Biometals (2009) 22:117–130
DOI 10.1007/s10534-008-9195-y

Microbial responses to environmental arsenic

David Páez-Espino · Javier Tamames · Víctor de Lorenzo · David Cánovas

Received: 5 November 2008 / Accepted: 7 December 2008 / Published online: 7 January 2009

Abstract Microorganisms have evolved dynamic mechanisms for facing the toxicity of arsenic in the environment. In this sense, arsenic speciation and mobility is also affected by the microbial metabolism that participates in the biogeochemical cycle of the element. The *ars* operon constitutes the most ubiquitous and important scheme of arsenic tolerance in bacteria. This system mediates the extrusion of arsenite out of the cells. There are also other microbial activities that alter the chemical characteristics of arsenic: some strains are able to oxidize arsenite or reduce arsenate as part of their respiratory processes. These type of microorganisms require membrane associated proteins that transfer electrons from or to arsenic (AoxAB and ArrAB, respectively). Other enzymatic transformations, such as methylation-demethylation reactions, exchange inorganic arsenic into organic forms contributing to its complex environmental turnover. This short review highlights recent studies in ecology, biochemistry and molecular biology of these processes in bacteria, and also provides some examples of genetic engineering for enhanced arsenic accumulation based on phytochelatins or metallothionein-like proteins.

Keywords Arsenic · Bioremediation · Bacteria · Metallothioneins · Heavy metals · *ars* genes

D. Páez-Espino · V. de Lorenzo (✉)
Centro Nacional de Biotecnologia CSIC, Campus de Cantoblanco, 28049 Madrid, Spain
e-mail: vdlorenzo@cnb.csic.es

J. Tamames
Institut Cavanilles de Biodiversitat i Biologia Evolutiva, University of Valencia, 46071 Valencia, Spain

D. Cánovas
Departamento de Genética, Facultad de Biología, Universidad de Sevilla, 41012 Sevilla, Spain

Introduction

Arsenic was one of the first chemicals recognized as carcinogen (Rosen 1971). However, the mechanisms responsible for its oncogenic effects are known only in part (Pott et al. 2001). As a consequence, the levels of environmental arsenic assigned to mortality risk are subject to occasional re-evaluations. For instance, while 50 μg/l of arsenic in water was considered for some time the reference contaminant level causing a cancer incidence of 1%, the figure was recently lowered by the US Environmental Protection Agency to 10 μg/l (Smith et al. 2002; Singh et al. 2007), thus reflecting some uncertainties on the matter. An estimated over 40 million people in the world are at risk from drinking arsenic-contaminated water (Nordstrom 2002). One of the reasons is that the management of arsenic wastes is difficult. The fact that arsenic has a variety of valence states (+V, +III, 0, −III) hinders

the treatment and/or removal of the compound. In addition, arsenic is highly abundant and worldwide distributed through the Earth crust. As an example, many geothermal waters contain high concentrations of arsenic i.e. in Kamchatka, New Zealand, Japan, and USA. The main sources of arsenic are natural (organic-rich or black shales, mineralized and mined areas, volcanogenic areas, thermal springs…) and also originates as the result of human activities (mining activities, waste processing, pesticides, industrial activity; Nordstrom 2002). Most of arsenic uses in agriculture and industry have been discontinued nowadays, but residues from such activities have left a heritage of arsenic-contaminated sites (Leist et al. 2000). This is dramatized by the fact that arsenic is often associated to pyrite, one of the most ubiquitous minerals, and is commonly concentrated in mineral deposits containing sulfides. Under anoxic conditions, nitrate influences the As cycling by oxidizing ferrous iron to produce As-sorbing particles of hydrous ferric oxides and increasing the more particle-reactive As(V) (Senn and Hemond 2002). However, depending on the physico-chemical conditions, the arsenic in these particles can be easily solubilized in ground waters (Oremland and Stolz 2005). Therefore, a case-by-case study is required prior to the selection of the appropriate remediation strategy. For example, the pentavalent form of the element is the arsenic species that is most effectively removed by coagulation with iron (Leist et al. 2000).

Not only most living organisms have developed arsenic resistance mechanisms but some of them are able to utilize, or even require arsenic for their ordinary physiology (see Fig. 1 for a summary of microbial-mediated biochemistry of arsenic). These can use As for respiration (Macy et al. 1996; Newman et al. 1997b, c; Stolz and Oremland 1999; Macy et al. 2000; Silver and Phung 2005) and/or as electron donor (Santini et al. 2000; Gihring and Banfield 2001; Silver and Phung 2005). Apart of altering the redox state of arsenic, some microbes can also methylate inorganic species (Qin et al. 2006) or demethylate organic counterparts (Silver and Misra 1984; Cullen and Reimer 1989; Silver and Phung 2005). Such microbial activities in water and

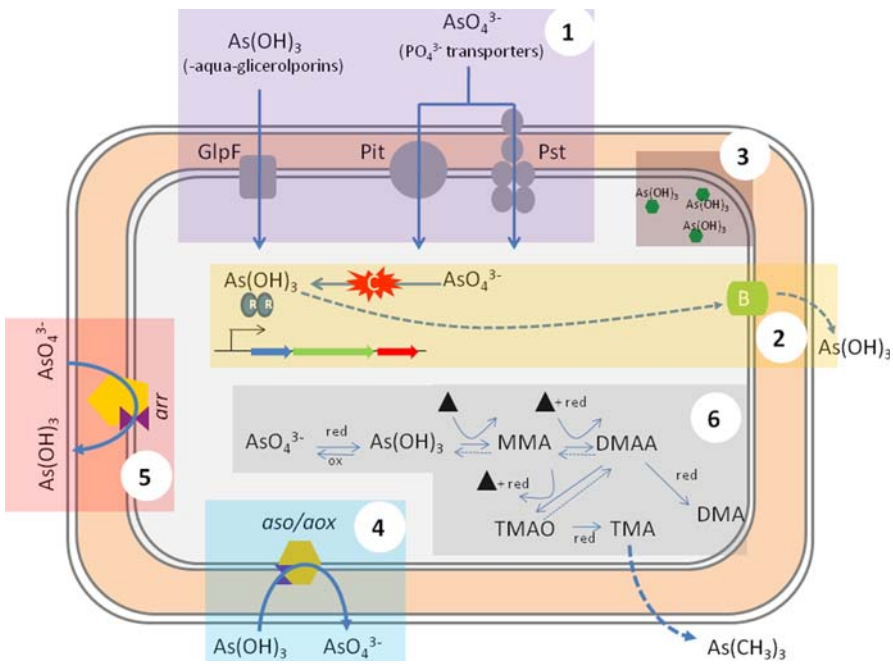

Fig.1 Diagram of the different microbial processes involved in arsenic biochemistry in the environment. (*1*) Arsenic enters the cells through the phosphate transporters (arsenate) or the aqua-glyceroporins (arsenite). (*2*) Once inside the cells, arsenate is reduced to arsenite by ArsC, which further extruded out of the cell by the specific pump ArsB. (*3*) Arsenite can also be detoxified by complexation with Cys-rich peptides. In addition, (*4*) arsenite can serve as electron donor by oxidation to arsenate. (*5*) Arsenate can be used as the ultimate electron acceptor during respiration. (*6*) Inorganic arsenic can also be transformed into organic species in a methylation cascade

sediments strongly influence the speciation and bioavailability of arsenic (Oremland and Stolz 2005), participating actively in its environmental cycle. Although yeast and fungi do display intriguing biological responses to As exposure (Cánovas et al. 2004; Cánovas and de Lorenzo 2007), this short review will deal predominantly with bacteria in view of their potential as environmental bioremediation agents.

Speciation and biochemistry of arsenic in the environment

Arsenic in Nature can occur in four oxidation states, arsenate As(V), arsenite As(III), elemental As(0) and arsenide (As^{-III}). The two highest oxidation states are the most common in nature, whereas the two lowest are rare (Rosen 2002; Oremland and Stolz 2003). There are several reports that clearly establish a direct link between microbial activities and the arsenic speciation in the environment. Depending on the physico-chemical characteristics of the site and the structure of the microbial population, distinct arsenic species may predominate. For example, in Meager Creek hot springs, (BC, Canada) the major arsenic species found in the microbial mats were arsenate and arsenite along with small amounts of arsenosugars (Koch et al. 1999). In other niches, for instance, the rhizosphere, accumulation of arsenic in wetland plants originates the formation of iron oxyhydroxides precipitates on the surface of the roots, thereby forming distinctive structures called iron plaques (Otte et al. 1995).

The geothermal springs in Yellowstone National Park, USA, often contains arsenic concentrations of 10–40 μM. One acid thermal spring (pH 3.1, 58–62°C) in this park exhibited rapid rates of arsenite oxidation between 2.7 and 5.6 m downstream from the spring source, corresponding to a brown Fe/As-rich mat. It was found that the filamentous microbial mat contained iron (III) oxyhidroxide co-precipitated with As(V) (Langner et al. 2001). In this site, changes in the microbial community developed along with the arsenite oxidation activity. The appearance of archeal 16S rDNA sequences belonging to *Crenarchaeota* and *Euryarchaeota* was simultaneous with the start of arsenite oxidation, suggesting that these microorganisms are responsible of such an activity. These microbial communities were dominated by microorganisms phylogenetically related to *Hydrogenobacter acidophilus* and *Desulphurella* sp. (Jackson et al. 2001). In a different environment, denaturing gradient gel electrophoresis (DGGE) coupled to 16S rDNA sequencing was instrumental for revealing that exposure to mine tailings stimulated the numbers of *Caulobacter*-like, *Sphingomonas*-like, and *Rhizobium*-like populations. Contrary to what happens in the geothermal springs above, these communities have the ability to reduce arsenate rapidly (Macur et al. 2001). This arsenate-respiring bacteria are able to release arsenite from sediments (Hemond 1995) and from arsenate-containing minerals (Newman et al. 1997b, c) or from adsorptive sites of aluminum oxides or ferrihydrite (Appelo et al. 2002). Furthermore in an interesting case, *Alcalilimnicola ehrlichei* strain MLHE-1 was described as a bacterium capable of nitrate and arsenate reduction coupled to arsenite oxidation (Oremland et al. 2002).

Laboratory studies have also shed some light on the various microbial activities on environmental arsenic. In one interesting case, leaching and run-off of arsenic from a contaminated soil was analyzed in soil-water-sediment mesocosms. 7.5% of the total load of arsenic in this instance remained in water as As(V); 44% settled down to the shallow sediment zone as As(V) together with some dimethyl arsenic acid (DMAA); and 48% went to the deeper sediment zone which corresponded mainly to As(III) (Ruokolainen et al. 2000). In a separate study, five environmental bacterial isolates were found to transform arsenate into arsenite and volatile methylarsines. The strains belong to the *Proteus*, *Escherichia*, *Flavobacterium*, *Corynebacterium* and *Pseudomonas* genera (Shariatpanahi et al. 1981). This documents the widespread ability of diverse bacteria to act on arsenic with many different biochemical results.

Arsenic can also appear naturally in the form of organic derivatives, which implies the presence of C-As bounds. A number of microorganisms are able to methylate arsenic giving rise to mono-, di-, and/or tri-methyl derivatives. One example of this is *Rhodopseudomonas palustris* (Qin et al. 2006). Strains of this species are able to catalyze the formation of a number of methylated intermediates from As(III), with trimethylarsine as the end product. Reduced methylated species are volatile, and therefore released to the atmosphere upon biosynthesis. Although these species are more genotoxic than the

Table 1 Toxicity of the different arsenic species

Arsenic species	LD_{50} (g/kg)[a]	Genotoxic response (μg/ml)[b]	In vitro genotoxicity (mM)[c]	Chemical structure
Inorganic				
Arsine	0.003[d]			AsH_3
Arsenite	0.014[d], 0.034[e]	1–2	>300 mM	AsO_2H
Arsenate	0.020[d], 0.041[e]	10–14	>1,000 mM	AsO_4H_3
Methylarsine (MMA)			30 mM	CH_3AsH_2
Dimethylarsine (DMA)			150 μM	$(CH_3)_2AsH$
Trimethylarsine (TMA)	7.87[g]			$(CH_3)_3As$
Organic				
Methylarsonic acid (MMAA)	0.7–1.8[d], 0.7[e]	2,500–5,000	>3,000 mM	$CH_3AsO(OH)_2$
Dimethylarsinic acid (DMAA)	0.7–2.6[d], 3.3[e]	10,000	>300 mM	$(CH_3)_2AsO(OH)$
Trimethylarsine oxide (TMAO)	>10[e]			$(CH_3)_3AsO$
Tetramethylarsonium ion (TMA^+)	0.89[e]			$(CH_3)_4As^+$
Arsenocholine	>10[e]			$(CH_3)_3As^+CH_2CH_2OH$
Arsenobetaine	>10[e,f,g]			$(CH_3)_3As^+CH_2COOH$

[a] Lethal Dose 50; [b] Moore et al. (1997); [c] Mass et al. (2001); [d] Tamaki and Frankenberger (1992); [e] Eguchi et al. (1997); [f] Kaise et al. (1985); [g] Yamauchi et al. (1990)

other derivatives (inorganic and organic), they are rapidly converted to their corresponding oxidized derivatives, which are less noxious (Table 1). But organoarsenicals can also be demethylated (Notti et al. 2007). For example, strain ASV2, an unidentified Gram-negative bacterium, was found to utilize arsonoacetate or arsonochloroacetate as sole carbon and energy sources. The end arsenic species in this case is likely to be arsenite because an inducible arsenite-oxidizing activity was found in the strain as well (Quinn and McMullan 1995). Finally, conversion of arsenobetaine into trimethyl arsine oxide (TMAO; Kaise et al. 1985) was found in some bottom sediments collected from coastal waters.

Arsenic uptake in bacteria

Despite its utilization by some bacteria as electron donor or acceptor (see above), As does not play any metabolic or nutritional role in the cell cytoplasm. Thus, cells do not require intracellular arsenate or arsenite (the two most frequent oxidation states in nature), and therefore they have not developed any dedicated arsenic uptake system. Instead, arsenic enters the cells through existing transporters due to the analogy of its chemical species to other molecules (Rosen and Liu 2008). Specifically, arsenate is an oxianion very close chemically to the widespread and life-essential phosphate. It thus comes as no surprise that the Pit (phosphate transporter) and the Pst (specific phosphate transporter) systems of *E. coli* (Harold and Baarda 1966; Rosenberg et al. 1977; Rosen 2002) turn out also to be the major conduits for arsenate uptake in this bacterium (Willsky and Malamy 1980), with a strong preference for the Pit system. On the other hand, at physiological pH arsenite is present as non-charged $As(OH)_3$ rather than as its oxianionic form (Ramirez-Solis et al. 2004). In this case, aqua-glycerolporin (GlpF in *E.coli*)—a branch of the aquaporin superfamily of transporters—was found to bring arsenite inside the cells (Meng et al. 2004). *E. coli* GlpF homologues have been also described in other organisms such as *Leishmania major* (Gourbal et al. 2004) or *Pseudomonas putida* (Páez-Espino, unpublished) and they are likely to transport arsenite as well. In addition, some types of sugar uptake systems transport arsenite in yeast (hexose permeases; Liu et al. 2004) and mammalian cells (glucose permeases; Liu et al.

2006). These observations leave the possibility open that other transport systems for thus far unidentified solutes can be operative in bacteria for surrogate arsenite uptake.

Arsenite oxidation

Arsenite is more noxious to cells than arsenate (Table 1). Due to its avidity for thiol groups, arsenite binds to cysteine residues in enzymes. In this context, it generally believed that biological oxidation of arsenite could be a detoxification mechanism (Tamaki and Frankenberger 1992), linked to an ancient origin of arsenite oxidases (Lebrun et al. 2003). Under certain physico-chemical conditions, microbial-mediated oxidation of arsenite can be crucial for the deliberate removal of arsenic in solution: arsenite is more soluble than arsenate, and arsenate is much more effectively removed than arsenite by coagulation with Fe(III) (Leist et al. 2000).

In addition, arsenite oxidation can serve as an electron donor. A strain belonging to the *Agrobacterium*/*Rhizobium* branch of α-Proteobacteria can utilize arsenite as the sole source of electrons with a doubling time of 7.6 h. This strain was found to be the most rapidly growing chemolitotrophic arsenite oxidizer known. The microorganism was isolated from a gold mine in the Northern Territory of Australia (Santini et al. 2000). Arsenite oxidizers include one chemoorganotrophic Gram-negative motile rod, exhibiting a high minimum inhibitory concentration for As(III) of 6.65 mM (Weeger et al. 1999). Also, *Thermus aquaticus* and *T. thermophilus* were able to oxidize arsenite to arsenate 100-fold faster than abiotic controls in laboratory experiments. On this basis, it was suggested that such species could play an important role in the speciation and bioavaliability of arsenic in thermal environments (Gihring and Banfield 2001; Gihring et al. 2001). Yet another *Thermus* strain was found to both oxidize and reduce (for respiration) arsenic. However, no energy was obtained out of arsenite oxidation in this case (Gihring and Banfield 2001).

The intimate mechanism of oxidation of arsenite is becoming clear owing to the growing availability of arsenite oxidase structures. The one from *Alcaligenes faecalis* was crystalized and the structure solved in 2001 (Ellis et al. 2001). Similar enzymes were later purified from *Rhizobium* sp. strain NT-26 (Santini and vanden Hoven 2004) and *Hydrogenophaga* sp. strain NT-14 (vanden Hoven and Santini 2004). Regardless of the origin, the enzyme is a member of the DMSO reductase family of molybdenum enzymes with two subunits, one large (AoxB, ~90 kDa) containing a Mo atom bound to two pyranopterin cofactors and a small subunit containing a Rieske-type site [2Fe-S] (AoxA, ~14 kDa) carrying a TAT leader sequence (Ellis et al. 2001). The predicted binding site for of arsenite places the ion adjacent to the Mo center in the large subunit. After the reduction of the Mo from Mo(VI) to Mo(IV), the electrons are transferred to the [3Fe–4S] center of the large subunit, then to the [2Fe–2S] center of the small subunit, and finally to the first coupling protein of the respiratory chain (Anderson et al. 1992; Ellis et al. 2001).

The genes encoding the arsenite oxidase subunits have been identified in the genomes of different microorganisms. Unfortunately, different authors assign unlike names to otherwise homologous genes and enzymes. While the designation *aoxA*/*aoxB* has been kept for *Cenibacterium arsenoxidans* strain ULPAs1 (Muller et al. 2003) and *Agrobacterium tumefaciens* (Kashyap et al. 2006), alternative nomenclatures have been given to the enzymes/genes of *Alcaligenes faecalis* (*asoB*/*asoA*; Stolz et al. 2006) and the *Rhizobium* sp. strain NT-26 (*aroB*/*aroA*; Santini and vanden Hoven 2004). In the known operons, the relative gene order *aoxAB* is always kept, but the gene pair is associated, extra ORFs and regulatory genes in the same operon depending of the species. For instance, one *aoxRSABC* operon was identified in both *A. tumefaciens* (Kashyap et al. 2006) and *Rhizobium* sp. strain NT-26 (Santini and vanden Hoven 2004). While *aoxC* is the gene for a c_2-type cytocrome isoform, *aoxR* encodes a NtrC-like protein acting as a regulator of the system, and *aoxS* determines a periplasmic arsenite-binding receptor which is the sensor histidine kinase of the system.

Arsenate reduction

Two different arsenic reduction activities have been found in bacteria. One is the dissimilatory reduction for anaerobic respiration, which involves arsenate as the terminal acceptor of electrons. The second one has the purpose of detoxifying arsenic by converting

arsenate to arsenite, which is the substrate of the arsenic efflux pumps (see below). The rapid appearance of arsenate-respiring bacteria in enrichment cultures suggest that they are widespread and metabolically active in nature. The first report of an arsenic-respiring strain was MIT-13 strain, a microorganism displaying the shape of a vibrio (Ahmann et al. 1994) and later named *Geospirillum arsenophilus* (Lovley and Coates 1997). Lactate disappearance in the culture medium was observed to be propertional to the conversion of As(V) to As(III). Sediment samples from the site where MIT-13 was isolated caused the dissolution of solid iron arsenate and the reduction of arsenate to arsenite (Ahmann et al. 1994). After this report on MIT-13, the number of known arsenic-respiring microorganisms has grown considerably. The isolation of arsenate-respiring bacteria belonging to different phylogenetic groups (Gram-positive, β-, γ- and ε-proteobacteria and *Chrysiogenes arsenatis*) suggests that they are spread throughout the whole bacterial domain (Macy et al. 1996; Newman et al. 1997c; Stolz and Oremland 1999; Silver and Phung 2005). The electron donor used for arsenic respiration varies from one species to the other (Stolz and Oremland 1999). Strains belonging to the Aquificales order were isolated from a subsurface hot aquifer of a Japanese gold mine and found to display versatile energy-generating systems including the utilization of arsenate, selenate or molecular oxygen as electron acceptors. These isolates dominated the ecosystem, perhaps reflecting the geochemical conditions of their habitat (Takai et al. 2002). An obligate anaerobic, hyperthermophilic arsenic-respiring microorganism, named *Pyrobaculum arsenaticum*, was isolated from a hot spring at the Pisciarelli solfatara, Naples, Italy. It could respire arsenate, forming arsenite, as well as sulfur or thiosulfate, originating sulfide. Interestingly, this strain was able to precipitate As_2S_2/As_4S_4 (a mineral called *realgar*) when grown on arsenate and thiosulfate or L-cysteine. Another member of the genus, *P. aerophilum*, was also able to grow on arsenate as electron acceptor (Huber et al. 2000). One *Thermus* species isolated from arsenic-rich terrestrial geothermal environment was able to both oxidize and reduce arsenic. Under aerobic conditions, this strain oxidizes As(III) at a rate 100-fold greater than abiotic rates without gaining metabolic energy. In the absence of oxygen, the strain can utilize arsenate as electron acceptor with lactate oxidation (Gihring et al. 2001). Other extremophiles capable of arsenic respiration are *Bacillus arsenicoselenatis* and *Bacillus selenitireducens* isolated from the anoxic muds of Mono Lake, California, an alkaline, hypersaline, arsenic-rich water body. Both isolates are alkaliphiles and halophiles and could also grow by dissimilatory reduction of Se(VI) or (IV) (Switzer Blum et al. 1998). Many other bacteria with respiratory arsenate reductase activity have been recently described: *Shewanella* sp. strain ANA-3 (Malasarn et al. 2008), *Sulfurospirillum barnesii* (Malasarn et al. 2004), *Desulfuroporosinus* sp. strain Y5 (Perez-Jimenez et al. 2005), *Wollinella succinogenes* (Stolz et al. 2006), *Alkaliphilus metalliredigenes* (Stolz et al. 2006), *Clostridium* sp. strain OhILAs (Stolz et al. 2006), *Alkaliphilus oremlandii* (Fisher et al. 2008), the vibrio shaped bacteria *Sulfurospirillum barnessi* and *S. arsenophilum* (Stolz et al. 1999), and *Desulfotomaculum auripigmentum* a Gram-positive bacterium with a hexagonal S-layer on its cell wall (Newman et al. 1997a).

In the cases where the issue has been examined in detail, arsenate reduction is carried out by a membrane-bound enzyme (Macy et al. 2000) encoded in the *arr* operon which always includes the *arrA* and *B* genes in its genomic configuration. To date, only the respiratory arsenate reductase (Arr) from *C. arsenatis* and *B. selenitireducens* have been purified and characterized (Krafft and Macy 1998; Afkar et al. 2003). *C. arsenatis* was isolated from a reed bed at Ballarat Goldfields in Australia. It is a strictly anaerobe microorganism, able to respire arsenate and nitrate but not sulfate (Macy et al. 1996). The respiratory arsenate reductase of *C. arsenatis* is a heterodimer periplasmic protein composed of two subunits (ArrA, 87 kDa and ArrB, 29 kDa). The larger subunit is a molybdopterin subunit containing an iron–sulfur center [4Fe–4S], and the smaller one contains a [Fe–S] center protein (Krafft and Macy 1998), and it seems to be a protein related to DmsB of DMSO reductase and NrfC of nitrite reductase. The enzyme is very specific for its substrate and does not use nitrate, sulfate, selenate or fumarate. Little is known re. the genetic control of expression of these enzymes. Genome data have shown a considerable diversity in the genes that cluster adjacent to the *arr* operons, some of them are likely to be regulatory. Furthermore, some strains, i.e. *D. hafniense*, *A. metalliredigens* or *W. succinogenes*, posses a third

membrane integral subunit (ArrC). In the case of the Gram-positive *B. selenitireducens* the structure of the respiratory arsenate reductase *ArrA*/*ArrB* heterodimer is basically the same than that of *C. arsenatis* although the catalytic subunit shares only a 47% of identity. In this example, the reductase is also a membrane-associated protein and can *inter alia* reduce arsenite, selenate and selenite (Afkar et al. 2003).

The concomitant reduction of sulfate and arsenate is not frequent, as only a fraction of the microbial isolates tested to that end are able to do it. The importance of such a joint reduction resides in that stimulating sulfate reduction and the ensuing generation of sulfide is an effective mechanism for precipitation of metals, including arsenite. However, the stability of As_2S_3 is highly dependent on the pH and the concentration of sulfide. If sulfate reduction occurs too quickly, this could result in the lack of arsenic precipitation. Therefore, not all microorganisms capable of sulfate reduction can successfully precipitate arsenic trisulfide (Newman et al. 1997b). Others, however, run the process very effectively. For instance, *Desulfomicrobium* strain Ben-RB and *Desulfotomaculum auripigmentum* can reduce concomitantly, As(V) to As(III) and S(VI) to S(II), through which they form both intracellular and extracellular arsenic trisulfide precipitates (Newman et al. 1997b).

In addition to the properties of the arsenic-respiring microorganisms just described, other strains display additional interesting activities. For example, some *Desulfitobacterium*-type strains posses the ability of reducing As(V), Fe(III), Se(VI), Mn(IV) and a variety of oxidized sulfur species, and reductively dechlorinating chlorophenols (Niggemyer et al. 2001). These properties may open new possibilities of biological remediation of sites co-contaminated with arsenic and chlorophenols.

Bacterial arsenic tolerance mechanisms: the *ars* operon

The *ars* operons, encoding arsenic resistance/tolerance in bacteria, have been extensively studied (Wu ?tul?> and Rosen 1993; Rosen 1995; Oremland and Stolz 2003). The *ars* genes are widespread in nature and appear systematically co-transcribed by a large variety of genomic configurations arranged in a fashion that depends on the specific strain (Fig. 2). The core genes of the system include the transcriptional repressor ArsR, the arsenite efflux pump ArsB, and the arsenate reductase ArsC (Xu et al. 1998).

ars genes have been found in chromosomal locations and/or plasmid-encoded in a large number of Gram-negative bacteria belonging to the α- and γ-Proteobacteria as well as in Gram-positive Firmicutes. The minimal gene set arranged in one operon *arsRBC* is found in the chromosome of *E. coli* (Carlin et al. 1995; Diorio et al. 1995) and *P. fluorescens* MSP3 (Prithivirajsingh et al. 2001b; Prithivirajsingh et al. 2001a), as well as in the *Staphylococcus* plasmids pI258 and pSX267 (Silver 1998). An enlarged version of the core genes is found in some plasmids of *E. coli* (R773 and R46; Silver 1998) and *Acidophulus multivurum* AIU301, (pKW301; Suzuki et al. 1997; Suzuki et al. 1998). These enlarged operons are arranged as a gene cluster *arsRDABC*. ArsA is an ATPase that provides energy to ArsB for the extrusion of arsenite and antimonite. ArsD is identified as an arsenic chaperone for the ArsAB pump, transferring the trivalent metalloids As(III) and Sb(III) to the ArsA subunit of the pump (Lin et al. 2007) and increasing the affinity of ArsA for As(III). ArsD is a homodimer with three vicinal cysteine pairs in each monomer. Other strains harbor the core *ars* genes in different arrangements (Fig. 2). In the case of *Acidithiobacillus ferroxidans*, an acidophilic, chemolithoautotrophic, bio-mining bacterium the *ars* genes are arranged in two divergently transcribed operons, *arsRC* and *arsBH*. The gene *arsH* was not required for and did not enhance arsenic resistance when expressed in *E. coli* (Butcher et al. 2000). On the other hand, the *arsH* gene of the *Tn*2502 transposon of the *Yersinia* virulence plasmid pYV was required to confer full arsenic resistance. In this transposon, *arsH* was transcribed divergently from the *arsBC* genes. No definitive role for ArsH has been established so far in direct connection to arsenic resistance. Crystallization of the protein from *Shigella flexneri* and *Sinorhizobium meliloti* exposed its ability of NADPH:FMN-dependent reduction of molecular O_2 to hydrogen peroxide and its catalysis of the reduction of azo dyes (Vorontsov et al. 2007; Ye et al. 2007). *P. putida* ArsH was found to show the same NADPH:FMN-dependent oxydoreduction

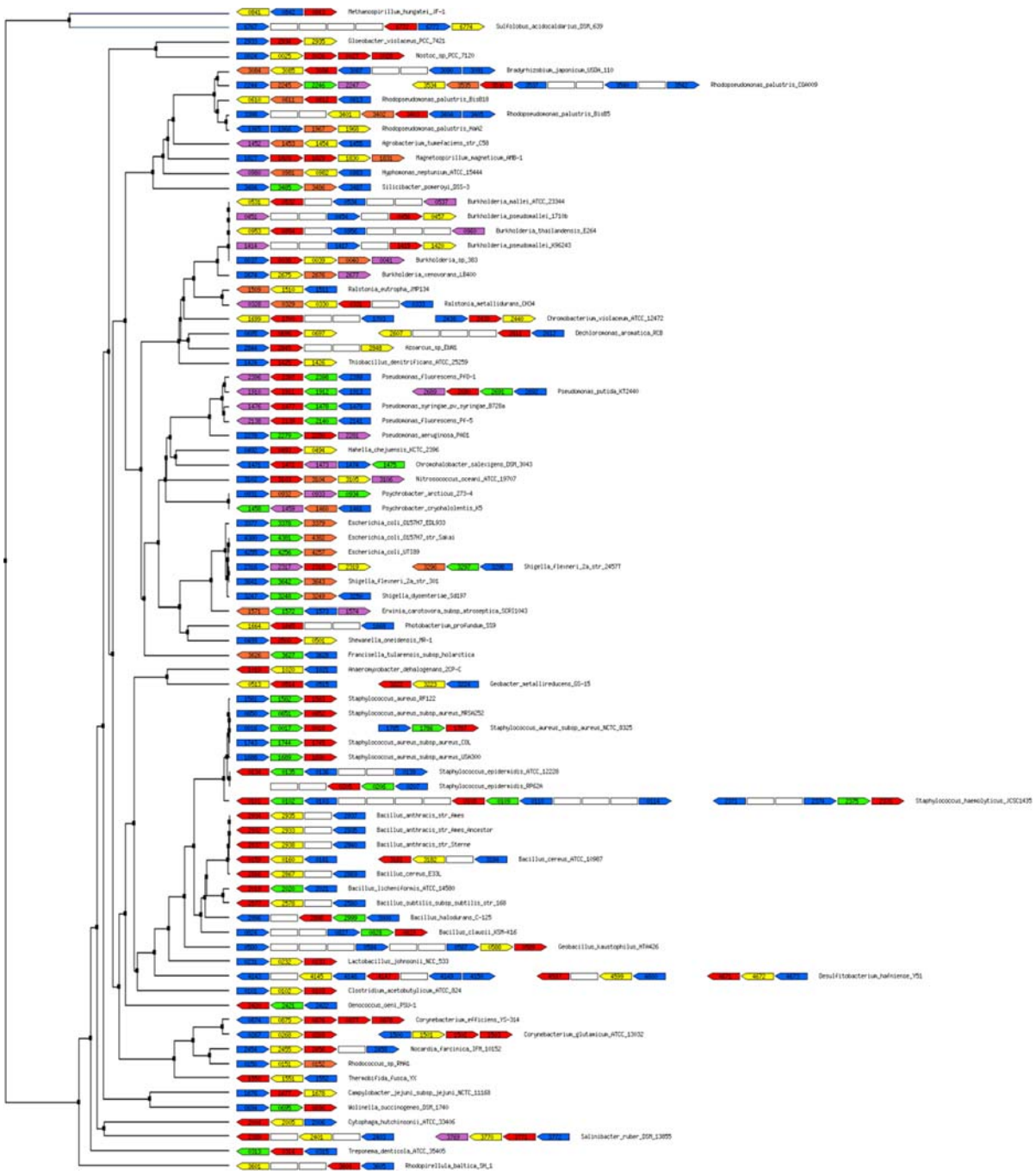

Fig. 2 Phylogenetic tree of bacteria harboring the *ars* genes. The phylogenetic tree was constructed with the 16S ribosomal DNA gene sequences by using Clustalw alignments. Diagrams depicting the organization of the *ars* operons for each species are shown. Color code: *arsR* in *blue*, *arsB* in *light green*, *ACR3*-like in *yellow*, *arsC* (glutaredoxin-coupled) in *orange*, *arsC* (thioredoxin-coupled) in *red*, *arsH* in *purple*. Other genes found in or adjacent to the *ars* clusters are shown as *empty boxes*

activity (Páez-Espino, unpublished). The *arsRBCH* operons of *P. putida* and *P. aeruginosa* display an organization different of that found either in *A. ferroxidans* or in the *Yersinia* plasmid pYV. Instead, the *Pseudomonas* genes look more similar to those of *E. coli* in that all genes are co-transcribed in

the same orientation (Canovas et al. 2003). Whether ArsH is required to confer full arsenic resistance in *Pseudomonas* (as in *Yersinia*) or not (as in *A. ferroxidans*) is not known yet. Interestingly, as shown in Fig. 2, *arsH* gene is present in almost all Gram-negative bacteria that contain the *ars* operon but there is no evidence of its presence in Gram-positives. *P. aeruginosa* harbors one chromosomal *ars* operon homologous to the *arsRBC* system of *E. coli*. Transcription of such *ars* orperons is activated by arsenite in *E. coli* (Cai et al. 1998) and *P. putida* (Páez-Espino, unpublished data).

In prokaryotes two different families of efflux pumps can be found, the so-called ArsB protein and also the ACR3 arsenite carrier gene family. There is a prevalence of *arsB* genes in *Firmicutes* and *Gammaproteobacteria*, while ACR3 are mostly present in *Actinobacteria* and *Alphaproteobacteria* (Achour et al. 2007; Fig. 2). Something similar happens to the cytoplasmic arsenate reductases in that there are two major protein families that differ in their structures, reduction mechanisms and the location of their catalytic cysteine residues. On one hand, there is the thioredoxin-coupled arsenate reductase class includes the ArsC proteins borne by *Staphylococcus aureus* plasmid pI258, *Bacillus subtilis,* and *P. putid*a. The second major type of reductases use glutaredoxin (not thioredoxin) in the corresponding biochemical reaction. Within this group, ArsC from the *E. coli* plasmid R773 and the eukaryotic ACR2p reductase from *Saccharomyces cerevisiae* represent two distinct enzyme classes (Fig. 2). Regardless of their specific type and origin all arsenate reductases are small cytoplasmic redox enzymes that convert arsenate to arsenite by the sequential involvement of three different thiolate nucleophiles that function as a redox cascade (Messens and Silver 2006).

Arsenic methylation

Methylation of arsenic is a widespread phenomenon in nature. From bacteria to humans, many organisms can methylate the element to different extents. Yet, while arsenic methylation by fungi and other eukaryotes has been well documented, less is known in bacteria. Yet, the methylation pathway(s) proposed for prokaryotes are the same as those described in the early studies (Challenger 1951) on the subject with the fungus *Scopulariopsis brevicaulis*. The general picture is that methylation pathway(s) involve a series of steps in which the reduction of the pentavalent form of As is followed by the oxidative addition of a methyl group (Dombrowski et al. 2005) thereby generating a growingly methylated series of As chemical species: methyl arsenite (MMA), dimethyl arsenate (DMA-V), dimethyl arsenite (DMA-III) and trimethyl arsine oxide (TMAO). While glutathione and other thiol-containing compounds participate in the reduction steps, anaerobic bacteria may use methylcobalamin as the electron donor (Krautler 1990; Stupperich 1993). The methylation reactions do require otherwise *S*-adenosylmethionine (SAM) as the source of methyl groups.

The environmental significance of As methylation and its potential for bioremediation of polluted sites through volatilization is still under considerable debate. In one study (Turpeinen et al. 2002) the speciation of arsenic in contaminated soil was examined under laboratory conditions and corroborated in a field study of a waste area. It was concluded that microbial transformation of As(V) to As(III), MMAA, DMAA and TMA by the microbial species which were dominant in the site was less than 0.5%. Regardless of this somewhat poor performance in situ, arsenic methylation has been found in aerobic and anaerobic bacteria (Bentley and Chasteen 2002), including *Clostridium collagenovorans*, *Desulfovibrio vulgaris* and *Desulfovibrio gigas* (which produce measurable amounts of TMA; Michalke et al. 2000) and *Methanobacterium formicium* (arsine). A distinct gene (*arsM*) determining all the SAM-dependent methylation steps in bacteria has been recently identified in more than 120 prokaryotic species (and different archaea), and further characterized in *Rhodopseudomonas palustris* (Qin et al. 2006). Since soluble methylated compounds are much less noxious than inorganic arsenic, these microorganisms have obviously a potential for bioremediation/biomitigation strategies based on the methylation and ensuing mobilization of the target pollutant (Yamanaka et al. 1989, 1997; Yamauchi et al. 1990; Moore and Kukuk 2002; Mass et al. 2001). However, the approach has evident downsides as well. The lifetime of volatile arsines in air is short, as they are quickly oxidized back to water-soluble species. Unfortunately, mono- and dimethylated arsines are very genotoxic (see Table 1). They target directly DNA without the

concourse of any other compounds (Mass et al. 2001). Despite these caveats, volatilization is considered a possible strategy for bioremediation of other toxic metals such as mercury (Rugh et al. 1998; Bizily et al. 2000) and has also been proven as a cheap and effective method for decontamination of industrial wastes in bioreactors (Essa et al. 2002). Whether or note the same will apply to arsenic remains an open question.

Engineering bacteria for enhanced accumulation of arsenic

One attractive possibility for removing arsenic from waste water or for immobilizing the element in a non-bioactive form is the use of microbial biomass as an adsorbant specific for the various As forms. Although bacteria generally accumulate very low arsenic levels because of the highly efficient *ars* export systems (Silver and Phung 1996; see above), some genetic strategies have been proposed to enhance this otherwise appealing possibility. In one case, the phytochelatin (PC) synthase of *Arabidopsis thaliana* (AtPCS) was successfully expressed in *E. coli* cells (Sauge-Merle et al. 2003). Phytochelatins are thiol-rich small molecules consisting of 2–5 repeats of the γ-Glu-Cys dipeptide with a terminal Gly residue. Their production is one of the mechanisms used by plants (and other eukaryotes) for neutralizing arsenite inside the cells (Schmoger et al. 2000). Expression of such PCs in the bacterial host increased the accumulation of intracellular arsenic by 50-fold by sequestering arsenic ions (likely arsenite) in a non-toxic form and competing with the arsenic efflux transporter. Another scenario for increasing As accumulation in bacterial cells involves expression of arsenic-binding proteins with metallothionein (MT)-like properties. *Bona fide* MTs are low-molecular-weight Cys-rich proteins, which bind metal ions such as Zn^{2+}, Cd^{2+}, Cu^{2+}, Hg^{2+} and Ag^{2+} (Hamer 1986). Some As-binding MTs have been occasionally described, e.g. in the As-tolerant marine alga *Fucus vesiculosus* (Merrifield et al. 2004), but none thus far in bacteria. However, the transcriptional regulator *arsR* present in the bacterial *ars* operon (see above) can be considered a As-specific metallothionein-like protein endowed with an unambiguous binding site for arsenite. It was thus logical that ArsR overexpression in *E. coli* allowed intracellular accumulation of As(III) and As(V) to an extent 13–60 fold higher than control cells (Kostal et al. 2004). The approach is not devoid of precedents, as overexpression of the metallo-regulatory protein MerR has been also reported for the sequestration of mercury (Bae et al. 2003). In the case of ArsR, the success of this strategy is due to the high binding affinity of the ArsR dimer for intracellular arsenite. Similarly to the instance with PCs discussed above, ArsR also seems to compete with the extrusion system for available arsenite in the cell cytoplasm. The difference of this system (in contrast with the synthesis of PCs above) is the high degree of specificity and affinity for arsenic. The concept of using metallothioneins for As removal has been recently upgraded by combining in *E. coli* the above mentioned As-binding MT of *Fucus vesiculosus* (Merrifield et al. 2004) with overexpression of the specific As transporter GlpF (Singh et al. 2008). These results open the possibility of designing As-specific bioadsorbants by merging dedicated transport systems with naturally-occurring or evolved intracellular As-binding polypeptides. The interesting part is that the thereby engineered resting cells can be used to completely remove 35 ppb of As(III) in 20 min, making this a low-cost option for arsenic removal from water (Singh et al. 2008).

Conclusion

Environmental pollution by arsenic brought about global and local geochemical cycles to many places of Earth has been often enhanced by irresponsible industrial and mining practices in given sites. Yet, intrinsic toxicity of Arsenic is one of the earlier challenges that biological systems had to cope with since the emergence of Life in the Biosphere. The amazing diversity of microbial responses to environmental As species just sketched in this short (and by no means exhaustive) review cannot thus come as a surprise. The most pressing challenge is how to translate the fundamental knowledge derived from the many studies carried out on this element into science-based interventions to combat its noxious effects on humans and the wider ecosystem. Despite the somewhat timid attempts discussed above on possible prevention and remediation strategies using bacteria, most advances are still in a very small-scale

stage. Fortunately, the room for improving the properties of biological agents for multi-scale As removal or neutralization is still ample, and new conceptual frameworks such as Systems and Synthetic Biology (de Lorenzo 2008) will surely help to this end.

Acknowledgments The work in Authors' Laboratory is funded by contracts of the 7th Framework Programme of the European Union and grants of the Spanish Ministery of Science and Innovation.

References

Achour AR, Bauda P, Billard P (2007) Diversity of arsenite transporter genes from arsenic-resistant soil bacteria. Res Microbiol 158:128–137. doi:10.1016/j.resmic.2006.11.006

Afkar E, Lisak J, Saltikov C, Basu P, Oremland RS, Stolz JF (2003) The respiratory arsenate reductase from *Bacillus selenitireducens* strain MLS10. FEMS Microbiol Lett 226:107–112. doi:10.1016/S0378-1097(03)00609-8

Ahmann D, Roberts AL, Krumholz LR, Morel FM (1994) Microbe grows by reducing arsenic. Nature 371:750. doi:10.1038/371750a0

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

Appelo CA, Van Der Weiden MJ, Tournassat C, Charlet L (2002) Surface complexation of ferrous iron and carbonate on ferrihydrite and the mobilization of arsenic. Environ Sci Technol 36:3096–3103. doi:10.1021/es010130n

Bae W, Wu CH, Kostal J, Mulchandani A, Chen W (2003) Enhanced mercury biosorption by bacterial cells with surface-displayed MerR. Appl Environ Microbiol 69: 3176–3180

Bentley R, Chasteen TG (2002) Microbial methylation of metalloids: arsenic, antimony, and bismuth. Microbiol Mol Biol Rev 66:250–271. doi:10.1128/MMBR.66.2.250-271.2002

Bizily SP, Rugh CL, Meagher RB (2000) Phytodetoxification of hazardous organomercurials by genetically engineered plants. Nat Biotechnol 18:213–217. doi:10.1038/72678

Butcher BG, Deane SM, Rawlings DE (2000) The chromosomal arsenic resistance genes of *Thiobacillus ferrooxidans* have an unusual arrangement and confer increased arsenic and antimony resistance to *Escherichia coli*. Appl Environ Microbiol 66:1826–1833. doi:10.1128/AEM.66.5.1826-1833.2000

Cai J, Salmon K, DuBow MS (1998) A chromosomal *ars* operon homologue of *Pseudomonas aeruginosa* confers increased resistance to arsenic and antimony in *Escherichia coli*. Microbiology 144:2705–2713

Cánovas D, de Lorenzo V (2007) Osmotic stress limits arsenic hyper-tolerance in *Aspergillus* sp. P37. FEMS Ecol 61:258–263. doi:10.1111/j.1574-6941.2007.00344.x

Canovas D, Cases I, de Lorenzo V (2003) Heavy metal tolerance and metal homeostasis in *Pseudomonas putida* as revealed by complete genome analysis. Environ Microbiol 5:1242–1256. doi:10.1111/j.1462-2920.2003.00463.x

Cánovas D, Vooijs R, Schat H, de Lorenzo V (2004) The role of thiol species in the hyper-tolerance of *Aspergillus* sp. P37 to arsenic. J Biol Chem 279:51234–51240. doi:10.1074/jbc.M408622200

Carlin A, Shi W, Dey S, Rosen BP (1995) The *ars* operon of *Escherichia coli* confers arsenical and antimonial resistance. J Bacteriol 177:981–986

Challenger F (1951) Biological methylation. Adv Enzymol Relat Subj Biochem 12:429–491. doi:10.1002/9780470122570.ch8

Cullen WR, Reimer KJ (1989) Arsenic speciation in the environment. Chem Rev 89:713–764. doi:10.1021/cr00094a002

de Lorenzo V (2008) Systems biology approaches to bioremediation. Curr Opin Biotechnol 19:579–589. doi:10.1016/j.copbio.2008.10.004

Diorio C, Cai J, Marmor J, Shinder R, DuBow MS (1995) An *Escherichia coli* chromosomal *ars* operon homolog is functional in arsenic detoxification and is conserved in Gram-negative bacteria. J Bacteriol 177:2050–2056

Dombrowski PM, Long W, Farley KJ, Mahony JD, Capitani JF, Di Toro DM (2005) Thermodynamic analysis of arsenic methylation. Environ Sci Technol 39:2169–2176. doi:10.1021/es0489691

Eguchi N, Kuroda K, Endo G (1997) Metabolites of arsenic induced tetraploids and mitotic arrest in cultured cells. Arch Environ Toxicol 32:141–145. doi:10.1007/s002449900166

Ellis PJ, Conrads T, Hille R, Kuhn P (2001) Crystal structure of the 100 kDa arsenite oxidase from *Alcaligenes faecalis* in two crystal forms at 1.64 A and 2.03 A. Structure 9:125–132. doi:10.1016/S0969-2126(01)00566-4

Essa AM, Macaskie LE, Brown NL (2002) Mechanisms of mercury bioremediation. Biochem Soc Trans 30:672–674. doi:10.1042/BST0300672

Fisher E et al (2008) Transformation of inorganic and organic arsenic by *Alkaliphilus oremlandii* sp. nov. strain OhILAs. Ann N Y Acad Sci 1125:230–241. doi:10.1196/annals.1419.006

Gihring TM, Banfield JF (2001) Arsenite oxidation and arsenate respiration by a new *Thermus* isolate. FEMS Microbiol Lett 204:335–340. doi:10.1111/j.1574-6968.2001.tb10907.x

Gihring TM, Druschel GK, McCleskey RB, Hamers RJ, Banfield JF (2001) Rapid arsenite oxidation by *Thermus aquaticus* and *Thermus thermophilus*: field and laboratory investigations. Environ Sci Technol 35:3857–3862. doi:10.1021/es010816f

Gourbal B et al (2004) Drug uptake and modulation of drug resistance in *Leishmania* by an aquaglyceroporin. J Biol Chem 279:31010–31017. doi:10.1074/jbc.M403959200

Hamer DH (1986) Metallothionein. Annu Rev Biochem 55:913–951

Harold FM, Baarda JR (1966) Interaction of arsenate with phosphate-transport systems in wild-type and mutant *Streptococcus faecalis*. J Bacteriol 91:2257–2262

Hemond HF (1995) Movement and distribution of arsenic in the Aberjona watershed. Environ Health Perspect 103(Suppl 1):35–40. doi:10.2307/3432010

Huber R, Sacher M, Vollmann A, Huber H, Rose D (2000) Respiration of arsenate and selenate by hyperthermophilic archaea. Syst Appl Microbiol 23:305–314

Jackson CR, Langner HW, Donahoe-Christiansen J, Inskeep WP, McDermott TR (2001) Molecular analysis of microbial community structure in an arsenite-oxidizing acidic thermal spring. Environ Microbiol 3:532–542. doi:10.1046/j.1462-2920.2001.00221.x

Kaise T, Hanaoka K, Tagawa S (1985) The formation of trimethylarsine oxide from arsenobetaine by biodegradation with marine microorganisms. Chemosphere 16:2551–2558. doi:10.1016/0045-6535(87)90313-4

Kashyap DR, Botero LM, Franck WL, Hassett DJ, McDermott TR (2006) Complex regulation of arsenite oxidation in *Agrobacterium tumefaciens*. J Bacteriol 188:1081–1088. doi:10.1128/JB.188.3.1081-1088.2006

Koch I, Feldmann J, Wang L, Andrewes P, Reimer KJ, Cullen WR (1999) Arsenic in the Meager Creek hot springs environment, British Columbia, Canada. Sci Total Environ 236:101–117. doi:10.1016/S0048-9697(99)00273-9

Kostal J, Yang R, Wu CH, Mulchandani A, Chen W (2004) Enhanced arsenic accumulation in engineered bacterial cells expressing ArsR. Appl Environ Microbiol 70:4582–4587. doi:10.1128/AEM.70.8.4582-4587.2004

Krafft T, Macy JM (1998) Purification and characterization of the respiratory arsenate reductase of *Chrysiogenes arsenatis*. Eur J Biochem 255:647–653. doi:10.1046/j.1432-1327.1998.2550647.x

Krautler B (1990) Chemistry of methylcorrinoids related to their roles in bacterial C1 metabolism. FEMS Microbiol Rev 7:349–354

Langner HW, Jackson CR, McDermott TR, Inskeep WP (2001) Rapid oxidation of arsenite in a hot spring ecosystem, Yellowstone National Park. Environ Sci Technol 35:3302–3309

Lebrun E et al (2003) Arsenite oxidase, an ancient bioenergetic enzyme. Mol Biol Evol 20:686–693. doi:10.1093/molbev/msg071

Leist M, Casey RJ, Caridi D (2000) The management of arsenic wastes: problems and prospects. J Hazard Mater 76:125–138. doi:10.1016/S0304-3894(00)00188-6

Lin YF, Yang J, Rosen BP (2007) ArsD: an As(III) metallochaperone for the ArsAB As(III)-translocating ATPase. J Bioenerg Biomembr 39:453–458. doi:10.1007/s10863-007-9113-y

Liu Z, Carbrey JM, Agre P, Rosen BP (2004) Arsenic trioxide uptake by human and rat aquaglyceroporins. Biochem Biophys Res Commun 316:1178–1185. doi:10.1016/j.bbrc.2004.03.003

Liu Z, Sanchez MA, Jiang X, Boles E, Landfear SM, Rosen BP (2006) Mammalian glucose permease GLUT1 facilitates transport of arsenic trioxide and methylarsonous acid. Biochem Biophys Res Commun 351:424–430. doi:10.1016/j.bbrc.2006.10.054

Lovley DR, Coates JD (1997) Bioremediation of metal contamination. Curr Opin Biotechnol 8:285–289. doi:10.1016/S0958-1669(97)80005-5

Macur RE, Wheeler JT, McDermott TR, Inskeep WP (2001) Microbial populations associated with the reduction and enhanced mobilization of arsenic in mine tailings. Environ Sci Technol 35:3676–3682. doi:10.1021/es0105461

Macy JM et al (1996) *Chrysiogenes arsenatis* gen. nov., sp. nov., a new arsenate-respiring bacterium isolated from gold mine wastewater. Int J Syst Bacteriol 46:1153–1157

Macy JM, Santini JM, Pauling BV, O'Neill AH, Sly LI (2000) Two new arsenate/sulfate-reducing bacteria: mechanisms of arsenate reduction. Arch Microbiol 173:49–57. doi:10.1007/s002030050007

Malasarn D, Saltikov CW, Campbell KM, Santini JM, Hering JG, Newman DK (2004) *arrA* is a reliable marker for As(V) respiration. Science 306:455. doi:10.1126/science.1102374

Malasarn D, Keeffe JR, Newman DK (2008) Characterization of the arsenate respiratory reductase from *Shewanella* sp. strain ANA-3. J Bacteriol 190:135–142. doi:10.1128/JB.01110-07

Mass MJ et al (2001) Methylated trivalent arsenic species are genotoxic. Chem Res Toxicol 14:355–361. doi:10.1021/tx0002511

Meng YL, Liu Z, Rosen BP (2004) As(III) and Sb(III) uptake by GlpF and efflux by ArsB in *Escherichia coli*. J Biol Chem 279:18334–18341. doi:10.1074/jbc.M400037200

Merrifield ME, Ngu T, Stillman MJ (2004) Arsenic binding to *Fucus vesiculosus* metallothionein. Biochem Biophys Res Commun 324:127–132. doi:10.1016/j.bbrc.2004.09.027

Messens J, Silver S (2006) Arsenate reduction: thiol cascade chemistry with convergent evolution. J Mol Biol 362:1–17. doi:10.1016/j.jmb.2006.07.002

Michalke K, Wickenheiser EB, Mehring M, Hirner AV, Hensel R (2000) Production of volatile derivatives of metal(loid)s by microflora involved in anaerobic digestion of sewage sludge. Appl Environ Microbiol 66:2791–2796. doi:10.1128/AEM.66.7.2791-2796.2000

Moore AJ, Kukuk PF (2002) Quantitative genetic analysis of natural populations. Nat Rev Genet 3:971–978. doi:10.1038/nrg951

Moore MM, Harrington-Brock K, Doerr CL (1997) Relative genotoxic potency of arsenic and its methylated metabolites. Mutat Res 386:279–290

Muller D, Lievremont D, Simeonova DD, Hubert JC, Lett MC (2003) Arsenite oxidase *aox* genes from a metal-resistant beta-proteobacterium. J Bacteriol 185:135–141. doi:10.1128/JB.185.1.135-141.2003

Newman D, Beveridge T, Morel F (1997a) Precipitation of arsenic trisulfide by *Desulfotomaculum auripigmentum*. Appl Environ Microbiol 63:2022–2028

Newman DK et al (1997b) Dissimilatory arsenate and sulfate reduction in *Desulfotomaculum auripigmentum* sp. nov. Arch Microbiol 168:380–388. doi:10.1007/s002030050512

Niggemyer A, Spring S, Stackebrandt E, Rosenzweig RF (2001) Isolation and characterization of a novel as(v)-reducing bacterium: implications for arsenic mobilization and the genus *Desulfitobacterium*. Appl Environ Microbiol 67:5568–5580. doi:10.1128/AEM.67.12.5568-5580.2001

Nordstrom DK (2002) Worldwide occurrences of arsenic in ground water. Science 296:2143–2145. doi:10.1126/science.1072375

Notti A, Fattorini D, Razzetti EM, Regoli F (2007) Bioaccumulation and biotransformation of arsenic in the Mediterranean polychaete *Sabella spallanzanii*: experimental observations. Environ Toxicol Chem 26:1186–1191. doi:10.1897/06-362R.1

Oremland RS, Stolz JF (2003) The ecology of arsenic. Science 300:939–944. doi:10.1126/science.1081903

Oremland RS, Stolz JF (2005) Arsenic, microbes and contaminated aquifers. Trends Microbiol 13:45–49. doi:10.1016/j.tim.2004.12.002

Oremland RS, Hoeft SE, Santini JM, Bano N, Hollibaugh RA, Hollibaugh JT (2002) Anaerobic oxidation of arsenite in Mono Lake water and by a facultative, arsenite-oxidizing chemoautotroph, strain MLHE-1. Appl Environ Microbiol 68:4795–4802. doi:10.1128/AEM.68.10.4795-4802.2002

Otte ML, Kearns CC, Doyle MO (1995) Accumulation of arsenic and zinc in the rhizosphere of wetland plants. Bull Environ Contam Toxicol 55:154–161. doi:10.1007/BF00212403

Perez-Jimenez JR, DeFraia C, Young LY (2005) Arsenate respiratory reductase gene (arrA) for *Desulfosporosinus* sp. strain Y5. Biochem Biophys Res Commun 338:825–829. doi:10.1016/j.bbrc.2005.10.011

Pott WA, Benjamin SA, Yang RS (2001) Pharmacokinetics, metabolism, and carcinogenicity of arsenic. Rev Environ Contam Toxicol 169:165–214

Prithivirajsingh S, Mishra SK, Mahadevan A (2001a) Detection and analysis of chromosomal arsenic resistance in *Pseudomonas fluorescens* strain MSP3. Biochem Biophys Res Commun 280:1393–1401. doi:10.1006/bbrc.2001.4287

Prithivirajsingh S, Mishra SK, Mahadevan A (2001b) Functional analysis of a chromosomal arsenic resistance operon in *Pseudomonas fluorescens* strain MSP3. Mol Biol Rep 28:63–72. doi:10.1023/A:1017950207981

Qin J, Rosen BP, Zhang Y, Wang G, Franke S, Rensing C (2006) Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite *S*-adenosylmethionine methyltransferase. Proc Natl Acad Sci USA 103:2075–2080. doi:10.1073/pnas.0506836103

Quinn JP, McMullan G (1995) Carbon-arsenic bond cleavage by a newly isolated gram-negative bacterium, strain ASV2. Microbiology 141:721–725

Ramirez-Solis A, Mukopadhyay R, Rosen BP, Stemmler TL (2004) Experimental and theoretical characterization of arsenite in water: insights into the coordination environment of As-O. Inorg Chem 43:2954–2959. doi:10.1021/ic0351592

Rosen P (1971) Theoretical significance of arsenic as a carcinogen. J Theor Biol 32:425–426. doi:10.1016/0022-5193(71)90178-0

Rosen BP (1995) Resistance mechanisms to arsenicals and antimonials. J Basic Clin Physiol Pharmacol 6:251–263

Rosen BP (2002) Biochemistry of arsenic detoxification. FEBS Lett 529:86–92. doi:10.1016/S0014-5793(02)03186-1

Rosen P, Liu Z (2008) Transport pathways for arsenic and selenium: a minireview. Environ Int. doi:10.1016/j.envint.2008.07.023

Rosenberg H, Gerdes RG, Chegwidden K (1977) Two systems for the uptake of phosphate in *Escherichia coli*. J Bacteriol 131:505–511

Rugh CL, Senecoff JF, Meagher RB, Merkle SA (1998) Development of transgenic yellow poplar for mercury phytoremediation. Nat Biotechnol 16:925–928. doi:10.1038/nbt1098-925

Ruokolainen M, Pantsar-Kallio M, Haapa A, Kairesalo T (2000) Leaching, runoff and speciation of arsenic in a laboratory mesocosm. Sci Total Environ 258:139–147. doi:10.1016/S0048-9697(00)00521-0

Santini JM, vanden Hoven RN (2004) Molybdenum-containing arsenite oxidase of the chemolithoautotrophic arsenite oxidizer NT-26. J Bacteriol 186:1614–1619. doi:10.1128/JB.186.6.1614-1619.2004

Santini JM, Sly LI, Schnagl RD, Macy JM (2000) A new chemolithoautotrophic arsenite-oxidizing bacterium isolated from a gold mine: phylogenetic, physiological, and preliminary biochemical studies. Appl Environ Microbiol 66:92–97

Sauge-Merle S, Cuine S, Carrier P, Lecomte-Pradines C, Luu DT, Peltier G (2003) Enhanced toxic metal accumulation in engineered bacterial cells expressing *Arabidopsis thaliana* phytochelatin synthase. Appl Environ Microbiol 69:490–494. doi:10.1128/AEM.69.1.490-494.2003

Schmoger ME, Oven M, Grill E (2000) Detoxification of arsenic by phytochelatins in plants. Plant Physiol 122:793–801. doi:10.1104/pp.122.3.793

Senn DB, Hemond HF (2002) Nitrate controls on iron and arsenic in an urban lake. Science 296:2373–2376. doi:10.1126/science.1072402

Shariatpanahi M, Anderson AC, Abdelghani AA, Englande AJ, Hughes J, Wilkinson RF (1981) Biotransformation of the pesticide sodium arsenate. J Environ Sci Health B 16:35–47

Silver S (1998) Genes for all metals-a bacterial view of the periodic table. The 1996 Thom Award Lecture. J Ind Microbiol Biotechnol 20:1–12

Silver S, Misra TK (1984) Bacterial transformations of and resistances to heavy metals. Basic Life Sci 28:23–46

Silver S, Phung LT (1996) Bacterial heavy metal resistance: new surprises. Annu Rev Microbiol 50:753–789. doi:10.1146/annurev.micro.50.1.753

Silver S, Phung LT (2005) Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. Appl Environ Microbiol 71:599–608. doi:10.1128/AEM.71.2.599-608.2005

Singh N, Kumar D, Sahu AP (2007) Arsenic in the environment: effects on human health and possible prevention. J Environ Biol 28:359–365

Singh S, Mulchandani A, Chen W (2008) Highly selective and rapid arsenic removal by metabolically engineered *Escherichia coli* cells expressing *Fucus vesiculosus* metallothionein. Appl Environ Microbiol 74:2924–2927

Smith AH, Lopipero PA, Bates MN, Steinmaus CM (2002) Arsenic epidemiology and drinking water standards. Science 296:2145–2146. doi:10.1126/science.1072896

Stolz JF, Oremland RS (1999) Bacterial respiration of arsenic and selenium. FEMS Microbiol Rev 23:615–627. doi:10.1111/j.1574-6976.1999.tb00416.x

Stolz JF, Ellis DJ, Blum JS, Ahmann D, Lovley DR, Oremland RS (1999) *Sulfurospirillum barnesii* sp. nov. and *Sulfurospirillum arsenophilum* sp. nov., new members of the *Sulfurospirillum* clade of the epsilon *Proteobacteria*. Int J Syst Bacteriol 49:1177–1180

Stolz JF, Basu P, Santini JM, Oremland RS (2006) Arsenic and selenium in microbial metabolism. Annu Rev Microbiol 60:107–130. doi:10.1146/annurev.micro.60.080805.142053

Stupperich E (1993) Recent advances in elucidation of biological corrinoid functions. FEMS Microbiol Rev 12:349–365. doi:10.1111/j.1574-6976.1993.tb00027.x

Suzuki K, Wakao N, Sakurai Y, Kimura T, Sakka K, Ohmiya K (1997) Transformation of *Escherichia coli* with a large

plasmid of *Acidiphilium multivorum* AIU 301 encoding arsenic resistance. Appl Environ Microbiol 63:2089–2091

Suzuki K, Wakao N, Kimura T, Sakka K, Ohmiya K (1998) Expression and regulation of the arsenic resistance operon of *Acidiphilium multivorum* AIU 301 plasmid pKW301 in *Escherichia coli*. Appl Environ Microbiol 64:411–418

Switzer Blum J, Burns Bindi A, Buzzelli J, Stolz JF, Oremland RS (1998) *Bacillus arsenicoselenatis*, sp. nov., and *Bacillus selenitireducens*, sp. nov.: two haloalkaliphiles from Mono Lake, California that respire oxyanions of selenium and arsenic. Arch Microbiol 171:19–30. doi: 10.1007/s002030050673

Takai K, Hirayama H, Sakihama Y, Inagaki F, Yamato Y, Horikoshi K (2002) Isolation and metabolic characteristics of previously uncultured members of the order Aquificales in a subsurface gold mine. Appl Environ Microbiol 68:3046–3054. doi:10.1128/AEM.68.6.3046-3054.2002

Tamaki S, Frankenberger WT Jr (1992) Environmental biochemistry of arsenic. Rev Environ Contam Toxicol 124:79–110

Turpeinen R, Pantsar-Kallio M, Kairesalo T (2002) Role of microbes in controlling the speciation of arsenic and production of arsines in contaminated soils. Sci Total Environ 285:133–145. doi:10.1016/S0048-9697(01)00903-2

vanden Hoven RN, Santini JM (2004) Arsenite oxidation by the heterotroph *Hydrogenophaga* sp. str. NT-14: the arsenite oxidase and its physiological electron acceptor. Biochim Biophys Acta 1656:148–155

Vorontsov II et al (2007) Crystal structure of an apo form of *Shigella flexneri* ArsH protein with an NADPH-dependent FMN reductase activity. Protein Sci 16:2483–2490

Weeger W et al (1999) Oxidation of arsenite to arsenate by a bacterium isolated from an aquatic environment. Biometals 12:141–149

Willsky GR, Malamy MH (1980) Characterization of two genetically separable inorganic phosphate transport systems in *Escherichia coli*. J Bacteriol 144:356–365

Wu J, Rosen BP (1993) Metalloregulated expression of the *ars* operon. J Biol Chem 268:52–58

Xu C, Zhou T, Kuroda M, Rosen BP (1998) Metalloid resistance mechanisms in prokaryotes. J Biochem (Tokyo) 123:16–23

Yamanaka K, Ohba H, Hasegawa A, Sawamura R, Okada S (1989) Mutagenicity of dimethylated metabolites of inorganic arsenics. Chem Pharm Bull (Tokyo) 37:2753–2756

Yamanaka K et al (1997) Metabolic methylation is a possible genotoxicity-enhancing process of inorganic arsenics. Mutat Res 394:95–101

Yamauchi H, Kaise T, Takahashi K, Yamamura Y (1990) Toxicity and metabolism of trimethylarsine in mice and hamsters. Fundam Appl Toxicol 14:399–407

Ye J, Yang HC, Rosen BP, Bhattacharjee H (2007) Crystal structure of the flavoprotein ArsH from *Sinorhizobium meliloti*. FEBS Lett 581:3996–4000