

Original Article

Arsenic adsorption by Bacterial Extracellular Polymeric Substances

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The main objective of this work was to isolate arsenic resistant bacteria from contaminated soil, followed by screening for their ability to adsorb arsenic. Six bacterial isolates (S1 to S6) were obtained from arsenic contaminated soil samples and among these, five (S1, S2, S3, S5 and S6) were characterized as bacillus and the rest one (S4) was cocci depending on shape. All the isolates except S6 produced extracellular polymeric substances (EPS) in the culture medium and displayed arsenic adsorbing activities demonstrated by adsorption of around 90% from initial concentration of 1 mg/L sodium arsenite. To clarify the role of EPS, we killed the bacteria that produced EPS and used these killed bacteria to see whether they could still adsorb arsenic or not. We found that they could adsorb arsenic similarly like that of EPS produced live bacterial isolates. From the observation it is concluded that these isolates showed potentiality to adsorb arsenic and hence might be used for bioremediation of arsenic.

Keywords: *arsenic, extracellular polymeric substance, arsenic resistant bacteria, bioadsorption.*

Introduction

Arsenic is a toxic heavy metal that commonly remains in the environment in trivalent and pentavalent form. The natural occurrence of arsenic in ground water is related to the arsenic complexes present in the soil. The main reason of arsenic in water is thought to be from geological sources rather than from mining or agricultural sources such as fertilizer or pesticide¹. Toxicity of elements depends on various factors such as physical state, particle size, adsorption rate, solubility etc.². Arsenic toxicity has become a global concern owing to the increasing contamination of soil, water and crops in many regions of the world including Bangladesh. In Bangladesh, the people in 59 out of 64 districts are suffering at various degrees of arsenic contamination. Seventy five million people are at risk and among them 24 million are potentially exposed to arsenic contamination³⁻⁴. Arsenic is known to induce skin lesions, cancers, immunosuppression and other symptoms⁵⁻⁶. Currently available physico-chemical methods used for removing arsenic from contaminated environment have many disadvantages because of the vast polluted area, high cost, generation of secondary contaminants etc. Therefore, development of technologies to reduce costs involving biotechnological approaches for treatment of contaminated environment has stimulated serious interest in studies of the bioremediation of toxic metals.

Biological remediation using live or dead cells or biosynthesized molecules are in report⁷. In various ways such as transportation

across the cell membrane, bioadsorption in cell wall, entrapment in extracellular capsule, oxidation-reduction reaction, precipitation into plants and microorganisms were able to accumulate metals⁸. Moreover, algae, fungi and bacteria are capable to transform arsenite to arsenate and vice versa during their growth⁹⁻¹⁰. Some reports also showed that bacteria can accumulate metals¹¹⁻¹². Although arsenic is a toxic metalloid, some bacteria develop resistance to arsenic due to an efflux system¹³⁻¹⁴. On the other hand, some microorganisms use arsenate as a terminal electron acceptor in an aerobic respiration¹⁵⁻¹⁶ or as a means of generating energy through chemoautotrophic arsenite oxidation¹⁷. In this study, we demonstrated the isolation of bacteria from arsenic contaminated soil and determining their resistance against arsenic. We also examined the arsenic removal ability of the bacterial isolates *in vitro*.

Material and Methods

Collection of soil samples

Surface (from 0-15 cm in depth) soil that had been continuously contaminated by exposing to arsenic containing tube-well water for more than 15 years were collected from Sonargaon area of Narayanganj district, Bangladesh. After collecting, soil samples were placed in plastic containers and kept on ice until further analysis.

Isolation and culture of bacteria from arsenic contaminated soil
Five grams of each soil samples was dissolved in 50 ml 0.9%

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autoclaved NaCl and then homogenized and filtered. Five milliliter of each filtered sample was inoculated into 50 ml Luria broth (LB) medium and incubated at room temperature on rotary shaker at 120 rpm for 2-3 hrs. Then 5 ml of soil suspension was inoculated into 100 ml LB medium containing Na-arsenite (0.5mg/L) and incubated at 120 rpm for 2 days. Then 200 ml from this medium was spread over LB plates and incubate at room temperature for 2 days. Through repeated sub culturing, pure cultures of six bacterial isolates (designated S1 to 6) were finally obtained.

Morphological characteristics and extracellular polymeric substances (EPS) staining

Gram staining and cell morphology was observed under light microscope. For EPS staining, loopful organisms were spread over slide and air-dried to fix the organism. Smear was stained with Crystal Violet for 2 minutes. Crystal Violet was gently washed off with water. Slide was blotted dry with bibulous paper and examined with oil immersion objective (100X).

Arsenic removal ability of bacterial isolates

The selected bacterial isolates were inoculated into LB medium with Na-arsenite (1.0 mg/L) and then incubated at room temperature, 120 rpm for 24 hours. Samples were collected at every 6 hrs for the measurement of growth and arsenite concentration in the medium. Bacterial growth was determined by measuring the O.D. (A_{600}). For determining the arsenite concentration, 3 ml of culture media was taken and centrifuged at 7000 rpm for 5 min. Arsenite concentration at different growth stages were then determined by graphite furnace atomic absorption spectrometer (A_{800}) in the culture supernatant.

Arsenic removal ability of EPS producing killed bacteria

After growing up to mid stationary phase where a maximum extracellular polymeric substance was produced, bacterial cells

(isolate S1 and S3) were placed on a flat open container and exposed to UV light for 6 to 8 hrs to kill them. To confirm bacterial death, subcultures were performed on LB plate and incubated overnight. Na-arsenite solution (1.0 mg/L) was added to the UV irradiated bacterial suspension and then incubated at room temperature, 120 rpm for 3 hrs. After centrifugation arsenite concentration in the culture supernatant was determined as described earlier.

Result and Discussion

Isolation and characterization of arsenic resistant bacteria from contaminated soil

Indigenous bacteria from arsenic-contaminated soils were first enriched and then cultured in presence of sodium arsenite (0.5 mg/L) in LB broth. Six bacterial isolates were obtained that grew in presence of sodium arsenite. This result suggested that the bacterial isolates might have developed the mechanism(s) for arsenic resistance to protect sensitive cellular processes. It was observed that colonies of the five isolates (S1, S2, S3, S5 and S6) showed off-white, circular and translucent appearance with flat and smooth surface whereas the rest one (S4) showed pale-orange appearance. Morphological characteristics of all isolates were determined by Gram staining. The results indicated that isolates S1, S2, S3, S5 and S6 were rod shaped bacteria like bacilli and the S4 isolate was cocci in shape (Table 1). Moreover, all the isolates irrespective of their shapes were found Gram positive. Generally, Gram-positive bacteria are more resistant to stress due to the presence of thick peptidoglycan layer. Further studies showed that five isolates (S1, S2, S3, S4 and S5) produced extracellular polymeric like substances (EPS) during stationary phases of their growth (Table 1 and Figure 1). EPS is composed of carbohydrates, protein, DNA, and adsorbed abiotic constituents¹⁸. This EPS is shown to be generated by various environmental stresses to help cell survival¹⁹.

Table 1: Morphological characteristics and EPS production of bacterial isolates from arsenic contaminate soil samples.

Bacterial isolates	Colony character	Shape	Gram reaction	EPS production
S1	off-white, circular, translucent, smooth surface	bacilli	Gram positive	positive
S2	off-white, circular, translucent, smooth surface	bacilli	Gram positive	positive
S3	off-white, circular, translucent, smooth surface	bacilli	Gram positive	positive
S4	pale-orange, smooth surface	cocci	Gram positive	positive
S5	off-white, circular, translucent, smooth surface	bacilli	Gram positive	positive
S6	off-white, circular, translucent, smooth surface	bacilli	Gram positive	negative

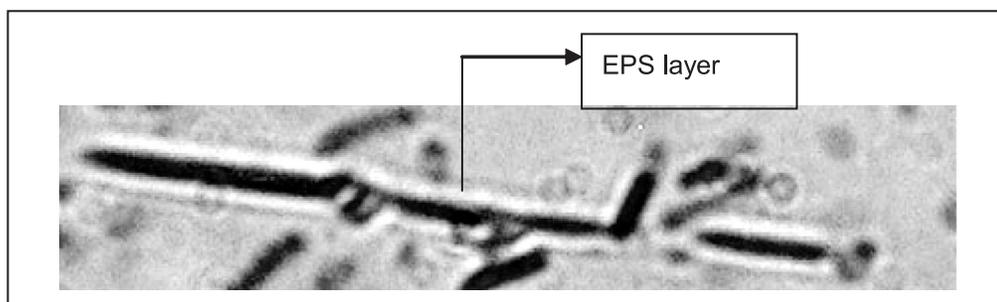


Figure 1: Microscopic observation (100X) of EPS production during stationary growth phase of isolated bacterial sample S3.

Arsenic removal by bacterial isolates

All the selected isolates showed relatively prolonged lag and log phase of growth when grown with 1mg/L sodium arsenite compared to control (culture without arsenite) (data not shown). This might be due to the effect of arsenite, which slowed down bacterial growth. During cultivation, the level of arsenite in the medium of each isolate did not decrease significantly up to log phase (data not shown). However, we observed that arsenite adsorption by the isolates mainly occurred during stationary phase of growth. All the isolates except S6 removed arsenite significantly (Figure 2). Interestingly, the isolates that removed arsenite produced EPS in the stationary phase of their growth. Therefore, we have reason to believe that EPS might, in part be responsible for arsenite adsorption, thereby reducing metal toxicity. This argument was supported by other reports that demonstrated microbial metal removal in mid log and stationary phase involving production of extracellular carbohydrates under environmental stress²⁰⁻²².

To clarify the role of EPS alone for arsenic adsorption, we killed the bacterial isolates S1 and S3 by exposing them to UV light followed by growing on LB plate. No visible growth was observed after overnight incubation, confirming death of the bacterial isolates (data not shown). These killed cells with EPS were then incubated with Na-arsenite (1mg/L) with shaking for 3 hrs followed by centrifugation to separate culture supernatant. To our surprise, arsenite concentration in the supernatant of S1 and S3 were drastically reduced to 0.075 and 0.17 respectively compared to control (1mg/L) (Figure 3). This result strongly argued that EPS present in killed bacteria adsorbed arsenite in similar extent with that of live cells.

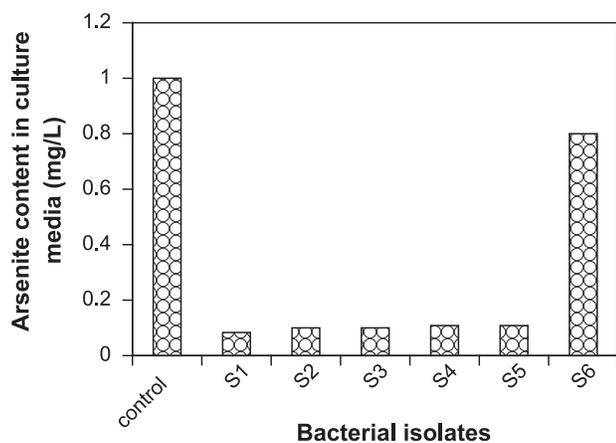


Figure 2. Removal of arsenite from culture media by the bacterial isolates. Arsenite concentration was determined after culturing bacterial isolates up to stationary phase of their growth. The result shown here is the average of triplicate experiments.

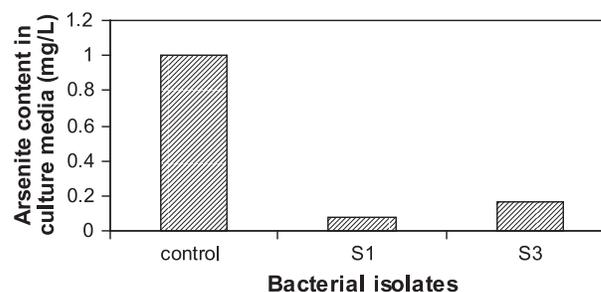


Figure 3. Removal of arsenite from culture media by the EPS produced-killed bacterial isolates (S1 and S3). Arsenite concentration was determined in culture supernatant after 3 hrs of shaking with EPS produced-killed bacterial isolates. The result shown here is the average of triplicate experiments.

Conclusion

In this study, we found that the isolated bacteria from arsenic contaminated soil were capable of adsorbing and removing arsenic from the culture media. They produced EPS during stationary phase of growth, which was particularly responsible for arsenic adsorption. Further research is necessary to identify these isolates and to explore the possibility to use these in the biofilm to remove arsenic from the contaminated environment.

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References

- Matschullat J. 2000. Arsenic in the geosphere—A review. *Sci. Total Environ.* **249**: 297–312.
- Anderson C and Cook GM. 2004. Isolation and characterization of arsenate-reducing bacteria from arsenic contaminated sites in New Zealand. *Curr. Microbiol.* **48**: 341-347.
- MacDonald R. 2001. Providing clean water: lessons from Bangladesh. *Br Med J.* **322**: 626-627.
- McLellan F. 2002. Arsenic contamination affects millions in Bangladesh. *Lancet.* **359**:1127.
- Mazumdar GDN, Gupta DJ and Santra A. 1997. Non-cancer effects of chronic arsenicosis with special reference to liver damage. In: CO Abernathy, RL Calderon, WR Chappell, eds. *Arsenic: Exposure and Health Effects*. London: Chapman & Hall. 112-123.
- Tsai SM, Wang TN and Ko YC. 1999. Mortality for certain diseases in areas with high levels of arsenic in drinking water. *Arch. Environ. Health.* **54**: 186–193.
- Katsoyiannis I, Zouboulis A, Althoff H and Bartel H. 2002. As(III) removal from groundwaters using fixedbed upflow bioreactors. *Chemosphere.* **47**: 325-332.
- Malik A. 2004. Metal bioremediation through growing cell. *Environ Int.* **30**: 261-278.
- Macy JM, Santini JM, Panling BV, O'Neil AH and Sly LI. 2000. Two new arsenate/sulfate-reducing bacteria: Mechanisms of arsenate reduction. *Arch Microbiol.* **173**: 49-57.
- Hasegawa H, Sohrin Y, Seki K, Sato M, Norisuye K, Naito K and Matsui M. 2001. Biosynthesis and release of methylarsenic compound during the growth of fresh water algae. *Chemosphere.* **43**: 265-272.

11. Srinath TT, Verma PW, Ramteke SK and Garg. 2002. Chromium (VI) biosorption and bioaccumulation by chromate resistant bacteria. *Chemosphere*. **48**: 427-435.
12. Hussein HS, Farah K, Kandil H and Moawad. 2005. Tolerance and uptake of heavy metals by *Pseudomonas*. *Process Biochem*. **40**: 955-961.
13. Diorio CJ, Cai J, Marmor R, Shinder MS and DuBow. 1995. An *Escherichia coli* chromosomal ars operon homolog is functional in arsenic detoxification and is conserved in Gram-negative bacteria. *J Bacteriol*. **177** (8): 2050-2056.
14. Cai JK, Salmon MS and DuBow. 1998. A chromosomal ars operon homologue of *Pseudomonas aeruginosa* confers increased resistance to arsenic and antimony in *Escherichia coli*. *Microbiol*. **144**: 2705-2713.
15. Ahmann D, Roberts AL, Krumholz L and Morel FMM. 1994. Microbe grows by reducing arsenic. *Nature*. **371**: 750.
16. Newman DK, Ahmann D and Morel FMM. 1998. A brief review of microbial arsenate respiration. *Geomicrobio J*. **15**: 255-268.
17. Santini, JM, Sly LI, Schnagl RD and Macy JM. (2000). A new chemolithoautotrophic arsenite-oxidizing bacterium isolated from a gold-mine: phylogenetic, physiological, and preliminary biochemical studies. *Appl Environ Microbiol*. **66**: 92-97.
18. Sutherland IW. 2001. Biofilm exopolysaccharides: a strong and sticky framework. *Microbiol*. **147**: 3-9.
19. Costerton, JW, Irvin RT and Cheng KJ. 1981. The bacterial glycocalyx in nature and disease. *Ann Rev Microbiol*. **35**: 299-324.
20. Wang CL, Michels PC, Dawson SC, Kitisakkul S, Baross JA, Keasling JD and Clark DS. 1997. Cadmium removal by a new strain of *Pseudomonas aeruginosa* in aerobic culture. *Appl Environ Microbiol*. **63**: 4075-4078.
21. Yilmaz EI. 2003. Metal tolerance and biosorption capacity of *Bacillus circulans* strain EB1. *Res Microbiol* **154**: 409-415.
22. Martin L, Reni O and Jean G. 1997. Uptake of lead, cadmium and zinc by a novel bacterial exopolysaccharide. *Water Res*. **31**(5): 1171-1179.