UFO1 genome. A similar gene (82 to 83% identity) was found as the only gene on a small contig in the draft genomes of A11, B4, HCF1, and R7. JBW45 contains 18 other genes annotated as transposases, many of which occur multiple times in the genome. Transposase and rRNA operon sequences resulted in breaks in assembly of the original JBW45 genome, underscoring the value of longer sequencing technologies, which is consistent with other reports (22, 25, 26).

The final assembly was circularized, resulting in a 5,380,816-bp chromosome with a G+C content of 39.5% and 250-fold sequence coverage. A total of 4,743 protein-coding, 77 tRNA, and 28 rRNA (ten 5S, nine 23S, and nine 16S) genes were identified. Four of the 16S rRNA genes contained a 100-bp insertion, which is consistent with previous findings of intragenomic 16S rRNA gene heterogeneity in UFO1 (21, 27). The average number of bacterial rRNA operons is four (28); however, UFO1 contains fifteen 16S rRNA genes (21). A higher number of rRNA operons may allow rapid adaptation and recovery from the stationary phase (29, 30). This may provide *Pelosinus* spp. with a competitive advantage upon nutrient stimulation.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession number CP010978. The version described in this paper is the first version, CP010978.1.

ACKNOWLEDGMENTS

PacBio sequencing was performed at the University of Maryland Institute for Genome Sciences. Microbial sequence and data analysis was supported by ENIGMA—Ecosystems and Networks Integrated with Genes and Molecular Assemblies (http://enigma.lbl.gov), a Scientific Focus Area Program at Lawrence Berkeley National Laboratory for the U.S. Depart-

ment of Energy, Office of Science, Office of Biological and Environmental Research under contract number DE-AC02-05CH11231. Microbial genome sequence assembly, annotation, and analysis by ENIGMA were supported by Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract no. DE-AC05-00OR22725.

REFERENCES

1. **Bowen De León K, Young ML, Camilleri LB, Brown SD, Skerker JM, Deutschbauer AM, Arkin AP, Fields MW.** 2012. Draft genome sequence of *Pelosinus fermentans* JBW45, isolated during *in situ* stimulation for Cr(VI) reduction. J Bacteriol **194:**5456–5457. http://dx.doi.org/10.1128/JB.01224-12.
2. **Beller HR, Yang L, Varadharajan C, Han R, Lim HC, Karaoz U, Molins S, Marcus MA, Brodie EL, Steefel CI, Nico PS.** 2014. Divergent aquifer biogeochemical systems converge on similar and unexpected Cr(VI) reduction products. Environ Sci Technol **48:**10699–10706. http://dx.doi.org/10.1021/es5016982.
3. **Burkhardt E-M, Akob DM, Bischoff S, Sitte J, Kostka JE, Banerjee D, Scheinost AC, Küsel K.** 2010. Impact of biostimulated redox processes on metal dynamics in an iron-rich creek soil of a former uranium mining area. Environ Sci Technol **44:**177–183. http://dx.doi.org/10.1021/es902038e.
4. **Fahrenfeld N, Zoeckler J, Widdowson MA, Pruden A.** 2013. Effect of biostimulants on 2,4,6-trinitrotoluene (TNT) degradation and bacterial community composition in contaminated aquifer sediment enrichments. Biodegradation **24:**179–190. http://dx.doi.org/10.1007/s10532-012-9569-2.
5. **Fuller ME, McClay K, Higham M, Hatzinger PB, Steffan RJ.** 2010. Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) bioremediation in groundwater: are known RDX-degrading bacteria the dominant players? Biorem J **14:**121–134. http://dx.doi.org/10.1080/10889868.2010.495367.
6. **Hansel CM, Fendorf S, Jardine PM, Francis CA.** 2008. Changes in bacterial and archaeal community structure and functional diversity along a geochemically variable soil profile. Appl Environ Microbiol **74:**1620–1633. http://dx.doi.org/10.1128/AEM.01787-07.
7. **Krzmarzick MJ, Miller HR, Yan T, Novak PJ.** 2014. Novel *Firmicutes* group implicated in the dechlorination of two chlorinated xanthones, analogues of natural organochlorines. Appl Environ Microbiol **80:**1210–1218. http://dx.doi.org/10.1128/AEM.03472-13.
8. **Martins M, Faleiro ML, da Costa AM, Chaves S, Tenreiro R, Matos AP, Costa MC.** 2010. Mechanism of uranium (VI) removal by two anaerobic bacterial communities. J Hazard Mater **184:**89–96. http://dx.doi.org/10.1016/j.jhazmat.2010.08.009.
9. **Men Y, Lee PKH, Harding KC, Alvarez-Cohen L.** 2013. Characterization of four TCE-dechlorinating microbial enrichments grown with different cobalamin stress and methanogenic conditions. Appl Microbiol Biotechnol **97:**6439–6450. http://dx.doi.org/10.1007/s00253-013-4896-8.
10. **Miura T, Yamazoe A, Ito M, Ohji S, Hosoyama A, Takahata Y, Fujita N.** 2015. The impact of injections of different nutrients on the bacterial community and its dechlorination activity in chloroethene-contaminated groundwater. Microbes Environ **30:**164–171. http://dx.doi.org/10.1264/jsme2.ME14127.
11. **Mosher JJ, Phelps TJ, Podar M, Hurt RA, Jr, Campbell JH, Drake MM, Moberly JG, Schadt CW, Brown SD, Hazen TC, Arkin AP, Palumbo AV, Faybishenko BA, Elias DA.** 2012. Microbial community succession during lactate amendment and electron acceptor limitation reveals a predominance of metal-reducing *Pelosinus* spp. Appl Environ Microbiol **78:**2082–2091. http://dx.doi.org/10.1128/AEM.07165-11.
12. **Newsome L, Morris K, Trivedi D, Atherton N, Lloyd JR.** 2014. Microbial reduction of uranium(VI) in sediments of different lithologies collected from Sellafield. Appl Geochem **51:**55–64. http://dx.doi.org/10.1016/j.apgeochem.2014.09.008.
13. **Penny C, Gruffaz C, Nadalig T, Cauchie H-M, Vuilleumier S, Bringel F.** 2015. Tetrachloromethane-degrading bacterial enrichment cultures and isolates from a contaminated aquifer. Microorganisms **3:**327–343. http://dx.doi.org/10.3390/microorganisms3030327.
14. **Somenahally AC, Mosher JJ, Yuan T, Podar M, Phelps TJ, Brown SD, Yang ZK, Hazen TC, Arkin AP, Palumbo AV, Van Nostrand JD, Zhou J, Elias DA.** 2013. Hexavalent chromium reduction under fermentative conditions with lactate stimulated native microbial communities. PLoS One **8:**e83909. http://dx.doi.org/10.1371/journal.pone.0083909.
15. **Chourey K, Nissen S, Vishnivetskaya T, Shah M, Pfiffner S, Hettich RL, Löffler FE.** 2013. Environmental proteomics reveals early microbial community responses to biostimulation at a uranium- and nitrate-contaminated site. Proteomics **13:**2921–2930. http://dx.doi.org/10.1002/pmic.201300155.
16. **Gihring TM, Zhang G, Brandt CC, Brooks SC, Campbell JH, Carroll S, Criddle CS, Green SJ, Jardine P, Kostka JE, Lowe K, Mehlhorn TL, Overholt W, Watson DB, Yang Z, Wu W-M, Schadt CW.** 2011. A limited microbial consortium is responsible for extended bioreduction of uranium in a contaminated aquifer. Appl Environ Microbiol **77:**5955–5965. http://dx.doi.org/10.1128/AEM.00220-11.
17. **Moe WM, Stebbing RE, Rao JU, Bowman KS, Nobre MF, da Costa MS, Rainey FA.** 2012. *Pelosinus defluvii* sp. nov., isolated from chlorinated solvent-contaminated groundwater, emended description of the genus *Pelosinus*, and transfer of *Sporotalea propionica* to *Pelosinus propionicus* comb. nov. Int J Syst Evol Microbiol **62:**1369–1376. http://dx.doi.org/10.1099/ijs.0.033753-0.
18. **Shelobolina ES, Nevin KP, Blakeney-Hayward JD, Johnsen CV, Plaia TW, Krader P, Woodard T, Holmes DE, VanPraagh CG, Lovley DR.** 2007. *Geobacter pickeringii* sp. nov., *Geobacter argillaceus* sp. nov. and *Pelosinus fermentans* gen. nov., sp. nov., isolated from subsurface kaolin lenses. Int J Syst Evol Microbiol **57:**126–135. http://dx.doi.org/10.1099/ijs.0.64221-0.
19. **Beller HR, Han R, Karaoz U, Lim H, Brodie EL.** 2013. Genomic and physiological characterization of the chromate-reducing, aquifer-derived firmicute *Pelosinus* sp. strain HCF1. Appl Environ Microbiol **79:**63–73. http://dx.doi.org/10.1128/AEM.02496-12.
20. **Brown SD, Podar M, Klingeman DM, Johnson CM, Yang ZK, Utturkar SM, Land ML, Mosher JJ, Hurt RA, Jr, Phelps TJ, Palumbo AV, Arkin AP, Hazen TC, Elias DA.** 2012. Draft genome sequences for two metal-reducing *Pelosinus fermentans* strains isolated from a Cr(VI)-contaminated site and for type strain R7. J Bacteriol **194:**5147–5148. http://dx.doi.org/10.1128/JB.01174-12.
21. **Brown SD, Utturkar SM, Magnuson TS, Ray AE, Poole FL, Lancaster WA, Thorgersen MP, Adams MWW, Elias DA.** 2014. Complete genome sequence of *Pelosinus* sp. strain ufo1 assembled using single-molecule real-time DNA sequencing technology. Genome Announc **2**(5)**:**e00881-14. http://dx.doi.org/10.1128/genomeA.00881-14.
22. **Brown SD, Nagaraju S, Utturkar S, De Tissera S, Segovia S, Mitchell W, Land ML, Dassanayake A, Köpke M.** 2014. Comparison of single-molecule sequencing and hybrid approaches for finishing the genome of *Clostridium autoethanogenum* and analysis of CRISPR systems in industrial relevant Clostridia. Biotechnol Biofuels **7:**40. http://dx.doi.org/10.1186/1754-6834-7-40.
23. **Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM.** 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One **9:**e112963. http://dx.doi.org/10.1371/journal.pone.0112963.
24. **Darmon E, Leach DRF.** 2014. Bacterial genome instability. Microbiol Mol Biol Rev **78:**1–39. http://dx.doi.org/10.1128/MMBR.00035-13.
25. **Treangen TJ, Salzberg SL.** 2012. Repetitive DNA and next-generation sequencing: computational challenges and solutions. Nat Rev Genet **13:**36–46. http://dx.doi.org/10.1038/nrg3117.
26. **Utturkar SM, Klingeman DM, Land ML, Schadt CW, Doktycz MJ, Pelletier DA, Brown SD.** 2014. Evaluation and validation of *de novo* and hybrid assembly techniques to derive high-quality genome sequences. Bioinformatics **30:**2709–2716. http://dx.doi.org/10.1093/bioinformatics/btu391.
27. **Ray AE, Connon SA, Sheridan PP, Gilbreath J, Shields M, Newby DT, Fujita Y, Magnuson TS.** 2010. Intragenomic heterogeneity of the 16S rRNA gene in strain UFO1 caused by a 100-bp insertion in helix 6. FEMS Microbiol Ecol **72:**343–353. http://dx.doi.org/10.1111/j.1574-6941.2010.00868.x.
28. **Stoddard SF, Smith BJ, Hein R, Roller BRK, Schmidt TM.** 2015. *rrn*DB: improved tools for interpreting rRNA gene abundance in bacteria and archaea and a new foundation for future development. Nucleic Acids Res **43:**D593–D598. http://dx.doi.org/10.1093/nar/gku1201.
29. **Condon C, Liveris D, Squires C, Schwartz I, Squires CL.** 1995. rRNA operon multiplicity in *Escherichia coli* and the physiological implications of *rrn* inactivation. J Bacteriol **177:**4152–4156.
30. **Klappenbach JA, Dunbar JM, Schmidt TM.** 2000. rRNA operon copy number reflects ecological strategies of bacteria. Appl Environ Microbiol **66:**1328–1333. http://dx.doi.org/10.1128/AEM.66.4.1328-1333.2000.