

## **Arsenic resistant microorganisms and their possible role in bioremediation**

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### **Abstract**

Arsenic, a metalloid which's ubiquitous in nature and widely distributed in environment. It's level in the environment has become a global concern due to its toxicity and adverse effects on human health; even at low concentrations, it is highly toxic and classified as a carcinogen. Although it often exists in soils and plants from natural sources such as igneous and sedimentary rocks, the largest anthropogenic source of arsenic is the use of chromated copper arsenate (CCA)-treated wood along with mining, smelting, refining, industrial processes etc. The arsenic menace in North-Indian belt especially Ganga basin comprising U.P. , Bihar, Jharkhand, West Bengal is due to fertilisers and leaches more coal ash, mining activities. It is not very clear that currently how grave is the contamination situation. This review provides a complete understanding of arsenic in the multimedia environment from source to removal. It is very important to raise awareness of the toxicity and the potential environmental impacts of arsenic exposure via various routes. Various microbes carry plasmid with them carrying Arsenic resistant genes who then impart chemical reactions in conversion of highly toxic form of arsenic to lesser toxic form. With emerging technologies bioremediation is a potent tool against Arsenic Contamination in environment. Methods like Phytoremediation and Nanobioremediation and advancement in Metagenomic, has provided scientific upper hand study of these microbes playing a critical role in arsenic biogeochemical cycling, so it is important to identify and characterise the microbial community which may enhance the mobility of arsenic in these soils. This knowledge can then be used to mitigate contamination levels along with designing appropriate clean up methods to alleviate the problem and prevent acute and chronic exposure to arsenic and provide sustainability for upcoming generation.

**Keywords:** Arsenic-resistant microbes, Genes, Arsenic-contamination, Phytoremediation, Nanobioremediation, toxicity, biogeochemical cycle.

### **INTRODUCTION**

Poison of Kings' Arsenic(As) is a metalloid with atomic number 33 and appears in Group 15 of periodic table below phosphorus.(Espinoza et al.1995, Saper et al. 2004, Tallman 2007) Albertus Magnus is credited with isolation of arsenic in elemental form from arsenic trisulphide in 1250. The primary oxidation states of inorganic Arsenic are Arsenide(As<sup>-2</sup>) Arsenite(As<sup>+3</sup>) Arsenate (As<sup>+5</sup>) forming trioxide (As<sub>2</sub>O<sub>3</sub>) pentaoxide (As<sub>2</sub>O<sub>5</sub>). Microbial process convert Arsenate to Arsenite which can then be methylated into various inorganic forms. Arsenate and Arsenite are both toxic in nature with Arsenite being more toxic than arsenate causing various types of cellular damage to biological system because there is a structural analogy between inorganic phosphate and Arsenic and hence it can be introduced in the cell exactly in the same system as phosphate is transported through cell membrane disrupting those phosphorylation and inhibit the synthesis of adenosine triphosphate. When people are exposed to arsenic concentration above permissible levels of 0.05 ppm it can cause various toxic effects.Symptoms include skin itching, skin cancer, lethargy, weakness, loss of energy appetite,limit working capacity, anorexia, Respiratory disorders, gastrointestinal disorders, enlarged liver, pain in abdomen etc.(Emsley 2000)

As Arsenic has high magnitude of solubility in water it's removal from Contaminated water is very difficult. There are conventional methods of removing Arsenic to less toxic form like coagulation, membrane filtration, Reverse osmosis, adsorption, filtration etc of Arsenic form As<sup>III</sup> to As<sup>V</sup>. Microorganisms can so carry out this conversion by using arsenic as a source of energy in their metabolic pathways. They have developed methods like Arsenic methylation and Oxidation from more toxic to less toxic form. Many microorganisms like Agrobacterium,Aerobacter, Acinetobacter, Deinococcus, Acidithiobacillus, Desulphotobacterium, Bacillus, Pseudomonas etc are used for carrying out these process of bioremediation.

## **Arsenic Uptake and Extrusion by Bacteria**

The similarity of molecular arsenic to substrates of membrane transporter proteins in prokaryotes permeates their uptake. In aqueous solution at physiological pH AsIII, exists as As(OH)<sub>3</sub> which is kind of similar to glycerol. Whereas, AsV acts as a structural analog of phosphate and is taken up by the membrane phosphate transporters. (Rahman A, Nahar N, Nawani NN, Jass J, Desale P, et al.) GlpF is an aquaglyceroprotein which involves in transport of AsIII across the cell membrane of which homologues of GlpF have been found in several bacteria. Pst and Pit are the phosphate transporter proteins which facilitate the uptake of AsV (Kruger MC, Bertin PN, Heipieper HJ, Arsène-Ploetze F 2013) Pst is specific transport system for phosphate whereas Pit is general transport system. The structural similarity of arsenate with phosphate leads to its easily uptake through Pit transport system (Bertin PN, Heinrich-Salmeron A, Pelletier E, Goulhen-Chollet F, Arsène- Ploetze F, et al. 2011) Pst transport AsV less efficiently hence the microbial community which are exposed to high level of arsenate only express Pst to reduce the AsV uptake (Kruger MC, Bertin PN, Heipieper HJ, Arsène-Ploetze F) In bacteria, arsenic extrusion is carried by two mechanisms. One is carrier-mediated efflux via an arsenite carrier protein, and the other by an arsenite-translocating ATPase (Rosen BP, Villadangos AF, Ordóñez E, Pedre B, Messens J, Gil JA, et al.) The bacteria having three gene operon arsRBC can extrude arsenite by ArsB alone whereas the five gene operon, arsRDABC, can extrude arsenite by the ArsAB pump. An ArsAB complex from when ArsA is co-expressed with ArsB which is obligatory coupled to ATP. E. coli synthesizing ArsA from plasmid R773 arsRDABC operon are more resistant to arsenite because the ArsAB ATPases can extrude arsenite more efficiently than ArsB alone (Rosen BP)

### **2.1. Genes Involved in Arsenic Transformation**

Arsenic tolerance in bacteria is mediated by the gene products of the ars operon. In E. coli genome arsRBC a three gene operon operates whereas in S. aureus arsRDABC a five gene operon is there (Rosen BP) The arsC encodes for an arsenate reductase, and arsB and arsA encodes for an arsenite efflux pump, i.e., ArsAB ATPase, and arsR encodes for a transcriptional regulator (Arsène-Ploetze F, Koechler S, Marchal M, Coppée JY, Chandler M, et al) ArsD acts as arsenic chaperone that transfers arsenite from the glutathione-bound complexes to ArsA subunit of the ArsAB complex and activates them Both operons may be present in one strain, as e.g. in T. arsenitoxidans 3As. The arr operon including the arrA and arrB genes is involved in arsenate reduction in the periplasm of the bacteria. The product of arsM gene is involved in the methylation of arsenite (Kruger MC, Bertin PN, Heipieper HJ, Arsène- Ploetze F) The four contiguous genes encoding arsenite oxidase from Centibacterium arsenoxidans, and named as aoxABCD. In A. faecalis, aoxA gene product is homologous to the aoxB gene product; similarly aoxB gene product is identical to the aoxA gene product. (Silver S, Phung LT) In

H. arsenoxidans, aoxS gene product is a sensor kinase and aoxR gene product acts as regulator for the aox operon, both are involve in quorum sensing (Koechler S, Cleiss- Arnold J, Proux C, Sismeiro O, Dillies Metal)

### **3. Arsenite Oxidation in Bacteria**

AsV the more toxic form of bacteria is oxidised into less toxic form AsIII. The arsenite oxidation is done in the periplasm of the bacteria AoxR, a regulator protein is activated by AoxS sensor kinase in presence of Arsenite. This AoxR control the expression of the aox operon in association with the RpoN. This RpoN is essential for the initiation of the arsenite oxidase transcription in A. tumefaciens.

### **4. Arsenate Reduction in Bacteria**

Cytoplasmic and periplasmic arsenate reduction are 2 types of Arsenate Reduction Pathways in Prokaryotes. When AsV is taken up by the pst and pit membrane transporters, arsC, an arsenate reductase enzyme involve in the reduction of the AsV to AsIII and leads to its extrusion through the arsAB pump. Whereas ArrA periplasmic respiratory arsenate reductase enzyme Homologues of the arsC gene is there in both the plasmids and chromosomes. ArsC utilizes glutaredoxins which acts as source of reducing potential in cytoplasmic reduction. In this reaction arsenate first binds

to the anion site in the ArsC forming arsenate thioester intermediary with active site Cys12 then it is reduced in 2 steps by glutaredoxin and glutathione, producing the Cystic2-S-As(III) intermediary, which hydrolyzes to release arsenite (Rosen BP). The reduced As(III) is extruded from the cell or sequestered in the intracellular compartments, either as free arsenite or as conjugates with glutathione or other thiols as such.

### 5. Arsenite Methylation

Arsenic methylation is understood in fungi and eukaryotes but less in bacteria.

The methylation pathway based on *Scopulariopsis brevicaulis* was first proposed by Challenger (1945) where arsenate reduction is followed by oxidative addition of methyl groups generating gaseous arsine like DMA, MMA, TMAO and TMA (Dombrowski et al. 2005). Glutathione participates in reduction in which is the source

of methyl groups is S-adenosinemethionine (SAM), and some anaerobic bacteria use methylcobalamin as electron donors (Páez-Espino et al. 2009). In 1971, McBride and Wolfe reported volatilisation of arsenicals by *Methanobacterium bryantii*. ArsM, a methyltransferase has been identified in over 100 Bacteria and 16 Archaea, and was characterized in *Rhodopseudomonas palustris* (Qin et al. 2006). Challenger (1945) also explained the cleavage of As-C to produce MMA in *Scopulariopsis brevicaulis* and *Penicillium notatum*. Demethylation of monomethyl and dimethyl-arsenic compounds has also been reported in *Alcaligenes*, *Pseudomonas*, and *Mycobacterium* species (Bentley and Chasteen 2002). A *Pseudomonas* bacterium uses DMA as carbon source releasing arsenic in the process (Maki et al. 2004).

### CONCLUSION

To quote an Indian proverb, *"Only when the Last Tree has died and the last*

**River has been poisoned and the last fish has been caught, we will realise that we cannot Eat Money."**

Arsenic is one of the most prevalent toxic chemicals found in various environmental media. This study reviews the current available literature on the occurrence, behavior, cycling, toxicity, and removal technologies of arsenic present in environmental media and is compiled to raise awareness of the toxicity and the potential environmental impacts of arsenic exposure via various routes. By reviewing the literature for arsenic toxicity and ways of remediation, following conclusion can be framed. Unlike organic pollutants, arsenic in nature cannot be biodegraded and thereby persists in environment for long time. Thus, the complete removal of arsenic from soil is difficult, but arsenic can be transformed from more toxic form to less toxic form. Microbes have developed several mechanisms of arsenic transformation like oxidation, reduction and methylation. Arsenite oxidation studies are significant because of extreme arsenite toxicity as compared to arsenate; hence detoxifying the arsenite polluted environment becomes essential. Behavior and mobility of arsenic species, both organic and inorganic, in the environment are influenced by a number of factors including, but not limited to pH, the presence of organic matter and complexing agents etc. More research efforts are needed to examine on the interaction of these factors under relevant environmental conditions to examine arsenic exposure and its related effects via ingestion and inhalation pathways, especially at low levels of arsenic. One of the major aspects of arsenic toxicity is the process of bioaccumulation of arsenic in plants. Since different plants have different genetic systems, there is a large variation of arsenic uptake by plants. The bioaccumulation and uptake of arsenic, along with other nutrients, in plants is very little known. Further studies must be devoted in this direction to understand the potential effects on human health and the effective removal mechanism in water and soil at both biochemical and molecular levels. Long-term field and economical studies of arsenic remediation are warranted to evaluate the efficiency and suitability of removal techniques for arsenic-contaminated water and soil.

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