Transcriptomic Analysis of Two 

*Thioalkalivibrio* Species Under Arsenite Stress Revealed a Potential Candidate Gene for an Alternative Arsenite Oxidation Pathway

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The genus *Thioalkalivibrio* includes haloalkaliphilic chemolithoautotrophic sulfur-oxidizing bacteria isolated from various soda lakes worldwide. Some of these lakes possess in addition to their extreme haloalkaline environment also other harsh conditions, to which *Thioalkalivibrio* needs to adapt. An example is arsenic in soda lakes in eastern California, which is found there in concentrations up to 3000 µM. Arsenic is a widespread element that can be an environmental issue, as it is highly toxic to most organisms. However, resistance mechanisms in the form of detoxification are widespread and some prokaryotes can even use arsenic as an energy source. We first screened the genomes of 76 *Thioalkalivibrio* strains for the presence of known arsenic oxidoreductases and found 15 putative ArxA (arsenite oxidase) and two putative ArrA (arsenate reductase). Subsequently, we studied the resistance to arsenite in detail in *Thioalkalivibrio jannaschii ALM2*¹, and *Thioalkalivibrio thiocyanoxidans ARh2*¹ by comparative genomics and by growing them at different arsenite concentrations followed by arsenic species and transcriptomic analysis. *Tv. jannaschii ALM2*¹, which has been isolated from Mono Lake, an arsenic-rich soda lake, could resist up to 5 mM arsenite, whereas *Tv. thiocyanoxidans ARh2*¹, which was isolated from a Kenyan soda lake, could only grow up to 0.1 mM arsenite. Interestingly, both species oxidized arsenite to arsenate under aerobic conditions, although *Tv. thiocyanoxidans ARh2*¹ does not contain any known arsenite oxidases, and in *Tv. jannaschii ALM2*¹, only arxB2 was clearly upregulated. However, we found the expression of a SoeABC-like gene, which we assume might have been involved in arsenite oxidation. Other arsenite stress responses for both strains were the upregulation of the vitamin B₁₂ synthesis pathway, which can be linked to antioxidant activity, and the up- and downregulation of different DsrE/F-like genes whose roles are still unclear. Moreover, *Tv. jannaschii ALM2*¹ induced the *ars* gene operon and
the Pst system, and *Tv. thiocyanoxidans* ARh2T upregulated the sox and apr genes as well as different heat shock proteins. Our findings for *Thioalkalivibrio* confirm previously observed adaptations to arsenic, but also provide new insights into the arsenic stress response and the connection between the arsenic and the sulfur cycle.

**Keywords:** RNA-Seq, arsenic, resistance, adaptation, sulfur-oxidizing bacteria, soda lake, soeABC

## INTRODUCTION

The genus *Thioalkalivibrio* comprises a group of metabolically diverse, haloalkaliphilic and chemolithoautotrophic sulfur-oxidizing bacteria thriving under extreme conditions in soda lakes. They are part of the family *Ectothiorhodospiraceae* within the Gammaproteobacteria (Sorokin et al., 2001a), and include 10 described species and more than 100 isolated strains (Foti et al., 2006; Sorokin et al., 2012). In *silico* analysis of the genomes of 76 strains classified *Thioalkalivibrio* in 25 genomic species, indicating a high genomic diversity within this genus (Ahn et al., 2017). Concomitantly, members of this genus are able to use different reduced sulfur compounds as electron donors such as sulfide, polysulfide, thiosulfate, polythionates, and elemental sulfur (Sorokin et al., 2001a, 2002b, 2003, 2004, 2012; Banciu et al., 2004). Moreover, the strains *Thioalkalivibrio paradoxis* ARh1T (Sorokin et al., 2002b), *Tv. thiocyanoxidans* ARh2T (Sorokin et al., 2002b) and *Tv. thiocyanodenitrificans* ARhD1T (Sorokin et al., 2004) are also able to oxidize thiocyanate (Sorokin et al., 2001b; Berben et al., 2017), and *Tv. denitrificans* ALJD1T (Sorokin et al., 2001a), *Tv. nitratireducens* ALEN2T (Sorokin et al., 2003), and *Tv. thiocyanodenitrificans* ARhD1T (Sorokin et al., 2004) can also grow anaerobically by denitrification. Recently, Andres and Bertin (2016) and Oremland et al. (2017) detected the presence of an arxA gene, which in other bacteria, is responsible for the anaerobic energy-generating oxidation of arsenite [As(III)] to arsenate [As(V)], in the genome of 11 *Thioalkalivibrio* strains. Furthermore, transcripts of the arxA gene that were highly similar to genes of *Thioalkalivibrio* were discovered in high abundance in Mono Lake, an arsenic-rich soda lake in eastern California (Edwardson and Hollibaugh, 2017). Soda lakes in this area possess, in addition to their characteristic extreme halokaline condition (Jones et al., 1977, 1998), elevated arsenic concentrations that range from 0.8 µM in Crowley Lake, over 200 µM in Mono Lake, to 3000 µM in Searles Lake (Oremland et al., 2004). However, despite the multi-extreme conditions, *Thioalkalivibrio* are found in abundance in these soda lakes (Stamps et al., 2018).

Numerous microorganisms developed mechanisms to detoxify their cells from arsenic and in some cases to even use it as an energy source. Arsenic is well known to be highly toxic to most organisms. It may contaminate soils and groundwater that are used for food production or as a drinking water source (Mandal and Suzuki, 2002; Cavalca et al., 2013) posing severe threats to human health (Kapaj et al., 2006). The most common forms in the environment are arsenite [As(III)] and arsenate [As(V)] (Smedley and Kinniburgh, 2002), of which the reduced form is more toxic (Hughes, 2002). This toxicity is due to the fact that As(III) is able to deactivate compounds by binding to sulfhydryl groups, as are present in glutathione (Scott et al., 1993) or in cysteines (Shen et al., 2013). As(V), however, can compete with phosphate in biochemical reactions due to its chemically similar structure and properties (Wolfe-Simons et al., 2009; Tawfik and Viola, 2011). To survive the presence of arsenic, prokaryotes can perform detoxification, which includes the reduction of As(V) to As(III) followed by As(III) methylation (Qin et al., 2006) and/or the active export of As(III) out of the cell (Ben Fekih et al., 2018). In the methylation process, the As(III) S-adenosylmethylamine methyltransferase ArsM transforms As(III) into methylated As(III) compounds. By this mechanism the cell forms even more toxic, highly volatile organic arsenic compounds that can escape from the cell (Qin et al., 2006). In the active transport system, bacteria pump arsenic out of the cell using the Ars gene system. It first reduces As(V) to As(III) by the arsenate reductase ArsC (Ji and Silver, 1992; Martin et al., 2001) and subsequently pumps the As(III) out by the efflux pump ArsB or ACR3 (arsenic compounds resistance) (Bobrowicz et al., 1997; Wysocki et al., 1997; Meng et al., 2004). The activity of these pumps can be augmented by an ATPase, the ArsA, which increases the resistance to arsenic even more (Rosen et al., 1988; Dey and Rosen, 1995; Rosen, 2002). ArsD is an As(III) chaperone that transfers As(III) to ArsA (Lin et al., 2006, 2007) and it also possesses a weak activity as transacting regulatory protein (Wu and Rosen, 1993). The main transacting regulatory protein of the Ars cluster is ArsR, which functions as a transcriptional repressor that activates transcription in the presence of As(III) (Wu and Rosen, 1991). In addition to detoxification, there are numerous prokaryotes that can generate energy by the oxidation of As(III) using arsenite oxidases Aio (Anderson et al., 1992) or Arx (Zargar et al., 2010), or by the anaerobic reduction of As(V) by the arsenate respiratory reductase Arr (Saltikov and Newman, 2003). These three proteins belong to the dimethyl sulfoxide (DMSO) reductase family of molybdoenzymes, also known as complex iron-sulfur molybdoenzymes (CISM) (McEwan et al., 2002; Rothery et al., 2008). They are composed by a heterodimer of a large subunit (AioA, ArxA, and ArrA) containing the molybdopterin binding site and a small subunit with an iron-sulfur cluster (AioB, ArxB, and ArrB) (Kraft and Macy, 1998; Ellis et al., 2001; Afkar et al., 2003; Zargar et al., 2010). AioC, ArxC, and ArrC are involved in electron transfer and in the case of the ArxC and the ArrC, are transmembrane proteins anchoring the protein to the periplasmic membrane (Stolz et al., 2006; Zargar et al., 2010, 2012; Van Lies et al., 2012; Kalimuthu et al., 2014; Andres and Bertin, 2016; Oremland et al., 2017; Glasser et al., 2018). Only recently, the clade of the Arx arsenite oxidase was discovered in *Alkalilimnicola ehrlichii* MLHE-1T (Hoeft et al., 2007; Richey et al., 2009; Zargar et al., 2010).
and in *Ectothiorhodospira* PHS-1 (Zargar et al., 2012), two haloalkaliphilic Gammaproteobacteria isolated from Mono Lake. These bacteria couple oxidation of As(III) as sole electron donor with nitrate reduction (Hoef et al., 2007; Zargar et al., 2010) or anoxygenic photosynthesis (Kulp et al., 2008; Hernandez-Maldonado et al., 2017), respectively. Interestingly, ArxA is more similar to ArrA than it is to AioA (Richey et al., 2009; Zargar et al., 2010).

The aim of our research was to understand the mechanisms of resistance and adaptation to arsenic within the genus *Thioalkalivibrio*. We first searched in 76 *Thioalkalivibrio* genomes for genes that potentially can be involved in arsenic metabolism. Subsequently, we grew two *Thioalkalivibrio* strains at different As(III) concentrations. For this, we chose *Tv. jannaschii* ALM2T, which was isolated from Mono Lake (Sorokin et al., 2002a) where arsenic is present at relatively high concentrations (Oremland et al., 2004), and *Tv. thiocyanoxidans* ARh2T, which was isolated from a Kenyan soda lake (Sorokin et al., 2002b). We measured the As(III) oxidation capacity of the two species and performed RNA-Seq analysis to study their gene expression under arsenite stress. To our knowledge, this is the first transcriptomic work done on the arsenite stress response in chemolithoautotrophic bacteria.

**MATERIALS AND METHODS**

**Strains and Growth Conditions**

Axenic cultures of *Tv. jannaschii* ALM2T and *Tv. thiocyanoxidans* ARh2T were grown in 200 ml batch cultures at 30°C on a shaker set at 100 rpm. The medium was composed of 17.5 g/l Na2CO3, 13.9 g/l NaHCO3, 6.1 g/l NaCl, 1 g/l K2HPO4, 0.2 g/l MgCl2, 40 mM Na2S2O3, 5 mM NH4Cl, and 1:1000 trace metals (Pfenning and Lippert, 1966). Sterile solutions of MgCl2, Na2S2O3 and trace elements were added from concentrated stock solutions after autoclaving. The final pH of the culture medium was adjusted to pH 9.8. As(III) as sodium arsenite (NaAsO2) (Sigma Aldrich, United States) was added to the medium just before inoculation of the bacteria. For the growth curves of *Tv. jannaschii* ALM2T and *Tv. thiocyanoxidans* ARh2T, the cultures were supplemented with 0.1, 0.5, 5, or 7.5 mM As(III). Cultures without As(III) were used as reference and growth of all cultures was monitored daily by measuring the OD at 600 nm. *Tv. thiocyanoxidans* ARh2T and *Tv. jannaschii* ALM2T grew up to a concentration of 0.1 and 5 mM As(III), respectively (Supplementary Figure 1). Therefore, cultures were prepared at 0.1 mM As(III) for *Tv. thiocyanoxidans* ARh2T, and at 0.1 and 5 mM for *Tv. jannaschii* ALM2T to study the As(III) resistance mechanism by transcriptomics. Again, cultures without As(III) were used as reference and their growth was followed by OD measurements at 600 nm. Samples for arsenic species and transcriptomic analysis were taken in the exponential growth phase at an OD600nm ~ 0.1, which corresponded to one [reference; 0 mM As(III)] and two [0.1 mM As(III)] days after inoculation for the *Tv. thiocyanoxidans* ARh2T cultures, and after one [reference; 0 mM As(III)], one [0.1 mM As(III)], and five [5 mM As(III)] days for the *Tv. jannaschii* ALM2T cultures. In addition, sterile culture medium was incubated under the same conditions to check for the possibility of chemical As(III) oxidation. To test the growth with As(III) as sole electron donor, *Tv. thiocyanoxidans* ARh2T was cultivated with 0.1 mM As(III), and *Tv. jannaschii* ALM2T with 0.1 and 2.5 mM As(III) in culture medium prepared as described above with the exception of containing 0.025 g/l MgSO4 × 7H2O and different Na2S2O3 concentrations depending on the culture (0, 1, 5, 10, and 40 mM). All experiments were done in triplicate.

**Arsenic Speciation by ICP-MS Analysis**

Culture supernatant was filtered through a 0.2 μm filter and arsenic species were determined according to Kim et al. (2007). To quantify the total As concentration, 5 ml of the filtrate was acidified prior the analysis with 200 μl of 2% (v/v) HNO3. For the determination of inorganic arsenic species As(III) and As(V), 5 ml of the filtrate was added to a Sep-Pak® Plus Acell Plus QMA cartridge (Waters, MA, United States). As(V) remained in the cartridge, whereas As(III) passed through. As(III) was collected and acidified with 200 μl of 2% (v/v) HNO3. The As(V) was then washed off the cartridge with 5 ml 0.16 M HNO3. Total As, As(III), and As(V) concentrations were measured by ICP-MS (Agilent Technologies, United States). Standard solutions ranging from 0 to 1 mg/l of As were prepared from a sodium arsenite (NaAsO2) solution (Sigma Aldrich, United States). All measurements were done in triplicate.

**Comparative Sequence Analysis**

The phylogenetic tree of ArxA, ArrA, and AioA was constructed based on a multiple alignment of amino acid sequences, which were selected by a BLASTp analysis of 76 *Thioalkalivibrio* genomes (Ahn et al., 2017) and of reference protein sequences. The selected sequences were aligned with MUSCLE (Edgar, 2004) and the tree was built with the software program MEGA7 (version 7.0.26; Kumar et al., 2016) using the Maximum Likelihood method with 1000 bootstrap replicates, the LG model as substitution model and a discrete gamma distribution (+G) as evolutionary rate differences amongst sites.

The phylogenetic tree of the two SoeA clusters was also built with aligned amino acid sequences found in 76 *Thioalkalivibrio* genomes and references, which were selected based on a previous BLASTp analysis. The alignment and the tree construction were calculated following the same protocol as described above.

**RNA-Sequencing**

The biomass was collected in 50 ml Greiner tubes and immediately placed into a centrifuge that was precooled to 4°C. The cells were pelleted by centrifugation at 7,000 × g for 4 min at 4°C. The supernatant was removed until approximately 2 ml, in which the cells were suspended and transferred to a 2 ml Eppendorf tube. The sample was then centrifuged at 15,000 × g for 1 min at 4°C. The supernatant was completely removed, and the cell pellet was immediately frozen in liquid nitrogen and stored at −80°C until further processing.
The frozen cell pellets were homogenized with a mortar and a pestle before being resuspended in QIAzol Lysis Reagent (Qiagen, Germany). Total RNA was extracted and purified with the RNase-free kit (Qiagen) following the manufacturer’s instructions. The purification step comprised a DNase treatment using the RNase-free DNase kit (Qiagen). The concentration was quantified with the NanoDrop ND2000 (Thermo Fisher Scientific) and the integrity of the RNA was checked on the 2200 TapeStation with Agilent RNA ScreenTapes (Agilent Technologies, Netherlands). Ribosomal RNA (rRNA) was removed by the Illumina Ribo-Zero rRNA Removal Kit (Bacteria) (Illumina, United States). Bar-coded RNA libraries were prepared using the Ion Total RNA-Seq kit v2 and the Ion Xpress RNA-Seq barcoding kit according to the supplier’s instructions (Thermo Fisher Scientific). Size distribution and yield were measured on the 2200 TapeStation using Agilent D1000 ScreenTapes (Agilent Technologies). Ribosomal RNA (rRNA) was quantified with the NanoDrop ND2000 (Thermo Fisher Scientific). The concentration was determined with the RNeasy kit (Qiagen) following the manufacturer’s instructions. The purification step comprised a DNase treatment with the RNase-free DNase kit (Qiagen). The concentration was determined with the NanoDrop ND2000 (Thermo Fisher Scientific). The quality of the reads was assessed by FastQC (version 0.11.7) and estimated to be sufficient. Therefore, no trimming or filtering was performed. Pseudonym sequences were generated in kallisto by mapping the reads from the fasta RNA-Seq files against the indexed reference and reads were quantified using 100 bootstrap samples. Subsequently, differential expression analysis was performed with the software program sleuth (Pimentel et al., 2017) (0.30.0) using the Wald test. The complete differential expression values are presented in Supplementary Table 1 and consists of the NCBI locus-tag, the b-value (beta-value), the P-value, the q-value, the raw counts and the annotation by NCBI for each gene. The b-value is a biased estimator of the log fold change and is on a natural-log scale (Pimentel et al., 2017).

The sequences were also analyzed with the RNA-Seq analysis module in the software program CLC Genomics Workbench 11.0.1 (QIAGEN). Proton Torrent fastq files were imported and trimmed using the following default settings: (i) removal of low-quality sequences with a limit of 0.05, (ii) removal of ambiguous nucleotides: maximum 2 nucleotides allowed, and (iii) discard reads below a length of 30 nucleotides. Subsequently, the trimmed reads were mapped to the reference genomes. Differential expression data includes the NCBI locus-tag, the max group mean, the log (fold change), the fold change, the P-value, the FDR P-value, and the Bonferroni value (Supplementary Table 2).

RESULTS AND DISCUSSION
Genomic Features of Arsenic Metabolism and Resistance in Thioalkalivibrio

We searched in Thioalkalivibrio for genes that can be used to grow on arsenic as an energy source. Therefore, a phylogenetic tree was constructed with the putative protein sequences of ArxA, AioA (arsenate oxidases), and ArrA (arsenate reductase) detected in the 76 available genome sequences of different Thioalkalivibrio strains (Figure 1A). In those, a putative ArxA was found in 14 Thioalkalivibrio strains and a putative ArrA in two. Genes coding for AioA were not detected in any of the strains. *Tv. nitratireducens* ALEN2\textsuperscript{T} was the only strain that contained both ArrA and ArxA. Previously, the presence of ArrA has only been reported in 11 *Thioalkalivibrio* strains (Andres and Bertin, 2016; Oremland et al., 2017) while the presence of ArrA has been never documented. For the strains used in the cultivation experiment, *Tv. jannaschii* ALM2\textsuperscript{T} possesses a putative ArxA while *Tv. thiocyanoxidans* ARh2\textsuperscript{T} lacks any of the known genes to generate energy from inorganic arsenic.

The genomes of *A. ehrlichii* MLHE-1\textsuperscript{T} (another member of the Ectothiorhodospiraceae), *Tv. jannaschii* ALM2\textsuperscript{T} and *Tv. thiocyanoxidans* ARh2\textsuperscript{T} encode different gene clusters for the detoxification (ars genes) and for the oxidation (arx genes) of arsenite (Figure 1B). *A. ehrlichii* MLHE-1\textsuperscript{T} (Zargar et al., 2010, 2012) and *Tv. jannaschii* ALM2\textsuperscript{T} possess an identical arx gene cluster for arsenite oxidation and a highly similar set of *ars* genes for arsenic resistance. For the *ars* genes, *A. ehrlichii* MLHE-1\textsuperscript{T} possesses the most complete gene cluster including arsADR, ACR3 and two detoxifying arsenite reductases arsC, one glutaredoxin-dependent arsC1 and one thioredoxin-dependent arsC2. In *Tv. jannaschii* ALM2\textsuperscript{T}, a more reduced set including an arsR, a glutaredoxin-dependent arsC and an ACR3 was present. Another annotated ACR3 efflux pump was found in ALM2\textsuperscript{T} outside the shown cluster (Locus-tag: F816_RS0108235) together with three uncharacterized membrane proteins. Interestingly, *A. ehrlichii* MLHE-1\textsuperscript{T} and *Tv. jannaschii* ALM2\textsuperscript{T} also encode for a universal stress protein (uspA) in their *ars* gene cluster. On the contrary, *Tv. thiocyanoxidans* ARh2\textsuperscript{T} only possesses a truncated *ars* gene cluster with an ACR3 and a glutaredoxin-dependent arsC, also subdivided in two operons, and without arsR. Outside of the operon, two putative ArsR for *Tv. thiocyanoxidans* ARh2\textsuperscript{T} were found by BLASTp, but with low identity values, using the ArsR of *A. ehrlichii* MLHE-1\textsuperscript{T} (Locus-tag: Mlg\_2713), and of *Tv. jannaschii* ALM2\textsuperscript{T} (Locus-tag: F816_RS0102085) (Supplementary Table 4) as subjects. Neither the genome of *Tv. thiocyanoxidans* ARh2\textsuperscript{T} nor of *Tv. jannaschii* ALM2\textsuperscript{T} contained the arsM gene necessary for the detoxification of the intracellular As(III) by methylation (Qin et al., 2006). Furthermore, the genomes of *Tv. jannaschii* ALM2\textsuperscript{T} and *Tv. thiocyanoxidans* ARh2\textsuperscript{T} were screened for the presence of arshiNLOPTX via BLASTp. In *Tv. jannaschii* ALM2\textsuperscript{T}, an ArsI [Locus-tag: F816_RS0102080; query cover of 93% and an identity of 41% with ArsI of *Bacillus* sp. MD1 (AI09488)] was found inside the *ars* gene operon as well.
as a second putative ArsI positioned directly besides the ars gene operon [Locus-tag: F816_RS14315 (78% query cover and 29% identity to AIA09488)]. In addition, putative sequences for ArsJ were detected in Tv. jannaschii ALM2T [Locus-tag: F816_RS1016725; query cover of 95% and a similarity of 60.3% with ArsJ of Pseudomonas aeruginosa (WP_003109849)] and in
Physiological and Transcriptomic Response to Arsenic Stress

Tv. jannaschii ALM2T and Tv. thiocyanoxidans ARh2T were cultivated in the presence of different concentrations of As(III) to determine their resistance. Tv. jannaschii ALM2T resists much higher As(III) concentrations than Tv. thiocyanoxidans ARh2T (Supplementary Figure 1). Tv. thiocyanoxidans ARh2T was only able to grow until a concentration of 0.1 mM As(III), whereas Tv. jannaschii ALM2T still grew up to 5 mM As(III). All cultures grew aerobically with thiosulfate as an electron donor and they could not grow with As(III) as their sole potential electron donor (Supplementary Figure 2).

To gain deeper insight in their resistance mechanism against arsenic, Tv. thiocyanoxidans ARh2T and Tv. jannaschii ALM2T were both cultivated in absence of As(III) (reference) and at 0.1 mM As(III). Furthermore, Tv. jannaschii ALM2T was also grown at 5 mM As(III). Cultures were harvested in their exponential growth phase to measure the arsenic species composition in the culture fluid and to determine gene expression in both Thioalkalivibrio species.

Arsenic species were measured in the culture medium at the beginning and at the end of the experiment to investigate the potential of both strains to oxidize As(III) to As(V) under aerobic conditions (Figure 2). Additional sterile samples were analyzed to determine the possibility of chemical oxidation of As(III) in the culture medium. Samples inoculated with Tv. jannaschii ALM2T and Tv. thiocyanoxidans ARh2T showed a decrease in As(III) and an increase in As(V) over time. During the same incubation time, As(III) and As(V) concentrations did not change significantly in the sterile samples indicating that As(III) oxidation was biologically induced. Tv. jannaschii ALM2T had a much stronger As(III) oxidizing capacity as compared to Tv. thiocyanoxidans ARh2T. When grown in the presence of 0.1 mM As(III), Tv. jannaschii ALM2T oxidized 57% of the present As(III) in 1 day, whereas Tv. thiocyanoxidans ARh2T only oxidized 26% after 2 days. Most importantly, when grown in the presence of 5 mM As(III), Tv. jannaschii ALM2T was able to oxidize 79% of As(III) within 5 days. These findings resemble previous incubation experiments of Mono Lake surface waters that showed a clear link between aerobic As(III) oxidation capacity and added sulfide or thiosulfate (Fisher et al., 2008). In their research, sulfide-amended lake brines showed the formation of thioarsenates compounds from As(III), which were fairly stable in sterile, oxic surface waters, but which were further oxidized to As(V) in samples containing sulfur-oxidizing bacteria. Molecular analysis of the enrichments identified bacteria closely related to Tv. jannaschii, Tv. versutus and Tv. nitratia. Furthermore, Edwardson et al. (2014) showed that pure cultures of Tv. jannaschii ALM2T and Tv. thiocyanoxidans ARh2T with As(III) in combination with thiosulfate. Whether Thioalkalivibrio can gain energy from As(III) or thioarsenate oxidation, or that this oxidation is only used for detoxification purposes, remains an open question. However, it can be excluded that these compounds support growth as a sole electron donor.

Transcriptomic analysis enabled screening for key genes in the metabolism of and in the resistance against arsenic, and it shows differences in gene expression between Tv. jannaschii ALM2T and Tv. thiocyanoxidans ARh2T. General information on the individual RNA-seq samples analyzed by kallisto and sleuth are presented in Supplementary Table 5 and the complete expression data can be found in Supplementary Table 1. In total, 57.4 million sequence reads were produced by the Ion Proton platform ranging from 2.3 million to 4.9 million sequence reads per sample. From those reads, between 57.9% and 72.5% could be assigned to an open reading frame (ORF) depending on the sample analyzed with kallisto. In Tv. jannaschii ALM2T, 2833 ORFs were detected, and 2716 ORFs in Tv. thiocyanoxidans ARh2T. For the analysis performed with sleuth, an ORF is considered differentially expressed if the P-value is greater than 0.7-fold and its P-value is lower than 0.1. The RNA-Seq data analyzed by sleuth gave 101 up- and 84 downregulated genes for Tv. thiocyanoxidans ARh2T at 0.1 mM As(III) [0.1 mM vs. 0 mM As(III)] (Supplementary Table 6), only two up- and one downregulated genes for Tv. jannaschii ALM2T at 0.1 mM As(III) [0.1 mM vs. 0 mM As(III)] (Supplementary Table 7), and 26 up- and 16 downregulated genes for Tv. jannaschii ALM2T at 5 mM As(III) [5 mM vs. 0 mM As(III)] (Supplementary Table 8). As certain pathways could not be completely revealed based on the sleuth results only, we decided to also analyze the RNA-Seq data with CLC Genomics Workbench (Supplementary Table 2). With CLC, between 77.74 and 82.2% of the reads could be allocated to an ORF. Here, an ORF was considered to be differentially expressed if the log2 fold change was higher than 1-fold and its P-value lower than 0.1. With this threshold, CLC found 99 up- and 91 downregulated genes for Tv. thiocyanoxidans ARh2T at 0.1 mM As(III) [0.1 mM vs. 0 mM As(III)], four up- and five downregulated genes for Tv. jannaschii ALM2T at 0.1 mM As(III) [0.1 mM vs. 0 mM As(III)], and 40 up- and 20 downregulated genes for Tv. jannaschii ALM2T at 5 mM As(III) [5 mM vs. 0 mM As(III)].

The quality of the RNA-Seq data analyzed by kallisto and sleuth was evaluated by principal component analysis and plotted in a graph with the first two principal components as axes (Supplementary Figure 3). On the first principal component, the samples of each condition cluster together and were well separated from the other conditions. Remarkably, the Tv. jannaschii ALM2T samples grown at 0, 0.1, and 5 mM As(III) are not ordered based on the increasing As(III) concentration on the
first principle component, but in the order of 0.1, 0, and 5 mM As(III). This phenomenon might be explained by a hormesis reaction, in which an agent, here As(III), at lower level exposes an beneficial effect on the organism and becomes only toxic at higher concentrations (Mattson, 2008).

We summarized the results of the gene expression under As(III) stress in a conceptual model (Figure 3), in which the groups correspond to the subgroups of the discussion: (1) Arsenic influx into the cell, (2) Arsenic metabolism and detoxification, (3) Response to oxidative damage by arsenite, (4) Sulfur metabolism, and (5) Recombination and energy generation.

(1) Arsenic Influx Into the Cell
The arsenic species As(III) and As(V) are able to enter the cell by transporters of molecules whose properties they mimic. In E.coli, As(III) can enter the cell by the aquaglyceroporin channel GlpF (Sanders et al., 1997; Meng et al., 2004). These channels normally transport small uncharged molecules such as glycerol (Heller et al., 1980; Borgnia and Agre, 2001), but also As(III) as non-charged As(OH)₃ under neutral pH (Ramírez-Solís et al., 2012). However, in environments with a pH higher than its pKa of 9.2, As(III) will be mostly present in its ionic form (Smedley and Kinniburgh, 2002). This is the case for soda lakes whose pH ranges from 9.5 to 11 and, from which most Thioalkalivibrio strains were isolated (Sorokin et al., 2014). Furthermore, thioarsenates are formed in oxic alkaline brines containing sulfide (Stauder et al., 2005; Planer-Friedrich et al., 2007, 2009; Fisher et al., 2008; Härtig and Planer-Friedrich, 2012), conditions present as well in Mono Lake (Hollibaugh et al., 2005). Until now, it is unknown how As(III) or thioarsenates enter the cells under these conditions. A possible porin involved in their influx could be F816_RS0109535 (Supplementary Table 2) in Tv. jannaschii ALM2ᵀ as it is highly downregulated at 0.1 and 5 mM As(III). However, no similar protein could be detected in the genome of Tv. thiocyanoxidans ARh2ᵀ. The downregulation of a porin responsible for As(III) or thioarsenate influx would keep the intracellular arsenic concentration lower in Tv. jannaschii ALM2ᵀ, thus conferring a higher As(III) resistance to the strain.

As(V) possesses a similar chemical structure and properties as phosphate (Wolfe-Simon et al., 2009), and can therefore be taken up by the phosphate uptake systems Pit (inorganic phosphate transporter) and Pst (specific phosphate transporter).
FIGURE 3 | Continued
ARh2 genes were not differentially expressed in ars As(III) produced by the arsenate reductase ArsC is pumped under microoxic/anoxic conditions. This is performed in combination with the oxidation of reduced sulfur compounds hypothesized that this strain uses Arx to reduce As(V) to As(III) incapable of this strain to perform denitrification, it could be Tv. jannaschii Due to the presence of Arx in to oxidize As(III) and to reduce As(V) (Richey et al., 2009). has been shown electron donor (Supplementary Figure 2) in the tested strains under aerobic condition with As(III) as sole production of Pst to increase phosphate uptake (Andres and Bertin, 2016). This would give another advantage for growth in combination with As(III) oxidation for Tv. jannaschii ALM2T. However, as both strains were able to tolerate 30 mM As(V) data not shown), it is possible that Tv. thiocyanoxidans ARh2T also possesses a similar mechanism, which was not upregulated with the low As(V) concentration deriving from the oxidation process.

(2) Arsenic Metabolism and Detoxification

Transcriptomic analysis of Tv. jannaschii ALM2T grown at 5 mM As(III) showed an upregulation of the arsenite oxidase arxB2 gene, but not of the structural component genes arxABC (Supplementary Tables 1, 2). The Arx protein is only known to work under anaerobic condition coupled to denitrification or anaerobic photosynthesis (Hoef et al., 2007; Kulp et al., 2008; Zargar et al., 2010; Hernandez-Maldonado et al., 2017). Moreover, as already discussed before, no growth was observed in the tested strains under aerobic condition with As(III) as sole electron donor (Supplementary Figure 2). Previously, Arx has been shown in vitro to function as a bidirectional enzyme able to oxidize As(III) and to reduce As(V) (Richey et al., 2009). Due to the presence of Arx in Tv. jannaschii ALM2T and the incapacity of this strain to perform denitrification, it could be hypothesized that this strain uses Arx to reduce As(V) to As(III) in combination with the oxidation of reduced sulfur compounds under microoxic/anoxic conditions.

Most organisms perform an active extrusion of As(III) as their main arsenic resistance mechanism. This is performed in prokaryotes by the arsenic resistance ars operon, where As(III) produced by the arsenate reductase ArsC is pumped out of the cell by ArsB/ACR3 (Ben Fekih et al., 2018). The ars genes were not differentially expressed in Tv. thiocyanoxidans ARh2T at 0.1 mM As(III) (Supplementary Table 1). For Tv. jannaschii ALM2T at 0.1 mM As(III), only the uspA gene was upregulated. In contrast, the ars gene cluster was highly expressed in Tv. jannaschii ALM2T at 5 mM As(III) including the ACR3 efflux pump, two hypothetical proteins and the uspA gene. An exception was the arsC, which explains the high concentration of As(V) observed in the medium at the time of sampling (Figure 2 and Supplementary Tables 1, 2). The uspA gene encodes a uspA that is known to be induced under different stress situations (Kvint et al., 2003), by which it increases the endurance of the cell (Nystrom and Neidhardt, 1994). Upregulation of this gene has been shown in bacteria under As(III) stress (Weiss et al., 2009; Cleiss-Arnold et al., 2010; Sacheti et al., 2013). Finally, an operon, which is located next to the arx and ars cluster in Tv. jannaschii ALM2T and which includes a putatively annotated glycosyl transferase involved in the cell wall biosynthesis and a rhodanese-related sulfurtransferase were highly upregulated at 5 mM As(III) in ALM2T. However, in Tv. thiocyanoxidans ARh2T, this operon is neither upregulated nor found next to the ars gene cluster.

(3) Response to Oxidative Damage by Arsenite

Arsenic has been shown to induce formation of reactive oxygen species (ROS) and nitric oxide (NO) inside the cell (Andres and Bertin, 2016; Zhang et al., 2016). These radicals can cause damage to nucleic acids, proteins, and lipids (Flora, 2011; Birken, et al., 2012; Ray et al., 2012; Espinosa-Diez et al., 2015). To reduce the oxidative damage by arsenic, bacterial cells have developed various responses including the upregulation of Fe- and Mn-superoxide dismutases, thiol peroxidases, thioredoxin reductases, thioredoxins, glutaredoxins, glutathione, organic hydroperoxide resistance proteins, and vitamin B6 (Andres and Bertin, 2016). In our experiments, however, we did not detect changes in expression for the two Thioalkalivibrio strains for any of the genes involved in known antioxidation pathways. However, the As(III) concentrations of 0.1 mM As(III) for Tv. thiocyanoxidans ARh2T and 5 mM As(III) for Tv. jannaschii ALM2T triggered upregulation of the complete vitamin B12 (cobalamin) synthesis pathway (Supplementary Tables 1, 2). Vitamin B12 has been shown to protect eukaryotic cells from oxidative damage by its antioxidant activity (Birch et al., 2009; Suarez-Moreira et al., 2009; Moreira et al., 2011; Alzoubi et al., 2012; Bito et al., 2017) as well as when it is generated by arsenic in hepatic rat cells (Chattopadhyay et al., 2012; Majumdar et al., 2012). In bacterial cells, vitamin B12 has also been shown to be an antioxidant able to protect the cell from oxidative stress in the acidophilic iron-oxidizing bacterium Leptospirillum group II CF-1 (Ferrer et al., 2016). Recently, Qin et al. (2018) discovered that the archaea Nitrosopumilus maritimus SCM1 produces vitamin B12 under Cu2+ stress. Moreover, cobSTU was shown to be expressed by
bacteria in an arsenic-rich acid mine drainage, but was only related to the activation of iron oxidation (Bertin et al., 2011). Here, we propose that vitamin B₁₂ is the main antioxidant produced under As(III) stress in *Thioalkalivibrio*.

In addition, *Tv. thiocyanoxidans* ARh₂⁺ significantly upregulates the expression of the chaperones dnaK and Hsp20, and slightly of groEL, grpE and dnaJ. In contrast, *Tv. jannaschii* ALM₂⁺ does not change the expression of those genes. Chaperones from the Hsp70 (DnaK, DnaJ and GrpE) and Hsp60 (GroEL and GroES) systems have been shown to be commonly induced as an arsenic stress response in bacteria (Andres and Bertin, 2016). These chaperones are essential for the cell viability and the survival under diverse stressful conditions as they facilitate the proper folding of newly translated proteins or maintain it for already translated ones (Houry, 2001; Hayer-Hartl et al., 2016; Hartl, 2017).

(4) Sulfur Metabolism

**Thioalkalivibrio** strains are sulfur-oxidizing bacteria that possess a high inter-genus diversity of different genes and pathways involved in sulfur oxidation (Berben et al., 2019). *Tv. thiocyanoxidans* ARh₂⁺ and *Tv. jannaschii* ALM₂⁺ differentiate from each other by the fact that *Tv. thiocyanoxidans* ARh₂⁺ possesses the TcDH pathway for thioarsonate oxidation, while *Tv. jannaschii* ALM₂⁺ does not (Berben et al., 2019). Interestingly, the sequences of the two SoeA in *Tv. thiocyanoxidans* ARh₂⁺ form two distinct clusters, i.e., one cluster grouping around SoeA of *Allochromatium vinosum* (cluster 1) (Dahl et al., 2013) and a second cluster forming a separate group (cluster 2), hereafter called "Soe-like" gene. Both copies are present together in *Tv. thiocyanoxidans* ARh₂⁺ under As(III) stress ([soe]YXXAB, aprA and soeABC (cluster 2)), whereas others were not differentially expressed, such as soeABC (cluster 1), sat and sorAB. In *Tv. jannaschii* ALM₂⁺, only the soeABC (cluster 2) was highly upregulated together with genes necessary for the molybdenum cofactor production of SoeA, the *monA* (GTP 3'-7-ocyclase) (Mendel and Leimkühler, 2015) and the molybdate ABC transporter.

As(III) was also oxidized in *Tv. thiocyanoxidans* ARh₂⁺ cultures, although this strain does not possess an arsenite oxidase in its genome (Figure 1). Therefore, another as yet unknown enzyme must exist besides the two known arsenite oxidases AioA and ArxA involved in As(III) oxidation under aerobic conditions. Comparing our results with the work of Fisher et al. (2008), we can hypothesize that thioarsonate species have also formed in our cultures, opening new possibilities for enzymatic pathways of As(III) oxidation in the presence of thiosulfate or sulfide. Some enzymes have already been hypothesized to be involved in the oxidation pathway of thioarsonate compounds. Edwardson et al. (2014) proposed the Sox pathway as a potential facilitator of thioarsonate oxidation based on the structural similarity between monothioarsenate and thiosulfate. In this system, Sox enzymes would be able to cleave the thiol group from monothioarsenate.

This hypothesis could be supported by the upregulation of the sox cluster in *Tv. thiocyanoxidans* ARh₂⁺, but is contradicted by the stable or even slight downregulation of these genes in *Tv. jannaschii* ALM₂⁺ where the strongest As(III) oxidation occurred. Furthermore, a sulfide-quinone oxoreductase and its operon were found upregulated in the presence of sulfide or As(III) in *Synechocystis* sp. strain PCC6803 (Nagy et al., 2014). As these genes are closely related to genes in *Tv. thiocyanodonitrificans* ARhD1⁻, although not found in a single operon in this strain, they proposed that these genes could be involved in thioarsonate oxidation in *Thioalkalivibrio*. However, no upregulation of these genes was detected in the dataset obtained with *Tv. jannaschii* ALM₂⁺ and *Tv. thiocyanoxidans* ARh₂⁺ growing with As(III). Couture et al. (2012) proposed in a review the involvement of the proteins SeI and SeU coupled to ArxC in thioarsonate production alongside to their normal activity of making selenophosphate and modifying RNA. However, we could not find any homologs to these proteins in the genomes of *Tv. jannaschii* ALM₂⁺ and *Tv. thiocyanoxidans* ARh₂⁺.

The only genes of the sulfur oxidation pathway that were induced in both strains at their highest respective As(III) concentration were the quinone-dependent sulfite oxidase soeABC (cluster 2). SoeABC is a molybdopterin oxireductase of the same family as the arsenic oxireductases Aio, Arr, and Arx (Kraft and Macy, 1998; Ellis et al., 2001; McEwan et al., 2002; Zargar et al., 2010; Dahl et al., 2013). Similar to those proteins, SoeA and SoeB form a heterodimer, which is anchored to the cytoplasmic membrane by SeoC. The difference between the SoeA and the arsenic oxireductase is that SoeA does not contain a TAT-signal peptide, and therefore it stays in the cytoplasm (Dahl et al., 2013). This TAT-signal peptide also does not exist in the SoeABC-like protein of the cluster 2. Until now, no activity and substrate specificity have been proven for this second Soe-like cluster. Therefore, we propose that this Soe-like protein as a possible candidate for co-oxidation of As(III) and sulfite (SO₃²⁻), or oxidation of thioarsonate. Moreover, we hypothesize that the observed oxidation has rather the aim of detoxifying the cell as both strains were unable to grow on As(III) as a sole electron donor (Supplementary Figure 2).

Multiple putative sulfurtransferases annotated as DsrE/F-like genes were found up- or downregulated in the presence of As(III). The sulfurtransferase DsrEFH binds to sulfur via a conserved cysteine of the DsrE and transports it to the DsrC in the reverse Dsr system of elemental sulfur oxidation to sulfite (Stockdreher et al., 2012). The function of a cysteine in an active site is known to be inactivated by the binding of As(III) to the sulhydryl group (Shen et al., 2013). One possibility for their change in expression could therefore be either their induction to compensate for the inactivation (upregulation) or their reduction to shut down the pathway (downregulation).

(5) Recombination and Energy Generation

Interestingly, genes for genetic recombination were downregulated. These include different transposases and
integrases. This is in contradiction with the findings of Gualco et al. (2004) where a rise in the amount of recombinants in conjugation and transduction, and transposition of the Tn9 was observed when As(III) at a sub-MIC (Minimal Inhibitory Concentration) was added. This is in agreement with the general understanding that stressful environmental conditions induce genetic variation in bacteria via mutations and recombination (Bjedov et al., 2003; Foster, 2005, 2007; Prudhomme et al., 2006; Schuurmans et al., 2014).

In addition, genes are induced that are involved in the various pathways for the electron transfer in oxidative phosphorylation including the NADH:ubiquinone oxidoreductase in both strains, a cytochrome c synthesis gene and a Na+/H+ antiporter subunit in Tv. thiocyanoxidans ARh2T. The transcriptional upregulation of these complexes are commonly observed in bacteria in the presence of arsenic (Andres and Bertin, 2016). One explanation could be that arsenic works as an uncoupler of the membrane potential and as an alternative substrate of ATPase, which could impair NADH and ATP production. To ensuring adequate NADH and ATP production, Tv. thiocyanoxidans ARh2T compensates this effect by the upregulation of the NADH:ubiquinone oxidoreductase, the Na+/H+ antiporter and by increased cytochrome c synthesis.

**CONCLUSION**

In this study, we identified the putative potential of arsenic metabolism by the presence of Arx in 14 Thioalkalivibrio strains, and of Arr in two. Furthermore, we investigated the main mechanisms of arsenite resistance for Tv. jannaschii ALM2T and Tv. thiocyanoxidans ARh2T. These strains do not share the same resistance to As(III), which is reflected in their growth response to different As(III) concentrations, in their repertoire of arsenic resistance genes, in their As(III)-oxidizing potential and in their transcriptome. From the gene expression, we discovered an involvement of vitamin B12 as the major player in the protection against arsenic-imposed oxidative stress, as well as the differential expression of DsrE/F-like proteins whose roles need to be elucidated in future research. Moreover, Tv. jannaschii ALM2T induced the transcription of the ars gene operon and the Pst system, and Tv. thiocyanoxidans ARh2T increased expression of the sox and apr genes as well as different heat shock proteins. Comparing our results with the work of Fisher et al. (2008), we can postulate the formation of thioarsenates in the Thioalkalivibrio cultures, which were then microbiologically further oxidized by an as yet unknown enzymatic pathway to As(V). We hypothesize that a Soe-like protein is responsible for this oxidation, but evidence must be obtained by future work.

**DATA AVAILABILITY**

The datasets generated for this study are deposited in the NCBI Sequence Read Archive under SRA accession numbers SRX5567239 to SRX5567253.

**AUTHOR CONTRIBUTIONS**

A-CA carried out the cultivation, the comparative sequence analyses, the RNA-Seq data analysis by sleuth, and drafted the manuscript. LC and MC carried out the analysis of the arsenic species. GM carried out the RNA-Seq data analysis by CLC. JS, DS, and GM assisted in the interpretation of the results, and together with LC provided a critical review of the manuscript. All authors read and approved the final version of the manuscript.

**FUNDING**

Financial support for A-CA, JS, and GM was provided by the ERC Advanced Grant Parasol (No. 322551). LC and MC were supported by the Cariplo Foundation project 2014-1301. DS was funded by the Russian Foundation for Basic Research (Grant No. 19-04-00401) and the Russian Ministry of Science and Higher Education. The sequencing platform was funded by the NWO Earth and Life Sciences (ALW) project 834.12.003.

**ACKNOWLEDGMENTS**

We would like to thank Eleanor Spring for the critical proofreading of the manuscript.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2019.01514/full#supplementary-material

**REFERENCES**

Afkar, E., Lisak, J., Saltikov, C., Basu, P., Oremland, R. S., and Stolz, J. F. (2003). The respiratory arsenate reductase from Bacillus selenitireducens strain MLS10. *FEMS Microbiol. Lett.* 226, 107–112. doi: 10.1016/S0378-1097(03)00709-8

Ahn, A. C., Meier-Kolthoff, J. P., Overmars, L., Richter, M., Woyke, T., Sorokin, D. Y., et al. (2017). Genomic diversity within the haloalkaliphilic genus *Thioalkalivibrio*. *PLoS One* 12:e0173517. doi: 10.1371/journal.pone.0173517

Alzoubi, K., Khabour, O., Hussein, N., Al-Azzam, S., and Mhaidat, N. (2012). Evaluation of vitamin B12 effects on DNA damage induced by pioglitazone. *Mutat. Res.* 748, 48–51. doi: 10.1016/j.mrgentox.2012.06.009

Anderson, G. L., Williams, J., and Hille, R. (1992). The purification and characterization of arsenite oxidase from *Alcaligenes faecalis*, a molybdenum-containing hydroxylase. *J. Biol. Chem.* 267, 23674–23682.

Andres, J., and Bertin, P. N. (2016). The microbial genomics of arsenic. *FEMS Microbiol. Rev.* 40, 299–322. doi: 10.1093/femsre/fuw050

Banciu, H., Sorokin, D. Y., Galinski, E. A., Muhayer, G., Kleerebezem, R., and Kuenen, J. G. (2004). *Thialkalivibrio* halophilus sp. nov., a novel obligately
chemolithoautotrophic, facultatively alkaliphilic, and extremely salt-tolerant, sulfur-oxidizing bacterium from a hypersaline alkaline lake. *Extremophiles* 8, 325–334. doi: 10.1007/s00792-004-0391-6

Ben Fekih, I., Zhang, C., Li, Y. P., Zhao, Y., Alwathnani, H. A., Saqib, Q., et al. (2018). Distribution of arsenic resistance genes in prokaryotes. *Front. Microbiol.* 9:2473. doi: 10.3389/fmicb.2018.02473

Berben, T., Overmars, L., Sorokin, D. Y., and Muyzer, G. (2017). Comparative genome analysis of three thioynenate oxidizing *Thioalkalivibrio* species isolated from soda lakes. *Front. Microbiol.* 8:254. doi: 10.3389/fmicb.2017.00254

Berben, T., Overmars, L., Sorokin, D. Y., and Muyzer, G. (2019). The diversity and distribution of sulfur oxidation-related genes in *Thioalkalivibrio*, a genus of chemolithoautotrophic and haloalkaliphilic sulfur-oxidizing bacteria. *Front. Microbiol.* 10:160. doi: 10.3389/fmicb.2019.00160

Berben, T., Sorokin, D. Y., Ivanova, N., Pati, A., Kyprides, N., Goodwin, L. A., et al. (2015). Partial genome sequence of the haloalkaliphilic soda lake bacterium *Thioalkalivibrio thiocyanoxidans* ARB 2T. *Stand. Genomic Sci.* 10:85. doi: 10.1186/s40793-015-0078-x

Bertin, P. N., Heinrich-Salmeron, A., Pelletier, E., Goulhen-Chollet, F., Arsène-Huot, M., et al. (2015). Oxidative stress and antioxidant defense. *World Allergy Organ. J.* 5, 9–19. doi: 10.1097/WOX.0b013e3182439613

Birch, C. S., Brach, N. E., McCaddon, A., and Williams, J. H. H. (2009). A novel role for vitamin B12: cobalamins are intracellular antioxidants in vitro. *Free Radic. Biol. Med.* 47, 178–184. doi: 10.1016/j.freeradbiomed.2009.04.023

Bito, T., Misaki, T., Yabuta, Y., Ishikawa, T., Kawano, T., and Watanabe, F. (2017). Arsenic-induced oxidative stress and its reversibility. *Free Radic. Biol. Med.* 11:709. doi: 10.1016/j.freeradbiomed.2015.07.008

Ferrer, A., Rivera, J., Zapata, C., Norambuena, J., Sandoval, Á., Chávez, R., et al. (2016). Cobalamin protection against oxidative stress in the acidophilic iron-oxidizing bacterium *Leptospirillum group II* CF-1. *Front. Microbiol.* 7:748. doi: 10.3389/fmicb.2016.00748

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Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and 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: 10.1007/s13382-017-0780

Edwardson, C. F., Planer-Friedrich, B., and Hollibaugh, J. T. (2014). Transformation of monothioarsenate by haloalkaliphilic, anoyxegenic photosynthetic purple sulfur bacteria. *FEMS Microbiol. Ecol.* 90, 858–868. doi: 10.1111/1574-6941.12440

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Edwinson-Diez, C., Miguel, V., Mennerich, D., Kitzmann, T., Sánchez-Pérez, P., Cadenas, S., et al. (2015). Antioxidant responses and cellular adjustments to oxidative stress. *Redox Biol.* 6, 183–197. doi: 10.1016/j.redox.2015.07.008

Ellis, P., J., Conrads, T., Hille, R., and Kuhn, P. (2001). Crystal structure of the 100 kDa arsenite oxidase from *Alcaligenes faecalis* in two-crystal forms at 1.64 Å and 2.03 Å. *Structure* 9, 125–132. doi: 10.1016/s0969-2126(01)00566-4

Enzimina, A., Antunes, C., Almeida, T., and Lourenço, W. (2018). ISME J. 11, 21–29. doi: 10.1093/ismej.1186/s40793-015-0078-x

Chehab, S., and Moreau, L. (2018). *Thioalkalivibrio* sp. strain CAJ2: a new role for sulfur in arsenic cycling. *Environ. Sci. Technol.* 42, 81–85. doi: 10.1021/acs.est.7b03196

Kumar, S., and Tamura, K. (2018). MEGA 7: Molecular evolutionary genetics analysis and phylogenetic inference. *Mol. Biol. Evol.* 35, 1547–1560. doi: 10.1093/molbev/msx098

Borgnia, M. J., and Agre, P. (2001). Reconstitution and functional comparison of purified GlpF and AqpZ, the glycerol and water channels from *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* 98, 12589–12594. doi: 10.1073/pnas.98.21.12589

Hoeft, S. E., Blum, J. S., Stolz, J. F., Tabita, F. R., Witte, B., King, G. M., et al. (2007). Transformation of arsenic-resistant crenarchaeotal archaeon *Crenarchaeon* isolates in anaerobic environments. *Environ. Microbiol.* 9, 125–132. doi: 10.1111/j.1462-2920.2006.00788.x

García, C. J., and Kuehn, C. H. (2007). *Review of Environmental Microbiology* 1, 19–33. doi: 10.1186/1476-4598-1-74

Birch, A. J., Christensen, S., and van der Wielen, W. P. (2010). *Nature Protocols* 5, 275–281. doi: 10.1038/nprot.2009.448

Chang, H., Chen, P., and Pande, K. (2010). Methodology for metagenomic analysis of environmental samples. *Methods Mol. Biol.* 679, 15–30. doi: 10.1007/978-1-60327-795-0_2

Clifford, R. M., Leong, L. C., Loughlin, H., and Watanabe, F. (2017). Arsenic stress response in *Thioalkalivibrio*. *Front. Microbiol.* 8:1276. doi: 10.3389/fmicb.2017.01276

Hernandez-Maldonado, J., Sanchez-Sedillo, B., Stoneburner, B., Boren, A., Miller, L. M., McCann, S., et al. (2017). *Environ. Microbiol.* 19, 130–141. doi: 10.1111/1462-2920.12309

Hartl, F. U. (2017). Unfolding the chaperone story. *Mol. Cell. Biol.* 28, 2919–2923. doi: 10.1099/mcb.0.024277-e

Hartl, F. U. (2017). The GroEL-GroES chaperonin machine: a nano-cage for protein folding. *Trends Biochem. Sci.* 41, 62–76. doi: 10.1016/j.tibs.2017.05.009

Höfle, S. E., Blum, J. S., Stolz, J. F., Tabita, F. R., Witte, B., King, G. M., et al. (2007). Alkaliphilic halophilic *Halobacterium* sp. nov., a novel, arsenite-oxidizing haloalkaliphilic gammaproteobacterium capable of chemostatotrophic or heterotrophic growth with nitrate or oxygen as the electron acceptor. *Int. J. Syst. Evol. Microbiol.* 57, 504–512. doi: 10.1099/ijs.0.03657-0

Hollibaugh, J. T., Caritat, S., Gislaved, H., Jellison, R., Joyce, S. B., LeClerg, C., et al. (2005). Arsenic speciation in Mono Lake, California: response to seasonal stratification and anoxia. *Geomicrobiol. J.* 26, 1925–1937. doi: 10.1111/j.1061-106X.2004.00111

Houy, W. A. (2001). Chaperone-assisted protein folding in the cell cytoplasm. *Curr. Protein Pept. Sci.* 2, 227–244. doi: 10.2174/1389203033381134
Smedley, P. L., and Kinniburgh, D. G. (2002). A review of the source, behaviour
Sorokin, D. Y., Berben, T., Melton, E. D., Overmars, L., Vavourakis, C. D., and
Shen, S., Li, X. F., Cullen, W. R., Weinfeld, M., and Le, X. C. (2013). Arsenic binding
Stolz, J. F., Basu, P., Santini, J. M., and Oremland, R. S. (2006). Arsenic and selenium in microbial metabolism. Annu. Rev. Microbiol. 60, 167–130. doi: 10.1146/annurev.micro.60.080805.142653
Scott, N., Hatlelid, K. M., MacKenzie, N. E., and Carter, D. E. (1993). Reactions of arsenic (III) and arsenic (V) species with glutathione. Chem. Res. Toxicol. 6, 102–106. doi: 10.1021/tr00016a016
Shen, S., Li, X. F., Cullen, W. R., Weinfeld, M., and Le, X. C. (2013). Arsenic binding to proteins. Chem. Rev. 113, 7767–7792. doi: 10.1021/cr300517d
Smedley, P. L., and Kinniburgh, D. G. (2002). A review of the source, behaviour and distribution of arsenic in natural waters. Chem. Rev. 17, 517–568. doi: 10.1021/0883-2927(02)00018-5
Sorokin, D. Y., Gorlenko, V. M., Tourova, T. P., Tsapin, A. N., Nealson, K. H., and Kuenen, G. J. (2002a). Thioalkalimicrobium cyclium sp. nov. and Thioalkalivibrio jannaschii sp. nov., novel species of haloalkaliphilic, obligately chemolithotrophic sulfur-oxidizing bacteria from hypersaline alkaline Mono Lake (California). Int. J. Syst. Evol. Microbiol. 52, 913–920. doi: 10.1099/ijs00207713-52-3-913
Sorokin, D. Y., Tourova, T. P., Lysenko, A. M., Mituyushina, L. I., and Kuenen, J. G. (2002b). Thioalkalivibrio thiocyanoxidans sp. nov. and Thioalkalivibrio paradoxus sp. nov., novel alkaliphilic, obligately autotrophic, sulfur-oxidizing bacteria capable of growth on thiocyanate, from soda lakes. Int. J. Syst. Evol. Microbiol. 52, 657–664. doi: 10.1099/ijs00207713-52-2-657
Sorokin, D. Y., Lysenko, A. M., Mituyushina, L. I., Tourova, T. P., Jones, B. E., Rainey, F. A., et al. (2001a). Thioalkalimicrobium aerophilum gen. nov., sp. nov. and Thioalkalimicrobium ibericum sp. nov., and Thioalkalivibrio versatilis gen. nov., sp. nov., Thioalkalivibrio nitritas sp. nov. and Thioalkalivibrio denitrificans sp. nov., novel obligately alkaliphilic. Int. J. Syst. Evol. Microbiol. 51, 565–580. doi: 10.1099/ijs00207713-51-2-565
Sorokin, D. Y., Tourova, T. P., Lysenko, A. M., and Kuenen, J. G. (2001b). Microbial thiocyanate utilization under highly alkaline conditions. Appl. Environ. Microbiol. 67, 528–538. doi: 10.1128/AEM.67.5.528-538.2001
Sorokin, D. Y., Muntyan, M. S., Panteleeva, A. N., and Muyzer, G. (2012). Thioalkalivibrio sulfidiphilus sp. nov., a halokailiphilic, sulfur-oxidizing gammaproteobacterium from alkaline habitats. Int. J. Syst. Evol. Microbiol. 62, 1884–1889. doi: 10.1099/ijs.0.034504-0
Sorokin, D. Y., Tourova, T. P., Antipov, A. N., Muyzer, G., and Kuenen, J. G. (2004). Anaerobic growth of the haloalkaliphilic denitrifying sulfur-oxidizing bacterium Thioalkalivibrio thiocyanodenitrificans sp. nov. with thiocyanate. Microbiology 150, 2435–2442. doi: 10.1099/mic.0.27015-0
Sorokin, D. Y., Tourova, T. P., Sjøllem, K. A., and Kuenen, J. G. (2003). Thioalkalivibrio nitratireducens sp. nov., a nitrate-reducing member of an autotrophic denitrifying consortium from a soda lake. Int. J. Syst. Evol. Microbiol. 53, 1779–1783. doi: 10.1099/ijs.0.02615-0
Stamps, B. W., Nunn, H. S., Petryshyn, V. A., Oremland, R. S., Miller, L. G., Rosen, M. R., et al. (2018). Metabolic capability and phylogenetic diversity of Mono Lake during a bloom of the eukaryotic phototroph Picocystis sp. strain ML. Environ. Microbiol. 20, 150, 2435–2442. doi: 10.1099/mic.0.27015-0
Stauder, S., Raue, B., and Sacher, F. (2005). Thioselenates in sulfidic waters. Environ. Sci. Technol. 39, 5933–5939. doi: 10.1021/es048034k
Stockdreher, Y., Venceslau, S. S., Josten, M., Sahl, H. G., Pereira, I. A. C., and Dahl, C. (2012). Cytoplasmic sulfurtransferases in the purple sulfur bacterium Allochloromatum vinsonum: evidence for sulfur transfer from DsrEFH to DsrC. PLoS One 7:e40785. doi: 10.1371/journal.pone.0040785
Stola, J. F., Basu, P., Santini, J. M., and Oremland, R. S. (2006). Arsenic and selenium in microbial metabolism. Annu. Rev. Microbiol. 60, 167–130. doi: 10.1146/annurev.micro.60.080805.142653
Suarez-Moreira, E., Yun, J., Birch, C. S., Williams, J. H. H., McCaddon, A., and Brasch, N. E. (2009). Vitamin B12 and redox homeostasis: cob(II)alamin reacts with superoxide at rates approaching superoxide dismutase (SOD). J. Am. Chem. Soc. 131, 15078–15079. doi: 10.1021/ja904670x
Tawfik, D. S., and Viola, R. E. (2011). Arsenate replacing phosphate - alternative life chemistries and ion promiscuity. Biochemistry 50, 1128–1134. doi: 10.1021/bi200002a
Van Lies, R., Nitsche, W., Carewlow, T. P., Capowiez, L., Santini, J. M., and Schoepp-Cothenet, B. (2012). Heterologously expressed arsenite oxidase: a system to study biogenesis and structure/function relationships of the enzyme family. Biochim. Biophys. Acta 1817, 1701–1708. doi: 10.1016/j.bbapap.2012.06.001
Willsky, G. R., and Malamy, M. H. (1980a). Characterization of two genetically separable inorganic phosphate transport systems in Escherichia coli. J. Bacteriol. 144, 356–365.
Willsky, G. R., and Malamy, M. H. (1980b). Effect of arsenate on inorganic phosphate transport in Escherichia coli. J. Bacteriol. 144, 366–374.
Wysocki, R., Bobrowicz, P., and Ułaszewski, S. (1997). The S. acidivorans arsC3 gene encodes a putative membrane protein involved in arsenate transport. J. Biol. Chem. 272, 30061–30066. doi: 10.1074/jbc.272.48.30061
Zhang, Y., Chen, S., Hao, X., Su, J. Q., Xue, X., Yan, Y., et al. (2016). Transcriptomic analysis reveals adaptive responses of an Enterobacteriaceae strain F547 to arsenic exposure. Front. Microbiol. 7:636. doi: 10.3389/fmicb.2016.00636
Zhang, Y., Chen, S., Hao, X., Su, J. Q., Xue, X., Yan, Y., et al. (2016). Transcriptomic analysis reveals adaptive responses of an Enterobacteriaceae strain L547 to arsenic exposure. Front. Microbiol. 7:636. doi: 10.3389/fmicb.2016.00636
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