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# Draft Genome Sequence of the Anaerobic, Nitrate-Dependent, Fe(II)-Oxidizing Bacterium *Pseudogulbenkiania ferrooxidans* Strain 2002

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***Pseudogulbenkiania ferrooxidans* strain 2002 was isolated as a lithoautotrophic, Fe(II)-oxidizing, nitrate-reducing bacterium. Phylogenetically, it is in a clade within the family *Neisseriaceae* in the order *Nessieriales* of the class *Betaproteobacteria*. It is anticipated that comparative genomic analysis of this strain with other nitrate-dependent, Fe(II)-oxidizing bacteria will aid in the elucidation of the genetics and biochemistry underlying this critically important geochemical metabolism.**

*Pseudogulbenkiania ferrooxidans* strain 2002 (formerly *Lutiella nitroferum* strain 2002) is a non-spore-forming, Gram-negative, motile rod belonging to the family *Neisseriaceae* in the order *Nessieriales* of the class *Betaproteobacteria* (13). Strain 2002 was isolated as part of a study of nitrate-dependent Fe(II) oxidation in freshwater lake sediments and characterized as the first mesophilic, nitrate-dependent, lithoautotrophic Fe(II) oxidizer (13). The 16S rRNA gene sequence had 99.3% similarity to the type species *Pseudogulbenkiania subflava* (10), whose genomic sequence is not publically available, and 99.8% similarity to *Pseudogulbenkiania* sp. strain NH8B, whose complete genomic sequence is available (8). The latter nitrate-reducing strain was isolated from agricultural paddy rice and soybean fields in Japan (12). It is anticipated that comparative genomics of *P. ferrooxidans* strain 2002 with other nitrate-dependent, Fe(II)-oxidizing bacteria will aid in the elucidation of the genetics underlying this globally important metabolism.

The draft genome of strain 2002 consisted of 4.2 Mb in 20 contigs with an average G+C content of 64.66%. A total of 3,927 protein-encoding genes were predicted. Sequencing performed at the Joint Genome Institute (JGI) included Sanger sequencing and 454 pyrosequencing to a depth of 20× coverage. All JGI library construction and sequencing techniques can be found at <http://www.jgi.doe.gov/>. Sequence assembly, quality assessment, and annotation were performed using the Phred/Phrap/Consed (CodonCode Corporation, Dedham, MA) (4, 5), Dupfinisher (6), PRODIGAL (7), and GENERATION software and the JGI Integrated Microbial Genomes site (11).

Early comparative genomics revealed little synteny between strain 2002 and the genome of *Acidovorax ebreus* strain TPSY, the first nitrate-dependent iron oxidizer for which there is a publically available genome sequence (2; K. G. Byrne-Bailey et al., unpublished data). In addition, preliminary annotation did not reveal a known carbon fixation pathway that would allow strain 2002 to survive by a lithoautotrophic lifestyle.

Type *c* cytochromes have been implicated in the mechanism of nitrate-dependent Fe(II) oxidation (3), with further biophysical evidence for strain 2002 (13) and *A. ebreus* strain TPSY (Byrne-Bailey et al., unpublished). Automated annotation of the genome of strain 2002 identified 41 genes predicted to encode *c*-type cytochromes, only 1 of which was a diheme, with no predicted proteins

containing multiple heme domains. This is equivalent to both the predicted number of cytochrome proteins in *A. ebreus* strain TPSY and other organisms known to metabolize iron (Byrne-Bailey et al., unpublished). However, manual curation of the genome identified a number of low-similarity homologues to *pio* genes and MtrA proteins which have defined multiple heme domains, none of which were clustered in strain 2002. Iron oxidation in the phototroph *Rhodospirillum rubrum* TIE-1 was shown to involve a number of proteins encoded by the *pio* genes (9), whereas iron reduction by *Shewanella* spp. involves the Mtr proteins (1). It is hoped that further investigation of these and other genes will aid in the understanding of nitrate-dependent iron oxidation in neutrophilic bacteria.

**Nucleotide sequence accession number.** The genomic sequence of *P. ferrooxidans* strain 2002 reported here has been deposited in the GenBank database under accession number NZ\_ACIS01000000.

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