UC Merced UC Merced Previously Published Works

Title

Draft Genome Sequence of Mn(II)-Oxidizing Bacterium Oxalobacteraceae sp. Strain AB_14

Permalink

https://escholarship.org/uc/item/2qn5h3fk

Journal Microbiology Resource Announcements, 8(43)

ISSN

2169-8287

Authors

Bushman, Timothy J Akob, Denise M Bohu, Tsing <u>et al.</u>

Publication Date

2019-10-24

DOI

10.1128/mra.01024-19

Peer reviewed





Draft Genome Sequence of Mn(II)-Oxidizing Bacterium Oxalobacteraceae sp. Strain AB_14

Timothy J. Bushman,^a Denise M. Akob,^a Tsing Bohu,^b Andrea Beyer,^c Tanja Woyke,^e Nicole Shapiro,^e Alla Lapidus,^{e,f} Hans-Peter Klenk,^g Kirsten Küsel^d

^aU.S. Geological Survey, Reston, Virginia, USA

^bMineral Resources, CSIRO, Kensington, Western Australia, Australia

^cInstitute for Microbiology, Friedrich Schiller University Jena, Jena, Germany

^dInstitute of Biodiversity, Friedrich Schiller University Jena, Jena, Germany

^eJoint Genome Institute, U.S. Department of Energy, Walnut Creek, California, USA

^fThe Center for Algorithmic Biotechnology, St. Petersburg State University, St. Petersburg, Russia

⁹School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom

ABSTRACT Biological Mn(II) oxidation produces reactive manganese oxides that help to mitigate metal contamination in the environment. Here, we present the genome of *Oxalobacteraceae* sp. strain AB_14, a species of Mn(II)-oxidizing bacteria (MOB) that is notable for its ability to catalyze Mn oxidation at low pH (5.5).

B iological Mn oxidation produces highly reactive and abundant Mn oxide phases in the environment that can mitigate metal contamination (1). However, little is known about Mn oxidation in low-pH environments, where metal contamination is often a problem due to acid mine drainage (AMD). Here, we present the genome of *Oxalobacteraceae* sp. strain AB_14, isolated from AMD-affected Gessen Creek sediment located in the former Ronneburg uranium mining area in Germany (2). Notably, this bacterium catalyzes Mn oxidation at low pH (5.5). It is hypothesized that Mn(II)oxidizing bacteria (MOB), such as strain AB_14, can reduce the aqueous metal load in heavy-metal-containing AMD environments (2, 3).

AB_14 was isolated on BM5.5 plates containing MnCO₃ (modified *Leptothrix* medium according to Mulder and van Veen [4]), as described by Akob et al. (2), then maintained indefinitely on solid or liquid BM5.5 medium by transferring every 1 to 2 months. Sequencing of the 16S rRNA gene showed that the isolate was most closely related to type strain *Duganella zoogloeoides* IAM 12670 (97.8% identity). For genome sequencing, strain AB_14 was grown to high cell density in liquid BM5.5 media (modified *Leptothrix* medium according to Mulder and van Veen [4]), as described by Akob et al. (2). Biomass was harvested by centrifugation, frozen at -20° C, and shipped to the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany) for DNA extraction. DNA was extracted using the U.S. Department of Energy (DOE) Joint Genome Institute (JGI) cetyltrimethylammonium bromide (CTAB) procedure for isolating high-molecular-weight genomic DNA (gDNA) (5). Isolated gDNA was analyzed using agarose gel electrophoresis to evaluate the quantity and quality, including molecular weight, of the extract according to the JGI guidelines (https://jgi.doe.gov/user-programs/pmo-overview/project-materials-submission-overview/).

Extracted DNA was sent to the JGI (Walnut Creek, CA) for whole-genome sequencing. An Illumina standard shotgun library and a long-insert mate pair library were constructed and sequenced using the Illumina HiSeq 2000 platform, resulting in 23,039,042 reads totaling 3,455.9 Mb and 30,522,994 reads totaling 2,777.6 Mb, respectively. Additionally, a PacBio SMRTbell library was constructed from the same gDNA extract and sequenced on

Citation Bushman TJ, Akob DM, Bohu T, Beyer A, Woyke T, Shapiro N, Lapidus A, Klenk H-P, Küsel K. 2019. Draft genome sequence of Mn(II)-oxidizing bacterium Oxalobacteraceae sp. strain AB_14. Microbiol Resour Announc 8:e01024-19. https://doi.org/10.1128/MRA .01024-19.

Editor David A. Baltrus, University of Arizona This is a work of the U.S. Government and is not subject to copyright protection in the United States. Foreign copyrights may apply. Address correspondence to Denise M. Akob, dakob@usgs.gov.

Received 21 August 2019 Accepted 30 September 2019 Published 24 October 2019 the PacBio RS platform (6); 82,778 raw PacBio reads yielded 129,789 adapter-trimmed and quality-filtered subreads totaling 282.8 Mb. All raw Illumina sequence data were passed through the DUK filtering program, which removes known Illumina sequencing and library preparation artifacts (7). Default parameters were used for all software unless otherwise specified. Filtered Illumina and PacBio reads were then assembled using ALLPATHS-LG version R37654 (PrepareAllpathsInputs: PHRED 64 = 1 PLOIDY = 1 FRAG COVERAGE = 125 JUMP COVERAGE = 25; RunAllpathsLG: THREADS = 8 RUN=std pairs TARGETS=standard VAPI WARN ONLY = True OVERWRITE = True) (8). The final assembly was based on 3,438.6 Mb of Illumina standard paired-end (PE), 2,777.4 Mb of Illumina Cre-LoxP inverse PCR (CLIP) PE read, and 282.8 Mb of PacBio postfiltered data, which provided an average of 888.0× Illumina coverage and $40.4 \times$ PacBio coverage of the genome.

The final draft assembly contained 8 contigs (N_{50}/L_{50} , 1/3.7 Mb) in 6 scaffolds (N/L_{50} , 1/3.8 Mb), with a total genome size of 7.0 Mb and a G+C content of 64.32%. The AB_14 genome was annotated in the Integrated Microbial Genomes (IMG) database (9). One round of manual curation was performed using GenePRIMP (10). The *Oxalobacteraceae* sp. AB_14 genome contained 6,242 genes, with 6,125 of them coding for proteins. Annotation identified a total of 117 RNA genes, including 21 rRNA genes (7 copies each of the 55 rRNA, 16S rRNA, and 23S rRNA genes), 82 tRNA genes, and 14 genes for noncoding RNAs. The genome also contains one likely clustered regularly interspaced short palindromic repeat (CRISPR) array.

Data availability. The AB_14 draft genome is available from the IMG database under genome identifier 2522125015 and from the NCBI database under BioProject number PRJNA183351, BioSample number SAMN02440486, and Sequence Read Archive accession number SRP024932.

ACKNOWLEDGMENTS

We thank Lynne Goodwin and Linda Meincke (JGI) for project management, Maria Fabisch (FSU Jena) for cultivation assistance, and Beatrice Trümper (DSMZ) for performing DNA extractions.

The work conducted by the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, is supported by the Office of Science of the U.S. Department of Energy under contract number DE-AC02-05CH11231. A.L. was partially supported by the Russian Science Foundation (grant 19-16-00049).

Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

We declare no competing financial interest.

REFERENCES

- Tebo BM, Johnson HA, McCarthy JK, Templeton AS. 2005. Geomicrobiology of manganese(II) oxidation. Trends Microbiol 13:421–428. https://doi.org/10.1016/j.tim.2005.07.009.
- Akob DM, Bohu T, Beyer A, Schaffner F, Handel M, Johnson CA, Merten D, Buchel G, Totsche KU, Küsel K. 2014. Identification of Mn(II)-oxidizing bacteria from a low-pH contaminated former uranium mine. Appl Environ Microbiol 80:5086–5097. https://doi.org/10.1128/AEM.01296-14.
- Bohu T, Akob DM, Abratis M, Lazar CS, Küsel K. 2016. Biological low pH Mn(II) oxidation in a manganese deposit influenced by metal-rich groundwater. Appl Environ Microbiol 82:3009–3021. https://doi.org/10.1128/AEM .03844-15.
- Mulder EG, van Veen WL. 1963. Investigations on the Sphaerotilus-Leptothrix group. Antonie Van Leeuwenhoek 29:121. https://doi.org/10 .1007/BF02046045.
- Joint Genome Institute (JGI). 2012. Bacterial genomic DNA isolation using CTAB. http://lofdmq2n8tc36m6i46scovo2e-wpengine.netdna-ssl .com/wp-content/uploads/2014/02/JGI-Bacterial-DNA-isolation-CTAB -Protocol-2012.pdf.
- Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, Peluso P, Rank D, Baybayan P, Bettman B, Bibillo A, Bjornson K, Chaudhuri B, Christians F, Cicero R, Clark S, Dalal R, Dewinter A, Dixon J, Foquet M, Gaertner A, Hardenbol P, Heiner C, Hester K, Holden D, Kearns G, Kong XX, Kuse R, Lacroix Y, Lin S, Lundquist P, Ma CC, Marks P, Maxham M, Murphy D, Park I, Pham T, Phillips M, Roy J,

Sebra R, Shen G, Sorenson J, Tomaney A, Travers K, Trulson M, Vieceli J, Wegener J, Wu D, Yang A, Zaccarin D, Zhao P, Zhong F, Korlach J, Turner S. 2009. Real-time DNA sequencing from single polymerase molecules. Science 323:133–138. https://doi.org/10.1126/science.1162986.

- Mingkun L, Copeland A, Han J. 2011. DUK—a fast and efficient kmer based sequence matching tool. Joint Genome Institute, Walnut Creek, CA. http://duk.sourceforge.net/.
- Gnerre S, MacCallum I, Przybylski D, Ribeiro FJ, Burton JN, Walker BJ, Sharpe T, Hall G, Shea TP, Sykes S, Berlin AM, Aird D, Costello M, Daza R, Williams L, Nicol R, Gnirke A, Nusbaum C, Lander ES, Jaffe DB. 2011. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. Proc Natl Acad Sci U S A 108:1513–1518. https:// doi.org/10.1073/pnas.1017351108.
- Chen IMA, Chu K, Palaniappan K, Pillay M, Ratner A, Huang JH, Huntemann M, Varghese N, White JR, Seshadri R, Smirnova T, Kirton E, Jungbluth SP, Woyke T, Eloe-Fadrosh EA, Ivanova NN, Kyrpides NC. 2019. IMG/M v.5.0: an integrated data management and comparative analysis system for microbial genomes and microbiomes. Nucleic Acids Res 47:D666–D677. https://doi.org/10.1093/nar/gky901.
- 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.