

Genome Sequence of Deep-Sea Manganese-Oxidizing Bacterium *Marinobacter manganoxydans* MnI7-9

Haifang Wang,^a Hang Li,^a Zongze Shao,^c Shuijiao Liao,^{a,b} Laurel Johnstone,^d Christopher Rensing,^e and Gejiao Wang^a

State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology^a and College of Basic Science,^b Huazhong Agricultural University, Wuhan, People's Republic of China; Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, State Oceanic Administration, Xiamen, People's Republic of China; and University of Arizona Genetics Core^d and Department of Soil, Water and Environmental Science,^e Tucson, Arizona, USA

Here we report the draft genome of *Marinobacter manganoxydans* MnI7-9, isolated from a deep-sea hydrothermal vent in the Indian Ocean and capable of oxidizing manganese even when there is a very high concentration of Mn²⁺. The strain also displayed high resistance and adsorption ability toward many metal(loid)s.

Bacteria of the genus *Marinobacter* (*Gammaproteobacteria*) are Gram-negative, motile, halophilic or halotolerant, and ubiquitous in various marine environments (8). All known strains have the ability to use petroleum hydrocarbons as sole carbon sources and interact with marine algae and plankton (1, 6). *Marinobacter manganoxydans* MnI7-9 was isolated from a heavy-metal-rich sediment sample collected from a deep-sea hydrothermal vent in the Indian Ocean (lat 25.32, long 70.04; depth, 2,474 m). It was assigned to the *Marinobacter* genus based on its 16S rRNA sequence and fatty acid analyses. *M. manganoxydans* MnI7-9 can tolerate high levels of metal(loid)s, oxidize manganese [Mn(II)] to Mn(III)/Mn(IV), even under 10 mM MnCl₂ conditions, and adsorb many metal(loid)s. So far, the complete genome sequences of 4 *Marinobacter* species, *M. adhaerens* HP15 (GenBank accession numbers CP001978 to CP001980), *M. algicola* DG893 (GenBank accession number ABCP00000000), *M. aquaeolei* VT8 (GenBank accession numbers CP000514 to CP000516), and *Marinobacter* sp. ELB17 (GenBank accession number AAXY00000000), have been published; however, a genome sequence for a highly metal-resistant and Mn(II)-oxidizing *Marinobacter* species from a deep-sea environment has not been reported.

Genome sequencing was performed using the 454 GS FLX sequencer (9), producing 314,378 random shotgun reads with an average length of 373 bp. The approximate genome coverage was 24-fold. The sequence reads were assembled into 88 contigs with a total of 4,549,590 bp using the GS De Novo Assembler, version 2.5.3. The rapid annotations using subsystems technology (RAST) server was used to annotate the assembled contigs (3). A total of 4,171 protein-coding genes (CDSs) and 51 structural RNAs were annotated, and 2,562 proteins have orthologs with *M. adhaerens* HP15 and *M. aquaeolei* VT8 in RAST (identities of >60%). A total of 3,128 proteins could be assigned to cluster of orthologous groups (COG) families. The GC content was 57.3% when analyzed using CLC Main Workbench 5 (<http://www.clcbio.com>). The NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) was employed for the gene annotation that was submitted to GenBank (<http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html>).

We particularly analyzed genes possibly responsible for Mn(II) oxidation, such as the multicopper oxidase genes *mofA*, *moxA*, and *mnxG* (4, 10). We found 5 genes encoding multicopper oxidases, 3 annotated as *copA*, 1 annotated as a twin-arginine translocation pathway signal gene, and 1 of unknown function, but none of them had significant homology with *mofA*, *moxA*, and *mnxG*. In addition,

genes encoding cytochrome *c* synthetase, tryptophan biosynthesis, parts of the tricarboxylic acid (TCA) cycle, and the secretory pathway (7) with a possible accessory role in Mn(II) oxidation were found in the genome of MnI7-9. However, the genes encoding heme-binding peroxidase (2) and sensor kinases (*MnxS1* and *MnxS2*) (7) with a possible involvement in Mn(II) oxidation were not found.

We also found genes encoding proteins associated with resistance to metal(loid)s based on the high tolerance of *M. manganoxydans* MnI7-9 to many metal(loid)s. We identified genes putatively involved in nickel, mercury, copper, chromate, arsenic, zinc, cobalt, and cadmium resistance. In addition, genes important for degradation of aliphatic and polycyclic aromatic hydrocarbons were found in the genome (5, 12). The draft genome sequence of *M. manganoxydans* MnI7-9 from a metal-rich, deep-sea hydrothermal vent might aid in understanding evolutionary processes in different *Marinobacter* species to adapt to variable marine environments (11).

Nucleotide sequence accession numbers. The genome sequence has been deposited at DDBJ/EMBL/GenBank under the accession number AGTR00000000. The version described in this paper is the first version, accession number AGTR00000000.

ACKNOWLEDGMENTS

This project was supported by a Major International Collaborative Project of the National Natural Science Foundation of China (31010103903) and the National Fundamental Fund of Personnel Training of China (J0730649).

Sequencing was performed at the University of Arizona Genetics Core.

REFERENCES

- Amin SA, Green DH, Waheeb DA, Gärdes A, Carrano CJ. 6 September 2011. Iron transport in the genus *Marinobacter*. *Biometals* doi:10.1007/s10534-011-9491-9.
- Anderson CR, et al. 2009. Mn(II) oxidation is catalyzed by heme peroxidases in "*Aurantimonas manganoxydans*" strain SI85-9A1 and *Erythrobacter* sp. strain SD-21. *Appl. Environ. Microbiol.* 75:4130–4138.
- Aziz RK, et al. 2008. The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* 9:75.
- Brouwers GJ, et al. 1999. *cumA*, a gene encoding a multicopper oxidase,

Received 18 November 2011 Accepted 29 November 2011

Address correspondence to Gejiao Wang, gejiaow@yahoo.com.cn.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JB.06551-11

- is involved in Mn^{2+} oxidation in *Pseudomonas putida* GB-1. Appl. Environ. Microbiol. 65:1762–1768.
5. Chen SX, Shao ZZ. 2009. Isolation and diversity analysis of arsenite-resistant bacteria in communities enriched from deep-sea sediments of the Southwest Indian Ocean Ridge. Extremophiles 13:39–48.
 6. Gärdes A, et al. 2010. Complete genome sequence of *Marinobacter adhaerens* type strain (HP15), a diatom-interacting marine microorganism. Stand. Genomic Sci. 3:97–107.
 7. Geszvain K, Tebo BM. 2010. Identification of a two-component regulatory pathway essential for Mn(II) oxidation in *Pseudomonas putida* GB-1. Appl. Environ. Microbiol. 76:1224–1231.
 8. Kaye JZ, Sylvan JB, Edwards KJ, Baross JA. 2011. *Halomonas* and *Marinobacter* ecotypes from hydrothermal vent, seafloor and deep-sea environments. FEMS Microbiol. Ecol. 75:123–133.
 9. Margulies M, et al. 2005. Genome sequencing in open microfabricated high density picoliter reactors. Nature 437:376–380.
 10. Ridge JP, et al. 2007. A multicopper oxidase is essential for manganese oxidation and laccase-like activity in *Pedomicrobium* sp. ACM 3067. Environ. Microbiol. 9:944–953.
 11. Singer E, et al. 2011. Genomic potential of *Marinobacter aquaeolei*, a biogeochemical “opportunitroph.” Appl. Environ. Microbiol. 77:2763–2771.
 12. Yakimov MM, Timmis KN, Golyshin PN. 2007. Obligate oil-degrading marine bacteria. Curr. Opin. Biotechnol. 18:257–266.