Microbial Strategies for the Bioremediation of Arsenic in Polluted Water

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Objectives

The aim of this review is to know bacterial strategies against high concentrations of arsenic in water and which of these mechanisms are useful for bioremediation. Also, it compares natural and genetically modified bacteria for their application in bioremediation.

Background

Arsenic contamination is a global problem. According to WHO, arsenic is one of the **most dangerous** substances for public health. In addition, inorganic arsenic is considered carcinogen.

It is estimated that **150 million people are exposed** to this metalloid and the most affected regions are Bangladesh, West Bengal and Taiwan. In these zones the arsenic levels in groundwater are higher than WHO's recommendation, which has stablished a recommended limit of 10 µg/L (10 ppb) of arsenic in drinking water.

Arsenic as a Contaminant

Arsenic is found in more than 200 minerals and it reaches the water due to the erosion of rocks and several anthropogenic activities that have intensified arsenic contamination in water too.

In natural waters, inorganic arsenic is found as **arsenite** $\mathbf{As^{+3}}$ and **arsenate** $\mathbf{As^{+5}}$. The trivalent form is considered the most toxic.

Bacteria play an important role in arsenic biogeochemical cycle due to the oxidation and reduction reactions with inorganic arsenic.

Thus, arsenic is a causative of severe epidemiological, cytotoxic and genotoxic affectations.

Table 1. Summary of the most important arsenic affectations in humans [1]

Epidemiology	Cytotoxicity	Genotoxicity
Dermal disease	Problems in cell differentiation	Deletions
Cardiovascular disease	Apoptosis	Oxidative DNA damage
Skin and bladder cancer	Excessive cell proliferation	DNA strand breaks
	Autophagy	Chromosomal aberrations

Pathways of Arsenic Uptake and Resistance

As^{+3} and As^{+5} uptake

- By non-specific phosphate transporters GlpF and Pit or Pst respectively
- Arsenate uptake is increased in low phosphate concentrations

Oxidation

- By Aio/Arx arsenite oxidase
- As^{+3} oxidation to As^{+5}
- *Ochrobactrum tritici, Gallionella ferruginea* and *Leptothrix ochracea*

Reduction

- By Arr arsenate reductase
- As^{+5} reduction to As^{+3}
- *Geospirillum arsenophilus, Bacillus arsenicoselenatis* and *Crysiogenes arsenatis*

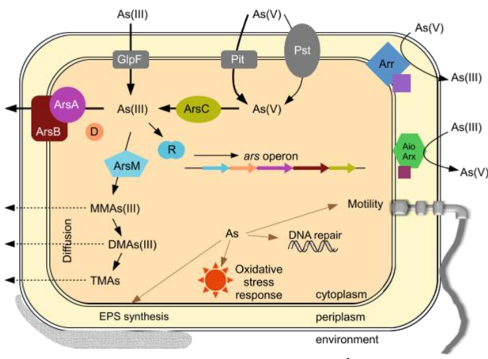

Figure 1. Bacterial interactions with arsenic [2]

ars operon

- ArsA: ATPase found together with ArsB
- ArsB: integral membrane permease that forms an efflux pump and extrudes arsenite outside the cell
- ArsC: cytosolic arsenate reductase
- ArsR: operon regulator
- *Escherichia coli, Herminiimonas arsenicoxydans, Thiomonas sp.* and *Corynebacterium glutamicum*

Methylation

- By ArsM methyltransferase
- Formation of volatile products
- *Penicillium sp., Aeromonas sp., Corynebacterium sp., E. coli* and *Methanobacterium bryantii*

Isolation of Arsenic Resistant Bacteria

Abbas et al. (2014). Samples of contaminated water from Pakistan

- Three bacterial species isolated: *Enterobacter* sp., *Klebsiella pneumoniae* 1 and *Klebsiella pneumoniae* 2
- All were arsenite oxidizing bacteria

Sarkar et al. (2013). Samples of contaminated water from West Bengal

- 64 arsenic-resistant bacteria were isolated, with a predominance of *Agrobacterium, Ochrobactrum* and *Achromobacter*
- Resistant to concentrations of 40 mM of As^{+3}

Paul et al. (2015). Samples of contaminated water from West Bengal

- Bacterial community formed by *Pseudomonas, Flavobacterium, Brevudimonas, Polaromonas, Rhodococcus, Methyloversatilis* and *Methylotenera*

Development of Genetically Modified Bacteria

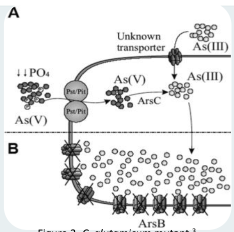

Figure 2. *C. glutamicum* mutant [3]

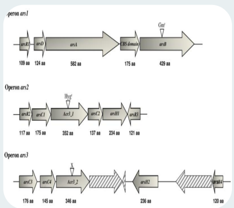

Figure 3. *ars* operons from *O. tritici* [5]

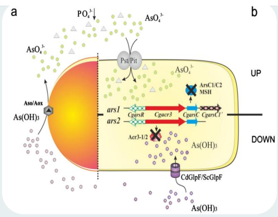

Figure 4. Consortium of *O. tritici* wild type and *C. glutamicum* mutant [4]

Corynebacterium glutamicum modification by Mateos *et al.* (2006)

- Increase of arsenate uptake → low phosphate concentrations (A)
- Mutation on the efflux pump gene → absence of ArsB permease
- Inability to extrude arsenite (B)
- Accumulation of arsenite in the cell → bioaccumulation
- Resistant to 60 mM of As^{+3}

Ochrobactrum tritici modification by Sousa *et al.* (2015)

- 6 different mutants were obtained
- Double mutant *arsB/Acr3_1* accumulates more arsenite than the others → useful as a bioaccumulator

Corynebacterium glutamicum modification by Villadangos *et al.* (2015)

- Mutation on the efflux pump gene → absence of Acr3 permease
- Mutation on *arsC* gene → no presence of arsenate reductase
- Consortium with two detoxification phases:
  1. *O. tritici* oxidizes As^{+3} to As^{+5} (a)
  2. As^{+5} and As^{+3} are accumulated by *C. glutamicum* double mutant (b)

Discussion

A large group of bacteria is **naturally resistant** to high concentrations of arsenic in water. Arsenite oxidizing bacteria are useful for bioremediation, since they transform As^{+3} to As^{+5} → biotransformation assembled to a conventional As^{+5} absorbance method.

It is also proposed a water treatment based in **bioreactors** with *H. arsenicoxydans* biofilms: the arsenic is sequestered due to the segregation of exopolymers.

On the other hand, **genetically modifications** in the *ars* operon allows the achievement of improved bacteria capable to optimize the assimilation, resistance and accumulation of inorganic arsenic → great bio-tool for bioremediation.

Genetically modified organisms are more effective as arsenic bioaccumulators and they can resist higher arsenic levels than autochthonous bacteria.

Future Prospects & Conclusions

Most studies are made in laboratories → need to prove them on an industrial scale to verify its economic viability.

Difficulty to implement modified microorganisms. Branco *et al.* proposed a method for the **immobilization** of *O. tritici* double mutant obtained in Sousa's *et al.* study → surface as arsenic biofilter for bioremediation processes

Biological treatment → promising option due to many advantages of bacteria:

- Specificity
- Economical
- Environmentally friendly
- Safe
- Reduction of waste

Relevant References

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