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Complete Genome Sequence of *Metallosphaera cuprina*, a Metal Sulfide-Oxidizing Archaeon from a Hot Spring▽

Li-Jun Liu,[1] Xiao-Yan You,[1,2] Huajun Zheng,[3] Shengyue Wang,[3] Cheng-Ying Jiang,[1,2]* and Shuang-Jiang Liu[1,2]*

State Key Laboratory of Microbial Resource[1] and Environmental Microbiology Research Center,[2] Institute of Microbiology, Beijing 100101, and Shanghai-MOST Key Laboratory of Health and Disease Genomics, Chinese National Human Genome Center, Shanghai,[3] People's Republic of China

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The genome of the metal sulfide-oxidizing, thermoacidophilic strain *Metallosphaera cuprina* Ar-4 has been completely sequenced and annotated. Originally isolated from a sulfuric hot spring, strain Ar-4 grows optimally at 65°C and a pH of 3.5. The *M. cuprina* genome has a 1,840,348-bp circular chromosome (2,029 open reading frames [ORFs]) and is 16% smaller than the previously sequenced *Metallosphaera sedula* genome. Compared to the *M. sedula* genome, there are no counterpart genes in the *M. cuprina* genome for about 480 ORFs in the *M. sedula* genome, of which 243 ORFs are annotated as hypothetical protein genes. Still, there are 233 ORFs uniquely occurring in *M. cuprina*. Genome annotation supports that *M. cuprina* lives a facultative life on CO_2 and organics and obtains energy from oxidation of sulfidic ores and reduced inorganic sulfuric compounds.

Extremely thermoacidophilic archaea play important roles in mobilizing metal sulfide deposits in natural bioleaching environments (5, 9). Due to the ability to oxidize reduced inorganic sulfur compounds (RISCs) under high-temperature conditions, *Metallosphaera* has attracted increasing interest from the biomining industry (5, 6, 10–13). The bioleaching *Metallosphaera sedula* was explored at the genomic level (2). Here, we present the complete genome of a newly isolated, bioleaching, and thermoacidophilic *Metallosphaera cuprina* strain (8).

Genomic DNA of *M. cuprina* Ar-4 was purified from cells grown in modified Allen medium (3). The whole genome was sequenced by a Roche 454 genome sequencer FLX instrument. A total of 295,139 shotgun reads were produced and assembled into 55 contigs, providing 67-fold coverage. Gaps were closed by multiplex PCR and primer-walking methods. The gap-spanning PCR products were sequenced with an ABI 3730 DNA analyzer, and the resulting sequences were assembled using Phred/Phrap/Consed software. The final consensus quality level of each base was above 64. Protein-coding genes were identified with the Glimmer 3.02 program (4). Protein function was predicted by either homology searches in the GenBank and UniProt protein databases, function assignment searches in the CDD (COG) database, or domain/motif searches in the Pfam databases. The KEGG tool was used to reconstruct metabolic pathways. Membrane proteins were predicted by the LipoP, SignalP, and ConPred II programs. The tRNA genes were identified by using the tRNAScan-SE tool, and the rRNA genes were identified by using the RNAmmer 1.2 and BLASTn programs.

M. cuprina Ar-4 grew chemolithotrophically on CO_2 with metal sulfide and RISCs as energy sources or chemoheterotrophically on various organics (8). Its genome consisted of a 1,840,348-bp circular chromosome. The genome carried 2,029 open reading frames (ORFs) in total. Genome annotation and metabolic reconstruction supported the idea that *M. cuprina* lived a facultative life. The *M. cuprina* strain fixed CO_2 via the 3-hydroxypropionate/4-hydroxybutyrate cycle, and this strain assimilated carbohydrates via the nonphosphorylated Entner-Doudoroff (ED) pathway. It had a complete tricarboxylic acid (TCA) cycle and an incomplete phosphate pentose pathway. Oxidation of RISCs by the heterodisulfide reductase complex, sulfide:quinone oxidoreductase, thiosulfate:quinone oxidoreductase, tetrathionate hydrolase, and sulfite:acceptor oxidoreductase in *M. cuprina* was proposed. The terminal oxidase complexes of *M. cuprina* that channel electrons from RISC oxidation to oxygen were similar to those of "*Metallosphaera yellowstonensis*" (7) and *M. sedula* (1).

The *M. cuprina* genome was 16% smaller than the *M. sedula* genome. Analysis indicated that the counterpart genes of about 480 ORFs in the *M. sedula* genome were not found in the *M. cuprina* genome. Still, there were 233 ORFs uniquely occurring in *M. cuprina*. Most of those ORFs were annotated as hypothetical protein genes. Gene redundancy in *M. cuprina* was apparently kept low. For example, there was only one copy of the 4-hydroxybutyryl–coenzyme A (CoA) dehydratase gene in *M. cuprina*, but duplication of this function was observed in the *M. sedula* genome (2). The information provided in the *M. cuprina* genome sequence will facilitate additional researches on this organism, as well as defining the core genome and key physiological features of the genus *Metallosphaera*.

* Corresponding author. Mailing address: Institute of Microbiology, Chinese Academy of Sciences, Bei-Chen-Xi-Lu No. 1, Chao-Yang District, Beijing 100101, People's Republic of China. Phone for Shuang-Jiang Liu: 86-10-64807423. Fax: 86-10-64807421. E-mail: liusj@sun.im.ac.cn. Phone for Cheng-Ying Jiang: 86-10-64807581. Fax: 86-10-64807421. E-mail: jiangcy@im.ac.cn.

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REFERENCES

1. **Auernik, K. S., and R. M. Kelly.** 2008. Identifcation of components of electron transport chains in the extremely thermoacidophilic crenarchaeon *Metallosphaera sedula* through iron and sulfur compounds oxidation transcriptomes. Appl. Environ. Microbiol. **74:**7723–7732.
2. **Auernik, K. S., Y. Maezato, P. H. Blum, and R. M. Kelly.** 2008. The genome sequence of the metal-mobilizing, extremely thermoacidophilic archaeon *Metallosphaera sedula* provides insights into bioleaching-associated metabolism. Appl. Environ. Microbiol. **74:**682–692.
3. **Brock, T. D., K. M. Brock, R. T. Belly, and R. L. Weiss.** 1972. *Sulfolobus*: a new genus of sulfur-oxidizing bacteria living at low pH and high temperature. Arch. Mikrobiol. **84:**54–68.
4. **Delcher, A. L., K. A. Bratke, E. C. Powers, and S. L. Salzberg.** 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics **23:**673–679.
5. **Han, C. J., and R. M. Kelly.** 1998. Biooxidation capacity of the extremely thermoacidophilic archaeon *Metallosphaera sedula* under bioenergetic challenge. Biotechnol. Bioeng. **58:**617–624.
6. **Inskeep, W. P., et al.** 2010. Metagenomes from high-temperature chemotrophic systems reveal geochemical controls on microbial community structure and function. PLoS One **5:**e9773.
7. **Kozubal, M. A., M. Dlakic, R. E. Macur, and W. P. Inskeep.** 2011. Terminal oxidase diversity and function in "*Metallosphaera yellowstonensis*": gene expression and protein modeling suggest mechanisms of Fe(II) oxidation in the *Sulfolobales*. Appl. Environ. Microbiol. **77:**1844–1853.
8. **Liu, L.-J., X.-Y. You, X. Guo, S.-J. Liu, and C.-Y. Jiang.** 2010. *Metallosphaera cuprina* sp. nov., a novel species of acidothermophilic metal-mobilizing Archaeon. Int. J. Syst. Evol. Microbiol. doi:10.1099/ijs.0.026591-0.
9. **Macur, R. E., H. W. Langner, B. D. Kocar, and W. P. Inskeep.** 2004. Linking geochemical processes with microbial community analysis: successional dynamics in an arsenic-rich, acid-sulfate-chloride geothermal spring. Geobiology **2:**163–177.
10. **Mikkelsen, D., U. Kappler, A. G. McEwan, and L. I. Sly.** 2006. Archaeal diversity in two thermophilic chalcopyrite bioleaching reactors. Environ. Microbiol. **8:**2050–2056.
11. **Mikkelsen, D., U. Kappler, A. G. McEwan, and L. I. Sly.** 2009. Probing the archaeal diversity of a mixed thermophilic bioleaching culture by TGGE and FISH. Syst. Appl. Microbiol. **32:**501–513.
12. **Romano, P., et al.** 2001. Comparative study on the selective chalcopyrite bioleaching of a molybdenite concentrate with mesophilic and thermophilic bacteria. FEMS Microbiol. Lett. **196:**71–75.
13. **Zhu, W., et al.** 2011. Sulfur oxidation activities of pure and mixed thermophiles and sulfur speciation in bioleaching of chalcopyrite. Bioresour. Technol. **102:**3877–3882.