Comparative Genomic Analysis Reveals the Metabolism and Evolution of the Thermophilic Archaeal Genus *Metallosphaera*

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Members of the genus *Metallosphaera* are widely found in sulfur-rich and metal-laden environments, but their physiological and ecological roles remain poorly understood. Here, we sequenced *Metallosphaera tengchongensis* Ric-A, a strain isolated from the Tengchong hot spring in Yunnan Province, China, and performed a comparative genome analysis with other *Metallosphaera* genomes. The genome of *M. tengchongensis* had an average nucleotide identity (ANI) of approximately 70% to that of *Metallosphaera cuprina*. Genes *sqr*, *tth*, *sir*, *tqo*, *hdr*, *tst*, *soe*, and *sdo* associated with sulfur oxidation, and gene clusters *fox* and *cbs* involved in iron oxidation existed in all *Metallosphaera* genomes. However, the adenosine-5′-phosphosulfate (APS) pathway was only detected in *Metallosphaera sedula* and *Metallosphaera yellowstonensis*, and several subunits of *fox* cluster were lost in *M. cuprina*. The complete 3-hydroxypropionate/4-hydroxybutyrate cycle and dicarboxylate/4-hydroxybutyrate cycle involved in carbon fixation were found in all *Metallosphaera* genomes. A large number of gene family gain events occurred in *M. yellowstonensis* and *M. sedula*, whereas gene family loss events occurred frequently in *M. cuprina*. Pervasive strong purifying selection was found acting on the gene families of *Metallosphaera*, of which transcription-related genes underwent the strongest purifying selection. In contrast, genes related to prophages, transposons, and defense mechanisms were under weaker purifying pressure. Taken together, this study expands knowledge of the genomic traits of *Metallosphaera* species and sheds light on their evolution.

**Keywords:** comparative genomics, *Metallosphaera*, metabolic potential, evolution, horizontal gene transfer

**INTRODUCTION**

Extremely acidophilic archaea of the genus *Metallosphaera* belong to the order Sulfolobales within the Crenarchaeota. Together with the genera *Acidianus* and *Sulfolobus*, physiologically versatile *Metallosphaera* spp. contribute significantly to biogeochemical element cycling and biomining processes. *Metallosphaera* spp. stand out due to their ability for facultative autotrophic growth and...
tolerance to high concentrations of metal ions (Ai et al., 2016; Wheaton et al., 2016). Members of the genus *Metallosphaera* grow aerobically at low pH values (<4) and high temperatures (>60°C). They are found in sulfur-rich and metal-laden environments including solfataric areas (*Metallosphaera sedula* DSM 5348 and strains from laboratory evolution) (Huber et al., 1989; Ai et al., 2016, 2017), hot springs (such as *Metallosphaera hakonensis* HO1-1 = JCM8857, *M. cuprina Ar-4*, *M. tengchongensis* Ric-A, *Metallosphaera yellowstonensis* MK-1, *Metallosphaera* sp. UBA165, and *Metallosphaera* sp. Obs4) (Takayanagi et al., 1996; Kozubal et al., 2008, 2011; Liu et al., 2011a; Peng et al., 2015; Parks et al., 2017), and bioleaching-related heaps (*Metallosphaera prunae* sp. Obs4) (Takayanagi et al., 1996; Kozubal et al., 2008, 2011; Liu et al., 2011a; Peng et al., 2015, 2017), and *R. sulfidophilum* Ron 12/II) (Fuchs et al., 1995). *Metallosphaera* species are of great potential in the extraction of base and precious metals from ores exploiting their ability to oxidize reduced organic sulfur compounds (RISCs) and ferrous ion, a process mediated by a set of terminal oxidases that are attached to their cell membranes (Auernik and Kelly, 2008, 2010; Orell et al., 2010). Previous studies showed that application of thermophilic archaea in bioleaching could achieve faster dissolution rates and higher copper leaching yields in comparison to widely used mesophilic bioleaching bacteria because the formation of the surface passivation and diffusion layer of chalcopyrite was significantly reduced at high temperature (Rawlings, 2005; Uribeta et al., 2015; Castro and Donati, 2016).

*Metallosphaera* species can grow on peptides in a heterotrophic mode, fix carbon dioxide using reduced sulfur compounds as reductant in an autotrophic mode, or grow on casamino acids and FeSO₄ or metal sulfides in a mixotrophic mode (Peeples and Kelly, 1995; Ulrike et al., 2005; Alber et al., 2006; Auernik and Kelly, 2008, 2010; Michael et al., 2010; Han and Kelly, 2015). Autotrophic carbon fixation through the 3-hydroxypropionate/4-hydroxybutyrate (HP/HB) cycle in *Metallosphaera* has been confirmed by genomics, transcriptomics, proteomics, and biochemical assays (Sebastian et al., 2011; Jiang et al., 2014). Reduced Fe and S (Fe²⁺, HS⁻, S⁰, SO₄²⁻, and SO₂⁺) are important electron donors for iron or sulfur-oxidizing microorganism. Unlike Fe²⁺ oxidation by the bacteria *Acidithiobacillus ferroxidans* and *Leptospirillum ferrooxidans*, which require the blue copper protein rusticyanin and various c-type cytochromes (Rohwerder et al., 2003; Singer et al., 2008), the Fe oxidation mechanism of iron-oxidizing archaea is yet unconfirmed, although potential proteins were noticed in the genomes of *Ferrophasia*, *Sulfobolus*, and *Metallosphaera* species (Dopson et al., 2005; Bathe and Norris, 2007; Auernik et al., 2008). The genes involved in iron oxidation (Kozubal et al., 2011) such as fox, cbs, rusticyanin, and sulfoocyanin have been detected in the genomes of *M. sedula* and *M. yellowstonensis*, but their pervasiveness in *Metallosphaera* is yet unknown. RISC oxidation mechanisms are complex and diverse in extremely thermoacidophilic archaea. Sulfur oxygenase reductase genes are present in the genus *Acidianus*, in the species *Sulfobolus tokadaii*, and in bacteria, but are absent from the genomes of *M. sedula* and *M. cuprina* (Auernik et al., 2008; Liu et al., 2011a), which raised the question how sulfur oxidation is initiated in *Metallosphaera*. Of the species within the genus *Metallosphaera*, genome sequences and genomic analyses have been reported for four isolates (*M. cuprina* Ar-4, *M. hakonensis* HO1-1 = JCM8857, *M. sedula* DSM_5348, and *M. yellowstonensis* MK1) (Auernik et al., 2008; Liu et al., 2011a). *M. tengchongensis* Ric-A, the newest member of *Metallosphaera*, which was isolated from a sulfuric hot spring in Tengchong, Yunnan Province, China, showed an excellent performance in copper extraction from chalcopyrite (Peng et al., 2015). To better understand the unique metabolism, adaptation for extreme thermal and acidic conditions, roles in biogeochemical cycling, and the evolutionary history of the genus *Metallosphaera*, we performed genome sequencing of *M. tengchongensis* Ric-A and compared its genome with 18 available *Metallosphaera* genomes, of which 12 are from Genbank database of National Center for Biotechnology Information (NCBI) (*M. sedula* DSM5348, ARS50-1, ARS50-2, ARS120-1, ARS120-2, SARC-M1, and CuR1; *M. hakonensis* HO1-1 and JCM8857; *M. cuprina* Ar-4; *M. yellowstonensis* MK-1; *Metallosphaera* sp. UBA165) and six scaffold genomes are from the Integrated Microbial Genomes and Microbiomes (IMG/M) system of DOE Joint Genome Institute (JGI) (*Metallosphaera* spp. My-r02, My-r05, My-r06, YNP_08, YNP_14, and Obs4) (Table 1). In this work, we performed comprehensive analyses of genome-based phylogenetic relationships, metabolic pathway and gene function, heavy metal resistance, adhesion and motility, as well as mobile genetic elements (MGEs) and selective pressure. These findings will improve our understanding of the adaptive strategies of the organisms to their harsh environment and provide clues to design biomining or bioremediation processes in the future.

**Materials and Methods**

Sample Collection and Sequencing

*Metallosphaera tengchongensis* strain Ric-A was isolated from the muddy water samples of sulfuric hot springs (24.57 N and 98.26 E, with the temperature range of 55–96°C and a pH range of 2.5–7.5, dissolved oxygen range of 0.01–1.00 mg/L) in Tengchong county of Yunnan Province, China. The hot springs are rich in S (SO₄²⁻, 701.20–22.46 mg/L), Fe (13.89–0.05 mg/L), Ca (71.55–1.12 mg/L), K (22.64–63.01 mg/L), Al (42.43–0.13 mg/L), and other elements (Liu et al., 2011a; Peng et al., 2015; Qin et al., 2019). The sample was concentrated by tangential flow ultrafiltration through a hollow fiber membrane (Tianjin MOTIMO Membrane Technology, China). An aerobic broth culture was subsequently established in an R2A medium (Tianjin MOTIMO Membrane Technology, China) with 1 ml of trace element solution (FeCl₂, 0.2 g; MnSO₄ · H₂O, 0.2 g; Na₂MoO₄ · 2H₂O, 0.08 g; CoCl₂ · 6H₂O, 0.06 g; ZnSO₄ · 7H₂O, 0.09 g in 1 L of distilled water). After static culture for 5–7 days at 65°C, samples of the grown culture were spread on BSM solid plates with potassium tetrathionate (K₂S₂O₄, 10 mmol/L) or yeast extract (1 g/L) as energy source. The plates were incubated for 5–7 days at 65°C, samples of the grown culture were spread on BSM solid plates with potassium tetrathionate (K₂S₂O₄, 10 mmol/L) or yeast extract (1 g/L) as energy source.
### TABLE 1 | The genomic statistics information and source of 19 strains used in this study.

| Organism/name   | Strain     | Genbank or JGI accession number | Level and database | Size (Mb) | Coding density (%) | GC% | Gene | Protein | Environment | Address                     |
|-----------------|------------|---------------------------------|--------------------|-----------|--------------------|-----|------|---------|-------------|-----------------------------|
| *M. tengchongensis* | Ric-A      | 2821472399/CP049074             | Complete NCBI      | 2.10      | 85.4               | 44.8 | 2331 | 2295    | Acidic hot spring          | China: Yunnan                |
| *M. sedula*      | DSM 5348   | CP000682.1                      | Complete NCBI      | 2.19      | 89.3               | 46.2 | 2377 | 2298    | Solfataric field           | Italy                       |
| *M. sedula*      | ARS50-1    | CP012172.1                      | Complete NCBI      | 2.19      | 89.4               | 46.2 | 2375 | 2297    | Lab                        | United States               |
| *M. sedula*      | ARS50-2    | CP012173.1                      | Complete NCBI      | 2.19      | 89.4               | 46.2 | 2377 | 2298    | Lab                        | United States               |
| *M. sedula*      | ARS120-1   | CP012174.1                      | Complete NCBI      | 2.19      | 89.3               | 46.2 | 2376 | 2298    | Lab                        | United States               |
| *M. sedula*      | ARS120-2   | CP012175.1                      | Complete NCBI      | 2.19      | 89.3               | 46.2 | 2376 | 2298    | Lab                        | United States               |
| *M. sedula*      | SARC-M1    | CP012176.1                      | Complete NCBI      | 2.19      | 89.3               | 46.2 | 2379 | 2301    | Lab                        | United States               |
| *M. sedula*      | CSR1       | CP008822.1                      | Complete NCBI      | 2.19      | 89.2               | 46.2 | 2373 | 2289    | Lab                        | United States               |
| *M. hakonensis*  | HO1-1      | CP029287.1                      | Complete NCBI      | 2.54      | 80.3               | 43.7 | 2785 | 2736    | Acidic hot spring          | Japan                       |
| *M. hakonensis*  | JCM 8857   | GCA_001315825.1                 | Draft NCBI         | 2.39      | 81.5               | 43.3 | 3312 | 3292    | Acidic hot spring          | Japan                       |
| *M. cuprina*     | Ar-4       | CP002656.1                      | Complete NCBI      | 1.84      | 87.9               | 42.0 | 1968 | 1894    | Acidic hot spring          | China: Yunnan               |
| *Metallosphaera* | sp. UBA165 | GCA_001652185.1                 | Draft NCBI         | 1.83      | 88.5               | 45.7 | 3186 | 2821    | Hot spring                 | Taiwan                      |
| *M. yellowstonensis* | MK1       | GCA_000243315.1                 | Draft NCBI         | 2.82      | 82.4               | 47.7 | 3411 | 3356    | Acidic hot spring          | United States: Yellowstone National Park |
| *Metallosphaera* | sp. My-r02 | 2522215033                      | Draft IMG-M        | 1.85      | 82.5               | 47.4 | 2419 | 2366    | Acidic hot spring          | United States: Yellowstone National Park |
| *Metallosphaera* | sp. My-r05 | 2551306706                      | Draft IMG-M        | 1.85      | 87.9               | 47.4 | 1561 | 1520    | Acidic hot spring          | United States: Yellowstone National Park |
| *Metallosphaera* | sp. My-r06 | 2551306703                      | Draft IMG-M        | 1.85      | 89.0               | 48.3 | 1496 | 1455    | Acidic hot spring          | United States: Yellowstone National Park |
| *Metallosphaera* | sp. YNP_14 | 2502873002                      | Draft IMG-M        | 1.38      | 84.5               | 47.2 | 2093 | 2061    | Thermal springs            | United States: Yellowstone National Park |
| *Metallosphaera* | sp. YNP_08 | 2502894001                      | Draft IMG-M        | 1.10      | 78.4               | 43.9 | 2275 | 2252    | Thermal springs            | United States: Yellowstone National Park |
| *Metallosphaera* | sp. Obs4   | 2770939403                      | Draft IMG-M        | 2.70      | 83.2               | 50.4 | 3060 | 2968    | Hot spring sediment        | United States: Yellowstone National Park |
for 7 days at 65°C. Colonies were picked and purified by re-plating. The purified strain Ric-A was grown at 70°C in BSM (pH 2.0) supplemented with 1 g/L yeast extract. The stationary-phase cells were harvested by centrifugation. The genomic DNA was extracted from the concentrated cells according to the instruction of “JGI Bacterial DNA isolation CTAB-2012.” After checking its quality, DNA was fragmented and the fragments were end-repaired and polyadenylated, and then ligated to sequencing adapter. SMRTbell DNA library was constructed by using Blue Pippin Size-Selection System; library quality was evaluated by Qubit 3.0 Fluorometer (Life Technologies, Grand Island, NY, United States) and sequenced by PacBio Biosciences (PacBio) RSII and Genome Analyzer IIx sequence platforms at Chinese National Human Genome Center at Shanghai (CHGC). After sequencing, the low-quality reads were filtered by the SMRT 2.3.0 (Chin et al., 2013), and the filtered reads were assembled to generate one contig without gaps. The hierarchical genome-assembly process (HGAP.3) pipeline implemented in SMRT Analysis 2.3.0 was used to correct for random errors in the long seed reads (seed length threshold 6 Kb) by aligning shorter reads from the same library against them. The resulting corrected, preassembled reads were used for de novo assembly. Genome data of 18 previously sequenced strains belonging to Metallosphaera were collected from NCBI and IMG-M database. The detailed genomic statistics information and source of 19 Metallosphaera strains used in this study were summarized in Table 1.

Average Nucleotide Identity and Whole Genome Alignments
Comparisons of average nucleotide identity (ANI) based on Blast algorithm were conducted using the pyani module1 with default parameters. We applied the “progressive Mauve program” within Mauve v 2.3.0 (Darling et al., 2004) for constructing and visualizing multiple genome alignments of M. tengchongensis Ric-A with four other available complete genome sequences of M. sedula DSM 5348, M. hakonensis HO1-1, M. cuprina Ar-4, and M. yellowstonensis MK1. BlastN-based whole-genome comparison of strains M. cuprina Ar-4, M. hakonensis HO1-1, Metallosphaera sp. My-r06, M. sedula DSM 5348, M. tengchongensis Ric-A, and M. yellowstonensis MK1 were performed and represented with BRIG-0.95 (Alikhan et al., 2011), and these strains were used as references, respectively. GC content and GC skew of each genome were also indicated.

Phylogenomic Analyses
We constructed a phylogenetic tree of the 19 Metallosphaera spp. genomes based on whole-genome sequences with CVTree3 (Xu and Hao, 2009). The phylogenetic tree of the 19 genomes based on concatenation of the 85 core genes in a genome was constructed with the neighbor-joining (NJ), UPGMA, and maximum-likelihood (ML) method using MEGA-X (Kumar et al., 2018) with 1000 bootstrap replications.

Genome Annotation
We applied Prokka (Seemann, 2014) and IMG Annotation Pipeline v.4.16.0 (Chen et al., 2017) for genome annotation and putative horizontally transferred gene detection. We performed Diamond BlastP v0.9.24 (Buchfink et al., 2015) with a cutoff e-value of 1e−5 together with the dbCAN database v2.0 (Le et al., 2018) for identification of genes related to carbohydrate activity enzyme (CAZymes). The BacMet v2.0 database (Pal et al., 2014) that contained genes with experimentally confirmed metal resistance function was used to identify the genes associated with metal resistance in Metallosphaera genomes. Gene annotations based on COG (Tatusov et al., 2001), Pfam (Finn et al., 2014), and TIGRFAM (Selengut et al., 2007) databases were performed via WebMGA (Wu et al., 2011) using Blast with a cutoff e-value of 1e−5.

Prediction of Mobile Genetic Elements
We applied the ISFinder (Siguié et al., 2006)4 to predict and classify insertion sequences (IS) and transposases within Metallosphaera genomes with Blastp v0.9.24 (cutoff e-value of 1e−5). We applied the IslandViewer 4 (Bertelli et al., 2017),5 which integrated prediction methods including IslandPath-DIMOB and SIGI-HMM that analyzed sequence composition, and another comparative genomic islands (GIs) prediction method IslandPick, to detect putative GIs distributed over Metallosphaera genomes. We applied PHASTER (Phage Search Tool Enhanced Release) (Arndt et al., 2016)6 for detection and annotation of prophage and prophage remnant sequences within Metallosphaera genomes. We also applied CrisprCasFinder (Couvin et al., 2018)7 for detection of CRISPRs and Cas genes within Metallosphaera genomes.

Comparative Genomic Analyses of Metallosphaera
The Bacterial Pan Genome Analyses tool (BPGA) pipeline (Chaudhari et al., 2016) was used to perform pan/core-genome analyses and calculation applying default parameters. The size of the Metallosphaera pan-genome was fitted into a power law regression function \( P_n = k n^\gamma \) with a built-in program of BPGA (Chaudhari et al., 2016), in which \( P_n \) was the total number of gene families, \( n \) stood for the number of tested genomes, and \( \gamma \) was free parameters. If exponent \( \gamma < 0 \), then the pan-genome of Metallosphaera was suggested to be “closed.” In this case, the size of the pan-genome is relatively constant, even if new genomes were added into the analysis. On the contrary, the pan-genome was suggested to be “open” in the case of \( 0 < \gamma < 1 \). In addition, the size of the core-genome of Metallosphaera was fitted into an exponential decay function \( F_n = k \exp(-n/\tau_c) \) with a built-in program of BPGA pipeline (Chaudhari et al., 2016), in which \( F_n \) stood for the number of core genes families, while \( k \) and \( \tau_c \) were free parameters. Gene family clustering followed by genome-wide comparisons of five

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1https://jgi.doe.gov/user-programs/pmo-overview/protocols-sample-preparation-information/
2https://www.pacb.com/support/software-downloads/
3https://pypi.org/project/pyani/
4http://www.is-biotoul.fr
5https://www.pathogenomics.sfu.ca/islandviewer/
6http://phaster.ca/
7https://crisprcas.i2bc.paris-saclay.fr/CrisprCasFinder/Index
Metallosphaera-type strains including *M. tengchongensis* Ric-A, *M. sedula* DSM 5348, *M. hakonensis* HO1-1, *M. cuprina* Ar-4, and *M. yellowstonensis* MK1 together with UniProt search, Gene Ontology (GO) Slim annotation, and GO enrichment analyses (default cutoff p-value is 0.05) were performed via OrthoVenn (Wang et al., 2015) with default parameters.

**Gene Family Evolution Analyses**

Count is a software designed to analyze numerical profiles of homologous gene families on a phylogeny, which can execute ancestral reconstructions and predict family- and lineage-specific characteristics along the evolutionary tree (Miklós, 2010). We used Count software, combined BPGA v2.0 pipeline, and Wagner parsimony algorithm (Farris, 1970) for gene family clustering, ancestor genome size estimation, and detecting gene family gain and loss events, together with family expansion and contraction events with penalty ratio set to 1. We conducted the analyses only on five *Metallosphaera*-type strains, including *M. tengchongensis* Ric-A, *M. sedula* DSM 5348, *M. hakonensis* HO1-1, *M. cuprina* Ar-4, and *M. yellowstonensis* MK1 taken into consideration, due to the estimation requiring complete sets of testing gene families available only in species with high-quality genome databases.

**Selective Pressure Analyses**

We detected the numbers of sites under negative (purifying) or positive (diversifying) selection and estimated global dN/dS values of each gene family that contained more than three non-identical sequences (due to limitation of HyPhy) based on multiple alignments of orthologous codon sequences and a tree topology by means of HyPhy (Wang et al., 2015) based on multiple alignments of orthologous than three non-identical sequences (due to limitation dN/dS values of each gene family that contained more or positive (diversifying) selection and estimated global.

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**RESULTS AND DISCUSSION**

**General Genome Features of *M. tengchongensis* and Other Strains**

A total of 2331 CDS, including 32 tRNA and 4 rRNA, were predicted in the genome of *M. tengchongensis* strain Ric-A using Prokka (Seemann, 2014). Whole-genome BLASTN-based ANI analyses showed only an ANI of 72.3% compared with the closest *Metallosphaera* genome (Supplementary Table S1). A summary of features for the 19 *Metallosphaera* genomes is listed in Table 1. *M. yellowstonensis* MK1 possessed the largest genome (2.82 Mb).

Core- and Pan-Genome and Phylogenomic Analysis of *Metallosphaera*

The phylogenetic trees based on the concatenated alignment of 85 core genes inferred with NJ, ML, MP, and UPGMA methods were congruent with each other, and the phylogenetic tree based on whole-genome analyses was congruent with the core-gene phylogenetic tree (Supplementary Figures S4, S5). Strain Obs4 is located on a clade apart from other *Metallosphaera* strains (Supplementary Figures S1, S4, S5). However, strain Obs4 should still be considered a member of genus *Metallosphaera* based on percentage of conserved proteins (POCP). Strain Obs4 had a POCP of 50.2% against *M. yellowstonensis* MK1, within the genus cutoff value of 50% (Qin et al., 2014). The pan-genome of 19 *Metallosphaera* strains possessed 6499 gene families, while the core-genome possessed only 85 gene families. Core- and pan-genome analyses of the 19 *Metallosphaera* genomes revealed an “open” pan-genome fitted into a power law regression function \( \beta (\gamma) = 1955.63 n^{0.372819} \) with a parameter (\( \gamma \)) of 0.372819 falling into the range 0 < \( \gamma \) < 1. The core-genome was fitted into an exponential regression \( \beta (\gamma) = 1886.87 e^{-0.16335 n} \), which had a steep slope, reaching a minimum of 85 gene families after the 19th genome was added (Figure 2B). The result of COG annotation revealed that the core-genome had a higher proportion of genes involved in COG categories that are associated with central biological functions translation, ribosomal structure, and biogenesis (I); posttranslational modification, protein turnover, chaperones (O); and coenzyme transport and metabolism (H) than the accessory genome and strain-unique genome. In contrast, the accessory genome had a higher proportion of genes related to COG energy production and conversion (C) and lipid transport and metabolism (I). We found that strain-specific gene families had a higher proportion of genes categorized in COG replication, recombination and repair (L), cell wall/membrane/envelope.
We propose that these genes are associated with adaptive evolution within the genus *Metallosphaera*. The oligotrophic, metal-laden, and extremely acidic environments select for a highly efficient DNA injury repair system and a flexible trophic mode in *Metallosphaera*. The genomic diversity and specificity of different *Metallosphaera* strains are reflecting their distinct survival strategies in different environments.

**Metabolic and Functional Potential**

**Sulfur Metabolism**

Sulfur and hydrogen are important in energy flow in thermal environments, such as marine hydrothermal systems, continental solfataras, and hot springs, where many bacteria and archaea can grow by oxidizing hydrogen, sulfide, elemental sulfur, and thiosulfate (Amend and Shock, 2001; Zeldes et al., 2019). It was reported that *Metallosphaera* could utilize different sulfur compounds as energy sources for growth (Peng et al., 2015). The genes *sqr* encoding for sulfide:quinone oxidoreductase (SQR), which catalyzes the oxidation of hydrogen sulfide forming polysulfide, were detected in all 19 genomes of *Metallosphaera*. Unlike the genus *Acidianus* and certain members of *Sulfolobus*, the genes encoding the homologs of sulfur oxygenase/reductase (SOR), a key enzyme for archaean sulfur oxidation (Kletzin, 1992; Chen et al., 2005; Urich et al., 2006; Dai et al., 2016), were not found in the genomes of all strains of *Metallosphaera*. Unlike the genus *Acidianus* and certain members of *Sulfolobus*, the genes encoding the homologs of sulfur oxygenase/reductase (SOR), a key enzyme for archaean sulfur oxidation (Kletzin, 1992; Chen et al., 2005; Urich et al., 2006; Dai et al., 2016), were not found in the genomes of all strains of *Metallosphaera*. However, sulfur dioxygenases (SDO, *sdo*) were encoded in all strains except for *M. cuprina* and *Metallosphaera* sp. Obs4 (Figure 3). In addition, sulfite-acceptor oxidoreductases (SAOR, *saor*) genes were also detected in all strains (Figure 3). *Metallosphaera* spp. also harbored genes encoding for tetrathionate hydrolase (TTH, *tth*), sulfite reductase (SIR, *sir*), and genomic clusters...
FIGURE 2 | Pan-genome analyses of strains in the genus Metallosphaera. (A) NJ phylogenetic tree based on concatenated 85 core genes (1000 bootstrap replicates) of 19 Metallosphaera strains (left) and stack bar diagram showing sizes of orthologous genes shared by all strains (i.e., the core-genome), number of orthologous genes shared by partial strains (i.e., the accessory genome), and number of strain-specific gene families (i.e., the unique gene) in each strain. (B) The curve of the fitting function and the estimation of pan-genome and core-genome sizes of Metallosphaera. $Ps$ and $Fc$ stand for the total number of gene families and the number of core gene families, respectively. More details for modeling approaches are presented in Section 2.6 “Comparative Genomic Analyses of Metallosphaera”. (C) Bar chart showing proportions of COG categories of the different parts of Metallosphaera pan-genome (i.e., core, accessory, unique). COG categories description (J, translation, ribosomal structure and biogenesis; K, transcription; L, replication, recombination and repair; D, cell cycle control, cell division, chromosome partitioning; T, signal transduction mechanisms; M, cell wall/membrane/envelope biogenesis; N, Cell motility; U, intracellular trafficking, secretion, and vesicular transport; O, posttranslational modification, protein turnover, chaperones; C, energy production and conversion; G, carbohydrate transport and metabolism; E, amino acid transport and metabolism; F, nucleotide transport and metabolism; H, coenzyme transport and metabolism; I, lipid transport and metabolism; P, inorganic ion transport and metabolism; Q, secondary metabolites biosynthesis, transport and catabolism; R, general function prediction only; S, function unknown).

encoding for the thiosulfate-quinone oxidoreductase (TQO) subunits ($doxA/doxD$) and heterodisulfide reductase (HDR, $hdrC1-hdrB1A-hyp-hdrC2-hdrB2$) complex. The genes encoding for thiosulfate sulfurtransferase (TST, $tst$) were also detected in all species (Figure 3). According to the genome composition, we speculate that the pathway of sulfur metabolism for Metallosphaera might be SDO oxidized sulfur to sulfite, SAOR oxidized sulfite to sulfate, and TQO was responsible for the transformation of thiosulfate to tetrathionate, whereas TTH catalyzed the tetrathionate hydrolysis into thiosulfate, sulfur, and sulfate. However, the function of some proteins, especially for SDO and SAOR, had not been verified in Metallosphaera members. TST and HDR catalyzed the mutual conversion of thiol proteins (RSH) and sulfane sulfates (RSSH) in the...
hydrogen and sulfur-containing compounds were only detected that are potentially associated with electron transfer between the structural and auxiliary proteins of Ni/Fe hydrogenase. Genes (e.g., hynL, hynK, hypC, hypD, and hypY) were found on the genomes of all strains (Laska et al., 2003) (Supplementary Figure S6).

**Iron Metabolism**

The fox genes involved in iron oxidation (Kozubal et al., 2011) were detected in all Metallosphaera-type species, but *M. cuprina* may have lost the subunits foxD, foxE, foxF, and foxI (Supplementary Figure S7). The arrangement of fox genes was similar in *Metallosphaera* spp. following the pattern foxA′-I-B-C-D-E-F-J-G-H, but it is different from other members of iron-oxidizing Sulfolobales (*Figure 4*), and in *M. hakonensis* HO1-1, a third copy of foxA was found within this cluster, probably resulting from gene duplication (*Figure 4*). The transcription initiation directions of these fox genes were not consistent, and their open reading frames (ORFs) were separated by spacers (*Figure 4*). Genes encoding for terminal oxidase complex cbsAB/soxNL were found downstream of fox cluster in *M. sedula* and *M. yellowstonensis* Ric-A, these two clusters were separated by a genome region of about 180 kb (*Figure 4*). A gene for sulfocyanin (SoxE), a blue copper-containing protein that may function as a temporary electron storage or electron carrier in the iron-oxidizing electron transport chain (Kozubal et al., 2011), occurred in all *Metallosphaera* genomes in this study. Genes encoding for multicopper blue protein (mco) that contained two plastocyanin type I copper domains were only detected in *M. yellowstonensis* and *M. sedula* (Kozubal et al., 2011). These oxidases may couple the reduction of oxygen to proton translocation in cooperation with Fox complex mentioned-above (Auernik and Kelly, 2008; Kozubal et al., 2011).
FIGURE 4 | Synteny analysis of fox gene clusters and soxNL/cbsAB gene clusters derived from representative Metallosphaera strains and other representative species in Sulfolobales.

Carbon Metabolism

*Metallosphaera* spp. contained an abundant repertoire of carbohydrate-active enzymes (CAZymes) including carbohydrate esterases (CEs), carbohydrate binding molecules (CBMs), glycosyltransferases (GTs), glycoside hydrolases (GHs), auxiliary activity proteins (AAs), and a small number of polysaccharide lyases (PLs), of which GTs were most abundant, and *Metallosphaera* sp. Obs4 possessed the most carbohydrate active enzymes (Supplementary Figure S7). The diversity of encoding genes for CAZymes possibly reflected different carbohydrate metabolism strategies in *Metallosphaera*. A complete set of genes encoding for glycolysis, gluconeogenesis, the archaeal pentose phosphate pathway, as well as an atypical TCA cycle (replacing genes encoding the alpha-ketoglutarate dehydrogenase with those encoding 2-oxoacid:ferredoxin oxidoreductase) were detected in all *Metallosphaera*
species. *Metallosphaera* spp. also contained complete non-phosphorylative and semi-phosphorylative Entner–Doudoroff (ED) pathways. Genes encoding homologous enzymes for the complete 3-hydroxypropionate/4-hydroxybutyrate (HP/HB) cycle and the dicarboxylate/4-hydroxybutyrate (DC/HB) cycle involved in autotrophic carbon fixation (Berg et al., 2007, 2010; Jiang et al., 2014) were also found in all *Metallosphaera* genomes (Supplementary Figure S9). It is reported that carbon monoxide was ubiquitous in hydrothermal habitats (King and Weber, 2007). All members of *Metallosphaera* in this study possessed putative type I carbon monoxide dehydrogenase (CODH) encoded by gene cluster *coxLSM*; however, the typical active site motif (VAYRCSFR) of CODH (Dobbek et al., 2002) was not observed in these proteins, which indicated that these CODH probably do not use CO; alternatively, they could possess a novel active site motif (Supplementary Figure S9).

**Nitrogen Metabolism**

All *Metallosphaera* strains possessed genes encoding for nitrate reductase and nitrite reductase involved in assimilatory nitrate reduction. Except for *narG* genes in seven strains of *M. sedula*, the *narGHJI* operon encoding for dissimilatory nitrate reductase was only found in *M. yellowstonensis* MK1 (Supplementary Figure S10). This operon was located on a GI in *M. yellowstonensis* MK1, suggesting that it might be acquired through horizontal gene transfer (HGT) events (Supplementary Table S2; Bertelli et al., 2017). Urease gene clusters consisting of the functional subunits (*ureAB* and *ureC*) and accessory proteins (*ureE*, *ureF*, and *ureG*) were found in all *Metallosphaera* species except for *M. yellowstonensis* (Supplementary Figure S10), indicating that urea assimilation was employed by most *Metallosphaera* strains to provide sufficient ammonia by converting one urea molecule into one carbon dioxide molecule plus two ammonia molecules. Genes encoding for nitriase and formamidase, metabolizing organic nitrogen to ammonia, and genes encoding for ammonium transport (*amt*) and ammonia-dependent biosyntheses, such as carbamoyl-phosphate synthases (*carAB*), glutamate dehydrogenases (*gdha*), and glutamine synthetases (*gna*), were found in the genomes of all *Metallosphaera* species (Supplementary Figure S10). There is also a complete set of genes involved in arginine synthesis in all *Metallosphaera* species (Supplementary Figure S10). Polyamine derived from arginine can stabilize DNA by protecting DNA from free radical attacks and thermal denaturation (Abby et al., 2018). Polyamine biosynthesis-related genes that encoded for agmatinase (*speB*), S-adenosylmethionine decarboxylase (*speD*), and spermidine synthase (*speC*) were found in the genomes of all *Metallosphaera* species, while the arginine decarboxylase gene (*speA*) for the first two steps of putrescine biosynthesis was not detected; its function may have been substituted by S-adenosylmethionine decarboxylase (*speD*) (Giles and Graham, 2008).

**Heavy Metal Resistance**

*Metallosphaera* can survive natural and anthropogenic metal-rich environments, and BacMet database annotations revealed an abundant repertoire of heavy metal resistance genes in *Metallosphaera* spp., exhibiting diverse strategies to avert the deleterious effect of toxic metals on biological function (Supplementary Figure S11). Most of the genes related to arsenic resistance (*aioAB*: arsenite oxidase; *arsABR*: arsenical pump-driving ATPase; *arsM*: methyltransferase), divalent-cation resistance (*corABZ*, *cuaA*, *cutA*, *czzD*, *crrB*, *ncn*, *nikABC*, and *mntRH*), mercury resistance (*merA*: mercuric reductase), and iron regulation (*fcdE*, *furA*, *flipC*) were found in all tested *Metallosphaera* genomes. Alkylmercury lyase (encoded by gene *merB*) that cleaved mercury-alkyl bonds for mercury detoxification (Melnick and Gerard, 2007) was only detected in *M. yellowstonensis*. Genes encoding for “DNA-binding protein from starved cells” (Dps) (Blake et al., 2005), which can physically shield DNA against oxidative damage, were only detected in strains of *M. sedula* and *Metallosphaera* sp. UBA1654. Dps proteins were also able to control Fenton reaction through storing ferric oxide as a mineral core on their interior cage surface (Blake et al., 2005).

**Adhesion and Motility**

Etracellular polysaccharides (EPS) play a significant role in cell adhesion and biofilm formation, which is closely related to colonization, mineral solubilizing ability, and protection against adverse environmental conditions (Basak et al., 2014; Marino et al., 2018; Yu et al., 2019). Gene clusters encoding for d-TDP-glucose pyrophosphorylase (*rfbA*), d-TDP-glucose 4,6-dehydratase (*rfbB*), d-TDP-4-dehydrorhamnose 3,5-epimerase (*rfbC*), and d-TDP-4-dehydrorhamnose reductase (*rfbD*), which can convert glucose-1-phosphate to the EPS precursor d-TDP-rhamnose via a series of reactions, were found in all *Metallosphaera* genomes (Supplementary Figure S9). Motility conferred by flagella can provide a competitive advantage for microorganisms to move toward beneficial conditions. From the genomic and functional analysis, it is found that the sequenced archaea maintain a unique flagellum composition and mode of assembly, distinct from the bacteria. Archaeal flagellin commonly anchors on a fla locus, which was encoded by 7–13 flagella-related genes. Studies of the flagella system of the crenarchaeal model strain *Sulfolobus acidocaldarius* identified seven *fla* genes called *flaB*, *flaX*, *flaG*, *flaE*, *flaH*, *flaI*, and *flaJ*, and confirmed that all seven genes were essential for assembly and motility (Thomas et al., 2001; Lassak et al., 2012; Dominik et al., 2016). However, in the genomes of 19 strains of *Metallosphaera*, genes encoding for crenarchaeal flagellin (*flaB*) and flagella accessory proteins (*flaH*, *flaI*, *flaG*, *flaE*, and *flaJ*) were only detected in strains of *M. sedula*, *M. yellowstonensis*, and *Metallosphaera* sp. UBA165. Further, a unique crenarchaeal gene *flaX*, coding for a structural part of the archaean flagellum assembly apparatus only present in *Metallosphaera* sp. Obs4 and *Metallosphaera* sp. My-r06 (Supplementary Figure S12). The roles of FlaX and other accessory proteins in *Metallosphaera* members need further experimental verification. Genome comparative results indicated that the flagellum assembly apparatus did not present in every genome of these strains, which is consistent with the motility difference of *Metallosphaera* strains known by their physiological research (Peng et al., 2015). The genomes of *M. tengchongensis* Ric-A and *M. hakonensis* DSM 7519 only
Metallosphaera attack and mediate interactions between the host and phage. The associated genes systems are attested to confer resistance to viral extracellular translocation abilities associated with HGT and phages, are genome segments that display intra- and/or Mobile genetic elements, including GIs, IS, transposons, CRISPR-Cas Systems have motility (Peng et al., 2015).

Wang et al. Metabolism and Evolution of Metallosphaera in accessory genes families were related to electron transport functions including translation (GO:0006412), rRNA binding from 0 to 27 (M. sedula 2019). The number of prophages and prophage remnants ranged survival and protect functionalities endowed by HGT events may facilitate adaptive 487 (M. yellowstonensis sequences per genome ranged from 65 (M. cuprina Ar-4) to 283 (M. yellowstonensis MK1) and the number of GI-related sequences per genome ranged from 21 (M. cuprina Ar-4) to 487 (M. yellowstonensis MK1) (Supplementary Tables S2, S3). GI regions of Metallosphaera spp. harbored genes related to the formation of extracellular polysaccharides (EPS) that account for ~2.4% of all sequences, defense and DNA repair systems (~2.7%), and stress resistance (~2.0%). These additional functionalities endowed by HGT events may facilitate adaptive survival and protect Metallosphaera against DNA damage and protein denaturation in its acidic hot spring habitat (Yu et al., 2019). The number of prophages and prophage remnants ranged from 0 to 27 (M. sedula DSM5348) (Supplementary Table S3). Type I-A and/or type III-A/D CRISPR-Cas systems were found in all the genomes in this study except for Metallosphaera sp. My-r06. M. tengchongensis Ric-A contained the most (410) CRISPR-Cas-related genes or spacers. CRISPR-Cas-related sequences were detected in predicted GI regions of all Metallosphaera spp. except for M. cuprina Ar-4, reflecting the mobility of CRISPR-Cas systems (Koonin and Makarova, 2017; Krupovic et al., 2017; Peters et al., 2017).

Gene Ontology Enrichment and Evolutionary Analyses of Five Metallosphaera-Type Strains

We further applied the software OrthoVenn (Yi et al., 2015) and Count (Miklós, 2010) for gene clustering, GO enrichment analyses, and gene family evolutionary analyses of five Metallosphaera-type strains including M. tengchongensis Ric-A, M. sedula DSM 5348, M. hakonensis HO1-1, M. cuprina Ar-4, and M. yellowstonensis MK1. Results showed that 1614 (68.6%) out of 2353 identified gene families were shared by all species. M. sedula DSM 5348 had the most gene families (2133) in its genome, whereas M. cuprina Ar-4 had the fewest (1827), and M. yellowstonensis MK1 possessed the most strain-specific gene families (42) followed by M. tengchongensis Ric-A (18) (Figure 5). GO enrichment analyses showed that in significantly enriched (p-value < 0.05) commonly shared gene families, GO terms were associated with basic biological functions including translation (GO:0006412), rRNA binding (GO:0019843), and ribosome-related function (GO:0005840; GO:0003735). Functions significantly enriched (p-value < 0.05) in accessory genes families were related to electron transport chain (GO:0022900; GO:0008137; GO:0042773; GO:0048038; GO:0006741; GO:0008121; GO:0003951), lipid metabolism-related long-chain fatty acid-CoA ligase activity (GO:0004467), and nutrient transport and catabolic process of nitrogen sources such as urea (GO:0043419; GO:0090939) and carbon sources such as lactate (GO:0035873), mandelate (GO:0019596), glycolate (GO:0097339), benzoylformate (GO:0050695), and maleylacetate (GO:0018506) related to heterotrophic lifestyle. Proton symporter activity (GO:0015295; GO:0015538) involved in pH homeostasis, amino acid biosynthesis-related denitrification pathway (GO:0019333), and sulfur metabolism-related functions including sulfate assimilation (GO:0000103), hydrogen sulfide biosynthetic process (GO:0070814), SIR (GO:0050311) together with hydrogenase-related functions (GO:0033748; GO:0008901), arginine metabolism (GO:0016990; GO:0019547), and cysteine biosynthesis process (GO:0019344) were also enriched (p-value < 0.05) in accessory gene families (Figure 5). These functions and pathways probably reflected the adaptation of Metallosphaera spp. to acidic, sulfur-rich, heat, and metal-laden environments. Metallosphaera may take advantage of hydrogen sulfide for cysteine biosynthesis. Disulfide bonds in thermophilic proteins are omnipresent in thermophiles and help stabilize proteins against the harsh conditions (Beeby et al., 2005). In addition, cysteine-rich proteins such as disulfide oxidoreductase can be used by microorganisms to chelate heavy metal ions in the cytoplasm so as to reduce metal-induced reactive oxygen species (ROS), which is supported by previous results that Cu${}^{2+}$ exposure induced assimilatory sulfur metabolism for cysteine biosynthesis in Metallosphaera (Wheaton et al., 2016; Meslé et al., 2017). Interestingly, functions enriched (p-value < 0.05) in strain-specific gene families of M. yellowstonensis MK1 were related to aromatic compound metabolism such as catabolic process of benzoate (GO:0018623; GO:0043640), phthalate methyltransferase-related functions (GO:0007165); these genes are probably involved in arsenic detoxification by catalyzing the formation of volatile trimethylarsine from arsenite (Qin et al., 2006; Ai et al., 2017).

To decipher the evolutionary histories of the Metallosphaera species, gene family gain, loss, expansion, and contraction events were predicted by mapping the identified gene families onto the core-gene tree (Figure 6). A large number of gene family gain events occurred at node 1, accounting for ~6% of gene families, and at the branches of M. yellowstonensis and M. sedula, accounting for ~10 and 6% of gene families, respectively. By means of gene acquisition, members of Metallosphaera have largely expanded their genetic diversity, resulting in functional divergence, which was similar to other Archaea (Brügger et al., 2002). Of gene families undergoing gain events, about half of them were poorly characterized, and a considerable proportion of them were related to COG (X) Mobiliome: prophages, transposons (~6%), COG (K) Transcription (~3%),
and COG (V) Defense mechanisms (~3%, which mostly were associated with CRISPR-Cas system) (Figure 6). Several sulfate assimilation and archaeal flagella biosynthesis-related genes were gained at the branches of *M. yellowstonensis* and *M. sedula*, indicating that sulfate assimilation and flagella biosynthesis were derived features of these strains (Supplementary Table S4). Several DNA damage repair genes (e.g., *uve* and *spl*) that helped maintain DNA fidelity were gained at the branches of *M. hakonensis*, *M. tengchongensis*, *M. sedula*, *M. yellowstonensis*, and node 1, and genes associated with EPS synthesis (e.g., *rfa* and *gal*) that enhanced colonization were gained in the branches of *M. sedula* and *M. yellowstonensis* (Supplementary Table S4). Several oxidoreductase encoding genes that associated with carbohydrate metabolism (e.g., *porAB* and *acoAB*) were gained at the branches of *M. sedula* and *M. tengchongensis*. A few of oxidoreductase genes related to aromatic compound
FIGURE 6 | Ancestral genome content reconstruction of Metallosphaera species with COUNT software. The numbers of gene families of each strain were shown in brackets before the names of strains. The numbers of gain and loss events were marked at each lineage of the tree. “+”s represent gain events and “−”s represent loss events. The pie chart shows the numbers of gained genes by COG categories. A list of gained and lost genes for the Metallosphaera-type strains, and nodes 1 and 2 are shown in Supplementary Table S4. COG categories description (J, translation, ribosomal structure and biogenesis; K, transcription; L, replication, recombination and repair; D, cell cycle control, cell division, chromosome partitioning; V, defense mechanisms; T, signal transduction mechanisms; M, cell wall/membrane/envelope biogenesis; N, cell motility; U, intracellular trafficking, secretion, and vesicular transport; O, posttranslational modification, protein turnover, chaperones; X, mobility; prophages, transposons; G, energy production and conversion; E, amino acid transport and metabolism; F, nucleotide transport and metabolism; H, coenzyme transport and metabolism; I, lipid transport and metabolism; P, inorganic ion transport and metabolism; Q, secondary metabolites biosynthesis, transport and catabolism; R, general function prediction only; S, function unknown).

Degradation (e.g., hca and nfn) were gained at the branches of M. yellowstonensis, M. sedula, and node 2. Genes encoding for putative type I CODH were expanded in M. sedula but contracted in M. yellowstonensis (Supplementary Table S4). Sulfocyanin encoding genes were expanded in M. hakonensis, M. tengchongensis, and Heme/copper-type cytochrome/quinol oxidase encoding genes associated with sulfur and iron oxidation (Supplementary Table S4) were expanded in the branches of
node 1 and *M. yellowstonensis*. Indicating their importance in niche adaption, several genes involved in nitrogen transport and metabolism were expanded in several species: ammonia permease encoding gene *amnB* in *M. cuprina* and *M. tengchongensis*, nitrite reductase encoding gene *mirD* in *M. hakonensis* and *M. sedula*, and adenosylmethionine decarboxylase encoding gene *speD* involved in polyamine biosynthesis in *M. yellowstonensis*. However, the urease encoding gene operon *ureABCDEF* was lost at the branch of *M. yellowstonensis* (Supplementary Table S4). In contrast to conspicuous gene acquisitions in other *Metallosphaera* strains, gene family loss events occurred frequently in *M. cuprina* taking up ~6% of gene families, which mostly compromised genes belonging to COGs Energy production and conversion (C) and Carbohydrate transport and metabolism (G) such as permease of fucose, sugar, arabinose, dehydrogenase of succinate, tartrate, malate/L-lactate, aldehyde and alronate, and oxidase of sulfite (Supplementary Table S4). Genomic streamlining in adaptation to the acidic, thermal, and oligotrophic environment may be the main reason for gene family losses in *M. cuprina*; previous studies showed that growth temperature was negatively correlated with genome size in bacteria (Sabath et al., 2013) and that deletion of dispensable sequences from bacterial genomes led to dose-dependent growth (Kurokawa et al., 2016; Zhang et al., 2017; Ren et al., 2019).

**Selective Pressure Analyses**

Functions undergoing rapid evolution can be distinguished, taking into account the functional categories and selective pressure (Carretero-Paulet et al., 2015; Zhong et al., 2018). To gain insight into the conservation and evolution of various gene families in *Metallosphaera*, evolutionary pressure on each gene family that contained at least three non-identical sequences in five species of *Metallosphaera* was measured by calculating global substitution rates (dN/dS) of non-synonymous (dN) to synonymous (dS), as well as numbers of mutation sites under significant negative or positive selection in each gene family. Results indicated that there was pervasive strong purifying (negative) selection (dN/dS < 1) acting on the gene families of *Metallosphaera* with no gene families showing positive selection (dN/dS > 1), among them 86.6% gene families showing a dN/dS ratio lower than 0.1 (Figure 7 and Supplementary Table S5), which emphasized purifying selection contributing largely to the long-term stability of *Metallosphaera* genomes by removing deleterious mutations. Gene families of *Metallosphaera* exhibit different degrees of purifying selection pressure as shown by COG annotation. Genes undergoing the strongest purifying selection were those related to Transcription (K), indicating that these functions were highly conserved, while genes related to mobiome: prophages, transposons (X) and Defense mechanisms (V) were under weaker purifying pressure since they showed relatively higher dN/dS ratios and more sites under diversifying (positive) selection, which indicated that these genes could gain specific adaptive mutations associated with the acquisition of new or adaptive functions (Figure 7 and Supplementary Table S5).

**Genome Expansion Through HGT**

Horizontal gene transfer is one of the important engines that drive genomic diversity and adaptive evolution of microbes, especially in the case of those that inhabit extreme environments (Olga et al., 2009; Zhang et al., 2017; Li et al., 2019), which in this case also contributed considerably to genome contents of *Metallosphaera*. Based on COG annotation, the transferred genes in *Metallosphaera* spp. comprised mostly defensive and metabolic functions, with approximately 6.7% defense mechanisms (V), 6.4% energy production and conversion (C), 6.4% carbohydrate transport and metabolism (G), 4.9% amino acid transport and metabolism (E), 3.9% coenzyme transport and metabolism (H), 3.2% inorganic ion transport and metabolism (P), 2.1% lipid transport and metabolism (I), and 1.1% secondary metabolites biosynthesis, transport, and catabolism (Q) (Supplementary Figure S13). Informational proteins such as ribosomal proteins and RNA processing proteins experienced fewer HGT events in comparison with other gene families (Supplementary Figure S13). Most HGT events appeared to be acquired from the same domain (Archaea), which is typical among the identified HGTs (Figure 8 and Supplementary Table S6). Bacteria also contributed largely to the emerging genetic diversity of *Metallosphaera* through cross-domain HGT, and acquired genes such as hydrogenase and benzoate/toluate dioxygenase also appeared to facilitate adaption to different niches, resulting in the functional divergence within the genus of *Metallosphaera* (Figure 8 and Supplementary Table S6). However, it should be noted that though metagenome-assembled genomes (MAGs) included in this study have supplemented available genomic data of *Metallosphaera* and expanded sample size, these MAGs may contain contamination generated during the binning process. Thus, the results of MAGs should be interpreted carefully.
CONCLUSION

In this study, the genome of a Metallosphaera species (M. tengchongensis) was comparatively analyzed with 18 other genomes from Metallosphaera strains to enrich our understanding of the genetic traits, metabolism, and environmental adaption mechanism of the Metallosphaera and shed light on their evolutionary history. Metallosphaera microbes were widely found in sulfur-rich and metal-burdened environments, and all Metallosphaera strains analyzed in this study contained sulfur and iron oxidation genes. However, the APS pathway was only detected in M. sedula and M. yellowstonensis, and certain subunits of the fox cluster were lost in M. cuprina. Gene deficiency of SOR hinted that new unusual enzymes might undertake the S\textsuperscript{0} oxidation role. Complete TCA cycles and ED pathways coexisted with HP/HB and DC/HB cycles in all genomes of these strains and supported their heterotrophic, autotrophic, and mixotrophic growth modes. The genes for assimilatory nitrate reduction were present in all genomes of 19 strains; however, a complete dissimilatory nitrate reductase gene cluster was only found in M. yellowstonensis, which demonstrated that Metallosphaera species and strains have different abilities to use inorganic nitrogen. Genes encoding for flagellin and flagella accessory proteins were only detected in strains of M. sedula, M. yellowstonensis, Metallosphaera sp. Obs4, Metallosphaera sp. UBA165, and Metallosphaera sp. My\_r06. We also found that functions related to assimilatory sulfur metabolism and cysteine biosynthesis associated with ROS reduction were significantly enriched in accessory gene families. Evolutionary analyses showed that massive gene family gain events occurred at the branches of M. yellowstonensis and M. sedula, whereas considerable gene family loss events occurred in M. cuprina and pervasive strong purifying selection was found acting on the gene families of Metallosphaera. We also found that HGT played an important role in shaping the genetic and functional diversity of Metallosphaera. These findings provide a data basis for subsequent studies of metabolism and environmental adaption mechanisms in thermophilic Archaea, and for strategies to design cellular biocatalysts for the biomining process.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the genome sequence of Metallosphaera tengchongensis strain Ric-A has been deposited at JGI IMG-ER database under the IMG
AUTHOR CONTRIBUTIONS

C-YJ, S-JL, and HY designed and coordinated the study. PW, LZL, and LJL performed the bioinformatics analysis. YQ, ZL, and XL carried out the experiments and interpreted data for the work. PW and LZL wrote the manuscript. C-YJ and S-JL edited the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.01192/full#supplementary-material

TABLE S1 | Average nucleotide identity (ANI) based on whole genome alignments of Metallosphaera spp.

TABLE S2 | Statistics of genomic islands in the genomes of Metallosphaera spp.

TABLE S3 | Statistics of Mobile genetic elements (MGEs) (A), prophages and prophage remnants (B), Transposons (C), CRISPR-Cas related sequences (D) present in the genomes of Metallosphaera spp.

TABLE S4 | Gene families undergoing gain (A), loss (B), expansion (C), and contraction (D) events in Metallosphaera spp. detected with COUNT

TABLE S5 | Selection pressure on gene families of Metallosphaera spp.

TABLE S6 | Putative horizontally transferred genes of Metallosphaera spp.
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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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