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Complete Genome Sequence of *Leptospirillum ferrooxidans* Strain C2-3, Isolated from a Fresh Volcanic Ash Deposit on the Island of Miyake, Japan

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A diazotrophic, acidophilic, iron-oxidizing bacterium, *Leptospirillum ferrooxidans*, known to be difficult to cultivate, was isolated from a fresh volcanic ash deposit on the island of Miyake, Japan. Here, we report the complete genome sequence of a cultured strain, C2-3.

Leptospirillum ferrooxidans is an acidophilic, obligate chemolithoautotrophic ferrous-iron-oxidizing bacterium, known to have nitrogenase-encoding genes (9). Our recent molecular ecological study showed that *L. ferrooxidans* was abundant in volcanic ash deposits derived from the Mount Oyama eruption in the year 2000 on the island of Miyake (Miyake-jima), Japan, suggesting that *L. ferrooxidans* is a pioneer microbe involved in the fixation of carbon and nitrogen in early soil ecosystems (1, 11). *L. ferrooxidans* strain C2-3 was isolated from a 7.6-year-old volcanic ash deposit at Mount Oyama by chemostat enrichment, and its acetylene reduction activity was characterized (10). The isolation of the strain is highly significant because *L. ferrooxidans* is poorly characterized due to the difficulty of maintaining pure cultures. Here, we determined the whole-genome sequence of *L. ferrooxidans* C2-3 to obtain its genomic information.

Genomic DNA was isolated from *L. ferrooxidans* C2-3 using phenol-chloroform extraction as described previously (12) and sequenced by the whole-genome shotgun sequencing method with ABI 3730*xl* DNA analyzer (Applied Biosystems). Approximately 7.3-fold coverage reads of the genome were assembled by Phred/Phrap/Consed software (3). The following annotation programs were used: MetaGeneAnnotator (7) for the identification of protein-coding sequences, tRNAscan-SE (6, 8) for tRNA, and RNAmmer (4) for rRNA.

The circular chromosome of *L. ferrooxidans* C2-3 contains 2,559,538 bp, for which 2,421 open reading frames, 51 tRNA genes, and 3 rRNA operons were predicted. Average G+C content of the genome was 50.1%. Ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO) genes for the Calvin-Benson cycle were not found, but two copies of RubisCO-like protein were predicted. *L. ferrooxidans* C2-3 has genes for the reductive tricarboxylic acid (TCA) cycle, except for 2-oxoglutarate:ferredoxin oxidoreductase and ATP-citrate lyase. Instead, two copies of pyruvate ferredoxin oxidoreductase genes and succinyl-CoA synthetase were found, consistent with the near-complete genome sequences of "*Leptospirillum rubarum*" and "*Leptospirillum ferrodiazotrophum*" that were obtained by community genomic and proteomic analyses of acidic mine drainage environments (2). The *L. ferrooxidans* C2-3 genome contained all genes encoding the components of the pentose phosphate pathway and some of the genes for the Embden-Meyerhof-Parnas pathway; genes for the TCA cycle were found to be incomplete. A single operon of nitrogenase was found to contain the *nifH*, *-D*, *-K*, *-E*, *-N*, *-X*, *-B*, *-S*, *-Z*, *-U*, *-T*, *-W*, and *-A* genes, but the *nifL* gene was not predicted. This result suggested that *L. ferrooxidans* might have an NifL-independent nitrogenase regulation system. Furthermore, genes of the two-component nitrogen regulatory system in *L. ferrooxidans* C2-3 were identified: not only *ntrCB* but also *nrtXY* genes. *ntrXY* genes were also found in the complete genome sequence of *Leptospirillum ferriphilum*, which has not been deposited (5). Additionally, *L. ferrooxidans* C2-3 has some hydrogenase-encoding genes, which are considered to have potential for hydrogen oxidation.

The complete genome sequence of *L. ferrooxidans* C2-3 will provide significant genetic information of acidophilic, iron-oxidizing bacteria and be useful for further studies to obtain a clear understanding of the ecology of difficult-to-culture microbes.

Nucleotide sequence accession number. The complete genome sequence of *L. ferrooxidans* C2-3 has been deposited in the DDBJ/EMBL/GenBank databases under accession number AP012342.

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REFERENCES

1. **Fujimura R, et al.** 2012. Analysis of early bacterial communities on volcanic deposits on the island of Miyake (Miyake-jima), Japan: a 6-year study at a fixed site. Microbes Environ. **27**:19–29.

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2. **Goltsman DS, et al.** 2009. Community genomic and proteomic analyses of chemoautotrophic iron-oxidizing "*Leptospirillum rubarum*" (group II) and "*Leptospirillum ferrodiazotrophum*" (group III) bacteria in acid mine drainage biofilms. Appl. Environ. Microbiol. **75**:4599–4615.
3. **Gordon D, Desmarais C, Green P.** 2001. Automated finishing with autofinish. Genome Res. **11**:614–625.
4. **Lagesen K, et al.** 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. **35**:3100–3108.
5. **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.
6. **Lowe TM, Eddy SR.** 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. **25**: 955–964.
7. **Noguchi H, Taniguchi T, Itoh T.** 2008. MetaGeneAnnotator: detecting species-specific patterns of ribosomal binding site for precise gene prediction in anonymous prokaryotic and phage genomes. DNA Res. **15**:387–396.
8. **Ohtsubo Y, Ikeda-Ohtsubo W, Nagata Y, Tsuda M.** 2008. GenomeMatcher: a graphical user interface for DNA sequence comparison. BMC Bioinformatics **9**:376.
9. **Parro V, Moreno-Paz M.** 2004. Nitrogen fixation in acidophile iron-oxidizing bacteria: the nif regulon of *Leptospirillum ferrooxidans*. Res. Microbiol. **155**:703–709.
10. **Sato Y, et al.** 2009. Nitrogenase activity (acetylene reduction) of an iron-oxidizing *Leptospirillum* strain cultured as a pioneer microbe from a recent volcanic deposit on Miyake-jima, Japan. Microbes Environ. **24**:291–296.
11. **Sugawara T, et al.** 2008. Abstr. 7th Int. Symp. Subsurface Microbiol., abstr S10-P8.
12. **Wang GC, Wang Y.** 1997. Frequency of formation of chimeric molecules as a consequence of PCR coamplification of 16S rRNA genes from mixed bacterial genomes. Appl. Environ. Microbiol. **63**:4645–4650.