Chapter 10
Microbes Are Essential Components of Arsenic Cycling in the Environment: Implications for the Use of Microbes in Arsenic Remediation

Sudhakar Srivastava and Kavita Shukla

Abstract Arsenic (As) is a ubiquitously distributed toxic element, and it has been present in the environment since the very beginning of evolution. Hence, microbes to higher organisms possess mechanisms to tackle arsenic that include conversion of arsenic from one form to another including inorganic to organic and vice versa. Microbes present in different environments possess a number of pathways for arsenic conversion and therefore play a crucial role in arsenic cycling in the environment. Arsenic contamination has emerged as a serious problem in some parts of the world in the past few decades. These include Bangladesh, India, China, Vietnam, Pakistan, etc. The presence of arsenic in soil and groundwater in affected areas also leads to the entry of arsenic in plants. The level of arsenic accumulation in plants including edible portions depends on the arsenic species. In this scenario, microbes, which affect arsenic speciation, can play a role in regulating arsenic accumulation and consequently arsenic stress in plants. The microbes can therefore be utilized effectively to safeguard crop plants from arsenic. If the microbes also possess plant growth-promoting ability, this strategy can impart further benefits. A number of plant growth-promoting microbes (PGPMs) have been identified, characterized and utilized for the improvement of growth of plants in arsenic-contaminated environment as well as for the reduction of arsenic levels in plants. This review presents the role of microbes in arsenic cycling in the environment and discusses efforts for their utilization in the amelioration of arsenic stress in plants.

Keywords Arsenic · Plant growth-promoting microbes · Remediation

S. Srivastava (✉) · K. Shukla
Plant Stress Biology Laboratory, Institute of Environment and Sustainable Development, Banaras Hindu University, Varanasi, India
e-mail: sudhakar.iesd@bhu.ac.in

P. K. Arora (ed.), *Microbial Metabolism of Xenobiotic Compounds*,
Microorganisms for Sustainability 10,
https://doi.org/10.1007/978-981-13-7462-3_10

10.1 Introduction

Arsenic (As) is an element present ubiquitously in the environment since the beginning of evolution, and consequently the pathways of its detoxification are found in almost every organism from bacteria to humans (Yang and Rosen 2016). Arsenic occurs in the environment in different inorganic [arsine (As^{-3}), elemental arsenic (As^0), arsenite [As(III)] and arsenate [As(V)]] and organic forms [dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), trimethylarsine oxide (TMAO), arsenocholine, arsenobetaine, etc.]. These different inorganic and organic species of arsenic have variable toxicities to biological systems. Hence, the processes of arsenic detoxification include conversion of more toxic inorganic form either to less toxic inorganic or to organic forms, followed by efflux from cells or vacuolar sequestration in different microbes (Bentley and Chasteen 2002; Upadhyay et al. 2018). Arsenate reduction to As(III) and vice versa As(III) oxidation to As(V) are catalysed by arsenite oxidase and arsenate reductase, respectively. Arsenic methylation is performed by As(III) S-adenosylmethionine (SAM) methyltransferases (Yang and Rosen 2016). The final fate of arsenic conversion to various organic forms can be the formation of volatile arsenic species like arsine (AsH_3) and trimethylarsine (TMA) (Paez-Espino et al. 2009). The potential of microbes for the conversion of arsenic to different methylated and volatile arsenic species depends on a number of factors including soil chemistry, organic matter, etc. (Mestrot et al. 2011).

Arsenic contamination is a widespread problem today with threats associated with arsenic toxicity being global. The problem is severe in Southeast Asian countries including Bangladesh, India, China, Pakistan, etc. These causes of arsenic contamination in these areas are mainly natural biogeochemical processes, although enhanced and worsened by human intervention (Srivastava et al. 2012; Rodriguez-Lado et al. 2013; Podgorski et al. 2017). Arsenic levels in the groundwater and soil are higher than maximum permissible limits set by WHO. The major accepted hypothesis for the presence of high arsenic in groundwater states that chemical changes induced by digging of shallow tube wells and hand pumps stimulated microbial reactions. This in turn led to the mobilization of arsenic present in bound form in sediments in the groundwater. The role of the number of microbes was identified in the process of arsenic mobilization in groundwater. The use of contaminated groundwater for irrigation leads to the entry of arsenic in soil and subsequently in plants. Rice is one of the worst affected crop plants with arsenic accumulation in its grains (Awasthi et al. 2017). The hunt for feasible low-cost strategies to mitigate arsenic contamination of rice is on. However, no suitable technology is available. The use of plant growth-promoting microbes (PGPMs) for the amelioration of arsenic toxicity and accumulation in rice is a deserving eco-friendly and sustainable method (Upadhyay et al. 2018). Further, suitable options are required for the remediation of highly contaminated and even abandoned sites. To this end also, plant- and microbe-based remediation methods have been considered viable options that can give desirable results in more time but at an affordable cost.

Hence, microbial utilization, due to its unique potential to handle even the high arsenic concentrations, is an approach that needs to be studied and optimized for commercial purposes.

In the present review article, the basic scheme of microbial metabolism of arsenic is presented. The studies demonstrating the utilization of microbes or their inherent pathways (genes) are discussed, and future perspectives are outlined.

10.2 Microbes: Role in Arsenic Cycling

Similar to cycling of various elements like carbon, nitrogen, oxygen and sulphur, a toxic element like arsenic also undergoes various ecological spheres in a cyclic manner. The microbes hold a crucial position in arsenic cycling due to the fact that they possess several possible ways to either detoxify and efflux arsenic or utilize arsenic species for metabolic purposes (Mukhopadhyay et al. 2002; Yang and Rosen 2016). It is now well known that arsenic-related genes in microbes are present in several clusters (Andres and Bertin 2016). The most simple arsenic operon is *ars* operon with basic constituent genes as *arsRBC*, where ArsR is an As(III)-responsive repressor, ArsB is an As(III) efflux permease and ArsC is an As(V) reductase (Yang and Rosen 2016; Andres and Bertin 2016). In this operon, other genes may also be present like ArsA (the As(III)-stimulated ATPase) and ArsD (As(III) metallochaperone); the operon thus becomes large: *arsRDBAC*. The binding of ArsA to ArsB increases the efficiency of As(III) efflux by ArsB. The function of ArsC becomes important when As(V) is the major species entering the cells that has to be reduced to As(III) for the efflux. Yet more genes may be present in ars operon, e.g. arsH and arsP, having variable functions. Several variations of the set of genes in ars operon in different microbes have been found (Andres and Bertin 2016). The eukaryotic counterpart of *arsRBC* in *Saccahromycescerevisiase* includes *ACR1 (ARR1)*, *ACR2 (ARR2)* and *ACR3 (ARR3)* that encode for transcriptional activator, arsenate reductase and efflux pump, respectively (Ghosh et al. 1999).

Another important microbial arsenic-related operon system is *aioBA* that encodes two subunits of arsenite oxidase. Other genes like *aioC* (encoding c-type cytochrome), *aioD* (encoding molybdenum cofactor) and *nitR* (encoding nitroreductase) and regulatory genes, *aioXSR* (encoding As(III)-binding protein, histidine kinase and response regulator, respectively), are present in some proteobacteria (Andres and Bertin 2016). Another operon meant for the respiration of As(V) is *arrAB* (encoding two subunits of respiratory As(V) reductase), while an operon for anaerobic As(III) oxidation is *arxB'AB* (where *arxA* and *arxB* encode for two subunits of anaerobic As(III) oxidase). Two other important genes of arsenic metabolism in microbes include *arsM*, encoding for arsenite methyltransferase, and *arsI*, encoding for C-As lyase. These two genes carry out the function of arsenic methylation and demethylation and organoarsenical degradation. Hence, there is an array of pathways through which microbes can handle arsenic infiltration. A few microbes are arsenotrophs which means they utilize As(III) oxidation and As(V) reduction for

their growth (Zargar et al. 2012). The basic reactions of arsenic metabolism and/or detoxification are presented in Fig. 10.1.

In arsenic microbial cycling, the reduction of As(V) to As(III) is catalysed by respiratory or cytoplasmic arsenate reductase. Most of the microbes reduce As(V) to As(III) for the purpose of its subsequent efflux back to the medium as a part of arsenic detoxification mechanism. In this As(V) reduction, microbes do not get any energy. However, in respiratory As(V) reduction, microbes gain energy to support their growth. The microbes (dissimilatory arsenate-reducing prokaryotes, DARPs) involved in arsenic release in groundwater through reductive dissolution of minerals use arsenate as a terminal electron acceptor in arsenic respiration under reducing conditions (Laverman et al. 1995; Krafft and Macy 1998). In this way, microbes are able to synthesize ATP. The electron donors may be various small organic molecules like pyruvate, glycerol, malate, etc. (Newmann et al. 1998; Oremland and Stolz 2005; Paez-Espino et al. 2009). DARPs belong to diverse group like *Firmicutes* and proteobacteria. The two-enzyme complex of ArrA and ArrB acts as an arsenate reductase in DARPs. In case of As(III) oxidation, As(III) acts as the electron donor and the process is coupled to respiration. The As(III) is oxidized, while oxygen is reduced along with energy gain that is then utilized for cell growth (Santini and vanden Hoven 2004; Muller et al. 2003). Arsenate-reducing microbes and DARPs are important players in groundwater release of arsenic in the form of As(III).

Arsenic also goes through cycles of methylation and demethylation in its cycle. The methylation of arsenic in a stepwise manner leads to the formation of mono-, di- and tri-methylated species, which are less toxic than inorganic arsenic species. Hence, arsenic methylation is also a detoxification mechanism (Upadhyay et al. 2018). The methylation of arsenic has been detected in bacteria, archaea, fungi, protozoan, cyanobacteria and algae and higher animals and humans (Wang et al. 2014; Yin et al. 2011; Qin et al. 2006). The methylation is catalysed by ArsM. The last product of methylation is trimethylarsine [TMAs(III)] which is a gas. However, it was surprising for years that even with capabilities of arsenic methylation being present so widely, arsenic still exists mostly as inorganic species in nature. The reason for this was revealed upon discovery of arsenic demethylation (Feng et al. 2005; Maki et al. 2006). ArsI is a C-As lyase that cleaves the C-As bond converting MAs(III) into As(III). ArsI may also cleave C-As bond in aromatic arsenic compounds. Thus, the use of even organic arsenicals, like roxarsone and p-arsanilic acid that are being used presently, is harmful as their eventual fate is demethylation and conversion to inorganic arsenic species (Yang and Rosen 2016). Another enzyme, ArsH, which is an NADPH-dependent FMN reductase, can oxidize trivalent organic arsenicals like MA(III) and Rox(III) to their pentavalent less toxic forms. This enzyme is crucial for arsenic tolerance of some bacteria.

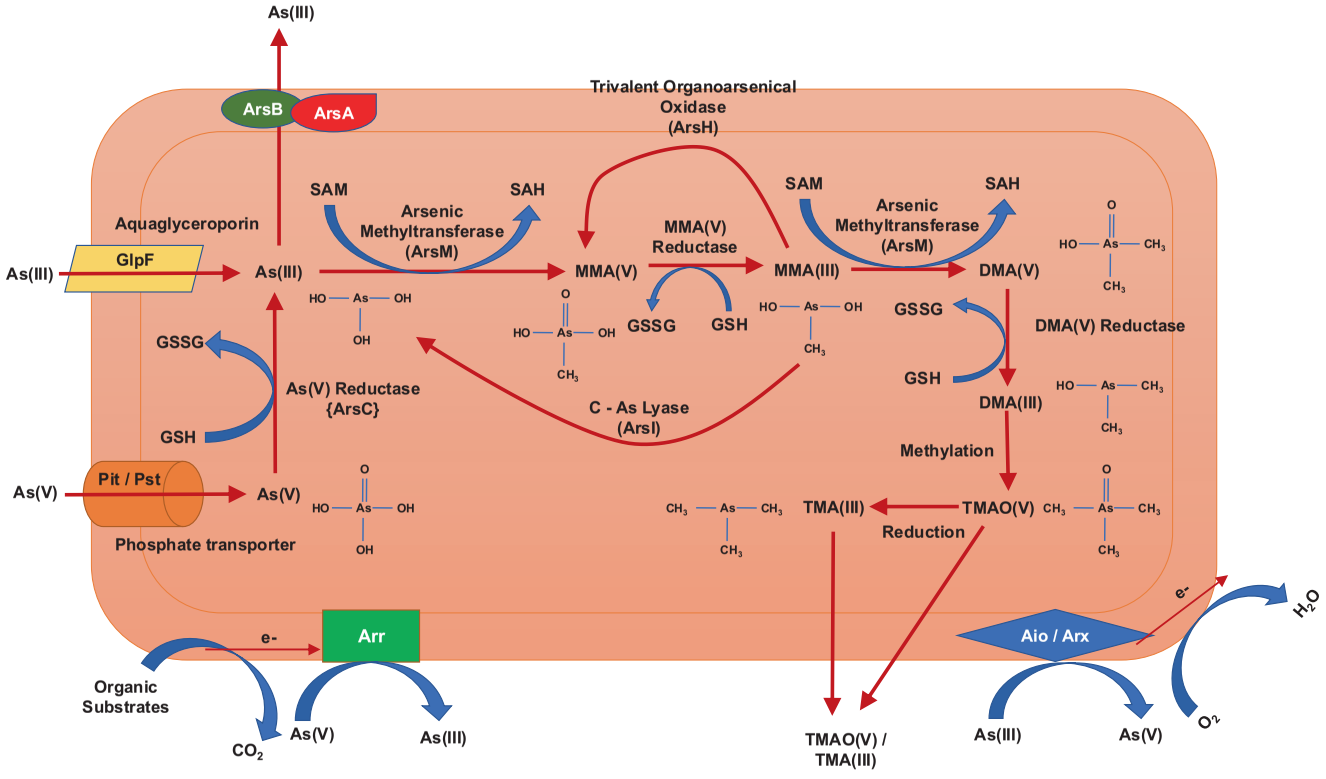

Fig. 10.1 Metabolism of arsenic in a microbial cell. There are different arsenic operons: Ars operon represents cytoplasmic arsenate reductase (ArsC), arsenite efflux ATPase (ArsA, ArsB) and arsenic methyltransferase (ArsM). There are other enzymes also like C-As lyase (ArsI) and trivalent organoarsenical oxidase (ArsH). Arr represents respiratory arsenate reductase, while Aio/Arx represents respiratory arsenite oxidase. Abbreviations: *As(V)* arsenate, *As(III)* arsenite, *MMA(V)* monomethylarsonic acid, *MMA(III)* monomethylarsonous acid, *DMA(V)* dimethylarsinic acid, *DMA(III)* dimethylarsinous acid, *TMAO(V)* trimethylarsine oxide, *TMA(III)* trimethylarsine, *GSH* reduced glutathione, *GSSG* oxidized glutathione, *SAM* S-adenosylmethionine, *SAH* S-adenosylhomocysteine

10.3 Role of Microbes in Arsenic Regulation in Plants in Lab and Field

Combating the problem of arsenic contamination of the environment requires simultaneous two-sided attention like the two sides of a coin. At one end, high arsenic present in abandoned land and water sites needs to be removed in a cost-effective manner, viz. through plant-based remediation. On the other end, arsenic present in agriculturally productive land and water needs to be prevented from entering plants. Since microbes can cycle arsenic into different inorganic and organic forms, they can potentially be utilized alone or in consortia to regulate arsenic accumulation and achieve desirable results. A number of studies have been performed to identify arsenic-tolerant microorganisms from arsenic-contaminated environments and have led to successful identification of several potential microbes that can be utilized to either increase the arsenic accumulation of phytoremediator plants or decrease the arsenic level in edible tissues of plants.

10.4 Microbe-Mediated Growth Improvement and Arsenic Level Reduction in Crop Plants

Roychowdhury et al. (2018) identified ten arsenic-resistant bacterial isolates from the fly ash (pond ash sample) belonging to genera like *Bacillus*, *Kytococcus*, *Staphylococcus*, etc. (e.g. *B. subtilis*, *B. thuringiensis*, *K. sedentarius*, *S. pasteuri*). These strains were capable of As(III) to As(V) and As(V) to As(III) conversion and store arsenic in their biomass. Prum et al. (2018) analysed the arsenic removal efficiency of several plants (*Echinodorus cordifolius*, *Cyperus alternifolius*, *Acrostichum aureum*, *Colocasia esculenta*) and found *E. cordifolius* to be the best arsenic remover. They also tested *E. cordifolius* along with an arsenic-tolerant microbe *Arthrobacter creatinolyticus* for arsenic removal in pot experiments. Authors found that dipping bacteria on plant roots for 5 min significantly increased plants' efficiency for arsenic removal both in lab and in field in a constructed wetland.

Bacterial inoculation has also been tested to reduce arsenic toxicity to and arsenic accumulation in rice seedlings. In a study, rice seedlings were inoculated with different strains of cadmium-tolerant bacteria (KKU2500-1, KKU2500-2, KKU2500-3, KKU2500-9, KKU2500-12, KKU2500-16 and KKU2500-22) and As(III)-oxidizing bacteria (4.25, 4.27, 4.40 and 4.44) in various combinations. These bacteria possessed As(III)-oxidizing ability and produced a high amount of inorganic sulphide and thiol compounds. The strain combinations like KKU2500-3/4.25 and KKU2500-3/4.44 not only improved the growth of rice seedlings in the presence of As(V)/As(III) but also significantly reduced arsenic translocation to shoot (Thongnok et al. 2018). Das and Sarkar (2018) isolated an arsenic-resistant microbe, *Acinetobacter lwoffii* (RJB-2), from arsenic-contaminated soil of North 24 Parganas, West Bengal, and used it as inoculum to *Vigna radiata*.

The bacterium inhibited arsenic uptake by plants and showed significant improvement in plant growth and also decreased oxidative stress to plants. Mallick et al. (2018) isolated *Kocuria flava* and *Bacillus vietnamensis* as resistant bacteria from Sunderban, West Bengal. The application of these bacteria led to improved growth and decreased arsenic in rice plants. An arsenic-tolerant microbe *Micrococcus luteus* has been found to improve growth and biomass of grapevine plants (Ivan et al. 2017).

Apart from bacteria, fungi and algae have also been tested as potential inoculants for improving the growth of plants and for reducing arsenic accumulation at the same time. The effect of the application of arbuscular mycorrhizal fungi (AMF), *Rhizoglomus intraradices* and *Glomus etunicatum*, on *Triticum aestivum* plants was analysed by Sharma et al. (2017). The mycorrhizal inoculation resulted in improved growth of plants, reduced arsenic translocation and maintained phosphorus to arsenic ratio. An improved growth and a decline in arsenic accumulation have also been found in soybean plants upon inoculation of *Rhizophagus intraradices* (Spagnoletti et al. 2016). *R. intraradices* has also been able to reduce inorganic/organic arsenic ratio in rice plants (Li et al. 2016). The application of the fungal strain of *Trichoderma*, *T. reesei* NBRI0716, was demonstrated to increase the yield of chickpea plants and reduce inorganic arsenic concentration when grown in arsenic (100 mg kg^{-1})-contaminated soil (Tripathi et al. 2013). Algal inoculants, *Chlorella vulgaris* and *Nannochloropsis* sp., have also been found to reduce arsenic accumulation in rice seedlings and ameliorate arsenic toxicity in a study by Upadhyay et al. (2016). In a recent work, Awasthi et al. (2018) studied a consortium of alga (*C. vulgaris*) and bacterium (*Pseudomonas putida*) against arsenic stress in rice seedlings. They demonstrated significant improvement in the growth of rice seedlings along with a decline in arsenic in root and shoot of seedlings when rice plants were grown with the consortium.

10.5 Microbe-Mediated Growth Improvement and Increased Arsenic Accumulation in Phytoremediator Plants

The use of microbes in increased arsenic removal and phytoremediation efficiency of plants has also been tested. In a recent work, Mukherjee et al. (2018) isolated endophytic microbes from arsenic-tolerant plant, *Lantana camara*, from Nadia, West Bengal, and applied these microbes as consortia in *Solanum nigrum*. These bacteria included *Enterobacter* sp., *Kocuria* sp. and *Kosakonia* sp. The microbial consortium helped plants maintain good growth and have higher shoot arsenic accumulation. The increased conversion of As(V) to As(III), glutathione levels and changes in transporter expression were major mechanisms behind the consortium-mediated increased arsenic tolerance of *Solanum* plants. Mesa et al. (2017) isolated four potential bacterial strains isolated from rhizosphere (*Ensifer adhaerens*) and roots (*Variovorax paradoxus*, *Phyllobacterium myrsinacearum*) of *Betula*

celtiberica in Spain and found that while *E. adhaerens* enhanced the growth of plants, *V. paradoxum* and *P. myrsinacearum* increased the accumulation of arsenic in plants. Arsenic-resistant microbes from rice fields (*Lysinibacillus* sp., *Bacillus altitudinis*, *B. megaterium*) have been reported to increase arsenic removal by *Pteris vittata* plants (Singh et al. 2015). Other potential arsenic-tolerant bacterial strains (*Pseudomonas* sp., *Delftia* sp., *Bacillus* sp., *Variovorax* sp. and *Pseudoxanthomonas* sp.) have also been found to enhance arsenic accumulation by *P. vittata* plants along with increase in growth, thus improving arsenic removal (Lampis et al. 2015). In *Populus deltoides*, the application of *Agrobacterium radiobacter* led to an increase in arsenic removal and root-to-shoot translocation (Wang et al. 2011).

10.6 Biotechnological Tools to Utilize Microbial Genes for the Regulation of Arsenic Accumulation in Plants

Extensive research on microbial strategies and pathways of arsenic tolerance has resulted in the identification of several potential genes that can be used for the development of transgenic plants for altering arsenic accumulation properties of plants. Such a strategy would reduce cost associated with microbial inoculation during each cropping period. Further, attempts have also been made to augment the arsenic resistance potential of microbes themselves through the expression of their own or foreign gene and then utilize such transgenic microbes for the amelioration of arsenic toxicity in plants.

A gene of huge interest for the past few years has been *arsM* whose enzyme product is involved in arsenic methylation and volatilization in microbes. Since organic methylated arsenicals have less toxicity and volatile arsenicals are expelled from plants, this gene has been the gene of choice in several studies. An *arsM* gene from *Chlamydomonas reinhardtii* (*CrarsM*) was expressed in the symbiotic bacterium, *Rhizobium leguminosarum*, that resulted in higher arsenic methylation potential of symbiont. The use of transgenic *R. leguminosarum* in symbiotic association with red clover plants resulted in an increased level of methylated arsenic in red clover plants. In addition, arsenic volatilization (0.01%–0.02% of total As) was also observed (Zhang et al. 2017). The *arsM gene* from the alga, *Chlamydomonas reinhardtii*, has also been used for the development of transgenic plants of *Arabidopsis thaliana*. It was found that the methylation of inorganic arsenic species to organic species and volatile arsenic species was increased in transgenic plants (Tang et al. 2016). A soil fungus, *Westerdykella aurantiaca*, has also been utilized for the isolation of *arsM* gene, *WaarsM*, by Verma et al. (2016). The *WaarsM* gene was expressed in the yeast, *S. cerevisiae*, which showed higher arsenic methylation as well as arsenic volatilization. The application of transgenic yeast cells to rice plants also improved the growth of rice in arsenic-stressed conditions. In an earlier work, Meng et al. (2011) utilized *arsM* gene from *Rhodopseudomonas palustris* to develop

transgenic rice plants and found an increased level of organic arsenic species including volatile arsenic.

10.7 Future Prospects

Microbes are the treasure of metabolic pathways and have several potential mechanisms that can be utilized to assist plants in handling arsenic stress more effectively. Most of the studies till date have studied microbial application with a single microbe or with a single group of microbes. Future studies need to focus on consortia containing different types of microbes like bacteria, algae and fungi so as to holistically improve plants' growth and alter arsenic accumulation. Further, microbial genes need to be utilized in combination to achieve better results in terms of arsenic level change in crop plants/phytoremediator plants.

References

Andres, J., & Bertin, P. N. (2016). The microbial genomics of arsenic. *FEMS Microbiology Reviews, 40*, 299–322.

Awasthi, S., Chauhan, R., Dwivedi, S., Srivastava, S., Srivastava, S., & Tripathi, R. D. (2018). A consortium of alga (*Chlorella vulgaris*) and bacterium (*Pseudomonas putida*) for amelioration of arsenic toxicity in rice: A promising and feasible approach. *Environmental and Experimental Botany, 150*, 115–126.

Awasthi, S., Chauhan, R., Srivastava, S., & Tripathi, R. D. (2017). The journey of arsenic from soil to grain in rice. *Frontiers in Plant Science, 8*, 1007.

Bentley, R., & Chasteen, T. G. (2002). Microbial methylation of metalloids: Arsenic antimony and bismuth. *Microbiology and Molecular Biology Reviews, 66*, 250–271.

Das, J., & Sarkar, P. (2018). Remediation of arsenic in mung bean (*Vigna radiata*) with growth enhancement by unique arsenic-resistant bacterium *Acinetobacter lwoffii*. *Science Total Environment, 624*, 1106–1118.

Feng, M., Schrlau, J. E., Snyder, G. H., Chen, M., Cisar, J. L., & Cai, Y. (2005). Arsenic transport and transformation associated with MSMA application on a golf course green. *Journal of Agricultural and Food Chemistry, 53*, 3556–3562.

Ghosh, M., Shen, J., & Rosen, B. P. (1999). Pathways of As (III) detoxification in *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 5001–5006.

Ivan, F. P., Salomon, M. V., Berli, F., Bottini, R., & Piccoli, P. (2017). Characterization of the As(III) tolerance conferred by plant growth promoting rhizobacteria to in vitro-grown grapevine. *Applied Soil Ecology, 109*, 60–68.

Krafft, T., & Macy, J. M. (1998). Purification and characterization of the respiratory arsenate reductase of *Chrysiogenes arsenatis*. *European Journal of Biochemistry, 255*, 647–653.

Lampis, S., Santi, C., Ciurli, A., Andreolli, M., & Vallini, G. (2015). Promotion of arsenic phytoextraction efficiency in the fern *Pteris vittata* by the inoculation of As-resistant bacteria: A soil bioremediation perspective. *Frontiers in Plant Science, 6*, 80.

Laverman, A. M., Blum, J. S., Schaerfer, J. K., Phillips, E., Lovley, D. R., & Oremland, R. S. (1995). Growth of strain SES-3 with arsenate and other diverse electron acceptors. *Applied and Environmental Microbiology, 61*, 3556–3561.

Li, H., Chen, X., & Wong, M. (2016). Arbuscular mycorrhizal fungi reduced the ratios of inorganic/organic arsenic in rice grains. *Chemosphere, 145*, 224–230.

Maki, T., Takeda, N., Hasegawa, H., & Ueda, K. (2006). Isolation of monomethylarsonic acid-mineralizing bacteria from arsenic contaminated soils of Ohkunoshima Island. *Applied Organometallic Chemistry, 20*, 538–544.

Mallick, I., Bhattacharyya, C., Mukherji, S., Dey, D., Sarkar, S. C., Mukhopadhyay, U. K., et al. (2018). Effective rhizoinoculation and biofilm formation by arsenic immobilizing halophilic plant growth promoting bacteria (PGPB) isolated from mangrove rhizosphere: A step towards arsenic rhizoremediation. *Science Total Environment, 610–611*, 1239–1250.

Meng, X. Y., Qin, J., Wang, L. H., Duan, G. L., Sun, G. X., Wu, H. L., Chu, C. C., Ling, H. Q., Rosen, B. P., & Zhu, Y. G. (2011). Arsenic biotransformation and volatilization in transgenic rice. *The New Phytologist, 191*, 49–56.

Mesa, V., Navazas, A., González-Gil, R., González, A., Weyens, N., Lauga, B., et al. (2017). Use of endophytic and rhizosphere bacteria to improve phytoremediation of arsenic-contaminated industrial soils by autochthonous *Betula celtiberica*. *Applied and Environmental Microbiology, 83*, e03411–e03416.

Mestrot, A., Feldmann, J., Krupp, E. M., Hossain, M. S., Roman-Ross, G., & Meharg, A. A. (2011). Field fluxes and speciation of arsines emanating from soils. *Environmental Science & Technology, 45*, 1798–1804.

Mukherjee, G., Chinmay, S., Naskar, N., Mukherjee, A., Mukherjee, A., Lahiri, S., Majumder, A. L., & Seal, A. (2018). An endophytic bacterial consortium modulates multiple strategies to improve arsenic phytoremediation efficiency in *Solanum nigrum*. *Scientific Reports, 8*, 6979.

Mukhopadhyay, R., Rosen, B. P., Phung, L. T., & Silver, S. (2002). Microbial arsenic: From geocycles to genes and enzyme. *FEMS Microbiology Reviews, 26*, 311–325.

Muller, D., Lievremont, D., Simeonova, D. D., et al. (2003) Arsenite oxidase aox genes from a metal-resistant betaproteobacterium. *Journal of Bacteriology 185,* 135–41.

Newmann, D. K., Ahmann, D., & Morel, F. M. M. (1998). A brief review of microbial arsenate respiration. *Geomicrobiology Journal, 15*, 255–268.

Oremland, R. S., & Stolz, J. F. (2005). Arsenic, microbes and contaminated aquifers. *Trends in Microbiology, 13*, 45–49.

Paez-Espino, D., Tamames, J., de Lorenzo, V., & Canovas, D. (2009). Microbial responses to environmental arsenic. *Biometals, 22*, 117–130.

Podgorski, J. E., Eqani, S. A. M. A. S., Khanam, T., Ullah, R., Shen, H., & Berg, M. (2017). Extensive arsenic contamination in high-pH unconfined aquifers in the Indus Valley. *Science Advances, 3*, e1700935.

Prum, C., Dolphen, R., & Thiravetyan, P. (2018). Enhancing arsenic removal from arsenic-contaminated water by *Echinodorus cordifolius*– Endophytic *Arthrobacter creatinolyticus* interactions. *Journal of Environmental Management, 213*, 11–19.

Qin, J., Rosen, B. P., Zhang, Y., Wang, G., Franke, S., & Rensing, C. (2006). Arsenic detoxification and evolution of trimethylarsine gasby a microbial arsenite S-adenosylmethionine methyltransferase. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 2075–2080.

Rodríguez-Lado, L., Sun, G., Berg, M., Zhang, Q., Xue, H., Zheng, Q., et al. (2013). Groundwater arsenic contamination throughout China. *Science, 341*, 866–868.

Roychowdhury, R., Roy, M., Rakshit, A., Sarkar, S., & Mukherjee, P. (2018). Arsenic bioremediation by indigenous heavy metal resistant bacteria of fly ash pond. *Bulletin of Environmental Contamination and Toxicology*. https://doi.org/10.1007/s00128-018-2428-z.

Santini, J. M., & vanden Hoven, R. N. (2004). Molybdenum-containing arsenite oxidase of the chemolithoautotrophic arsenite oxidizer NT-26. *Journal of Bacteriology, 186*, 1614–1619.

Sharma, S., Anand, G., Singh, N., & Kapoor, R. (2017). Arbuscular mycorrhizal augments arsenic tolerance in wheat (*Triticum aestivum* L.) by strengthening antioxidant defense system and thiol metabolism. *Frontiers in Plant Science, 8*, 906.

Singh, S., Shrivastava, A., Barla, A., & Bose, S. (2015). Isolation of arsenic-resistant bacteria from Bengal delta sediments and their efficacy in arsenic removal from soil in association with *Pteris vittata*. *Geomicrobiology Journal, 32*, 712–723.

Spagnoletti, F. N., Balestrasse, K., Lavado, R. S., & Giacometti, R. (2016). Arbuscular mycorrhiza detoxifying responses against arsenic and pathogenic fungus in soybean. *Ecotoxicology and Environmental Safety, 133*, 47–56.

Srivastava, S., Suprasanna, P., & D'Souza, S. F. (2012). Mechanisms of arsenic tolerance and detoxification in plants and their application in transgenic technology: A critical appraisal. *International Journal of Phytoremediation, 14*, 506–517.

Tang, J., Lv, Y., Chen, F., Zhang, W., Rosen, B. P., & Zhao, F. J. (2016). Arsenic methylation in *Arabidopsis thaliana* expressing an algal arsenite methyltransferase gene increases arsenic phytotoxicity. *Journal of Agricultural and Food Chemistry, 64*, 2674–2681.

Thongnok, S., Siripornadulsil, W., & Siripornadulsil, S. (2018). Mitigation of arsenic toxicity and accumulation in hydroponically grownrice seedlings by co-inoculation with arsenite-oxidizing and cadmium-tolerant bacteria. *Ecotoxicology and Environmental Safety, 162*, 591–602.

Tripathi, P., Singh, P. C., Mishra, A., Chaudhry, V., Mishra, S., Tripathi, R. D., et al. (2013). Trichoderma inoculation ameliorates arsenic induced phytotoxic changes in gene expression and stem anatomy of chickpea (*Cicer arietinum*). *Ecotoxicology and Environmental Safety, 89*, 8–14.

Upadhyay, A. K., Singh, N. K., Singh, R., & Rai, U. N. (2016). Amelioration of arsenic toxicity in rice: Comparative effect of inoculation of *Chlorella vulgaris* and *Nannochloropsis* sp. on growth, biochemical changes and arsenic uptake. *Ecotoxicology and Environmental Safety, 124*, 68–73.

Upadhyay, M. K., Yadav, P., Shukla, A., & Srivastava, S. (2018). Utilizing the potential of microorganisms for managing arsenic contamination: A feasible and sustainable approach. *Frontiers in Environmental Science, 6*, 24.

Verma, S., Verma, P. K., Meher, A. K., Dwivedi, S., Bansiwal, A. K., Pande, V., et al. (2016). A novel arsenic methyltransferase gene of *Westerdykella aurantiaca* isolated from arsenic contaminated soil: Phylogenetic, physiological, and biochemical studies and its role in arsenic bioremediation. *Metallomics, 8*, 344.

Wang, P., Sun, G., Jia, Y., Meharg, A. A., & Zhu, Y. (2014). A review on completing arsenic biogeochemical cycle: Microbial volatilization of arsines in environment. *Journal of Environmental Sciences, 26*, 371–381.

Wang, Q., Xiong, D., Zhao, P., Yu, X., Tu, B., & Wang, G. (2011). Effect of applying an arsenic-resistant and plant growth-promoting rhizobacterium to enhance soil arsenic phytoremediation by *Populus deltoides* LH05-17. *Journal of Applied Microbiology, 111*, 1065–1074.

Yang, H. C., & Rosen, B. P. (2016). New mechanisms of bacterial arsenic resistance. *Biomedical Journal, 39*, 5–13.

Yin, X. X., Chen, J., Qin, J., Sun, G.-X., Rosen, B. P., & Zhu, Y. G. (2011). Biotransformation and volatilization of arsenic by three photosynthetic cyanobacteria. *Plant Physiology, 156*, 1631–1638.

Zargar, K., Conard, A., Bernick, D. L., Lowe, T. M., Stolc, V., Hoeft, S., Oremland, R. S., Stolz, J., & Saltikov, C. W. (2012). ArxA, a new clade of arsenite oxidase within the DMSO reductase family of molybdenum oxidoreductase. *Environmental Microbiology, 14*, 1635–1645.

Zhang, J., Xu, Y., Cao, T., Chen, J., Rosen, B. P., & Zhao, F. J. (2017). Arsenic methylation by a genetically engineered *Rhizobium*-legume symbiont. *Plant and Soil, 416*, 259–269.