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

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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 CO₂ 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 bioremediation *Metallosphaera sedula* was explored at the genomic level (2). Here, we present the complete genome of a newly isolated, bioremediating, 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 RNAmer 1.2 and BLASTn programs.

*M. cuprina* Ar-4 grew chemolithotrophically on CO₂ 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 CO₂ 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*. 
Nucleotide sequence accession number. The *M. cuprina* genome sequence is available at GenBank under accession no. CP002656.

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REFERENCES


