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Surface binding and intracellular uptake of arsenic in bacteria isolated from arsenic contaminated site

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ABSTRACT

The present study deals with the surface binding and uptake of arsenic (As) in two bacterial strains (*Bacillus subtilis* and *Paenibacillus macerans*) isolated from the As contaminated soil. The selected As tolerant bacterial strains *B. subtilis* and *P. macerans* showed maximum removal of As from ambient medium by about 82.2 and 62.4%, respectively, at 50 $\mu g\,ml^{-1}$ initial concentration of arsenic (As III). Further, a comparison of results on As binding by cell surface and intracellular uptake across membrane at 50 $\mu g\,ml^{-1}$ concentration showed that contribution of cell surface binding in overall As removal by *B. subtilis* and *P. macerans* was about 90 and 82%, respectively. Only a small fraction of As was removed by intracellular uptake. Effects of various factors such as pH, temperature and time on the surface binding and intracellular uptake of arsenic revealed that both the strains removed more As in alkaline range at pH 8 (34.4 and 27.14 $\mu g\,mg^{-1}$ protein for surface binding by *B. subtilis* and *P. macerans*, respectively and 3.56 and 4.92 $\mu g\,mg^{-1}$ protein for intracellular uptake by *B. subtilis* and *P. macerans,* respectively) at initial concentration of 50 $\mu g\,ml^{-1}$. Optimum temperature for As removal was 30 °C for *P. macerans* (20.03 and 4.21 $\mu g\,mg^{-1}$ protein for surface binding and intracellular uptake, respectively), whereas *B. subtilis* exhibited maximum As removal between 35–40 °C (30.69 and 2.73 $\mu g\,mg^{-1}$ protein for surface binding and intracellular uptake, respectively). The kinetic parameter revealed that binding of As by both the bacterial strains followed pseudo-second-order kinetics. The adsorption behavior of the bacterial strains was better explained by Langmuir isotherm rather than Freundlich model. Results on FTIR spectra of As binding with bacterial strains revealed that the surface binding of As by bacterial strains was apparently facilitated by the lipids (2876 cm^{-1}), carbohydrates (1143, 1132 cm^{-1}), amines (3241 cm^{-1}), amides (3426, 3336, 1647, 1566 cm^{-1}) and aromatics groups (861, 814 cm^{-1}) unlike other metals.

1. Introduction

Arsenic is an extremely toxic metalloid widely distributed in the earth's crust with a concentration of ~1.5 to 2 ppm (National Research Council, 1977). Two forms of arsenic are common in natural water arsenate (As V) and arsenite (As III). However, human activities such as mining, smelting, metallurgy, use of As in pesticides, crop desiccants and in livestock feed had made the problem more serious (Nriagu and Azcue, 1990). Inorganic trivalent form of As is considered more toxic than pentavalent form (Saluja et al., 2011). Arsenic is known to have mutagenic and genotoxic effects on humans and it has been associated with increased risk of skin, kidney, lung and bladder cancers and is listed as class 'a' human carcinogen by the USEPA (Chen et al., 2002 and Karagas et al., 1998). Human exposure to arsenic compounds leads to serious health hazard, primarily due to ingestion of contaminated drinking water and sea food. Due to its harmful properties, the USEPA has set the permissible limit of arsenic in drinking water at10 ppb (USEPA, 1998). Arsenic toxicity due to natural contamination of drinking water has been noted as a significant public health problem in Bangladesh and India (Pearce, 2003). Thus, there is an urgent need to remove arsenic from contaminated water and soil.

Currently, there are several approaches that are employed to remove arsenic contamination from water and soil such as chemical precipitation, reverse osmosis and activated alumina. However, these procedures are very costly and there are negative externalities such as problematic side products. Microbial remediation is a novel approach where microorganisms can be employed not only for chemical transformation of hazardous chemicals to less toxic and more environmentally acceptable forms, but also for the physical removal of toxic species. Arsenic is lethal to most micro-organisms, yet certain bacteria are known to survive arsenic exposure (Patel et al., 2007). The ubiquity of arsenic

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in the environment has forced the microorganisms to evolve an arsenic defense mechanism (Rehman et al., 2007). Certain microorganisms are known to have developed the necessary genetic components which confer arsenic-tolerance mechanisms, allowing them to survive and grow in environment containing high levels of arsenic that would be toxic to most other organisms (Liao et al., 2011). Bioremediation of arsenic by arsenic tolerant microorganisms can be a potential tool in providing a cost-effective and environmental friendly technology for removal of arsenic (Valls and Lorenzo, 2002; Wang and Zhao, 2009).

Although various types of microbial biomass have been used as adsorbent to remove various metal ions including arsenic (Pokhrel and Viraraghavan, 2006; Teclu et al., 2008). A successful attempt to remove arsenic by using microorganisms has been hampered because arsenic usually exists as negatively charged ions (Cullen and Reimer, 1989) and does not bind with negatively charged membrane surface of microorganisms. For arsenic to be adsorbed by the microorganisms, membrane surface of the microorganisms must be changed by some form of pre-processing, which may be either chemical, such as the use of surfactants, or physical, such as in heat processing (Kapoor et al., 1999; Loukidou et al., 2003). With the exception of some marine bacteria and a few genetically modified *Escherichia coli* that have been reported previously, no bacteria has been yet successfully used for remediation of arsenic (Oremland and Stolz, 2003; Kostal et al., 2004; Takeuchi et al., 2007).

A study on the biosorption behavior of membrane and factors affecting its performance is considered important for designing of bioremediation technology. In the present study, attempts have been made to delineate arsenic sorption on the cell surface and taken up intracellularly by two arsenic tolerant bacterial strains (*B. subtilis* and *P. macerans*). Besides, an attempt has been made to study the role of important factors regulating the removal of arsenic.

2. Materials and methods

2.1. Chemicals and equipment

Stock of As(III) was prepared by dissolving sodium arsenite in sterile deionized Milli-Q water, stored at 4 °C in dark. For pH adjustment 0.1 N HCl and 0.1 N NaOH solutions were used. The solutions of arsenic were analyzed by using an atomic absorption spectrophotometer (AA 240 FS, Varian, Australia) at a wavelength of 193.7 nm.

2.2. Isolation of bacterial isolates

The bacterial strains selected for this study were isolated from arsenic affected areas of Parasia (N 25° 22′ 47.9834″ and E 78° 19′ 58.8565″) and Trilokpur village (N 28° 21′ 40.7149″ and E 80° 39′ 47. 5571″) of Lakhimpur Kheri district (27°57′0″N and 80°46′0″E), Uttar Pradesh, India. The soil samples were collected from a depth of 15 cm in sterile plastic bags. The arsenic tolerant colonies of bacteria were isolated and screened on nutrient agar plates containing 40 mM of sodium arsenite. The standard microbiological techniques were employed to screen the microorganism. Discrete arsenic tolerant bacterial colonies were picked up and maintained in nutrient broth containing 40 mM of arsenite.

2.3. Identification of bacterial isolates

The bacterial isolates were further identified first by using standard morphological and biochemical tests (Lechevalier, 1989), followed by 16S rDNA sequencing carried out at Genetech, Biotech Park, Lucknow (U.P., India). The gene sequences of the isolated As tolerant strains were submitted at NCBI. The bacterial strains along with their accession no. are as follows-*P. macerans* (accession no. KC633280), *B. subtilis* (accession no. KC625596). The bacterial strains *B. subtilis* and *P. macerans* could tolerate As (III) upto 60 and 40 mM, respectively.

2.4. Biosorption experiment

The stock solution of As(III) was prepared by dissolving sodium salt of this metal in Milli-Q water in calculated amount. The resulting stock solution was further diluted to get desired concentrations for biosorption experiments. Experiment was performed in 100 ml Erlenmeyer's flask containing 20 ml BSMY II. Exponentially growing culture was harvested by centrifugation

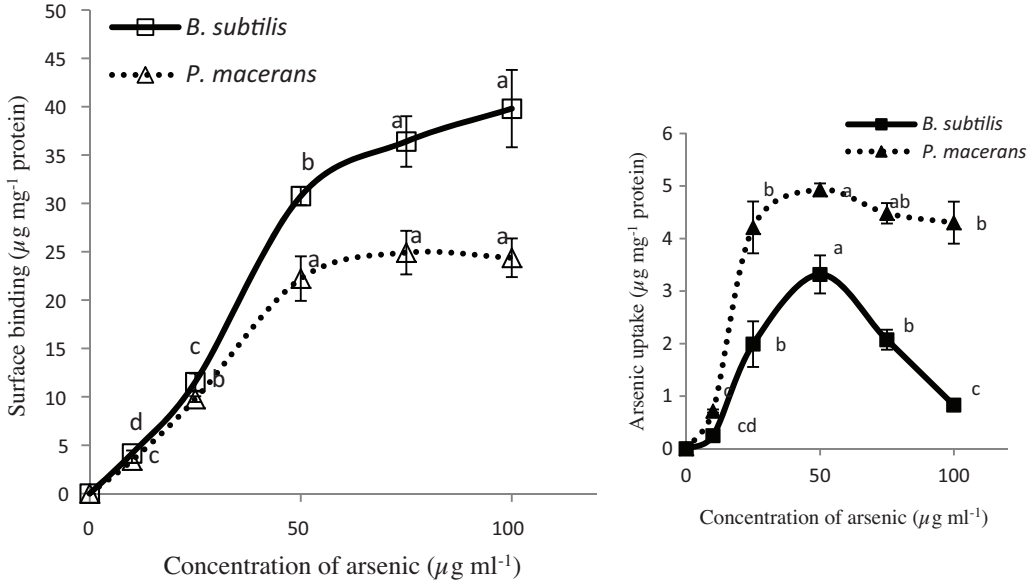

Fig. 1. Comparison between surface binding and intracellular uptake by *B. subtilis* and *P. macerans* at different concentrations of arsenic (0–100 μg ml^{-1}). Data are the mean of three replicates ± SD. Data was analyzed by one way analysis of variance (Duncan's multiple range test) at $p < 0.05$. Different alphabets show significant differences between the treatments.