Bioremediation of irrigation water contaminated with arsenical pesticides

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Introduction

Nowadays pollution by heavy metals of soils and water continues being a serious problem for both environment and human health due to its potential inclusion into food networks and its bioaccumulation [1].

The arsenic is a metalloid and in nature we can find different arsenic species (As^0, As^{-3}, As^{+3} i As^{+5}). Arsenic forms have different toxicities [2] [Fig. 1].

Currently arsenic used in pesticide fabrication and arsenic consumption derives to population intoxications through the irrigation of contaminated water [3] .

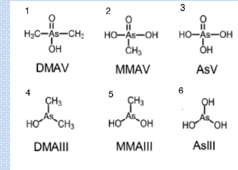

Fig. 1: Some arsenic species that it is arranged in descending order of toxicity: 1.Di-methyl arsenate (DMAV); 2.Monomethyl arsenate (MMAV); 3.Arsenate (AsV); 4.Di-methyl arsenite (DMAIII); 5.Monomethylarsenite (MMAIII); 6. Arsenite (AsIII) [2].

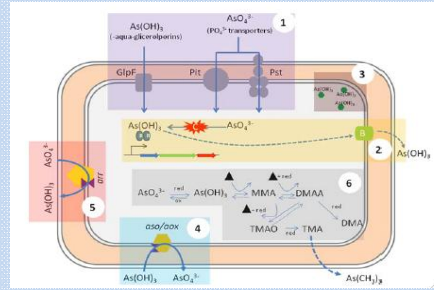

Fig.2. Diagram of the different microbial processes involved in arsenic biochemistry in the environment [4].

Bioremediation of arsenic can be conducted by different processes depending on the microorganism. The most used methods are: [Fig.2][4]:

1. Arsenic unspecific absortion: Arsenic enters the cells through the phosphate transporters (arsenate) or the aqua-glyceroporins (arsenite).
2. Arsenic resistance/tolerance: Once inside the cells, arsenate is reduced to arsenite by ArsC, which further extrude out of the cell by the specific pump ArsB.
3. Arsenic accumulation: Arsenite can also be detoxified by complexation with Cys-rich peptides.
4. Arsenite oxidation: Arsenite can serve as electron donor by oxidation to arsenate.
5. Arsenate reduction: Arsenate can be used as the ultimate electron acceptor during respiration.
6. Arsenic methylation: Inorganic arsenic can also be transformed into organic species in a methylation cascade.

Initial hypotesis

In the *Banerjee, S.* et al [5] article resolves that some microorganisms change the status of oxidation of the arsenate/arsenitet which does not . In this research we shall take some strains described in article by *Banerjee, S.* et al [5] and they will use for establishing targets.

Objectives

The project aims are:

- Define resistance/tolerance to arsenic of different strains.
- Check if the chosen strains actually retain the arsenic ions in polluted water contaminated by the environment.
- Check efficiency for a posterior use in bioremediation.

Expected outcomes

It is expected that the strains used in this project must have a high tolerance/resistance to arsenic and a high efficiency when retaining arsenate or arsenite which has oxidized or reduced, respectively, in the contaminated environment. They would be chosen to measure the concentration of arsenite and arsenate [Fig. 7].

Also the efficiency of each isolated bioremediate shall be compared separately or as a consortium [Fig.8]. Depending on the result they will be used in one way or another.

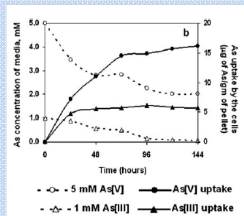

Fig. 7. Example of removal and uptake capacity *A. ludwffii* in presence of arsenate and arsenite ions [5].

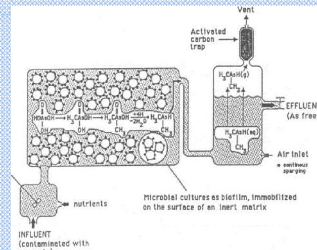

Fig. 8. Example of a bioreactor for arsenic elimination [14].

Materials and methods

Sample collection:

The samples will be taken from the delta of the River Ganges in India [Fig. 3] where an excess level of arsenic pollution higher than what FAO/OMS allows was detected (10µg/L).

Samples of river water will be put into sterilized bottles which shall be immersed 20 cm deep and then filled. Next the bottles will be labeled.

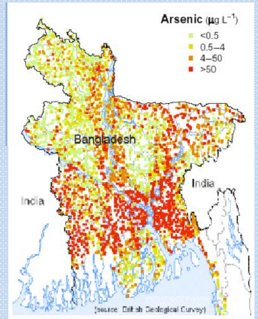

Fig. 3 Arsenic levels in delta Ganges river [6].

Measuring physiochemical parameters

The pH will be measured with pH meter (Cyberscan PC510, Eutech Instrumens; Netherlands) and arsenic concentration with an analytic test (Merck, Spectroquant 1.01747.0001).

Processing of the water:

Water will be filtered for removing particles in suspension, which could later interfere. Afterwards the water will be sterilized in an autoclave (ST200, Yamato Scientific Co.) at 121ºC during 20 minutes [7].

Research of resistant strains:

The strains that will be used in the study are [Fig. 4]:

- *Pseudomonas aeruginosa*
- *Bacillus circulants*
- *Acinetobacter lwoffii*

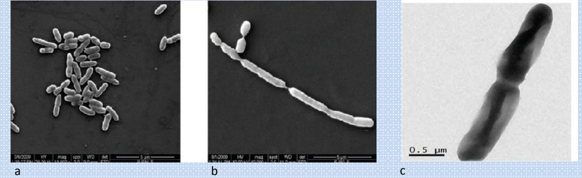

Fig. 4. (a) *A. lwoffii* in absence of arsenic, (b) *A. lwoffii* in presence of arsenic, (c) Transmission electron micrograph of *A. lwoffii* in presence of arsenic [5]

Physiologic characterization:

Temperaute optimum of growth will be achieved by incubing different cultures into different temperatures for 24 hours.

pH optimum will be measured by incubing the cultures into pH between 2 and 13 for 24 hours.

The growth is established by measuring the optical density of the culture in the spectrophotometer (UNICO, S-1200-E) at 600nm.

Evaluation of resistance to arsenic:

To evaluate arsenic resistance strains will be grown in an enrichment medium (LB) with different concentrations of arsenate (0-150mM), arsenite (0-50mM) and without the ion (control) during 18 hours. The growth will be measured using optic density at 600nm with spectrophotometer (UNICO, S-1200-E)[8].

To evaluate the maximum resistance levels of strains, the concentration of arsenate or arsenite necessary for inhibit the growth of any that is isolated will be analysed. This arsenite or arsenate concentration will be measured with a spectrophotometer (UNICO, S-1200-E)..

Defining bacterial activity:

The ability of oxiding arsenite and reducing arsenate of each strain will be tested with the use of silver nitrate ($AgNO_3$). This test will be made in a YEM Agar (Yeast Agar Manitol). To this medium 1mM of arsenite for the determination of the oxidation of arsenite and the same agar will be added, but adding 5mm of arsenate, for fixing the reduction of arsenate [Fig. 5].

The cultures will be incubated in 30ºC with 0,1M d'$AgNO_3$ for 7 days and the reaction, that is a chromogenic precipitate, will be measured *in visu*. The brown precipitate means that Ag3AsO4 is present in the medium, and therefore the strain has oxidase arsenite. In the same medium (without the arsenite), 5mm of arsenate will summed for fixing the reduction of arsenate [10].

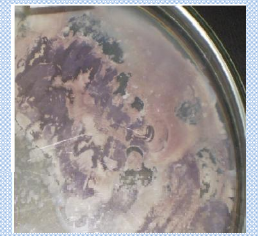

Fig. 5. Appearance of brownish precipitate after the application of $AgNO_3$ in agar plate supplemented with NaH_2AsO_3 in which *P. lubricans* was growing [9].

Evaluation of bioremediation:

Bioremediative potential will be determined by measuring absence/presence of arsenite or arsenate in water samples that have been taken and treated before.

300 ml will be taken from the different axenic cultures, and inoculated separately into 1L of different samples of environmental water; this culture will be maintained in an optimum pH and temperature and in a constant agitation [11].

After measuring separately the oxidative or reductive activity of the strains, this activity will be analysed as a consortia [Fig. 6]. To analyse the mentioned activity, a 100 mL of the axenic culture shall be taken and inoculated to a sole sample of environmental water. This culture will be maintained in the before mentioned pH and temperature parameters and in a constant agitation.

The initial and final concentration of arsenite/arsenate in the medium will be measured with a HPLC-ICP-MS (7500 Series, Agilent Technologies) [11] and the bacterial growth will be measured with the optical density by spectrophotometer (UNICO, S-1200-E) in 600nm. The duplication time and the constant rating of growth (k) during the logarithmic phase (of the growth curve) will be established by representing graphically [13].

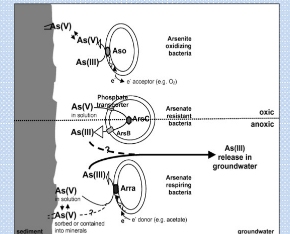

Fig. 6. Microbial interactions with As and impact on As(III) release in the groundwater [12].

Dissemination plan

The dissemination plan would follow these processes: first carry out a process of publication in various journals of the bioremediation field of microbes which have a high impact index such as Enviromental Microbiology, FEMS Microbiology reviews, Advances in Applied Microbiology or Journal of Hazardous Materials.

In addition the project will be presented at international conferences such as FEMS (Federation of European Microbiological Societies) or national of the SEM ((Sociedad Española de Microbiología)

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