



Draft Genome Sequence of the Iron-Oxidizing Acidophile Leptospirillum ferriphilum Type Strain DSM 14647

Juan Pablo Cárdenas,^{a,b} Marcelo Lazcano,^{a,b} Francisco J. Ossandon,^a Melissa Corbett,^c David S. Holmes,^{a,b} Elizabeth Watkin^c

Fundación Ciência & Vida, Santiago, Chileª; Facultad de Ciencias Biológicas, Universidad Andres, Bello, Santiago, Chile^b; School of Biomedical Sciences, Curtin University, Perth, Australia^c

The genomic features of the *Leptospirillum ferriphilum* type strain DSM 14647 are described here. An analysis of the predicted genes enriches our knowledge of the molecular basis of iron oxidation, improves our understanding of its role in industrial bioleaching, and suggests how it is adapted to live at extremely low pH.

Received 29 September 2014 Accepted 30 September 2014 Published 6 November 2014

Citation Cárdenas JP, Lazcano M, Ossandon FJ, Corbett M, Holmes DS, Watkin E. 2014. Draft genome sequence of the iron-oxidizing acidophile *Leptospirillum ferriphilum* type strain DSM 14647. Genome Announc. 2(6):e01153-14. doi:10.1128/genomeA.01153-14.

Copyright © 2014 Cárdenas et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license

Address correspondence to Elizabeth Watkin, e.watkin@curtin.edu.au

Leptospirillum ferriphilum is a Gram-negative chemolithoautotrophic bacterium, consistently isolated from metal-rich, mesophilic (25 to 40°C), and acidic environments (pH 1.3 to 2.0) where iron-bearing minerals are exposed to oxygen and water. Although the morphology of *L. ferriphilum* is variable, it is usually described as consisting of small (0.3 to 0.9 μ m), vibroid- to spiralshaped cells, with spirals of 3 to 12 cells. It possesses a single polar flagellum and is obligately chemolithotrophic, utilizing CO₂ as a carbon source. Its energy requirements are met by the aerobic oxidation of ferrous iron but not sulfur, and within industrial biooxidation tanks that possess high redox potentials, *L. ferriphilum* becomes the dominant iron oxidizer (1). The genomic sequencing of the *L. ferriphilum* type strain DSM 14647 provides important information regarding its bioleaching capabilities. The type strain (DSM 14647) was originally isolated from Peru (2).

The genome of *L. ferriphilum* strain DSM 14647^T was sequenced using the Roche GS-FLX 454 sequencing platform and assembled using Newbler (version 2.6), with *L. ferriphilum* ML-04 (accession no. CP002919) serving as a reference genome. The draft genome size is 2.4 Mb, with a median coverage depth of 23-fold and an average G+C content of 54.2%. It contains 20 large (>1,000 bp) contigs, with an N_{50} of 274,009, and 15 smaller contigs. The genes and RNA features were identified using RAST (3). The draft genome annotation predicts 44 tRNA sequences and 2,726 protein-coding genes, 53.5% of which were assigned putative functions (4).

The genome has genes predicted to encode enzymes of the reverse tricarboxylic acid (TCA) cycle (5). Nif genes, involved in nitrogen fixation, were not detected in this genome; however, it exhibits genes for ammonium assimilation. It also has genes that encode electron transfer proteins proposed to be involved in iron oxidation, including cytochrome₅₇₂, cytochrome₅₇₉, and 2 copies of the terminal electron acceptor *cbb3* complex (6). Genes involved in flagella biosynthesis and chemotaxis were also detected. As described previously for other members of the genus (7), it has genes encoding enzymes for the ectoine and trehalose biosynthetic pathways. It also has genes predicted to be involved in heavy metal

efflux (*czcABC*), arsenic resistance (*arsBC*), and mercury resistance (*merA*). The genome contains genes potentially encoding several components of a type IV secretion system involved in conjugative DNA transfer (8) and genes for a clustered regularly interspaced short palindromic repeat (CRISPR)-Cas system putatively involved in antiviral resistance (9).

Nucleotide sequence accession number. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. JPGK00000000.

ACKNOWLEDGMENTS

We acknowledge FONDECYT 1130683 and 1140048 and the Basal Program of CONICYT PFB16.

J.P.C. is a recipient of a graduate fellowship from CONICYT. Sequencing was funded by a Bioplatforms Australia Omics grant.

REFERENCES

- Coram NJ, Rawlings DE. 2002. Molecular relationship between two groups of the genus *Leptospirillum* and the finding that *Leptospirillum ferriphilum* sp. nov. dominates South African commercial biooxidation tanks that operate at 40 degrees C. Appl. Environ. Microbiol. 68:838–845. http:// dx.doi.org/10.1128/AEM.68.2.838-845.2002.
- 2. Sand W, Rohde K, Sobotke B, Zenneck C. 1992. Evaluation of *Leptospirillum ferrooxidans* for Leaching. Appl. Environ. Microbiol. 58:85–92.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. http://dx.doi.org/10.1186/1471-2164-9-75.
- Tatusov RL, Natale DA, Garkavtsev IV, Tatusova TA, Shankavaram UT, Rao BS, Kiryutin B, Galperin MY, Fedorova ND, Koonin EV. 2001. The COG database: new developments in phylogenetic classification of proteins from complete genomes. Nucleic Acids Res. 29:22–28. http://dx.doi.org/10.1093/nar/29.1.22.
- Levicán G, Ugalde JA, Ehrenfeld N, Maass A, Parada P. 2008. Comparative genomic analysis of carbon and nitrogen assimilation mechanisms in three indigenous bioleaching bacteria: predictions and validations. BMC Genomics 9:581. http://dx.doi.org/10.1186/1471-2164-9-581.
- Bonnefoy V, Holmes DS. 2012. Genomic insights into microbial iron oxidation and iron uptake strategies in extremely acidic environments. Environ. Microbiol. 14:1597–1611. http://dx.doi.org/10.1111/j.1462 -2920.2011.02626.x.

- Goltsman DSA, Denef VJ, Singer SW, VerBerkmoes NC, Lefsrud M, Mueller RS, Dick GJ, Sun CL, Wheeler KE, Zemla A, Baker BJ, Hauser L, Land M, Shah MB, Thelen MP, Hettich RL, Banfield JF. 2009. Community genomic and proteomic analyses of chemoautotrophic iron-oxidizing "*Lepto-spirillum rubarum*" (group II) and "*Leptospirillum ferrodiazotrophum*" (group III) bacteria in acid mine drainage biofilms. Appl. Environ. Microbiol. 75: 4599–4615. http://dx.doi.org/10.1128/AEM.02943-08.
- Trokter M, Felisberto-Rodrigues C, Christie PJ, Waksman G. 2014. Recent advances in the structural and molecular biology of type IV secretion systems. Curr. Opin. Struct. Biol. 27C:16–23. http://dx.doi.org/ 10.1016/j.sbi.2014.02.006.
- 9. Sorek R, Lawrence CM, Wiedenheft B. 2013. CRISPR-mediated adaptive immune systems in bacteria and archaea. Annu. Rev. Biochem. 82:237–266. http://dx.doi.org/10.1146/annurev-biochem-072911-172315.