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Microbial removal of arsenic: Mechanisms and Applications

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Abstract: With the ever increasing environmental pollution caused by toxic inorganic pollutants, human population is at a great health risk. According to EPA (Environment Protection Agency) silver (Au), arsenic (As), barium (Ba), cadmium (Cd), chromium (Cr) and copper (Cu) are the toxic inorganic components present in soil and water with arsenic being the most toxic. Over the course of evolution, microorganisms exposed to arsenic have developed various mechanisms for their survival. These mechanisms make the heavy metals less toxic by affecting their speciation, availability, mobility or volatilization. The genes involved in arsenic bioconversions are well studied. Arsenic bioremediation activity can be further improved by over-expressing genes or by using truncated pathways. This article gives an overview of all major microbial pathways employed in arsenic transformation and finally how the use of genetically engineered bacteria can overcome these limitations making the process more efficient and economical.

Keywords: Arsenic, bioaccumulation, bioconversion, bioremediation.

Introduction

In the past few decades, due to rapid increase in population, industrialization and urbanization; the problem of environmental pollution has escalated leading to the contamination of land and water (Mukhopadhyay, *et al.* 2000). According to EPA (Environment Protection Agency) Au, As, Ba, Cd, Cr, Cu are some of the toxic inorganic components present in soil and water (Sparks, *et al.* 2005; Liu, *et al.* 2011) and arsenic is considered to be the most toxic contaminant of all. Arsenic contamination is both due to natural and anthropogenic activities including volcanic eruptions, weathering of rocks, atmospheric precipitation, manufacturing of arsenic based compounds like pesticides and insecticides, mining and smelting of arsenic containing ores, combustion of fossil fuels *etc.* (Stolz, *et al.* 2010). The main environmental concern about arsenic is not related to its presence in the soil and sediments in anomalous amounts, but to its high concentration in surface water and its exposure to human beings (Nordstrom, *et al*. 2002; Zhao, *et al*. 2010). Arsenic exists in a variety of valence states with arsenite (As^{+3}) and arsenate (As^{+5}) being the most abundant forms (Liu, *et al*. 2011). Arsenite affects directly by producing reactive oxygen species (ROS) resulting in oxidative damage to DNA, proteins and lipids and indirectly by inactivating the cellular antioxidant system (Drewniak & Sklodowska, 2012). Also arsenite has high affinity for thiol groups and therefore it inhibits the enzymatic reactions based on thiol groups in the body (Hughes, *et al*. 2002). Arsenate inhibits enzymatic reactions by competing with phosphate ions. It also degrades iron-sulphur (Fe-S) clusters of proteins and acts as protein inhibitor (Kruger, *et al*. 2013). These metabolic interferences can lead to death from multi-system organ failure. Considering its toxic effects arsenic contamination has become a matter of concern in many parts of the world.

Prevalence, usage and toxicity of arsenic

Arsenic is ubiquitous in the environment and its presence in a region depends on its geography as well as its proximity to industries. Arsenic is reported to be naturally present in the sedimentary aquifers of Bangladesh, India, Argentina, Cambodia, Chile, China, Ghana, Hungary, Inner Mongolia, Mexico, Nepal, New Zealand, Philippines, Taiwan, United Statesand Vietnam (Nordstrom, *et al*. 2002). Bangladesh seems to be one of the biggest examples of mass arsenic poisoning (Bundschuh, *et al.* 2012). More than 150 million people use groundwater from these aquifers in Bangladesh and it is extensively used for cultivation of rice and other staple crops. Hence people living in these regions are exposed to arsenic in both their food and water (Nordstrom, *et al*. 2002; Zhao, *et al*. 2010). Tube-wells installed in Bangladesh, meant to provide clean drinking water contain 400 times the WHO's maximum permissible level of arsenic (0.01 ppm) (Stolz, *et al.*2010). Arsenic contamination is also as a result of anthropogenic activities such as use of pesticides, combustion of fossil fuels and leaching from mine tailings (Cheng, *et al*. 2009; Pa´ez-Espino, *et al*. 2009). A study conducted by Dr Guifan Sun, Dean of China Medical University, showed that in China, 14.7 million people are at risk of water contaminated with high levels of arsenic due to extensive coal

burning activities and consumption of contaminated food (Welch, *et al*. 2009). At the 8th World Congress on Environmental Health held in Durban, South Africa, it was noted that many communities on the African continent are ingesting arsenic well above the safety threshold level (Lado, *et al.* 2013). In these countries arsenic contamination is considered to be one of the foremost environmental causes of cancer (Ellis & Garelick, 2008).

Arsenic is used in industries as alloying agent in the manufacture of transistors, for lasers and doping in semiconductors, as well as in the processing of glass, pigments, textiles, paper, metal adhesives, wood preservatives and ammunition (Smith, *et al.* 2002). It is also used in the process of hide tanning and, to a limited extent, in pesticides, feed additives and pharmaceuticals. It is also used to synthesize salvarsan, a drug for syphilis (Singh, *et al*. 2008). But the continually increasing availability and abundance of arsenic induces population intoxication and poses a significant risk to human health. It is believed to be a human carcinogen and high levels of arsenic in drinking water increase the risk of skin cancer and tumors of the bladder, kidneys, liver and the lungs (Duker, *et al*. 2005). Short term and long term exposure to high levels of arsenic leads to the thickening and discoloration of the skin (also called skin pigmentation), numbness in the hands & feet and cramping or pain in the muscles and joints (Neubauer, 1947; Nicolis, *et al*. 2009; Platanias, 2009; Dani, *et al*. 2010). Metal ions of arsenic bind to various cellular structures and biomolecules, causing destabilization of enzymes, DNA and RNA and thus induce replication defects, mutagenesis, hereditary genetic disorders and eventually cancer (Gebel, 2000). The only available treatment of arsenic poisoning or arsenicosis involves removal of arsenic by dialysis, chelating agents, replacement of red blood cells, and bowel cleansing if ingested (Tokar, *et al.* 2010). Thus removal of arsenic from contaminated sites is extremely necessary for the benefit of human society and health.

Chemical and physical methods of remediation

Currently various physical and chemical methods are used for the remediation of arsenic-contaminated sites. One such method is the conversion of arsenic to insoluble forms by combining with hydroxide, carbonate or sulfide salts (Kim & Nriagu, 2000). Co-precipitation of arsenate with the floc formed from ferric and aluminum salts removes arsenate with 50% efficiency at neutral pH. This technique, however, requires the use of multiple chemicals thus adding to the cost of treatment and environmental pollution (Drewniak & Sklodowska, 2012). Another method uses adsorption where arsenic ions in solutions bind onto polyelectrolyte (adsorbents) through electrostatic interactions, van der Waals forces, covalent bonding *etc.* (Egal, *et al.*2010). The technique uses materials that have a strong affinity for soluble arsenic. such as aluminum oxide, activated carbon andiron-based media (Shih, 2005). But factors such as pH, oxidation state and the presence of competing ions affect the removal of arsenic through this method (Smedley & Kinniburgh, 2002). Ion exchange is another process by which arsenic anions are exchanged for chloride or other anions. It has been suggested as the best technology for arsenic removal from drinking water but the generation of toxic chemical reagents and their release into the environment reduces its efficiency (Stolz, *et al*. 2010).

Microbial transformation of arsenic

While various physico-chemical processes have been developed for treating arsenic pollutants; these approaches are often expensive and non-specific. Moreover, the chemicals used during physical and chemical processes are toxic themselves. As a result, there has been an increased interest in eco-friendly treatments like bioremediation(Stolz, *et al.* 2010). Overtime, microbes have devised certain strategies for surviving in the presence of arsenic including oxidation, reduction, methylation and bioaccumulation (Macy, *et al.* 2000; Silver, *et al.*2005).The redox transformations of arsenic species by micro-organisms could be utilized as a means of detoxification as well as to gain energy for growth. T*he mechanisms adopted for survival can be broadly classified as resistance mechanism and metabolic mechanisms. Employing these strategies for arsenic remediation is an attractive option as they are selective, cheaper, more efficient and comparatively harmless.*

Transport inside the cell

Arsenic enters inside the cells with the help of three different transporter proteins viz. Pst, Pit and GlpF (Figure 1). Arsenate is taken up by the phosphate inorganic transport protein (Pit) or phosphate specific transport protein (Pst) (Silver & Phung, 2005) due to its structural similarity to phosphate. Pit is a trans-membrane protein and the uptake of ions is coupled with ATP hydrolysis. It is a reversible process and allows for both influx and efflux of divalent ions. Pst is a periplasmic unidirectional transporter protein and consists of four subunits, a periplasmic ion-binding protein, two cytoplasmic associated proteins and a dimeric ATP binding protein (Wanner, 1993; Veen, 1997). It has been reported that Pst transports arsenate less efficiently than Pit. Microbes present near highly arsenic polluted sites and possessing only the Pst transporter are reported to have reduced

arsenate uptake. Arsenite is present as $As(OH)_3$ at neutral pH and enters the cell via GlpF. GlpF belongs to the aquaporin superfamily of transporters which promote the transport of molecules such as glycerol and urea across the cell membrane. In microbes with disrupted gene encoding GlpF, arsenic transport persists but with reduced efficiency. [Figure 1]

Resistance mechanisms

Micro-organisms show resistance to arsenic either by pumping arsenic out or by accumulating ions inside the cell and cell surface thus restricting its ability to interfere with metabolic functions. The uptake of ions can take place via active or passive mechanisms. The passive mechanisms involve adsorption of arsenic ions on the surface of the cell either due to attractive forces, exchange with other ions present on the cell wall or by surface polysaccharides through their carboxyl groups. Passive binding is rapid, reversible and independent of metabolism; it can occur within dead or living cell. The active mechanisms, on the other hand, utilize energy from ATP hydrolysis and are comparatively slow. Cyanobacterial species *M. aeruginosa* and *O. tenuisa* have been shown to accumulate As (V) in the cytoplasm and on the cell wall respectively (Huang, *et al.* 2014). Similarly, indigenous bacteria isolated from arsenic contaminated sites have been shown to possess biotransformation and bioaccumulation activity (Xie, *et al.* 2013; Banerjee, *et al.* 2013).

The efflux system for arsenic in prokaryotes is encoded by genes of *ars* operon present either on the chromosome or plasmid. The gene clusters in micro-organisms differ vastly in composition and arrangement with the core *ars* operon being constituted by three genes, *ars*RBC. The *arsR* gene codes for a trans-acting regulatory protein which controls the transcription of the *ars* operon. Binding of arsenic to the repressor protein induces conformational changes in *ars*R protein resulting in its dissociation from the operator and thus initiating transcription. The mechanism governing the allosteric regulation of *ars*R by transition metals was studied using *S. aureus* CzrA, a member of the *arsR* family (Campanello, *et al.* 2013). *Ars*C is involved in the conversion of pentavalent arsenate to arsenite and *ars*B is responsible for the transfer of arsenic across the membrane. Three families of bacterial arsenate reductase (*Ars*C) are known based on the protein structures and location of cysteine residues viz. the glutaredoxin/glutathione (Grx/GSH) clade (*E. coli* plasmid R773 as prototype), the thioredoxin (Trx) clade (*S. aureus* plasmid pI258 as prototype) and the mycothiol (MSH)/mycoredoxin (Mrx) clade (Actinobacteria as prototype) (Ordonez, *et al.* 2009).

Apart from these genes, several micro-organisms are reported to possess additional genes involved in arsenic resistance. The *ars*A gene in the *ars*RDABC operon encodes for an ATPase protein involved in arsenic extrusion which increases arsenite resistance levels significantly when coupled with *ars*B gene product. *Ars*D, a secondary regulator , acts as a chaperone protein that transfers arsenite to the *ars*A subunit of the *ars*AB complex activating it (Lin, *et al.* 2007). Another four-gene operon encoding a putative membrane permease (*ars*P) and a membrane transporter (ACR3) with homology to *ars*B was identified in thermophilic *Campylobacter* strain (Nakajima, *et al.* 2013). A recent study with *C. jejuni* demonstrated that *ars*P, in fact, functions as an efflux protein for removal of organic arsenic (Shen, *et al.* 2014). The transporter ACR3 was one of the three genes identified in *S. cerevisiae* to be involved in arsenic resistance (Bobrowicz, *et al.* 1997). The other two genes are ACR1 and ACR2 (now known as ARR1 and ARR2 respectively) where ACR1 is suspected to act as a transcriptional regulator. It is noteworthy that recent studies have found ACR3 to be widespread and it is fast becoming the predominant transporter in place of *ars*B.

An arsenate-resistant bacteria of *Ochrobactrum sp.* was also shown to contain two arsenate reductase genes *ars*C1 and *ars*C2 originating from different families of arsC; *ars*H and mfs (major facilitator transporters) (Yang, *et al.* 2013). Although no functional role has been assigned to *ars*H it seems to be important for arsenic resistance. Some of the recently discovered genes include *ars*K gene from B. subtilis (Sato & Kobayashi, 1998), *ars*T gene encoding a putative Trx reductase and *ars*O gene encoding a putative monooxygenase in an *ars*RBO operon from *Streptomyces sp.* strain (Wang, *et al.* 2006). The discovery of new genes in *ars* operon in different micro-organisms means that there are still several unreported genes involved in arsenic resistance. Table I presents a summary of the microbial genes involved in arsenic resistance along with the proteins encoded and their functions.[Table 1 here]

Metabolism of arsenic

Methylation of arsenic

Methylation of arsenic is a well-established phenomenon in which a methyl group or groups are attached to arsenic resulting in increased compound volatility. The *ars*M gene responsible for methylationthat was characterized in*Rhodopseudomonas palustris* belongs to the methyltransferase family. It encodes for the cytoplasmic enzyme methyltransferase, the key player in the cascade of reactions involving the conversion of As (V) to As (III) using reduced glutathione(Qin, *et al.* 2006). Arsenic methyltransferase generates mono- and di-methylated, pentavalent and trivalent arsenic

compounds sequentially (Figure 2).An alternative mechanism suggests that the trivalent methylated arsenic species are converted to pentavalent species as the later is less toxic (Stolz, *et al.* 2010). The toxicity of methylated arsenic compounds depends on their methylation status and valence state. Pentavalent species like monomethylarsonic acid[$CH_3AsO(OH)_2$], dimethylarsinic acid[$(CH_3)_2AsOOH$] and trimethylarsine oxide[$(CH_3)_3AsO$] are less toxic than arsenate while trivalent species like monomethylarsine [(CH_3AsH_2)], dimethylarsine[$(CH_3)_2AsH$] and trimethylarsine[$(CH_3)_3As$] are extremely toxic (Chen, *et al.* 2013). The *ars*M gene is also found in eukaryotes and its product results in the production of non-volatile compounds such as monomethylarsonate [MMA(V)], methylarsonite [MMA(III)], dimethylarsinate [DMA(V)], dimethylarsinite [DMA(III)]as well as several volatile arsines, including monomethylarsine, dimethylarsine and trimethylarsine oxide [TMAO].A consortium of *Dunaliella salina* and *Bacillus solisalsi*having high arsenic removal abilitywas shown to possess pathways for arsenic transformation including oxidation, reduction and methylation along with efflux pumps(Wang, *et al.* 2013).

Arsenite Oxidation

A number of bacterial species have been discovered to oxidize arsenic, including *Acinetobacter junni*, *Marinobacter sp.,Stenotrophomonas sp.*, *Geobacillus stearothermophilus* and *Alcaligenes faecalis* (Chang, *et al* 2011; Bahar, *et al* 2012; Majundar, *et al.* 2013).Oxidation of As(III) can be simply for detoxification as seen in many heterotrophs or for the purpose of energy production as in chemoautotrophs. Chemoautotrophs use arsenite as an electron donor to produce energy required for growth in aerobic conditions (Santini, *et al.* 2000, 2002). As(III) oxidation coupled to nitrate has also been reported to occur in anaerobic environments(Oremland, *et al.* 2002).

The *aio*operon (previously known as *aso/aox/aro* operon) encodes for the respiratory arsenate oxidase (*aio)* which converts arsenite to the less toxic arsenate (Figure 2). This periplasmic enzyme has two subunits-a substrate binding catalytic subunit (*aio*B) and a beta subunit (*aio*A) . The former contains an Fe-S cluster and a molybdenum atom joined to the four sulfur ligands via coordination bonds while the smaller *aio*A subunit contains one Rieske Fe-S cluster and a TAT (Twin Arginine Translocation) signal sequence. The signal sequence is useful in guiding arsenite oxidase to the periplasm. Recently a novel arsenite oxidase gene cluster has been identified in *Alphaproteobacterium Rhizobiumsp*. The hetedoimeric enzyme consists of a large subunit with *bis*-molybdopterin guanine dinucleotide and a 3Fe-4S cluster while the other subunit contains a Rieske 2Fe-2S cluster (Warelow, *et al.*2013). A new arsenite oxidase clade, *Arx*A has also been identified in a haloalkaliphilic bacterium belonging to *Alkalilimnicola sp.* (Zargar, *et al.* 2012).

The mechanism governing arsenite oxidation is still under speculation. A regulatory process involving an arsenic-sensitive *aio*S (a histidine kinase) and an *aio*R regulator was first identified in *Agrobacterium tumefaciens* (Kashyap, *et al.* 2006). Furthermore, a mechanism using *H. arsenicoxydans* was proposed(Koechler, *et al.* 2010) which stated that *aio*S autophosphorylates in the presence of As(III) followed by transfer of the phosphate to *aio*R. Downstream genes *aio*C and *aio*D encode cytochrome C and an enzyme for synthesis of molybdopterin respectively (Cai, *et al.* 2009). A recent study showed that a periplasmic arsenite binding protein encoded by *aio*X is upregulated in the presence of As(III) (Liu, *et al.* 2011). It is speculated to function by either transferring arsenic to *aio*S or interacting with *aio*S to generate a signal transduction. There is a need for more sophisticated systems that could further shed a light on the molecular regulation of arsenic oxidation pathway. [Figure 2 here]

Dissimilatory reduction of arsenic

Enzymes involved in the reduction of arsenic are either cytoplasmic or periplasmic in nature. Cytoplasmic arsenic reductase (*ars*C) used in arsenic detoxification by microbes has already been discussed in detail. The periplasmic arsenate reductase encoded by the *arr* operon (Figure 2) uses arsenate as the terminal electron acceptor resulting in the generation of energy. Both these reductases are expressed in a *Shewanellasp*. strain (*ars* operon under both aerobic and anaerobic conditions, whereas the *arr* operon is only expressed anaerobically) (Saltikov, *et al.* 2005).

The dissimilatory reduction of arsenic fromAs^{+5} to As^{+3} via the *arr* operon is accompanied by oxidation of organic or inorganic compounds such as acetate, lactate, butyrate, iron or sulphur (carried out by sulphur reducing bacteria) and synthesis of ATP. The resulting sulphides and alkalinity provide favourable conditions for arsenic precipitation. The *arr* operon encodes for a respiratory arsenate reductase (*arr*) whose components include a catalytic subunit *arr*A havinga single [4Fe-4S] cluster and an electron transfer protein *arr*B having four [4Fe-4S] clusters. Additional *arr* genes have been identified in *A. ehrlichii* which includes another *arr*B subunit (*arr*B2), a membrane-anchoring subunit (*arr*C) and a chaperone (*arr*D) (Stolz, *et al.* 2010).

In a study *arr* genes from, *Alkaliphilus oremlandii* and *Shewanella* sp. strain were also found to be capable of oxidizing arsenite (Stolz, *et*

al. 2010). This can be explained by the fact that both the respiratory arsenate reducatse (*arr*) and oxidase (*aio*) belong to the DMSO superfamily of molybdenum-containing oxidoreductases although they are quite different from each other (Castelle, *et al.* 2013). However, physiologically the enzyme can work only as an oxidase or reductase. In another study a haloalkaliphilic bacterium *Alkalilimnicola ehrlichii* was found to possess two operons encoding respiratory reductase that can act both as oxidase and reductase depending upon the electron potentials of the molybdenum and Fe–S clusters & other constituents of the electron transport chain (Richey, *et al.* 2009). Table II lists all the genes employed in the metabolism of arsenic by microbes along with the proteins encoded and their functions. [Table II here]

Arsenic-resistant microbes as biosensors

The systems developed by microorganisms for detoxification and removal of arsenic can also be employed for detecting its presence in the soil. In fact, arsenic biosensors are emerging as a safe and cheaper alternative to traditional detection methods using chemicals and expensive laboratory equipment. Early biosensors mostly employed luminescent enzymes which converted generated an electrical or optical signal proportionate to the target concentration. However, there are limitations of making enzyme-based biosensors; mostly the cost involved in enzyme purification and the need to give cofactors to generate a measurable signal. Alternatively, pH based arsenic detection system are being used as it allows for simpler detection via change in color (Aleksic, *et al.*2007; De Mora, *et al.* 2011). A chromogenic system using *B. subtilis* endospores has also been developed (Joshia, *et al.* 2010). It provides the advantage of accessibility and can be easily stored as it is in dried form. However, a thorough investigation of the specificity of the biosensor, its media and storage requirements, temperatures, etc. need to be done to ensure their successful application.

Nowadays whole cell biosensors are also being viewed an efficient method to detect presence of toxic ionsas they are stable and have low cost of production. Many of these only use the necessary components that allow the recognition of the toxic metal and begin transcription. In this context, arsenic resistant genes (such as *ars*R and *ars*D) along with a reporter gene can beinserted in ahost plasmidin *E. coli* wherein upon activation the reporter gene produces signal proportional to the amount of arsenic detected. In a study to develop strong arsenic biosensors, *ars*R was placed in trans under control of a T7 promoter while GFP expression was under the control of the *ars*R promoter (Tani, *et al.* 2009). Recently, a group found that a stronger constitutive *ars*R production would decrease the arsenite-dependent GFP signal (Merulla, *et al.* 2013). Hence, arsenic biosensors with higher expression levels and sensitivities are required for improving field-assays.

Genetic Engineering

Although highly diverse and specialized micro-organisms have been reported for the detoxification of arsenic; remediation using native microbes involves several challenges. These include low degradation rates resulting in the accumulation of the pollutant, presence of diverse or mixed contamination (Shukla, *et al.* 2010) and adverse biotic (antagonism, competition, predation) & abiotic stresses (temperature, pH, moisture) (Singh, *et al.* 2011) on site. These factors hamper the survival of native micro-organisms significantly. One strategy to increase the population of desirable micro-organisms is bioaugmentation. This can be achieved either by adding the native microbes possessing the desired genes or micro-organisms that have been genetically modified (GMO) to the soil. The use of GMOs is particularly advantageous since they possess desirable properties like increased enzyme specificity or affinity, enhanced metabolic activity and the use of heterologous strains promotes the survival of micro-organisms under stressful conditions (Ferrer, *et al.* 2005).

Over-expression of regulatory genes like *ars*R or *arr*R in bacteria offers immense potential for bioremediation due to their selectivity toward arsenic and their ability to control the transcription of the *ars/aio* operon. In a study, engineered bacterial cells over-expressing *ars*R under T7 promoter completely removed 50 ppb (parts per billion) of arsenite from contaminated water; accumulating 13-60 folds of it than the wild type (Kostal, *et al.* 2004). The over-expressed regulatory protein product of *ars*R enhances transcription of *ars* operon leading to increased reduction and extrusion of arsenic. Similarly, other genes could be targeted for over-expression especially the ones involved in the transformation (*ars*C) and efflux of arsenic (*ars*B and ACR3). Incidentally, the protein products of *ars*A and *ars*C are produced in higher amounts as compared to that of *ars*B due to differential gene expression. The expression of *ars*B can be increased as was done by constructing a chimeric *ars*A-*ars*BATPase which could efficiently exclude arsenite from cells (Dou, *et al.* 1992). Thus over-expression of the genes involved in arsenic resistance could enhance removal of arsenic from contaminated sites by micro-organisms considerably.

Several microorganisms have also been engineered to express *ars*M gene which enables removal of arsenic from soil by converting it to volatile species. A study with two genetically engineered strains of *Sphingomonas desiccabilis* and *Bacillus idriensis* showed 10 times increase in methylated arsenic gas as compared to the wild type strains (Liu, *et al.* 2010). Also recently, *Pseudomonas*

putida engineered to express gene for arsenic methylation showed 5 folds increase in arsenic resistance enabling their survival in arsenic-contaminated soils (Chen, *et al.* 2013). In another study *ars*M from *Rhodopseudomonas palustris* was expressed in an *E.coli* strain (Qin, *et al.* 2006) which transformed arsenic in the medium to dimethylarsenate [DMA(V)], trimethylarsenate oxide [TMAO] and trimethylarsine [TMA(III)]. The pentavalent products were hundred-thousand folds less toxic than arsenic and the volatility of TMA(III) reduced arsenic toxicity in the medium. Hence, the use of micro-organisms expressing *ars*M could prove as an efficient and inexpensive method for bioremediation of polluted sites along with enhancing their survival at such sites.

Genetic engineering techniques can also be employed in order to improve the sequestration of metal ions by peptides (Kang, *et al.* 2007). Metallothioneins (MTs) are low molecular weight peptides present mostly in animals and eukaryotes. The only prokaryotic MT discovered yet is in few *Synechococcus* strains encoded by the *smt*Agene (Ji & Silver, 1995). These peptides are rich in cysteine and form complexes with metallic ions through thiol residues (Figure II). They also act as antioxidants by reducing the oxidative stress caused by presence of ions. Recently, recombinant *E. coli* cells were made to express human metallothionein to increase bioaccumulation of arsenic (Su, *et al.* 2009). The cells showed 3 folds increase in the amount of intracellular arsenic from 76.3 to 316.9 µg/g dry cells compared to control. These findings suggest that genetically enhanced bacterial cells could be employed as efficient biosorbents for treating arsenic-contaminated water. Phtochelatins (PCs) are another class of metal-binding peptides that are present in plants. They are synthesized via phytochelatin synthase (PCS) by transfer of γ- Glu-Cys from glutathione (GSH) to another GSH (Singh, *et al*. 2010). In a study, phytochelatin synthesizing gene PC synthase from *Arabidopsis thaliana* (*AtPCS*) was expressed in *E. coli* resulting in 20-50 fold increase in cellular uptake of metal ions (Sauge-Merle, *et al.* 2003). Phytochelatins and metallothioneins containing multiple functional domains have also been constructed synthetically to enhance bioaccumulation of heavy metals. A multi-domain polypeptide expressed in *E. coli* had a removal capacity of 65-folds relative to the non-recombinant cells (Mauro & Pazirandeh 2000). Another study demonstrated that varying the length of phytochelatins could also increase the quantity of accumulated heavy metals (Bae, *et al.* 2000). The use of synthethic peptides is thus seen as an emerging tool for bioaccumulation of arsenic efficiently.

Another method for increasing arsenic accumulation inside the cells is by combining the over-expression of arsenic chelators and transporters like MTs, GlpF or Pit/Pst and deletion of efflux systems like *ars*AB or ACR3. In a study, the overexpression of a metallothioneinfrom *Fucus vesiculosus* (*fMT)* in *E.coli* increased the accumulation of arsenic species about 26-30 folds and the co-expression of the arsenite transporter GlpF completely removed arsenic (35 ppb) from the medium (Singh, *et al.* 2008).However, it is to be understood that several problems can occur due to over-expression of MTs in the cytoplasm such as metal-induced cellular toxicity, disturbance of intracellular redox environment, reduced metal uptake, *etc*. This can be overcome by either expressing the MTs on the surface or coupling their cytoplasmic expression with efficient metal ion transporters to extrude arsenic out. Thus, genetic engineering tools could be very useful in manipulating genes involved in arsenic resistance and metabolism to detoxify arsenic-contaminated sites by either enhancing its accumulation inside the cells or its removal from the medium.

Conclusion

With environmental pollution at its peak, suitable measures to combat it are required. Different methodologies for tackling arsenic pollutants can be employed butthe use of genetically engineered bacteria is most efficient as it bypasses nearly all limitations of physical and chemical methods and is a step-up from the use of native microorganisms alone. However, majority of the studies related to bioremediation of heavy metals have been conducted under the laboratory conditions. It is quite difficult to study the decontamination of the polluted sites in natural environments because of various factors in detoxification process. Before releasing a Genetically modified Organism (GMO) into the environment, the researchers should emphasize upon the ethical responsibilities to be considered. The stability of GMOs and the horizontal transfer of DNA are crucial issues regarding the potential impact of their release into the field for bioremediation Important parameters in this context are survival, number, activity, and dispersion of released GMOs. Ideally such methods should be applicable in the field and in real time, and should be simple and inexpensive while also being accurate. Therefore, future researches would be more focused on identifying the factors that enhance the *in situ* bioremediation by engineered bacteria. In addition to the GMO itself, it is useful to track the recombinant DNA with which the GMO has been engineered like suppression or overexpression of certain genes (*ars*M), so as to monitor potential loss or gain of these genes and their possible horizontal transfer to other microorganisms.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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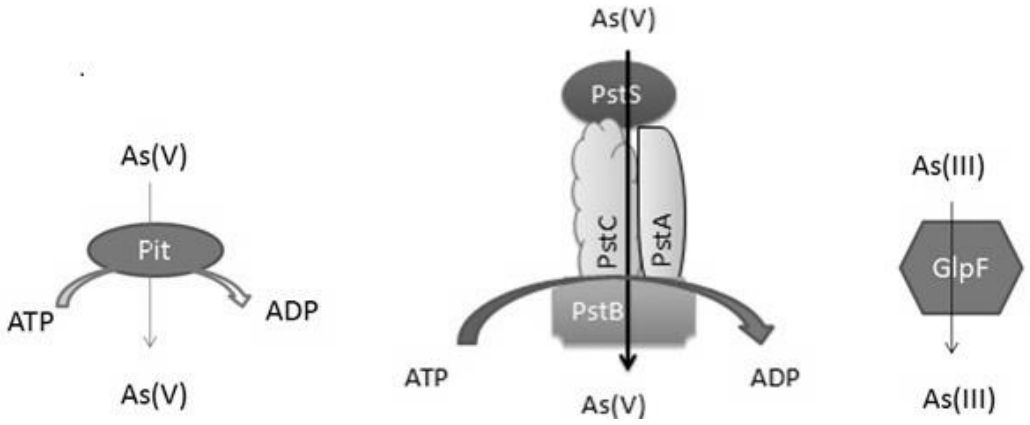

Figure 1 Uptake mechanisms for arsenic: The Phosphate inorganic transport (Pit) or the Phosphate specific transport (Pst) system is used for transporting As (V) into the cell coupled with ATP hydrolysis. The Pst system consist of an ATPase PstB, a transport channel PstA-PstC and a high-affinity phosphate binding protein PstS. The glycerol transporter (GlpF in *E.coli*) is also used to transport neutral As (III) into the cell (Chan and Torriani 1996)

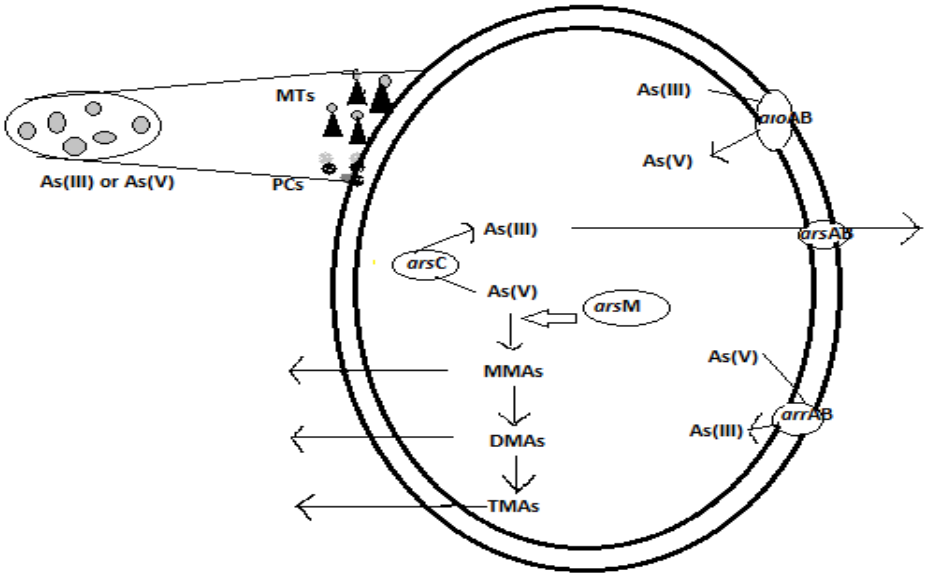

Figure2 Bioaccumulation and metabolism of Arsenic: Arsenic can be complexed by peptides rich in cysteine residues such as metallothioneins (MTs) or phytochelatins (PCs) expressed on the cell surface. Inside the cell, arsenate may be reduced to arsenite by arsenate reductase (*ars*C) and extruded out via the efflux pump (*ars*AB) encoded by *ars* operon.Inorganic arsenate may also be transformed into volatile organic species following a methylation cascade mediated by *ars*M. Furthermore, arsenite may be oxidized into less toxic arsenate via the periplasmic arsenate oxidase (*aio*AB) encoded by *aio* operon and arsenate may be reduced to arsenite via the periplasmic arsenate reductase (*arr*AB) encoded by *arr* operon. These transformations help in arsenic detoxification as well as in energy production.

Table I: Genes involved in resistance mechanisms: The genes involved in arsenic resistance mechanism via reduction are shown with a brief description of proteins encoded by them and their respective functions.

REDUCTION		
Genes	***Proteins encoded***	***Function***
*Ars*R	repressor protein	controls transcription of *ars* operon
*Ars*A	ATPase protein	Part of the *ars*AB efflux pump
*Ars*B	transmembrane efflux protein	extrusion of arsenite
*Ars*C	reductase protein	reduces arsenate to arsenite
*Ars*D	chaperone protein	transfers arsenite to *ars*A
*Ars*P	membrane efflux protein	extrusion of organic arsenic
*Ars*H	putative oxidoreducatse	function unknown

Table II: Genes involved in metabolic mechanisms: The genes involved in arsenic metabolism mechanisms viz. oxidation, dissimilatory reduction and methylation are shown with a brief description of proteins encoded by them and their respective functions.

OXIDATION		
Genes	***Protein encoded***	***Function***
*Aio*AB	periplasmic respiratory arsenate oxidase	oxidizes arsenite to arsenate
*Aio*S	hisidne kinase	phosphorylation
*Aio*R	regulatory protein	regulates transcription of *aio* opeon
*Aio*X	arsenic binding protein	involved in signal transduction during oxidation of arsenite
DISSIMILATORY REDUCTION		
*Arr*AB	periplasmic respiratory arsenate reductase (*Arr*)	reduces arsenate to arsenite coupled with generation of energy
METHYLATION		

*Ars*M	cytoplasmic methyltransferase	methylation of arsenic (arsenite or arsenate) to mono-, di- and tri- methylated arsenic compounds