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Isolation and characterization of arsenic-resistant bacteria and possible application in bioremediation

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ABSTRACT

Ground water arsenic contamination is a widespread problem in many developing countries including Bangladesh and India. In recent years development of modern innovative technologies for the removal of arsenic from aqueous system has become an interesting topic for research. In this present study, two rod shaped Gram-positive bacteria are being reported, isolated from arsenic affected ground water of Purbasthali block of Burdwan, West Bengal, India, which can tolerate arsenate concentration up to 4500 ppm and 550 ppm of arsenite concentration. From biochemical analysis and 16S rRNA sequencing, they were identified as *Bacillus* sp. and *Aneurinibacillus aneurinilyticus* respectively. The isolates SW2 and SW4 can remove 51.45% and 51.99% of arsenite and 53.29% and 50.37% of arsenate, respectively from arsenic containing culture media. Both of the isolate can oxidize arsenite to less toxic arsenate. These two arsenic resistant bacteria can be used as a novel pathway for the bioremediation of arsenic.
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1. Introduction

Arsenic is a toxic metalloid and it is present in the soil as insoluble sulfides and sulfosalts such as Arsenopyrite, Orpiment, Realgar, Lollingite and Tennantite [1]. Although arsenic is naturally present in the earth crust, arsenic contamination occurs mainly due to various anthropogenic activities, like excessive use of arsenic in pesticide, herbicide, wood preservatives and medicinal product [2].

Arsenic mainly exists in the environment as arsine(−III), elemental arsenic(0), arsenite(III) and arsenate(V). Among all these forms only arsenite and arsenate are more abundant in natural environment than the other two [3]. Arsenate and arsenite both are very toxic in nature, arsenite being more toxic than arsenate. They can induce various types of cellular damage in biological system [4]. There is structural analogy between arsenate and inorganic phosphate. So it can be introduced into the cell through the same system as phosphate is transported through the cell membrane, disrupting those metabolic reactions which require phosphorylation and inhibit the synthesis of adenosine triphosphate [5]. When people are exposed to arsenic concentration above permissible limit i.e., 0.05 ppm [6], it causes various toxic effects on human health. The most common symptoms of arsenic toxicity, are skin itching, skin cancer, weight loss, loss of appetite, weakness, lethargy and easily fatigued limits the physical activities and working capacities, chronic respiratory disorder, gastrointestinal disorders like anorexia, nausea, pain in abdomen, enlarged liver and spleen [7] and moderate to severe anemia was also reported in a few cases.

As arsenic has high magnitude of solubility, its removal from contaminated water is very difficult [8]. There are various conventional methods for the removal of arsenic from drinking water like coagulation, membrane filtration, reverse osmosis, adsorption, filtration, etc. [9]. In these conventional processes, the oxidation step, required for the transformation of As(III) to As(V) is gained either through the reaction with oxygen under normal atmospheric condition which is very slow, or through chemical oxidants like hydrogen peroxide, chlorine and ozone which is very costly and also produces harmful by-products [10]. Microorganisms can reduce the toxicity of the contaminants by using them in their metabolic processes as energy sources [11]. Microorganisms have developed different mechanisms like arsenite methylation, arsenite oxidation, etc. to transform more toxic form of arsenic i.e., arsenite to less toxic form, arsenate [12]. A special type of enzyme is present in the protoplasm of arsenic oxidizing bacteria, arsenic oxidase. With the help of this enzyme, the bacteria oxidize arsenite to arsenate [13].

Many arsenic resistant microbes were reported which can withstand high concentration of arsenic, can be potentially used

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for the bioremediation of arsenic from arsenic contaminated ground water. Chowdhury et al. [14] isolated a novel strain, *Planococcus* KRPC10YT from arsenic contaminated bore-well of West Bengal, India which can tolerate up to 30 mM arsenate and 20 mM arsenite. In 2005, Shivaji et al. [15] found a novel arsenic-resistant strain, *Bacillus arsenicus* from arsenic contaminated soils in Chakdah district of West Bengal, India which was able to grow in the presence of 20 mM arsenate and 0.5 mM arsenite.

But very limited works have been done toward bioremediation of arsenic using the arsenic resistant bacteria. Purbasthali block of Burdwan, West Bengal, India is severely affected with arsenic. According to Roy et al. [16], the arsenic concentration in the tube well water of this area is 0.076–0.205 ppm. But no research has been conducted to isolate arsenic resistant bacteria from this particular affected area and also to apply these bacteria in bioremediation of arsenic contaminated ground water till date. In this present study, two arsenic resistant bacteria are being reported which were resistant to very high concentration of arsenate and arsenite, from the arsenic contaminated water of Purbasthali and are also able to reduce arsenic concentration from contaminated water.

2. Materials and methods

2.1. Study area

Purbasthali block of Burdwan district, West Bengal, India was chosen for the present study which was previously reported for arsenic contamination [16]. The Study area is situated within 23°29′28.3″N–23°31′54″N latitude and 88°17′4.3″E–88°21′56.1″E longitude (Fig. 1).

2.2. Sampling

For the collection of water samples, twelve villages of Purbasthali block, namely Kalyanpur (A1, A2, A3, 23°30′15.4″N and 88°18′2.6″E), Misbahpur (B, 23°30′15.2″N and 88°18′17.7″E), Paschim Atpara (C, 23°30′24.9″N and 88°19′33.6″E), Purba Atpara (D, 23°30′41.6″N and 88°20′6″E), Kamalnagar (E, 23°31′35.2″N and 88°21′25.1″E), Laxmipur (F, 23°30′39.3″N and 88°19′26.4″E), Natun Laxmipur (G, 23°31′8.9″N and 88°19′26.3″E), Dhamas (H, 23°17′38.4″N and 87°49′5.4″E), Sinhari (I, 23°17′37.3″N and 87°47′5.8″E), Tamaghata (J, 23°31′58.2″N and 88°20′50.5″E), Majida (K, 23°31′59.5″N and 88°19′30.10″E) and Rukuspur (L, 23°31′59.6″N and 88°19′30.9″E), were selected. Five samples were collected from each sampling spot. Sterilized polythene bottles were used for the collection of ground water samples from the arsenic contaminated tube well and they were transported into thermo boxes immediately after collection. The samples were stored at 4 °C after taking to the laboratory for subsequent analysis.

2.3. Physico-chemical analysis of water samples

Immediately after collection, pH, Electrical conductivity (EC) and total dissolved solid (TDS) of water samples were measured in-situ by using Multi-parameter (PC Test 35). Other parameters like acidity, alkalinity, hardness, chloride, phosphate and iron were measured by following standard methods of APHA [17]. Arsenic content of the water samples was measured following SDDC method [16].

2.4. Isolation and enumeration of arsenic-resistant bacteria

Groundwater samples were serially diluted up to 10^{-6} dilution and inoculated following pour plate method into nutrient agar media and incubated at 30 °C ± 1 for 24 h. From all these water samples twelve distinct colonies were selected based on the colony morphology and they were tested for their arsenic resistant potentiality by culturing in nutrient broth media amended with 100–5000 ppm of sodium arsenate and 100–1000 ppm of sodium arsenite and the absorbance was measured at 650 nm at 24 h interval.

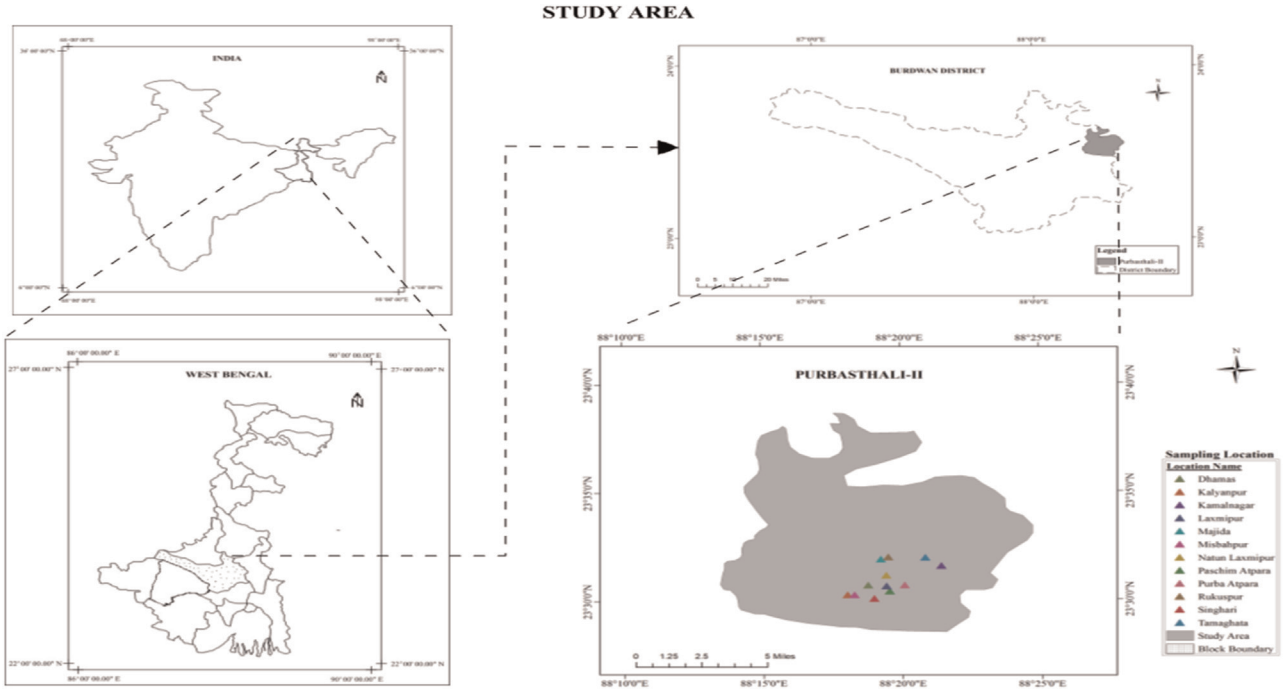

Fig. 1. Study area map.

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