Arsenite Oxidation by a Newly Isolated Betaproteobacterium Possessing arx Genes and Diversity of the arx Gene Cluster in Bacterial Genomes

Melody Cabrera Ospino*, Hisaya Kojima* and Manabu Fukui

The Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan

Microbes play essential roles in arsenic transformation in the environment. Microbial arsinite oxidation is catalyzed by either of two distantly related arsinite oxidases, referred to as AIO and ARX. The arx genes encoding ARX and its regulatory proteins were originally defined in the genomes of gammaproteobacteria isolated from an alkaline soda lake. The arx gene cluster has been identified in a limited number of bacteria, predominantly in gammaproteobacteria isolated from lakes characterized by high pH and high salinity. In the present study, a novel arsinite-oxidizing betaproteobacterium, strain M52, was isolated from a hot spring microbial mat. The strain oxidized arsinite under both microaerophilic and nitrate-reducing conditions at nearly neutral pH. Genome analysis revealed that the strain possesses the arx gene cluster in its genome and lacks genes encoding AIO. Inspection of the bacterial genomes available in the GenBank database revealed that the presence of this gene cluster is restricted to genomes of Proteobacteria, mainly in the classes Gammaproteobacteria and Betaproteobacteria. In these genomes, the structure of the gene cluster was generally well-conserved, but genes for regulatory proteins were lacking in genomes of strains belonging to a specific lineage. Phylogenetic analysis suggested that ARX encoded in the genomes can be divided into three groups, and strain M52 belongs to a group specific for organisms living in low-salt environments. The ArxA protein encoded in the genome of strain M52 was characterized by the presence of a long insertion, which was specifically observed in the same group of ARX. In clone library analyses with a newly designed primer pair, a diverse ArxA sequence with a long insertion was detected in samples of lake water and hot spring microbial mat, characterized by low salinity and a nearly neutral pH. Among the isolated bacterial strains whose arsinite oxidation has been demonstrated, strain M52 is the first betaproteobacterium that possesses the arx genes, the first strain encoding ARX of the group specific for low-salt environments, and the first organism possessing the gene encoding ArxA with a long insertion.

Keywords: arsenite oxidation, ARX, betaproteobacterium, arx gene cluster, freshwater, bacterial genomes
INTRODUCTION

Despite their toxic nature, compounds of arsenic are utilized by some prokaryotes. Microbial transformation of arsenic includes respiratory As(V) reduction and As(III) oxidation for autotrophic growth, which are referred to as “arsenotrophy” (Oremland et al., 2009). The interconversion of arsenate and arsenite in arsenotrophy is catalyzed by one of the three enzymes of the dimethyl sulfoxide (DMSO) reductase family, arsenite reductase (ARR) and two distantly related arsenite oxidases, AIO and ARX (Zargar et al., 2012).

ARX is encoded by the arxA genes, which were initially defined in Alkalilimnicola ehrlichii MLHE-1. This bacterium was isolated from anoxic bottom water of an alkaline-saline lake and can grow chemolithoautotrophically by anaerobic arsenite oxidation coupled with nitrate reduction (Oremland et al., 2002). The arx genes have also been identified in the genomes of phototrophic bacterial strains isolated from alkaline-saline environments, such as Ectothiorhodospira sp. PHS-1 and Ectothiorhodospira sp. BSL-9. These strains are capable of arsenite oxidation coupled to anoxygenic photosynthesis. The essential role of the arxA gene in chemooautotrophic and phototrophic arsenite oxidation was demonstrated by mutagenesis experiments with A. ehrlichii MLHE-1 and Ectothiorhodospira sp. BSL-9, respectively (Zargar et al., 2010; Hoef McCann et al., 2017). In the genome of A. ehrlichii MLHE-1, the arx genes form a gene cluster consisting of structural and regulatory components. The structural component is comprised of five genes, arxB2ABCD. The other group of genes transcribed in the opposite direction, arxXRS, are thought to encode a regulatory system (Zargar et al., 2010). The same arrangement of the arxA genes was also found in Ectothiorhodospira sp. PHS-1 (Zargar et al., 2012) and BSL-9 (Hernandez-Maldonado et al., 2016, 2017). To date, only a few arsenite-oxidizing bacteria have been reported to possess the arx genes or arxA. They are predominantly gammaproteobacteria isolated from lakes characterized by high pH and high salinity, including Halomonas sp. ANA-440 (Hamamura et al., 2014), Halorhodospira halophila SL1 (Challacombe et al., 2013; Hernandez-Maldonado et al., 2017), Ectothiorhodospira sp. MLW-1 (Hoeft McCann et al., 2017), and the three strains mentioned above. As an exceptional case, Desulfotomaculum sp. TC-1 (in the phylum Firmicutes) was isolated from a sulfidic hot spring at pH 5.5 (Wu et al., 2017).

As mentioned above, most current knowledge regarding ARX came from studies on gammaproteobacterial strains from environments with high pH and high salinity. On the other hand, however, the arx genes have also been identified in the genome of other bacteria whose ability for arsenite oxidation has not been demonstrated. One such strain, Sulfuricella denitrificans skB26, is a sulfur-oxidizing betaproteobacterium isolated from an artificial freshwater lake. In its genome, the arx genes form a gene cluster corresponding to that of A. ehrlichii MLHE-1. The protein encoded by the arxA gene of S. denitrificans skB26 is phylogenetically distinct from those of the halophilic and alkaliphilic gammaproteobacteria. In addition, it has a unique inserted amino acid sequence (Watanabe et al., 2014). In some previous studies, partial sequences of the arxA gene were obtained with culture-independent approaches. In one such study, arxA gene sequences closely related to that of S. denitrificans skB26 were frequently detected in samples characterized by low salinity and a nearly neutral pH (Ospino et al., 2018). These results suggest the presence of a lineage of ARX specific for freshwater environments, but arsenite oxidation by the ARX of S. denitrificans skB26 or other members of this lineage has not been demonstrated.

In this study, an arsenite-oxidizing betaproteobacterium possessing the arx genes was isolated. This isolate has the arxA gene belonging to the lineage frequently detected in freshwater environments. In addition, the diversity of the arx gene cluster was investigated by using genome sequences available in public databases.

MATERIALS AND METHODS

Isolation of an Arsenite-Oxidizing Bacterium, Strain M52

To obtain a novel arsenite-oxidizing bacterium, a dark green microbial mat developed on an inclined concrete wall was obtained from the Jozankei hot spring located in Hokkaido, Japan. The mat is identical to that used in previous studies (Kojima et al., 2017; Ospino et al., 2018). A piece of mat was inoculated in a synthetic basal medium (pH 7.0 and 341.0 mg/L of NaCl), which was prepared as described previously (Connon et al., 2008). Prior to sterilization, the medium was purged with N2 or CO2 gas to enrich anaerobic organisms. Just before inoculation, stock solutions of sodium arsenite and sodium chloride were added to the medium to final concentrations of 0.5 and 3 mM, respectively. The headspace of the bottle was filled with N2 gas, and incubation was performed in the dark at 32°C. The enrichment was subject to successive transfers to fresh media with an increased concentration of arsenite, 1 mM. During the enrichment culture procedure, the presence of organisms with the arxA gene was monitored by the PCR-based method previously described (Ospino et al., 2018). After the fifth transfer, a pure culture was obtained by repeated agar shake dilution (Widdel and Bak, 1992). For the isolation, another medium was used with Na2S2O3 and NaN3 as an electron donor and acceptor, as described previously (Kojima et al., 2017).

Genome Sequencing and Phylogenetic Analysis of Strain M52

Genomic DNA was extracted from strain M52 using a Wizard® Genomic DNA purification kit (Promega, Madison, WI, United States). Genome sequencing and assembly were performed as described previously (Umezawa et al., 2016). The obtained genome was annotated with Rapid Annotation using Subsystem Technology (RAST) (Overbeek et al., 2014). The annotation overview of the genome was made in the SEED Viewer version 2.0 (online). The assembled genome was subject
to inspection for genes involved in arsenic metabolism. The genome of strain M52 is available in GenBank under the Accession No. NZ_APO19373.

The closest cultured relatives of the novel strain were identified by comparison to the rRNA database in BLASTn, and a phylogenetic tree was constructed. The sequences were aligned with MAFFT version 7. Gaps and poorly aligned regions were excluded using Gblocks v0.91b. The tree topology was inferred by neighbor-joining using MEGA 6.

**Culturing Experiments With Arsenic**

Arsenite oxidation ability of strain M52 and effects of arsenic on its growth were examined with culturing experiments at 45°C, as described below.

To verify whether strain M52 was capable of oxidizing arsenite anaerobically, culturing experiments were performed under anoxic conditions by using the basal medium used for the enrichment culture. The medium was supplemented with 0.2% (w/v) NaCl, 1–2 mg/L yeast extract, 0.5 mM sodium molybdate, 1 mM sodium arsenite and 10 mM sodium nitrate. For measurements of arsenite and arsenate concentrations, approximately 500 μL of medium was aseptically collected from each bottle at 0, 6, and 18 days of incubation. The collected samples were immediately filtered through 0.22 μm pore size filters and stored at −30°C until the measurements. The concentrations of arsenite and arsenate were determined by high-performance liquid chromatography (HPLC), as described previously (Watanabe et al., 2017).

Arsenite oxidation was also examined in the basically same medium which contained no nitrate, under anoxic, microoxic, andoxic conditions. For test of anoxic conditions, the medium was prepared as above, and headspace was filled with N₂ gas. The medium for microoxic conditions was prepared as same as the above, but filter-sterilized air was added to the culture bottles after autoclaving, to obtain final oxygen concentrations of 1 or 2% (v/v) in the headspace. Foroxic conditions, the medium was prepared without purging with N₂ gas and head space was filled with air. Arsenite and arsenate were measured with HPLC just after inoculation and after 6 days incubation without shaking.

Effects of arsenic on growth were investigated under nitrate-reducing conditions, by monitoring turbidity along with concentrations of arsenate and nitrate. The experiments were performed with the medium same as that used for the first experiment, but concentration of yeast extract was 5 mg/L. Turbidity was measured as optical density at 660 nm, with a spectrophotometer. Arsenate and nitrate were quantified with ion chromatography equipped with conductivity detector (ICS-1500, Dionex), equipped with a column for anion analyses (IonPac AS12A, Dionex). The experiments were performed in the presence and absence of acetate, to assess heterotrophic and autotrophic growth. The experiment with acetate was started with medium containing 5 mM sodium acetate and 1 mM sodium arsenite. For comparison, bottles without arsenite were also prepared. In case of the experiments without acetate, the medium was initially supplemented with 0.65 mM sodium bicarbonate and 0.5 mM arsenite. These compounds were successively added to the medium during the incubation, by injection of dense solutions. At the latest stage of the experiments, 5 mM sodium acetate was added to the culture.

Tolerance to arsenite was tested in the basal medium, supplemented with sodium acetate (5 mM), sodium nitrate (5 mM), and 5 mg/L yeast extract. The strain M52 was cultured in presence of sodium arsenite with different concentrations (1, 2, 5, and 10 mM).

**Detection of the arx Gene Cluster in Prokaryotic Genomes**

To explore the arx genes in prokaryotic genomes, amino acid sequences encoded by the arxXRSB2ABCD genes of *A. ehrlichii* MLHE-1 were used as queries to identify homologous proteins in the non-redundant protein database of the National Center for Biotechnology Information (NCBI). The identifications were made using Diamond v 0.9.14 (Buchfink et al., 2014), and two proteins were considered to be homologous based on the following thresholds: an amino acid identity of ≥30%, a spanning alignment of ≥60% of the length of the query, and an e-value ≤1e⁻³. In addition to sequences in the protein database, good-quality genome sequences without functional annotation were collected for the analysis from the “Microbial Genomes resource” in NCBI. They were selected based on the following criteria: consist of contigs ≤300, have an N50 ≥20 kb and contain ≤10 kb of ambiguous base pairs. In the collected genomes, protein coding regions were deduced using Prodigal v2.6.3 in normal mode (with default parameters) (Hyatt et al., 2010), and the predicted proteins were used to build a custom database for identification of homologous proteins with the method described above. The data collection from NCBI databases occurred in January 2018. Furthermore, three genome sequences obtained in our laboratory were also included in the analysis. They are genomes of three strains isolated from the same microbial mat: strain M52 obtained in this study, *Sulfitortus calidifontis* J1A (Kojima et al., 2017) and *Sterolibacteriaceae* bacterium strain JSB (Watanabe et al., 2019). They were analyzed with the same methods used for the unannotated genome sequences as described above.

**Phylogenetic Analyses**

The identified proteins, homologous to ArxA of *A. ehrlichii* MLHE-1, were subjected to phylogenetic analysis, including the putative subunits of other enzymes from the DMSO reductase family. The amino acid sequences were aligned with MUSCLE (Edgar, 2004). Gaps were allowed within an appropriate block by Gblocks v0.91b (Talavera and Castresana, 2007). The tree was inferred with the distance criterion by the BioNJ algorithm using FastME 2.0 (Lefort et al., 2015). The inference included 1000 bootstraps replicates (Gascuel, 1997). Phylogenetic trees of proteins encoded by the *arxA*, *arxB*, and *arxD* genes were constructed as below. The amino acid sequences were separately aligned and curated as described above. The trees were reconstructed by the maximum-likelihood algorithm with the LG + G + I substitution model using PhyML.

---

1. ftp://ftp.ncbi.nlm.nih.gov/blast/db/
2. ftp://ftp.ncbi.nlm.nih.gov/genomes/genbank/bacteria/
(v3.0) (Guindon et al., 2010). The gamma shape parameter was estimated directly from the data. The aLRT test (SH-Like) was used to calculate the support values (Anisimova and Gascuel, 2006). To construct the ArxA-B consensus tree, the alignments of ArxA and ArxB were concatenated using MEGA version 6 (Tamura et al., 2013) and then subjected to the remaining steps as explained above. All of the phylogenetic analyses were performed on the Phylogeny.fr platform (Dereeper et al., 2008).

Phylogenetic analysis of organisms possessing the arx genes was conducted on the basis of ribosomal proteins (RPs). The following 10 RPs were used for the analysis: L2, L3, L4, L13, L23, S2, S4, S9, S10, and S11 (see Supplementary Table S1 for detailed information). One of the identified genomes lacked some of these proteins, and thus it was excluded from the analysis. Each ribosomal protein was independently aligned using MAFFT (Katoh et al., 2017) with default parameters. The poorly aligned regions were removed using the Gblocks v0.91b program, allowing gaps located in less than 50% of the sequences. The resulting 10 alignments were concatenated using MEGA version 6. The tree was inferred by neighbor-joining under the p-distance model in MEGA 6. Support values were calculated using 1000 bootstrap replicates. The final trees were visualized using the R package ggtree (Yu et al., 2017).

Identification of Conserved Motifs and Prediction of Secondary Structure

In the proteins encoded by arxAB genes, conserved motif regions were identified by Pfam online (Finn et al., 2014) and visualized in the multiple sequences alignment on MEGA 6. The presence and location of twin-arginine signal peptide cleavage sites were identified using the TatP 1.0 server online (Bendtsen et al., 2005). Secondary structure prediction was performed for the ArxA proteins with insertion, using the Jpred 4 server (Drozdzetskiy et al., 2015).

Clone Library Construction and Analysis of a Long Insertion in the arxA Gene

To explore the diversity of the ArxA proteins which have insertions, a set of degenerate primers was newly designed. The primers were designed based on the ArxA amino acid sequences characterized by the presence of a long insertion, encoded in the genomes of S. denitrificans skB26, Sulfuritortus calidifontis J1A, Gammaproteobacteria RIFOXYD12 FULL_61_37, Gammaproteobacteria RIFOXYA12 FULL_61_12, and Betaproteobacteria CG2_30_59_46. The primers were designed at positions flanking the insertion to obtain the full length of the insertion (Figure 8A). The primers are arxA_G2_F (AARCGTACCAAYCCSAAV AAGG) and arxA_G2_R (GTTCCTTGGCTAGTCTGCAT). PCR was performed in a 25 µL volume reaction mixture containing 0.5 µmol/L of each primer, 1× Ex Taq Buffer (Takara, Shiga, Japan), 0.2 mmol/L dNTPs (Takara), 0.625 U of Ex Taq (Takara), 3% DMSO and template DNA solution. The PCR conditions were as follows: 94°C for 3 min; 34 cycles of denaturing at 94°C for 45 s, annealing at 55°C for 45 s, extension at 72°C for 1.5 min; and then a final extension step at 72°C for 7 min.

Two DNA samples obtained in previous studies were used as templates for PCR amplification with newly designed primers: one water sample was obtained at a depth of 40 m in Lake Mizugaki (Watanabe et al., 2017), and a dark green microbial mat sample was retrieved from Jozankei hot spring (Ospino et al., 2018). The resulting PCR products were analyzed with agarose gel electrophoresis, and bands corresponding to expected sizes (ca. 1200 bp and ca. 900 bp, with and without insertion) were excised from the gel. From the pieces of gel, DNA was extracted with QIAquick Gel Extraction Spin Kit (Qiagen). The purified amplicons were cloned into pCR2.1-TOPO vector and transformed into TOP10 cells (Invitrogen), according to the manufacturer's instructions. The resulting clone libraries were analyzed as described previously (Ospino et al., 2018), and operational taxonomic units (OTUs) were assigned at a cutoff value of 0.02 in Mothur (Schloss et al., 2009). The most abundant sequence of each OTU was selected as representative for phylogenetic analysis. The representative sequences were aligned with ArxA reference sequences using MUSCLE. The alignment was trimmed manually, excluding gaps. Phylogenetic relationships were inferred based on distance criteria using the BioNJ algorithm to construct a Neighbor-joining tree with 1000 bootstrap replicates in FastME 2.0. The ArrA of Alkaliphilus oremlandii OhILAs and Hal arsenita bacter silvermannii were used as the outgroup. The nucleotide sequences obtained in this study have been deposited under the accession numbers LC439110 to LC439195.

Phylogenetic Analysis of Partial Sequences of the arxA Gene Reported in Previous Studies

In some previous studies, partial sequences of the arxA gene were obtained with the primer pair arxA_Deg_F_B/arxA_Deg_R_B (Zargar et al., 2012; Hamamura et al., 2014; Wu et al., 2017) (Table 1). The phylogenetic positions of these sequences were reexamined with reference ArxA sequences collected in this study. In the phylogenetic analysis, partial sequences obtained in this study were also included. The alignment was made using MUSCLE and was trimmed manually to exclude the gaps. A neighbor-joining tree was reconstructed using the BioNJ algorithm with 1000 bootstrap replicates in FastME 2.0. The arsenite reductase ArrA sequences were used as the outgroup.

RESULTS

Isolation and Characterization of Arsenite-Oxidizing Bacterium Strain M52

From the microbial mat, arsenite-oxidizing enrichment cultures were established. The presence of an arxA-carrying organism in culture was confirmed with PCR amplification and sequencing with a previously reported primer pair. By analyzing the 16S rRNA gene, it was revealed that the cultures were dominated by a bacterium related to Sterolibacteriaceae bacterium strain J5B (Watanabe et al., 2019). The dominating organism was isolated by using the same methods that were used for the isolation of
strain J5B, and the resulting isolate was designated strain M52. During the isolation procedures, strain M52 showed anaerobic and chemolithoautotrophic growth depending on nitrate and thiosulfate. The strain J5B is facultatively autotrophic bacterium which can grow on some organic acids (Watanabe et al., 2019). Among the organic substrates which support growth of strain J5B, acetate and lactate were tested with strain M52. Both these substrates supported anaerobic growth of strain M52 under nitrate reducing conditions.

The complete genome of strain M52 consisted of a single circular chromosome with a length of 2.74 Mb and a G+C content of 63.6%. In the genome, 2794 coding sequences were predicted. There are two copies of the rrr operons which contain the 16S rRNA genes with slightly different lengths. BLASTn searches revealed that the most closely related species with validly published names were Sterolibacterium denitrificans and Georgfuchsia toluolica, with sequence identities of 94–95%. By constructing a phylogenetic tree, it was confirmed that strain M52 is a novel member of the family Sterolibacteriaceae (Figure 1).

In the genome of strain M52, the arxA gene was identified, and its nucleotide sequence perfectly matched the partial sequence detected in the enrichment cultures. The arxA gene of strain M52 encoded a protein that has 92 extra amino acids compared to the proteins of strains J5B and acr3 (detoxifying arsenate reductase), and acr3 (arsenate-specific efflux pump). On the other hand, the arXM gene, which is involved in arsenic methylation, was absent in the genome. The aio genes, which encode another type of arsenite oxidase, and arrAB, which encodes respiratory arsenate reductase, were not identified in the genome of strain M52.

Further inspection of the genome revealed that strain M52 has genes required for respiratory nitrate reduction. They include the genes encoding catalytic subunits of periplasmic nitrate reductase (napA), nitrite reductase (nirS), nitric-oxide reductase (norB) and nitrous oxide reductase (nosZ). The genome also harbors genes required for respiration with oxygen, the ccoNOQP genes encoding cytochrome c bb3-oxidase and the cydAB genes encoding cytochrome bd oxidase. Although the strain was isolated from enrichment culture with chlorate, genes for chlorate reduction were not identified in the genome.

### Arsenic Metabolism of Strain M52

Under anaerobic conditions, strain M52 oxidized arsenite to arsene in the medium containing nitrate as sole electron acceptor (Figure 2A). In the unoinoculated control, changes in concentrations of arsenate or arsene were not observed. Arsenite oxidation by strain M52 was also tested in the medium without nitrate. In the absence of nitrate, arsenite oxidation was not observed under anaerobic conditions (Figure 2B). In the same medium, strain M52 oxidized arsene when incubation was performed under microoxic conditions with 1 or 2% oxygen in the headspace (Figure 2B). The oxygen-dependent arsenite oxidation was not observed under atmospheric oxygen level (20%).

To assess effects of arsenic on anaerobic growth of strain M52, cell density was monitored along with changes in concentrations of nitrate and arsenite. As shown in Figure 3A, 1 mM of arsenite showed negative effect on heterotrophic growth on acetate. In the presence of acetate, arsene was generated in the early stage of growth, but it seemed to be reduced back into arsene in the later stage of incubation possibly by the ars system. The effects on autotrophic growth was tested with gradual supplement of arsene to abate toxicity (Figure 3B). With a serial addition of arsene, continuous production of arsene was observed throughout the incubation. A small increase in turbidity was observed until day 5, but no obvious growth was observed during the period from day 5 to day 19. In this period, supplement of inorganic carbon source did not enhanced growth. After the measurements at day 19, acetate was added to the culture. After that, cell density increased within the following 5 days, suggesting that viability was retained during the experiment (Figure 3B). In these experiments, nitrite was monitored with ion chromatography which can detect nitrite as well. In all samples analyzed, however, nitrite was not detected.

### TABLE 1 Partial arxA gene sequences of uncultured organisms included in phylogenetic analysis in this study (Supplementary Figure S8).

| Prefix       | References | Characteristics of sampling site | Accession number |
|--------------|------------|----------------------------------|------------------|
|              |            | pH | Temperature (°C) | Salinity (g/L) | Cl- (mM) | Arsenic (µM) |
| HC           | Zargar et al., 2012 | 8.3a | Variablea | NA | 0.6–0.8a | 1.5–2.7 | JN624760–JN624765 |
| MLBX         | Zargar et al., 2012 | 9.8b | NA | 90d | 500d | 200 | JN624766–JN624770 |
| Paoha_Island | Zargar et al., 2012 | 9.4 | 43 | 23 | NA | 100 | JN624771 |
| _red_mat     |            |     |             |               |          |      |                  |
| HJ           | Hamamura et al., 2014 | 9.8 | NA | 6.2 | NA | 0.67–133 | KC852945–KC852951 |
| JZK1200      | This study | 7.8c | 42.6c | NA | 40.2c | 40c | LC439110–LC439121 |
| JZK900       |            |     |             |               |          |      |                  |
| MZG900       | This study | 7.0 | 103 | NA | 1.4 | 1.5d | LC439155–LC439195 |

Marked values were cited from references which are different from those for the sequences, as follows: a Wikie and Hering, 1998; b Onemland et al., 1993; c Ospino et al., 2018; d Watanabe et al., 2017. NA, not available.
Arsenite tolerance of strain M52 was tested in presence of nitrate and acetate under anoxic conditions. Anaerobic growth on acetate was observed in the presence of 5 mM or lower concentration of arsenite, and higher concentrations resulted in slower growth of the strain. No growth was observed in the presence of 10 mM arsenite.
the genomes (Figure 4). Among the genomes, 17 genomes had gene organization identical to that of the arx genes in A. ehrlichii MLHE-1 (Figure 4). For the proteins encoded by the gene homologous to arxA, a phylogenetic tree was constructed to identify their phylogenetic positions within the DMSO reductase family (Figure 5 and Supplementary Figure S1). In the tree, the proteins encoded in the identified genomes formed two distinct clades, corresponding to those of bacteria and archaea. The former encompasses ArxA of A. ehrlichii MLHE-1 and Ectothiorhodospira sp. PHS-1, which were included in the original definition of ARX. On the other hand, there is no evidence regarding the function of the proteins forming the latter cluster, and arsenic metabolism by Candidatus Methanoperedens has not been reported. Based on these results, the archaeal genomes were excluded from the following analyses. Hereafter, the bacterial genes homologous to the arx genes of A. ehrlichii MLHE-1 are simply referred to as arx genes.

The novel strain isolated in this study, strain M52, has arx gene cluster similar to that of A. ehrlichii MLHE-1, but the arxB2 gene is located downstream of arxABCD, similar to a MAG of Candidatus Muproteobacteria (Figure 4). The arxB2 gene was not identified in four bacterial genomes. The arxD gene was conserved in all the genomes except for three genomes from Alphaproteobacteria and Deltaproteobacteria.

Genes encoding regulatory proteins ArxXSR were commonly found upstream of the arxB2ABCD genes on the opposite strand. In the genomes of Thioalkalivibrio sp. ALMg11 and Thioalkalivibrio sp. AKL19, the arxSR genes were identified downstream of arxB2ABCD on the same strand as exceptional cases (Figure 4). Halorhodospira halophila SL1 can oxidize arsenite, but the arxXSR genes were not identified in its genome. The arxXSR genes were consistently absent in the genomes of three genera in the order Oceanospirillales (Halomonas and Marinospirillum and Nintricola laciesaponensis) (Figure 4).

The other genomes lacking arxXSR genes were distributed in Candidatus Muproteobacteria, Alphaproteobacteria, and Deltaproteobacteria.

**Phylogeny of Proteins Encoded by the Arx Proteins**

Phylogenetic relationships among the identified Arx proteins were further investigated in detail. At first, phylogenetic trees of ArxA and ArxB were separately constructed. The resulting trees showed phylogenetic congruence with minor exceptions, indicating the coevolution of these two subunits (Supplementary Figure S2). For a more reliable phylogenetic inference, an ArxAB consensus tree was constructed by concatenating the alignments of ArxA and ArxB (Figure 6). In the tree, the majority of ArxAB proteins were grouped into three groups corresponding to well-supported monophyletic clusters. In this study, these groups are referred to as group 1, group 2, and group 3, respectively (Figure 6). Correspondence between the grouping of ARX and the class-level taxonomy of the bacteria can be seen in Figures 4, 7. Direct comparisons between the phylogeny of bacteria (based on RPs) and ARX are shown in Supplementary Figure S3.

The ARX of A. ehrlichii MLHE-1 was classified into the group 1. The ARX of this group was identified only in genomes of gammaproteobacterial haloalkaliphiles (Figures 3, 5). Ectothiorhodospira sp. PHS-1 is closely related to organisms with the ARX of group 1, but its ARX is phylogenetically distinct from those of the group 1. It was classified into group 2, together with the ARX of bacteria belonging to the classes Gammaproteobacteria, Betaproteobacteria, Alphaproteobacteria, and Ca. Muproteobacteria. The ARX of strain M52 belonged to group 3. This group consists of ARX encoded in bacteria isolated at a relatively low pH and low salt concentrations and MAGs from such environments.
FIGURE 4 | Arrangement of the arx gene cluster found in the genomes. Arrows with different colors represent the following genes: yellow for arxAB, green for arxC, blue for arxD, gold for arxB2, peach for arxX, coral for arxS, purple for arxR, and gray for hypothetical proteins. The number of genomes for each class-level taxonomy of bacteria is shown in parentheses. Name of organisms with ARX of group 1, group 2, and group 3 are shown in blue, green and red respectively. Names with black square correspond to organism reported as arx-arsenite-oxidizing bacterium, including strain M52. Names with black triangle represent the metagenome-assembled genomes (MAGs). Black star represents the genes encoding products annotated as partial protein. Names of organisms affiliated to the Order Oceanospirillum are enclosed in a gray dashed square. The conserved arxABC genes are highlighted in beige color.
A phylogenetic analysis of ArxD was also constructed (Supplementary Figure S4). In the tree, ArxD proteins associated with the ARX of group 1 and group 3 formed exclusive clusters. Phylogeny discordance with ArxD proteins was apparent in the ARX of group 2, but it was also observed in that of group 1. For instance, the positions of *Thioalkalivibrio nitratireducens* DSM 14787, *Thioalkalivibrio denitrificans* ALJD, and *Thioalkalivibrio* sp. ALM2T are considerably different in the phylogenetic trees of ArxD and ArxAB. In contrast, the phylogeny of ArxD proteins associated with ARX of group 3 was consistent with that of ArxAB, as indicated by the same branching pattern in the two trees (Supplementary Figure S4).

Conserved Regions in the ArxAB Amino Acid Sequences

Comparison among amino acid sequences of the ArxAB encoded in the identified genomes indicated that they share some characteristics with those of *A. ehrlichii* MLHE-1 and *Ectothiorhodospira* sp. PHS-1 reported in previous studies (Zargar et al., 2010, 2012; Van Lis et al., 2013; Badilla et al., 2018), as described below. The ArxA sequences had three conserved regions commonly identified in subunit A of the DMSO reductase family enzymes: an iron–sulfur [4Fe-4S] motif; a catalytic binding pocket sequence similar to that suggested in the ArxA of *A. ehrlichii* MLHE-1 and *Ectothiorhodospira* sp. PHS-1 (Zargar et al., 2012); and a twin-arginine signal peptide on the N-terminus (Supplementary Figure S5). Additionally, all the sequences had an XGRGWG motif located near the putative catalytic binding pocket. This motif is argued to be one of the conserved motifs that distinguish ArxA from the closely related arsenate reductase ArrA, characterized by a corresponding motif of (R/K)GRY (Glasser et al., 2018). The ArxB also share similarities to subunit B of the enzymes from the DMSO reductase family with at least three conserved iron–sulfur [4Fe-4S] clusters (Supplementary Figure S6).

Diversity of arxA With Insertion

As reported previously, ArxA of *S. denitrificans* skB26 has a long insertion (Watanabe et al., 2014). Similar insertions were identified in five other genomes, including strain M52 isolated in this study. To evaluate the diversity of the insertion in ArxA, a new primer pair was designed (Figure 8A). The primers were tested with purified genomic DNA from *S. denitrificans* skB26, *Sulfuritortus calidifontis* J1A, *A. ehrlichii* MLHE-1, and *Nitrincola lacisaponensis* 4CA. Among them, strains skB26 and J1A have the *arxA* gene with a long insertion. As a bacterium possessing the *arrA* gene, *Sulfuratitea hydrogenivorans* sk43H was used to confirm the specificity of the primer pair. The primer pair, named arxA_G2_F/arxA_G2_R, generated PCR products of approximately 1200 bp in size from strains skB26 and J1A (Figure 8B, lanes 3 and 5). A product of approximately 900 bp was obtained from strain MLHE-1 (Figure 8B, lane...
8) but not from strain 4CA (Figure 8B, lane 4). No PCR products were obtained from the genomic DNA of strain sk43H (Figure 8B, lane 9). Although strains M52 and J5B have the insertions, their arxA gene sequences were not used for the primer design, because genomes of these strains were not available when the primers were designed. At a later time, the primer pair was tested with these strains but generated no PCR products.

Two environmental samples were analyzed with the new primer pair. They were water collected at a depth of 40 m in Lake Mizugaki (MZG) and a microbial mat obtained in Jozankei hot springs (JZK). A PCR product of the expected size with the insertion (1200 bp) was obtained only from the sample from JZK (Figure 8B, lanes 1 and 2). PCR amplicons of approximately 900 bp length were obtained in both JZK and MZG samples (Figure 8B, JZK: lanes 1 and 2; MZG: lane 7). With these PCR products, three clone libraries, named JZK1200, JZK900 and MZG900, were constructed (Table 1 and Supplementary Table S4). The phylogenetic tree in Supplementary Figure S7 shows the relationships between the partial ArxA sequences from the three libraries and the reference sequences. The neighbor-joining tree was reconstructed excluding gaps, using a final dataset of 310 compared amino acid positions. The tree indicated that five OTUs without insertions are clustered with ARX of group 2. The other OTUs were grouped with ARX of group 3. In the clone library of JZK1200, five OTUs with a long insertion were
detected. Four of them are closely related to the ArxA of strains isolated from the same microbial mat (Sulfuritortus caldifontis J1A, strain M52 and strain J5B). The other OTU detected in this library was phylogenetically distinct from them and harbored the insertion with a clearly different sequence. In some OTUs detected in the library of 900 bp, insertions of another type were identified (Supplementary Figure S7). The insertions of this type, consisting of 12 amino acid residues, were also identified in ArxA of group 3 encoded in some MAGs.

The analysis of full ArxA sequences indicated that the insertions are distantly located from the putative functional conserved regions. No sequence motif was identified within the long insertion, but some amino acids were abundant and a few of them were conserved, such as lysine, alanine, glutamate, and leucine (Supplementary Figure S7). The analysis with Jpred4 predicted that the insertion sequences form alpha helices, with a high probability to form a coiled-coil structure. Coiled-coil is a common structural motif in proteins, formed by two or more
strands of alpha-helices winded around each other in superhelical fashion (Lupas, 1997; Truebestein and Leonard, 2016). The above mentioned amino acids enriched in the insertions are known to be involved in formation of the coiled-coil frequently, because of their propensity to form alpha-helices (Monera et al., 2002; Kwok and Hodges, 2004; Surkont and Pereira-Leal, 2015). All the insertion sequences were cut out and individually subjected to BLAST analysis, but no homologous sequence was identified outside the ArxA.

Phylogenetic Reassessment of Partial arxA Gene Sequences Detected in Previous Studies

An additional phylogenetic analysis was performed to reassess the phylogenetic positions of previously reported partial sequences of the arxA gene. A neighbor-joining tree was reconstructed using a final dataset of 92 compared amino acid positions. The tree showed that all of the clones recovered from high-saline and high-pH samples are closely related to ArxA encoded in the genomes of haloalkaliphilic gammaproteobacteria (Supplementary Figure S8). The ArxA of Desulfitomaculum sp. TC-1 isolated from a hot spring was also related to these bacteria. In contrast, all the clones recovered from the Hot Creek riverbeded sediment (HC) fell within a strongly supported cluster together with ARX of group 3 encoded in the genomes of bacteria isolated from the Jozankei hot spring (Supplementary Figure S8).

DISCUSSION

To the best of our knowledge, strain M52 is the first arsenite-oxidizing betaproteobacterium which possesses the arx genes. It was isolated from a microbial mat of a hot spring which contained approximately 3 mg/L of total arsenic (Kubota et al., 2010; Ospino et al., 2018). From the same mat, partial arxA gene sequences were detected with culture-independent methods (Ospino et al., 2018). The arxA gene of strain M52 was not detected in that analysis, suggesting that culture-based approaches are still effective to explore diversity of arsenite-oxidizing bacteria with arx genes. Although strain M52 lacks aio genes in the genome, it oxidized arsenite under microaerophilic and nitrate-reducing conditions. Some genomic features of the strain are consistent with these observations, as the genome harbors genes for respiration with oxygen and nitrate. The two types of terminal oxidases encoded in the genome are both high-affinity terminal oxidases characterized by low K_m values for oxygen (Morris and Schmidt, 2013), suggesting that the strain M52 is adapted to a low-oxygen environment. The concentration of oxygen in the air may be too high for this organism, and it might have

FIGURE 8 | (A) Positions of PCR primers for the arxA gene and expected size of the PCR product. (B) Gel images showing the results of PCR with newly designed primers. Samples in lanes are: M, 10 kb DNA ladder as a size marker; (1–2) Jozankei microbial mat; (3) Sulfuritortus calidifontis J1A; (4) Nitrincola lasiasonensis 4CA; (5) Sulfuricella denitrificans skB26; (6) no template control; (7) Mizugaki water; (8) Alkalilimnicola ehrlichii MLHE-1; (9) Sulfuritalea hydrogenivorans sk43H; (10) no template control. In lanes 1 and 2, bands of expected sizes are marked with triangles.
suppressed arsenite oxidation activity (**Figure 2B**). As another organism which oxidizes arsenite under microoxic conditions, *Hydrogenobaculum* strain H55 was reported in a previous study (Donahoe-Christiansen et al., 2004). In contrast to strain M52, strain H55 has genes for AIO (Clingenpeel et al., 2009).

The *arx* gene cluster of strain M52 had the same set of genes as those found in *A. ehrlichii* MLHE-1 and *Ectothiorhodospira* sp. PHS-1, with some small differences in positions of the genes. The comparative analysis showed that the majority of the genes in the *arx* gene cluster are conserved in more than half of the genomes (**Figure 4**). Especially, the *arxABC* genes encoding the putative functional elements of the enzyme are highly conserved. For other enzymes involved in arsenic metabolism, AIO arsenite oxidase and ARO arsenate reductase, the *aioBA* and *arrAB* genes encode the constituent subunits, and they are well-conserved in the genomes (Van Lis et al., 2013; Andres and Bertin, 2016). In contrast, the *arxC* gene homologous in the *arr* and *aio* gene cluster is not well-conserved (Slyemi and Bonnefoy, 2012; Grimaldi et al., 2013; Andres and Bertin, 2016). The *arxB2* gene, predicted to encode a type of ferredoxin protein, appears to be specific to the *arx* gene cluster, since no homologous genes have been described for AIO or ARO. A previous study demonstrated the expression of the *arxB2* gene in *Ectothiorhodospira* sp. BSL-9 (Hernandez-Maldonado et al., 2017). Additionally, transcripts were detected in the southern basin of Mono Lake (Edwardson and Hollibaugh, 2017). However, the role of *arxB2* in the arsenite oxidation remains unclear. The *arxD* gene was also highly conserved and predicted to encode a TorD-like protein required to introduce the cofactor into the enzyme (Genest et al., 2009).

The occurrence of homologous genes in the *aio* or *arr* gene cluster (*aioD* and *arrD*) is variable as well (Van Lis et al., 2013; Andres and Bertin, 2016). The *arrXSR* genes are absent from the *arx* gene cluster of the genomes from members of the order *Oceanospirillales*** (**Figure 4**). Similar findings were reported in previous studies on the gene clusters of *aio* and *arr*, which indicated that the presence of *aioXSR* and *arrXSR* is variable among taxonomic groups (Slyemi and Bonnefoy, 2012; Van Lis et al., 2013; Badilla et al., 2018). The *aioXSR* genes regulate the expression of the *aioAB* genes in some arsenite-oxidizing strains (Kashyap et al., 2006; Koechler et al., 2010; Sardiwal et al., 2010; Liu et al., 2012; Li et al., 2013). A bacterium which lacks the *aioXSR* module, *Halomonas* sp. HAL1, seems to employ another two-component system to regulate expression of *aioAB* (Chen et al., 2015). Thus far, the involvement of the *arrXSR* genes in regulation of the expression of *arxAB* has not been demonstrated.

The incongruency between the phylogeny of ARX and RPs suggested that horizontal gene transfer events have affected the evolution of the ARX and arsenite-oxidizing bacteria (**Supplementary Figure S3**). In other arsenic-related enzymes, the involvement of horizontal gene transfer in their evolutionary history has been reported as well (Andres and Bertin, 2016). In the genomes harboring *arx* genes, some features suggestive of gene transfer were identified. Some of the *arx* gene clusters were located in mobile genetic elements such as plasmids, as in *Sulforicella demitriificans* skB26 (Watanabe et al., 2014) and *Halomonas* sp. A3H3 (NCBI Reference Sequence: NZ_HG423344.1) (Koechler et al., 2013), and in a genomic island in *Azoarcus* sp. CIB (Martin-Moldes et al., 2015). In strain M52, a gene encoding transposase (92% identity to the IS110 family transposase of *Thiomonas* sp.) was identified upstream of the *arxRSX*.

In this study, ARX were grouped into three groups based on phylogenetic analysis of *ArxAB*** (**Figure 6**). The three groups have some specific characteristics, as follows. Strains with ARX in group 1 are all haloalkaliphiles and belong to the orders *Chromatiales* and *Oceanospirillales*. The phylogenetic analysis of previously reported data confirmed that the partial *arxA* sequences clustered with group 1 were all detected in environments with high pH and salinity. The sole exception was *Desulfotomaculum* sp. TC-1 obtained by PCR amplification (Wu et al., 2017). This strain exhibits optimum growth at pH 6.8 and belongs to the phylum *Firmicutes*, although *arx* genes identified in this study were only in the genomes of *Proteobacteria*. More detailed analysis of this organism is necessary to obtain the full sequences of the *arx* genes. *Ectothiorhodospira* sp. PHS-1 is haloalkaliphile belonging to the order *Chromatiales*, but its ARX was classified into group 2. Group 2 seemed to be the most heterogeneous group, because the genes encoding this type of ARX were identified in seven genomes belonging to four classes in the phylum *Proteobacteria*. These genomes included those of phototrophic haloalkaliphiles, heterotrophs growing in low-salt medium, and MAGs obtained from freshwater environments. Partial sequences of *arxA*, related to group 2, were detected in a freshwater lake and an alkaline salt lake. The former is Lake Mizugaki, from which some OTUs were obtained in this study, and the latter is Mono Lake, where close relatives of *Ectothiorhodospira* sp. PHS-1 were detected in previous studies (**Figure 8**). In contrast to these groups, ARX of group 3 was not identified in the genome of phototrophic organisms. The isolated strains with group 3 ARX are all sulfur-oxidizing chemolithoautotrophs. The MAGs with this type of ARX were obtained from a CO₂-driven geyser (Probst et al., 2017) and groundwater sample (Anantharaman et al., 2016), suggesting that corresponding organisms do not depend on light. These findings also suggest that the ARX of group 3 is specific for low-salt environments. This idea is supported by phylogenetic analysis of the partial *arxA* gene sequences. Among the previously reported partial sequences, those from Hot Creek riverbed sediment were clustered with group 3 (**Supplementary Figure S8**). This sample is characterized by low salt concentrations in comparison to the other samples analyzed in the previous studies. The other partial sequences of group 3 were obtained in this study. Group 3 is characterized by a phylogenetic relationship that is highly congruent with the phylogeny of ArxD (**Supplementary Figure S4**).
affected by PCR bias and close relatives of strain M52 might be missed in the analysis. However, application of these primers resulted in the unexpected detection of arxA without insertions. One of the samples used in this analysis was the isolation source of strain M52, and the other one was used in a previous study to detect the arrA gene (Watanabe et al., 2017). In that study, arsenate respiration by *Sulfuritalea* in the deep water of Lake Mizugaki was suggested (Watanabe et al., 2017). In another sample of deep water obtained from the same lake in a different year, partial arrA gene sequences were detected with another primer pair (Ospino et al., 2018). It is plausible that anaerobic bacteria detected with forms of arxA and arrA are driving the arsenate cycle in the anoxic layer of water in this lake. In contrast to ARX of group 1 and group 2, involvement of the group 3 ARX in arsenite oxidation has not been demonstrated. Strain M52 is the first organism for which the presence of the genes for group 3 ARX and arsenite oxidation were both demonstrated (Figures 2, 3). The absence of the aio gene cluster in the genome of strain M52 suggests that the arx genes are responsible for its arsenite oxidation. However, further experimental evidence is required to confirm arsenite oxidation by group 3 ARX. Strain M52 also has a long insertion in ArxA and an unusual gene arrangement in the arx gene cluster characterized by the unique position of arxB2 (Figure 4). The role of these elements in arsenite oxidation by strain M52 will be important subject of further studies.

REFERENCES

Anantharaman, K., Brown, C. T., Hug, L. A., Sharon, I., Castelle, C. J., Probst, A. I., et al. (2016). Thousands of microbial genomes shed light on interconnected biogeochemical processes in an aquifer system. *Nat. Commun.* 7, 1–11. doi: 10.1038/ncomms13121

Andres, J., and Bertin, P. N. (2016). The microbial genomics of arsenic. *FEMS Microbiol. Rev.* 39:fu050. doi: 10.1093/femsre/fu050

Anisimova, M., and Gascuel, O. (2006). Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. *Syst. Biol.* 55, 539–552. doi: 10.1080/10635150600755453

Badilla, C., Osborne, T. H., Cole, A., Watson, C., Djordjevic, S., and Santini, J. M. (2018). A new family of periplasmic-binding proteins that sense arsenic oxyanions. *Sci. Rep.* 8, 1–12. doi: 10.1038/s41598-018-24951-w

Bendtsen, J. D., Nielsen, H., Widdick, D., Palmer, T., and Brunak, S. (2005). Prediction of twin-arginine signal peptides. *BMC Bioinform.* 6:167. doi: 10.1186/1471-2164-6-167

Buchfink, B., Xie, C., and Huson, D. H. (2014). Fast and sensitive protein alignment using DIAMOND. *Nat. Methods* 11, 596–602. doi: 10.1038/nmeth.3176

Challacombe, J. F., Majid, S., Deole, R., Brettin, T. S., Bruce, D., Delano, S. F., et al. (2013). Complete genome sequence of *Halorhodospira halophila* SL1. *Stand. Genomic Sci.* 8, 206–214. doi: 10.4056/sigs.3677284

Chen, F., Cao, Y., Wei, S., Li, Y., Li, X., Wang, Q., et al. (2015). Regulation of arsenite oxidation by the phosphate two-component system PhoBR in *Halomonas* sp. *HAL1*. *Front. Microbiol.* 6:923. doi: 10.3389/fmicb.2015.00923

Clingenpeel, S. R., D’Imperio, S., Oduro, H., Druschel, G. K., and McDermott, T. R. (2009). Cloning and in situ expression studies of the *Hydrogenobaculum* arsenite oxidase genes. *Appl. Environ. Microbiol.* 75, 3362–3365. doi: 10.1128/AEM.00336-339

Connon, S. A., Koski, A. K., Neal, A. L., Wood, S. A., and Magnuson, T. S. (2008). Ecophysiology and geochemistry of microbial arsenic oxidation within a high arsenic, circumneutral hot spring system of the Alvord Desert. *FEMS Microbiol. Ecol.* 64, 117–128. doi: 10.1111/j.1574-6941.2008.00456.x

SUPPORTING INFORMATION

Author Contributions

MO, HK, and MF designed the study. MO and HK performed experiments and wrote the manuscript. MO conducted data analysis. All authors contributed to manuscript revision, and approved the submitted version.

Funding

This study was supported by JSPS KAKENHI (Grant No. 15K07209).

Acknowledgments

We thank Arisa Shinohara, Tomohiro Watanabe, and Tomoya Iwata for their assistance.

Supplementary Material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2019.01210/full#supplementary-material

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., et al. (2008). Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res.* 36, 465–469. doi: 10.1093/nar/gkn180

Donohoe-Christiansen, J., D’Imperio, S., Jackson, C. R., Inskeep, W. P., and McDermott, T. R. (2004). Arsenite-oxidizing *Hydrogenobaculum* strain isolated from an acid-sulfate-chloride geothermal spring in Yellowstone National Park. *Appl. Environ. Microbiol.* 70, 1865–1868. doi: 10.1128/AEM.70.3.1865-1868.2004

Drozdzewski, A., Cole, C., Procter, J., and Barton, G. J. (2015). Prf4d: a protein secondary structure prediction server. *Nucleic Acids Res.* 43, W389–W394. doi: 10.1093/nar/gkv332

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32, 1792–1797. doi: 10.1093/nar/gkh340

Edwardson, C. F., and Hollibaugh, J. T. (2017). Metatranscriptomic analysis of prokaryotic communities active in sulfur and arsenic cycling in Mono Lake, California, USA. *ISME J.* 11, 2195–2208. doi: 10.1038/ismej.2017.80

Finn, R. D., Bateman, A., Clements, J., Coggill, P., Eberhardt, R. Y., Eddy, S. R., et al. (2014). Pfam: the protein families database. *Nucleic Acids Res.* 42, 222–230. doi: 10.1093/nar/gkt1223

Gascuel, O. (1997). BIOM: an improved version of the NJ algorithm based on a simple model of sequence data. *Mol. Biol. Evol.* 14, 685–695. doi: 10.1093/oxfordjournals.moleev.a025808

Genest, O., Méjean, V., and Jobbi-Nivol, C. (2009). Multiple roles of TorD-like chaperones in the biogenesis of molybdoenzymes. *FEMS Microbiol. Lett.* 297, 1–9. doi: 10.1111/j.1574-6968.2009.01660.x

Glasser, N. R., Oyala, P. H., Osborne, T. H., Santini, J. M., and Newman, D. K. (2018). Structural and mechanistic analysis of the arsenate respiratory reductase provides insight into environmental arsenic transformations. *Proc. Natl. Acad. Sci. U.S.A.* 115, E8614–E8623. doi: 10.1073/pnas.1807984115

Grimaldi, S., Schoepf-cothenet, B., Ceccaldi, P., Guigliarelli, B., and Magalon, A. (2013). The prokaryotic Mo / W- bis PGD enzymes family: a catalytic workhorse in bioenergetic. *Biochim. Biophys. Acta* 1827, 1048–1085. doi: 10.1016/j.bbadis.2013.01.011

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., and Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood
Widdel, F., and Bak, F. (1992). "Gram-negative mesotrophic sulfate-reducing bacteria," in The Prokaryotes, eds A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (New York, NY: Springer), 3352–3378. doi: 10.1007/978-1-4757-2191-1_21

Wilkie, J. A., and Hering, J. G. (1998). Rapid oxidation of geothermal arsenic(III) in streamwaters of the eastern Sierra Nevada. Environ. Sci. Technol. 32, 657–662. doi: 10.1021/es970637r

Wu, G., Huang, L., Jiang, H., Peng, Y., Guo, W., Chen, Z., et al. (2017). Thioarsenate formation coupled with anaerobic arsenite oxidation by a sulfate-reducing bacterium isolated from a hot spring. Front. Microbiol. 8:1336. doi: 10.3389/fmicb.2017.01336

Yu, G., Smith, D. K., Zhu, H., Guan, Y., and Lam, T. T. Y. (2017). Ggtree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol. Evol. 8, 28–36. doi: 10.1111/2041-210X.12628

Zargar, K., Conrad, A., Bernick, D. L., Lowe, T. M., Stolc, V., Høeft, S., et al. (2012). ArxA, a new clade of arsenite oxidase within the DMSO reductase family of molybdenum oxidoreductases. Environ. Microbiol. 14, 1635–1645. doi: 10.1111/j.1462-2920.2012.02722.x

Zargar, K., Høeft, S., Oremland, R., and Saltikov, C. W. (2010). Identification of a novel arsenite oxidase gene, arxA, in the haloalkaliphilic, arsenite-oxidizing bacterium Alkalilimnicola ehrlichii strain MLHE-1. J. Bacteriol. 192, 3755–3762. doi: 10.1128/JB.00244-10

Conflicts of Interest Statement: 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.

Copyright © 2019 Ospino, Kojima and Fukui. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.