Microbiology of inorganic arsenic: From metabolism to bioremediation

Shigeki Yamamura1 and Seigo Amachi2,*

Center for Regional Environmental Research, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan1 and Graduate School of Horticulture, Chiba University, 648 Matsudo, Matsudo, Chiba 271-8510, Japan2

Received 29 October 2013; accepted 11 December 2013

Arsenic (As) contamination of drinking water and soils poses a threat to a large number of people worldwide, especially in Southeast Asia. The predominant forms of As in soils and aquifers are inorganic arsenate [As(V)] and arsenite [As(III)], with the latter being more mobile and toxic. Thus, redox transformations of As are of great importance to predict its fate in the environment, as well as to achieve remediation of As-contaminated water and soils. Although As has been recognized as a toxic element, a wide variety of microorganisms, mainly bacteria, can use it as an electron donor for autotrophic growth or as an electron acceptor for anaerobic respiration. In addition, As detoxification systems in which As is oxidized to the less toxic form or reduced for subsequent excretion are distributed widely in microorganisms. This review describes current development of physiology, biochemistry, and genomics of arsenic-transforming bacteria. Potential application of such bacteria to removal of As from soils and water is also highlighted.

© 2013, The Society for Biotechnology, Japan. All rights reserved.

[Key words: Arsenate reduction; Arsenite oxidation; Arsenic contamination; Biogeochemical cycle of arsenic; Bioremediation]

Although arsenic (As) is commonly known as a toxin, it is ubiquitous in the environment (1); however, its abundance in the earth’s crust is low (0.0001%) and its background concentrations in soils are generally less than 15 mg kg⁻¹ (2,3). Nevertheless, local concentrations can vary depending on parent materials and geological history of the region. For example, Himalayan-derived sediment is the source of groundwater As contamination in large areas of south and southeast Asia (4). In Bangladesh and West Bengal, India, approximately 60–100 million people rely on drinking water that contains As in excess of the World Health Organization standard (5). Direct consumption of rice irrigated with As-contaminated water is another significant route of human exposure (6). Accordingly, health problems associated with exposure to As are a worldwide concern. Although As has both toxic and therapeutic properties, chronic exposure to As has caused a wide variety of adverse health effects including dermatological conditions and skin and internal cancers (7). In addition, recent studies have indicated that gestational As exposure is associated with increased cancer incidence in adulthood (8).

Anthropogenic discharges such as air emissions, soil amendments, mining operations, and wood preservation have also resulted in elevated As levels (9). Indeed, As has become a prevalent soil contaminant throughout the world (9,10). Accumulated As in contaminated soils has the potential to leach into ground and surface water, and direct exposure from ingestion, inhalation, and dermal routes can impact animal and human health. In Japan, the Soil Contamination Countermeasures Law was enacted in 2003 to address issues caused by harmful substances, including As.

Although As can exist in four oxidation states (V, III, 0, –III) with a variety of inorganic and organic forms, inorganic arsenate [As(V)] and arsenite [As(III)] predominate in aquatic and soil environments (11–13). As(V) is present as negatively charged oxyanions (H₂AsO₄⁻/HAso₄²⁻) at moderate pH. These oxyanions are strongly adsorbed to the surface of common soil minerals such as Fe and Al (hydr)oxides. As(III) primarily exists as uncharged H₃AsO₃ with a pKa of 9.2, and is therefore less adsorptive and more mobile than As(V) in most environments (12). In aerobic environments, As(V) is found to be the predominant species and immobilized in solid phase. In contrast, As(III) is more prevalent in anoxic environments, which leads to mobilization into the aqueous phase (14).

Microorganisms can mediate redox transformations of As via As(V) reduction and As(III) oxidation (2). To date, a wide variety of As(V)-reducing and As(III)-oxidizing prokaryotes have been isolated from various As-contaminated environments (2,15), and a recent study suggested that they are also distributed in the natural environment containing background levels of As (16,17). Because both As(V) reduction and As(III) oxidation directly affect the mobility and bioavailability of As, microbial activities play a key role in biogeochemical As cycling. Such microbial processes have the potential to promote As removal from contaminated soils/waters when used as intended. This article outlines the latest physiology and phylogeny for microbial metabolism of inorganic As and highlights their advances as bioremediation techniques.

AS(III) OXIDATION

Aerobic As(III) oxidizers The bacterial oxidation of As(III) was first reported in 1918, although the finding went largely unnoticed until 1949, when Turner isolated 15 strains of heterotrophic As(III)-oxidizing bacteria (18–20). Currently, physiologically...
diverse As(III) oxidizers are found in various groups of Bacteria and Archaea and include both heterotrophic As(III) oxidizers (HAOs) and chemolithoautotrophic As(III) oxidizers (CAOs) (21,22). Heterotrophic As(III) oxidation is generally considered a detoxification mechanism that converts As(III) into less toxic As(V), although it may be used as a supplemental energy source (23). In contrast, CAOs use As(III) as an electron donor during fixation of CO₂ coupled with reduction of oxygen (24). Anaerobic CAOs have also recently been isolated (see below). In addition, some researchers have reported curious facultative anaerobic HAOs capable of either aerobic As(III) oxidation or anaerobic As(V) reduction (25,26). In recent studies, As(III) oxidizers were isolated from As-rich environments (27,28), as well as metal-contaminated soil containing low levels of As and uncontaminated garden soil (29,30).

Arsenite oxidase, which was first isolated in 1992 (31), has been identified in both CAOs and HAOs (24,22). In both cases, the enzyme contains two subunits, a large subunit containing a molybdopterin center and a [3Fe-4S] cluster and a small subunit containing a Rieske [2Fe-2S] cluster (33,34). Although homologous genes encoding these two subunits were formerly assigned different names (aaxB-aaxA/arA-arB/arA-aas0, nomenclature for genes involved in prokaryotic aerobic As(III) oxidation was recently unified and the name aio was newly assigned; therefore, the large and small subunit are denoted aioA and aioB, respectively (35). AioA is similar to the molybdデン-constaining subunits in the DSMO reductase family and distantly related to the catalytic subunit of respiratory As(V) reductase (ArrA) (21,22).

Homologs of genes encoding AioA are found in phylogenetically diverse species including members of α-, β-, γ-Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes, Aquificae, Deinococcus-Thermus, Chlorobi, Chloroflexi, Nitrospira, and Crenarchaeota (Fig. 1). These genes are found clustered in several groups in the AioA-based tree, with strains in the phyla including thermophiles basically forming distinct phylogenetic branches from mesophiles. The major mesophiles are divided into two groups, group I, which is mainly composed of α-Proteobacteria, and group II, which is primarily composed of β- and γ-Proteobacteria. This pattern suggests that these groups probably originated from respective proteobacterial divisions. However, there are considerable inconsistencies between the AioA-based phylogeny and 16S rRNA-based classification, suggesting that horizontal gene transfer plays a role in the propagation of aio genes in prokaryotes (36). In some cases, two identical copies of the aioA gene have been found in the same strain. For example, the DGGE profile of Thiomonas arsenivorans DSM 16361 showed two bands corresponding to two distinct aioA-related sequences (37), whereas two copies of aioA in Ancylobacter sp. O1 were clustered more closely (38). AioA-like genes have been amplified from a variety of As-rich environments including mine, arsenical pesticide- or smelter-impacted sites, and geothermal sites (36,37,39-43). Additionally, Engel et al. (44) recently detected Chloroflexi and Proteobacteria-related aioA sequences from the same microbial mat collected at a geysier. Taken together, these investigations suggest that the diversity of aioA genes in prokaryotes is wider than previously suspected. Moreover, aioA-like genes have been obtained from soil or sediments containing background levels of As (16,45), indicating that diverse aerobic As(III) oxidizers reside in the environment, regardless of As contamination.

**Anaerobic As(III) oxidizers**

In 2002, Oremland et al. (46) isolated an anaerobic As(III)-oxidizing bacterium, strain MLHE-1, from anoxic bottom water of Mono Lake, CA, USA, which is an alkaline soda lake known for its high concentration of As(V) (200 μM). Strain MLHE-1, later proposed as Alkalilimnicola ehrlichii sp. nov. (47), is a chemolithoautotrophic bacterium that can couple As(III) oxidation to nitrate reduction under anaerobic conditions. A purple sulfur bacterium, Ectothiorhodospira sp. PHS-1, was also isolated from red-pigmented biofilms in Mono Lake (48). This strain can use As(III) as the electron donor for anaerogenic photosynthesis and produces As(V) anaerobically under light conditions. Interestingly, both of these bacteria appear to lack aioA genes and instead possess genes that are much more closely related to arrA (49,50) (Fig. 2). This gene, designated arrA, is required for chemoautotrophic growth on As(III) and nitrate by strain MLHE-1. In addition, arrA is strongly induced by As(III) in strain PHS-1. Thus, it is possible that ArrA is a novel type of As(III) oxidase that forms a distinct phylogenetic clade within the dimethyl sulfoxide (DMSO) reductase family. arrA-like genes have recently been found in a nearly complete genome sequence of uncultured bacterium within the candidate division OP1 (51) and in a reconstructed complete genome of the dominant organism (RBG-1) in deep sediment of the Colorado River, CO, USA (52). As(III)-oxidizing denitrifying chemoheterotrophs (strains DAO-1 and DAO-10 within the classes β- and α-Proteobacteria, respectively) have been isolated from As-contaminated soils, but it is still unclear which genes are required for As(III) oxidation (53).

**As(V) Reduction**

A wide variety of bacteria known as As(V)-resistant microbes (ARMs) can reduce As(V) via detoxification systems (15,54). As(V) usually enters bacterial cells through phosphate transporters (Por or Pst). Once inside, As(V) is reduced to As(III) by a cytoplasmic As(V) reductase (ArsC) with the aid of glutathione or ferredoxin as the reducing power. As(III) is finally excreted out of the cells via a membrane efflux pump, ArsB or Acr3 (55). In some cases, an ATPase ArsA is bound to ArsB to facilitate As(III) efflux, conferring an advantage to organisms exposed to high levels of As. As(III), which enters bacterial cells through aquaglyceroporin, may also be extruded by the same system.

In addition to the detoxifying As(V) reduction, certain bacteria can reduce As(V) as the terminal electron acceptor for anaerobic respiration. Such bacteria are defined as dissimilatory As(V)-reducing prokaryotes (DARPs). These bacteria are phylogenetically diverse, including members of Firmicutes, γ-, δ-, and ε-Proteobacteria (Fig. 2) (15). The respiratory As(V) reductase (ARR) consists of a larger catalytic subunit ArrA and a smaller subunit ArrB (56). ArrA is a member of the DMSO reductase family containing a molybdenum center and a [4Fe-4S] cluster, while ArrB contains three to four [4Fe-4S] clusters. ARR of Alkalifilus orenlandii and Shewanella sp. ANA-3, which are both As(V)-respiring bacteria, was recently found to be biochemically reversible (57), showing both As(III) oxidation and As(V) reduction activities upon in vitro gel assay. Richey et al. (57) suggested that the physiological role of ARR depends on the electron potentials of the molybdenum center and [Fe-S] clusters, additional subunits, or constitution of the electron transfer chain.

As sorption onto metal oxide minerals, especially on iron (hydr)oxides, is an important process controlling the dissolved concentration of As in various environments. As(V) is strongly associated with iron and aluminum (hydr)oxides, whereas As(III) is more mobile than As(V) (58). Thus, reductive dissolution of As-bearing iron (hydr)oxides by dissimilatory iron-reducing bacteria can cause As release (59,60). In addition, direct reduction of As(V) adsorbed onto soil minerals by DARPs may be another important mechanism of As mobilization (61,62). ARMs are generally not considered to be involved in As release because ArsC, the cytoplasmic As(V) reductase, is not able to directly reduce As(V) adsorbed onto soil minerals (63). Therefore, ARR is believed to be responsible for As(V) reduction in solid phase, although how periplasmic ARR transfers...
electrons to As(V) adsorbed onto mineral surface is still unclear. Below, we describe the physiological and biochemical aspects of representative DARPs, as well as the genomic organization of the genes involved in respiratory As(V) reduction and As resistance.

Sulfurospirillum spp. The first DARP, strain MIT-13, was isolated in 1994 from As contaminated sediments of the Aberjona Watershed, MA, USA (64). This strain, which was later proposed as Sulfurospirillum arsenophilum (65), is a member of the

![Diagram of phylogenetic tree of AsA sequences found in isolated strains. The AraA of Chrysiogenes arsenatis was used as the outgroup. Circles and triangles at the branch nodes represent bootstrap percentages (1000 replicates): filled circles, 90–100%; open circles, 70–89%; open triangles, 50–69%. Values <50% are not shown. The scale bar represents the estimated number of substitutions per site.](image-url)
**FIG. 2.** Neighbor-joining phylogenetic tree of *ArrA* and *ArxA* sequences found in isolated strains. The *AioA* of *Desulfotobacterium hafniense* DCB-2 (YP 002457721) was used as the outgroup. Circles and triangles at the branch nodes represent bootstrap percentages (1000 replicates): filled circles, 90–100%; open circles, 70–89%; open triangles, 50–68%. Values <.50% are not shown. The scale bar represents the estimated number of substitutions per site.

*ε*-Proteobacteria that can use As(V) as the electron acceptor when lactate is supplied as the electron donor and carbon source. Several other members of *Sulfurospirillum* spp., including *Sulfurospirillum barnesi* and *S. halorespirans*, are also capable of dissimilatory As(V) reduction (66). Many strains of *Sulfurospirillum* spp. can use nitrate, nitrite, Fe(III), elemental sulfur, thiosulfate, and oxygen (microaerobic) as the electron acceptors in addition to As(V). *S. barnesi* has been found to reduce As(V) adsorbed on ferrihydrite as well as on aluminum hydroxide (62). *S. arsophilum* also released As from sterilized Aberjona sediments (61).

**Chrysiogenes arsenatis**  
*C. arsenatis*, which is a phylogenetically unique member of the phylum *Chrysiogenetes*, was isolated from Australian gold mine wastewater as an acetate-using dissimilatory As(V)-reducer that also uses nitrate and Fe(III), elemental sulfur, thiosulfate, and oxygen (microaerobic) as the electron acceptors in addition to As(V). *S. barnesi* has been found to reduce As(V) adsorbed on ferrihydrite as well as on aluminum hydroxide (62). *S. arsophilum* also released As from sterilized Aberjona sediments (61).

**Bacillus spp.**  
To date, at least five *Bacillaceae*, *Bacillus arsenicobolu* (69), *B. selenitetigecans* (69), *B. macyae* (70), *B. selenobrunensis* (71), and *B. weideri* (72), have been isolated as dissimilatory As(V)-reducing bacteria. Among these, *B. selenobrunensis* strain SF-1 is able to release As from iron and aluminum (hydr)oxides coprecipitated with As(V), as well as from As-contaminated soils (73). The membrane-bound ARR of *B. selenobrunensis* has been purified and characterized (74).

**Desulfotobacterium spp.**  
Niggemeyer et al. (75) isolated a dissimilatory As(V)-reducing bacterium, *Desulfotobacterium* sp. strain GBFH, from As-contaminated sediments. They also demonstrated that *Desulfotobacterium hafniense* DCB-2 and *D. frappieri* PCP-1, which are both known to be reductively dechlorinating bacteria, can also respire As(V). These *Desulfotobacterium* strains are metabolically versatile and can use a wide variety of electron acceptors including Fe(III), Se(IV), Mn(IV), fumarate, elemental sulfur, sulfite, and thiosulfate (75). In a genome of *D. hafniense* DCB-2, arsenic-metabolizing genes are encoded on an arsenic island (Fig. 3). In addition to *arrA* and *arrB* genes, genes encoding a NrfD-like membrane protein (*ArrC*), a TorD-like chaperone protein (*ArrD*), and putative regulatory proteins involved in the two-component signal transduction system (*ArrR*, *ArrS*, and *ArrT*) are present. Furthermore, As detoxification genes encoding a putative transcriptional repressor (*ArrR*), an As(III) chaperone protein (*ArrD*), and an ATPase (*ArrA*) are present, but are encoded on the opposite strand.

**Shewanella sp. ANA-3**  
*Shewanella* sp. ANA-3 was isolated from an As-treated wooden pier located in a brackish estuary (76). This organism can also use nitrate, Fe(III), Mn(IV), thiosulfate, fumarate, and oxygen as electron acceptors. Because *Shewanella* spp. are genetically tractable, comprehensive molecular characterization of dissimilatory As(V) reduction has been performed using this strain. ARR of *Shewanella sp. ANA-3* is a periplasmic enzyme that is expressed during the exponential and stationary phases (77). Interestingly, ARR is finally released from the cells into the surrounding environment (77). The expression dynamics of *arrA* in strain ANA-3 were determined and compared by Saltikov et al. (78), and the results revealed that it is only expressed anaerobically, while it is repressed by other electron acceptors such as oxygen, nitrate, and fumarate. Conversely, *arsC* is expressed under both aerobic and anaerobic conditions. *arrA* is also induced by 100 nM As(III), while 1000 times more As(III) is required for the induction of *arsC*. A gene encoding a membrane-associated tetraheme c-type cytochrome, *cymA*, is required for ANA-3 to grow on As(V) (79). This cytochrome is also indispensable for growth on Fe(III), Mn(IV), and fumarate. When incubated with As(V)-adsorbed ferrihydrite, a Fe(III) reduction deficient mutant of strain ANA-3 effectively reduced solid-phase As(V), while an As(V) reduction deficient mutant did not (80). These findings strongly suggest that the Fe(III) reduction pathway is not required for ferrihydrite-adsorbed As(V) reduction. The organization of *arrA* and *arrB* genes in the genome of strain ANA-
3 is shown in Fig. 3. Genes encoding ArsD, ArsA, ArsB (a membrane efflux pump), and ArsC are also present, which may confer strain ANA-3 As resistance (76).

**Geobacter spp.** Geobacter spp., which are the most common and ubiquitous iron-reducing bacteria, play a pivotal role in dissimilatory iron reduction in terrestrial and freshwater environments (81). Recent studies have demonstrated that Geobacter spp. also function as dissimilatory As(V)-reducing bacteria, and may play a role in As reduction and mobilization. Islam et al. (60) found that As release from West Bengal sediments occurred when they were incubated anaerobically with acetate as the electron donor, and subsequent 16S rRNA gene clone library analysis indicated that 70% of clones in the acetate-amended sediments were affiliated with the family Geobacteraceae. Similar results were obtained upon microbial community analysis of Cambodian sediments (82). In addition, dissimilatory As(V) reductase genes (arrA) closely related to Geobacter spp. have frequently been detected in As-contaminated sediments (83–86). Until recently, only two Geobacter species, Geobacter uranireducens Rf4 and G. lovleyi SZ, had been known to possess putative arrA and arrB genes in their genomes. However, neither of these strains is able to grow using As(V) as the electron acceptor, even though resting cells of G. lovleyi SZ reduce As(V) in the presence of acetate (86). Various arr genes with an operon-like structure are present in the G. lovleyi SZ genome, but it appears to lack the arrA and arrD genes (Fig. 3). An arsenic island found in the genome of G. uranireducens Rf4 also lacks the arrA and arrD genes. Considering that both of these As detoxifying genes are frequently found in other dissimilatory As(V)-reducing bacterial genomes, their incapacity to grow on As(V) might be in part due to the absence of arrA and arrD genes.

In 2013, Ohtsuka et al. (87) isolated a new dissimilatory As(V)-reducing bacterium, Geobacter sp. OR-1, from As-contaminated Japanese paddy soil. Strain OR-1 also used nitrate, Fe(III), and fumarate as electron acceptors and catalyzed the release of As from As(V)-adsorbed ferrihydrite or sterilized paddy soil. Furthermore, As K-edge X-ray absorption near-edge structure (XANES) analysis suggested that strain OR-1 reduced As(V) directly in the soil solid phase. Because strain OR-1 is the first Geobacter species with the capacity to grow using As(V) as the sole electron acceptor, it may be useful as a model microorganism influencing mobilization of As in flooded soils and anoxic sediments (87). Preliminary draft genome analysis of strain OR-1 identified two arsenic islands containing multiple arrA and arrD genes (Ehara and Amachi, unpublished data).

**Anaeromyxobacter sp. PSR-1** Anaeromyxobacter dehalogenans was isolated by Sanford et al. (88) as a reductively dechlorinating δ-Proteobacterium that grows using 2-chlorophenol as an electron acceptor. This organism has a gliding motility, forms a spore-like structure, and can also use nitrate, Fe(III), U(VI), Se(IV), fumarate, and oxygen (microaerobic) as electron acceptors (89–91). Kudo et al. (92) recently isolated a dissimilatory As(V)-reducing bacterium, strain PSR-1, from As-contaminated soil and found that it had 99.7% 16S rRNA gene similarity with A. dehalogenans. As release was observed when strain PSR-1 was incubated with As(V)-adsorbed ferrihydrite or sterile As-contaminated soil. Multiple attempts to amplify the putative arrA gene from genomic DNA of strain PSR-1 were unsuccessful, indicating that strain PSR-1 may harbor an atypical arrA gene that cannot be amplified using previously designed degenerate primers (92). Although complete genome sequences of several strains of Anaeromyxobacter spp. have been released, none possess putative genes for arrA and arrB.

**APPLICATION TO BIOREMEDIATION**

**Removal from water** As described above, As(III) is less adsorptive than As(V); therefore, As(III) oxidation is an important pretreatment process for adsorption and coprecipitation using Al/Fe(III) minerals. Because chemical oxidation of As(III) via oxygen is very slow (93), application of aerobic As(III) oxidizers can be an effective remediation strategy for removal of As from contaminated water. For example, Ike et al. (94) successfully obtained an enrichment culture with a soil free from As contamination that showed high As(III)-oxidizing activity and greatly enhanced As adsorption by activated alumina. Three aerobic HAOs, presumably classified as Haemophilus spp., Micrococcus spp., and Bacillus spp., were isolated from their enrichment culture. Andrianisa et al. (95) demonstrated that an activated sludge collected from a treatment plant receiving no As-contaminated wastewater rapidly oxidized As(III). Furthermore, they isolated an aerobic CAO through subculture of the sludge
without an organic carbon source. They also found that biological As(III) oxidation can occur in a full-scale plant based on field investigations of an oxidation ditch activated sludge process receiving As-contaminated wastewater. In their investigation, oxidized As in the effluent was shown to be effectively removed by coprecipitation with ferric hydroxide. In a recent study by the same group, a continuous-flow bioreactor with immobilized aerobic As(III)-oxidizing bacteria was performed as a pretreatment step for As removal from groundwater (98). Anaerobic CAOs can be also used as an alternative process for pre-oxidation of As(III) (100). Yamamura et al. (107) investigated the removal of As from two contaminated soils containing 250 and 2400 mg/kg of As, respectively. Soda et al. (108) proposed application of a slurry bioreactor using the DARP for remediation of As-contaminated soil and developed a mathematical model that provides a framework for understanding and predicting the dissolution of As from soil. Lee et al. (109) applied a combination of microbial As mobilization and electrokinetic remediation to As-contaminated mine tailing soils. In both batch- and column-type bioleaching reactors, stimulation of indigenous bacteria by the injection of organic carbon sources led to the reductive dissolution of As. Subsequent electrokinetic treatment enhanced As removal efficiency, and the combined process achieved about 67% removal of As from soil containing 4023 mg/kg of As. Although similar removal efficiency was only observed during electrokinetic treatment, the preliminary microbial As mobilization reduced the duration of electrokinetics, with the combined process resulting in a 26.4% cost reduction. Because the treatment methods available for As-contaminated soils are mainly soil replacement, containment, and solidification/stabilization, these studies indicate a great potential for application of DARPs as a novel bioremediation strategy for As removal from soils.

In conclusion, the history of the interaction between As and prokaryotes considerably exceeds that of its interaction with human beings. Although the microbiological study of As is a century old, we still have only a limited understanding of the ancient processes by which prokaryotes utilize or tolerate As. The more we learn about them, the more complex they appear to become. However, we believe that the development of research in this field can provide the key to handling this toxic and ubiquitous metalloid.

ACKNOWLEDGMENTS

This work was partly supported by JSPS KAKENHI Grant Numbers 23681005 for S. Y. and 23580103 for S. A. In addition, S. A. is grateful to A. Ebara and M. Tonomura (Chiba University) for providing technical assistance with informatics.

References


