



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 $30^{\circ}\text{C} \pm 1$ 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.

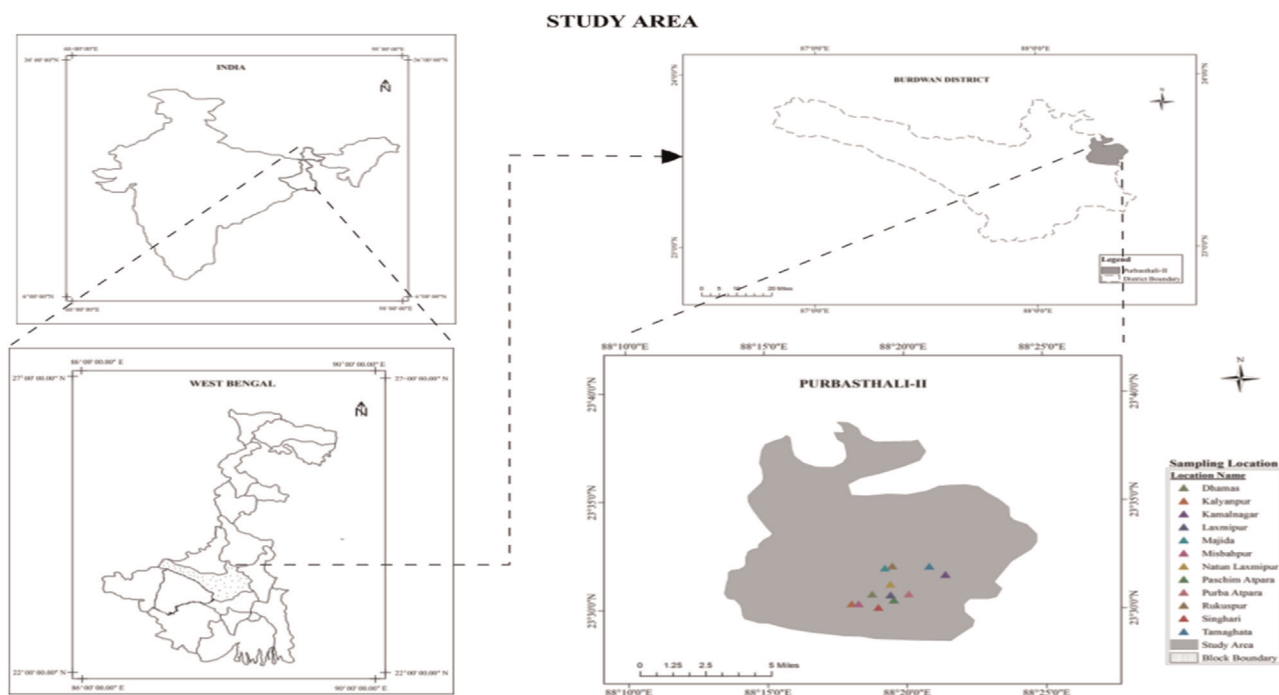


Fig. 1. Study area map.

2.5. Phenotypic and biochemical characterization

Morphological and physiological characterization of the isolated bacterial colony was done following the standard methods of Pelczar et al. [18]. Different biochemical properties of the bacterial isolates such as enzyme activity (indole, catalase, urease and oxidase), methyle red test, Voges–Proskauer test, nitrate reductibility, citrate, ability to produce hydrogen sulphide, utilization of different carbon sources, and utilization of gelatin, starch and lipid were tested by following the standard methods [18]. To study the antibiotic sensitivity of the isolated bacteria, different standard antibiotic discs such as, ofloxacin, gentamicin, chloramphenicol, vancomycin, nalidixic acid, rifampicin, ciprofloxacin, levofloxacin and ampicillin were used [19]. For scanning electron microscopy, bacterial smear was prepared on a cover glass and heat fixed over a flame for 1–2 s followed by fixation of the smear by 2.5% glutaraldehyde (aqueous) for 45 min. The slides were then dehydrated passing through 50–90% of alcohol solutions and finally through absolute alcohol for 5 min each. The bacteria on the cover glass were gold coated and observed under a 15 kV scanning electron microscope (HITACHI, S-530, SEM and ELKO Engineering) for surface morphology study.

2.6. 16S rDNA sequencing of the bacterial isolates

From the pure culture pellet, genomic DNA was isolated using Chromous Genomic DNA isolation kit. The ~1.5 kb 16S-rDNA fragment was amplified using high fidelity PCR polymerase and the PCR product was sequenced bi-directionally by Genetic Analyzer (ABI 3130 Genetic Analyzer) using the forward and reverse primer (*Bacillus* specific universal primer). The NCBI BLAST program was used to analyze the sequence data of the isolates and these were aligned using the “ClustalW Submission Form” (<http://www.ebi.ac.uk/clustalw/>) and analyzed by ClustalW24. Evolutionary distances between the sequences were calculated using the method of Jukes and Cantor [20] and the phylogenetic tree was prepared following the “neighbor joining” method [21].

2.7. Bioremediation test

Isolated bacteria were inoculated in the nutrient broth media prepared in 100 ppm arsenate and arsenite solution and incubated at 30 °C ± 1. After 24 h, 48 h and 72 h of incubation the solution were centrifuged at 10,000 rpm for 10 min to separate the bacterial biomass from the media [22] and the arsenic concentration of the media was measured following SDDC method [16].

2.8. Oxidation and reduction of arsenic by the isolates

The ability of the bacterial isolates to reduce and oxidize arsenic (V) and(III) respectively was tested by using silver nitrate solution [7]. Isolates were cultured in the agar plate (peptic digest of animal tissue 5 g/l, beef extract 1.5 g/l, yeast extract 1.5 g/l and agar powder 15 g/l) containing either As(V) or As(III). After 72 h, small amount of silver nitrate was added to the media. If the media turned brown, it confirms the presence of silver arsenate and if it turns yellow, the presence of silver arsenite was confirmed.

3. Results

3.1. Physicochemical properties of water

The physicochemical properties of all the water samples are presented in Table Sup 1. All the ground water samples of the study area were found to be alkaline in nature with average pH value of

7.23. Electrical conductivity of the water samples range from 0.21 mS/cm to 0.65 mS/cm. Average hardness and chloride content of the water were 76.11 ppm and 39.49 ppm, respectively. The average phosphate content was 0.352 ppm. A few water samples were above the permissible limit of iron i.e., 1 ppm [23]. The arsenic concentration of three samples, namely, A1, A2 and A3 were recorded highest i.e., 0.119 ppm, 0.104 ppm and 0.117 ppm respectively.

3.2. Arsenic resistance of the bacterial isolates

A total of twelve colonies were isolated from all the water samples plated and they were cultured in nutrient broth media amended with 100–5000 ppm arsenate and 100–1000 ppm arsenite solution. From the recorded optical density it was observed that the growth of two isolates (SW2 and SW4) increased with increasing arsenate concentration up to 3000 ppm and then slowly decreased and ultimately stopped growing at 4500 ppm of arsenate. The minimum inhibitory concentration (MIC) of these two isolates was 4500 ppm for arsenate. But both the isolates showed decreased growth pattern with increasing concentration of arsenite, and finally stopped growing in 600 ppm arsenite solution. These two isolates, namely SW2 and SW4, were chosen for further characterization.

3.3. Phenotypic and biochemical characterization of the isolates

Both the isolates were white in color, have smooth surface, and flat colonies. The colony of isolate SW4 was round in shape while SW2 had varied shape and size. SW4 colony was concentric with transparent center and opaque periphery but SW2 colony was opaque all over. Both the isolates were Gram-positive and rod shaped (Fig. 2A and B). Different biochemical properties of the two isolates are presented in Table 1. The two isolates were positive for catalase enzyme production and were not able to produce indole enzyme. Isolate SW2 showed positive result for MR test, nitrate reductase test, oxidase test and negative result for Voges–Proskauer test, citrate utilization, ureas test, H₂S production, gelatin hydrolysis and lipid hydrolysis. Although the isolates did not require Na⁺ ions for growth, SW2 can tolerate sodium chloride up to 10% whereas SW4 can tolerate sodium chloride up to 8% but the optimum growth of the two isolates occurred in the media containing 4–6% of sodium chloride. SW4 was positive for gelatin and lipid hydrolysis but showed negative result for MR test, Voges–Proskauer test, citrate utilization, ureas test and H₂S production. The isolate SW2 can utilize different carbon sources like glucose, sucrose, arabinose, fructose, maltose, salicin, inositol, mannose and aesculin but can't utilize lactose and manitol. SW4 can ferment glucose, sucrose, lactose, manitol, arabinose, fructose and mannose but can't grow in the medium amended with maltose, salicin, inositol and aesculin. This particular isolate can also produce gas by fermenting glucose, sucrose, lactose, manitol and arabinose. Maximum growth of isolate SW2 occurred in the media amended with sucrose, fructose and salicin, and for SW4, maximum growth occurred in the media amended with fructose, mannose and arabinose. Both the isolates showed sensitivity to recommended doses of some antibiotics like doxycyclin, ofloxacin, gentamicin, chloramphenicol, vancomycin, nalidixic acid, rifampicin, ciprofloxacin, levofloxacin and were resistant toward ampicillin.

3.4. Identification of the isolates

Based on phenotypic, biochemical and phylogenetic analysis (Fig. 3), the isolates SW2 and SW4 were identified as *Bacillus* sp. KM02 and *Aneurinibacillus aneurinilyticus* strain BS-1 respectively.

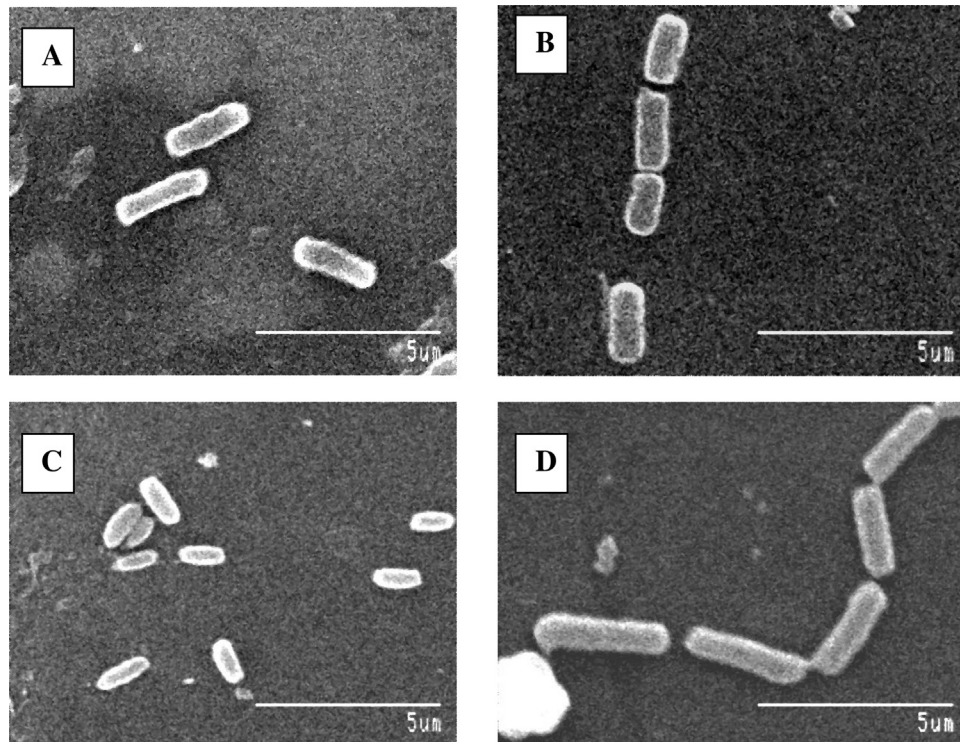


Fig. 2. Scanning electron micrograph of (A) isolate SW2 (control), (B) isolate SW2 treated with arsenite, (C) isolate SW4 (control) and (D) isolate SW4 treated with arsenite.

3.5. Bioremediation of arsenate and arsenite by the isolates

The two isolates were resistant against high concentration of both As(III) and As(V). They also exhibit the potentiality to reduce arsenic concentration from nutrient broth media containing 100 ppm of arsenate and arsenite (Fig. 4). The non-significant removal was recorded between the two isolates SW2 (51.45%) and SW4 (51.99%) of arsenite. However, significant removal of arsenate between SW2 (53.29%) and SW4 (50.37%) was observed at 5% level of significance from the media after 72 h of incubation.

Table 1
Biochemical properties of the isolates.

Parameters	SW2	SW4
Catalase enzyme	+ve	+ve
Indole enzyme	–ve	–ve
MR test	+ve	–ve
VP test	–ve	–ve
Citrate test	–ve	–ve
Salt tolerance	Up to 10%	Up to 8%
Nitrate reductase	+ve	–ve
Oxidase test	+ve	–ve
Urease test	–ve	–ve
H ₂ S production	–ve	–ve
Glucose fermentation	+ve	+ve (gas)
Sucrose fermentation	+ve	+ve (gas)
Lactose fermentation	–ve	+ve (gas)
Manitol fermentation	–ve	+ve (gas)
Arabinose	+ve	+ve (gas)
Fructose	+ve	+ve
Maltose	+ve	–ve
Salicin	+ve	–ve
Inositol	+ve	–ve
Mannose	+ve	+ve
Aesculin	+ve	–ve
Gelatin hydrolysis	–ve	+ve
Lipid hydrolysis	–ve	+ve
Gram stain	+ve	+ve

3.6. Oxidation and reduction of arsenic

After the addition of silver nitrate into 72 h old culture plate containing arsenite, the media slowly turned brown (Fig. 5) which confirmed the presence of silver arsenate in the media. But when silver nitrate was mixed with the culture containing arsenate, it also turned brown confirming the presence of silver arsenate. Hence it is observed that none of the bacteria has the ability to reduce arsenate to arsenite, but both of them can oxidize arsenite to arsenate.

4. Discussion

From the experimental data it can be observed that there are wide variations in the level of all physicochemical properties of the ground water samples studied including arsenic concentration. All the water samples were neutral to alkaline in nature which is suitable for bacterial growth [22]. High range of electrical conductivity of all the water samples indicates the presence of high amount of soluble electrolytes in the ground water [24]. All the water samples can be classified as soft water as the value of total hardness and chloride were far below the permissible limit (300 ppm and 500 ppm, respectively) according to WHO [23]. Phosphate concentration of all the water samples was exceeding the permissible limit of 0.1 ppm [25]. Arsenic concentration of three of the water samples was above the permissible limit i.e., 0.05 ppm for India and Bangladesh.

Growth of the two isolated strains increased with increasing arsenic concentration. Such increasing growth pattern of bacteria in presence of arsenate was also reported by Honschopp et al. [26]. When a microbial community stays under a selective stress condition like high concentration of arsenic for a very long period, they must develop some mechanism to detoxify it and overcome the restriction for growth [27]. It is reported that sometimes microbial community gain protection against toxic agents through

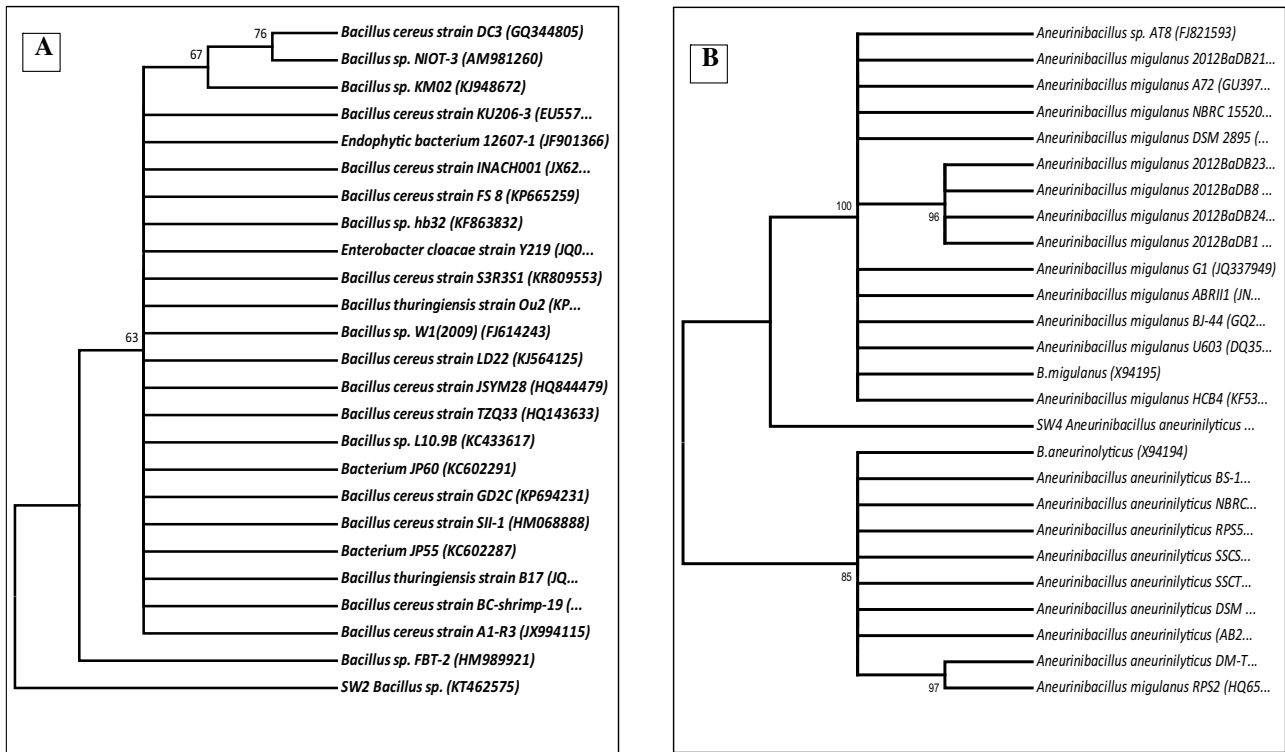


Fig. 3. Phylogenetic tree of (A) isolate SW2 and (B) isolate SW4.

the formation of microbial biofilms [28]. Hence it may be possible that through the formation of biofilms the isolated bacterial strains can resist the toxicity of arsenic. The arsenic resistance mechanism of bacteria can be plasmid associated [29], or by ars operon, containing the genes arsRBC [30] when it can resist higher concentrations. A carrier protein, arsB also helps in the extrusion of arsenic from cell. The bacterium which possesses arsA gene also coupled with arsB and significantly increases the arsenic resistant capacity of the bacteria [31]. In 2004, Anderson and Cook [32] have isolated seventeen bacterial strains including *Bacillus licheniformis*,

Bacillus polymyxa, etc. which were able to resist up to 100 ppm arsenic. Incidence of an arsenic hyper tolerant bacterium from well water which was able to tolerate up to 2000 ppm arsenate was also reported by Zelibor [33]. Other bacteria from *Bacillus* groups reported over time also showed the gene mediated arsenic resistance potentiality [34–36]. In this study, as both the bacteria were *Bacillus*, it can be speculated that they possess ars operon, containing the genes arsRBC and the carrier protein also by which they extrude the arsenate from the cell. By either oxidizing or reducing the toxic heavy metals, bacteria sometimes gained energy

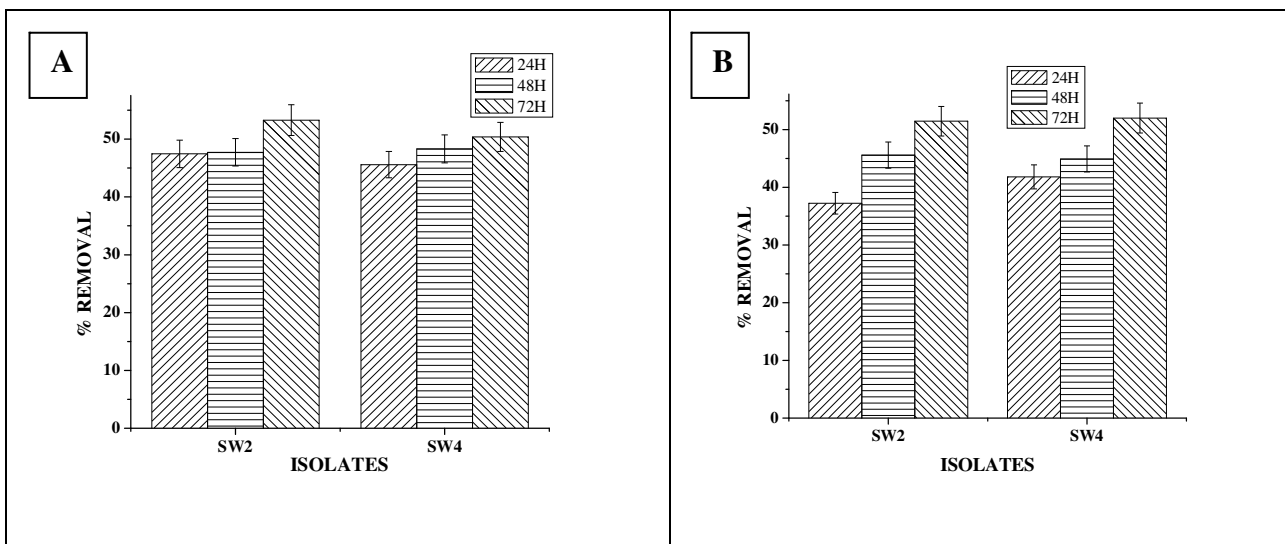


Fig. 4. (A) Arsenite removal by isolates SW2 and SW4, (B) arsenate removal by isolates SW2 and SW4.

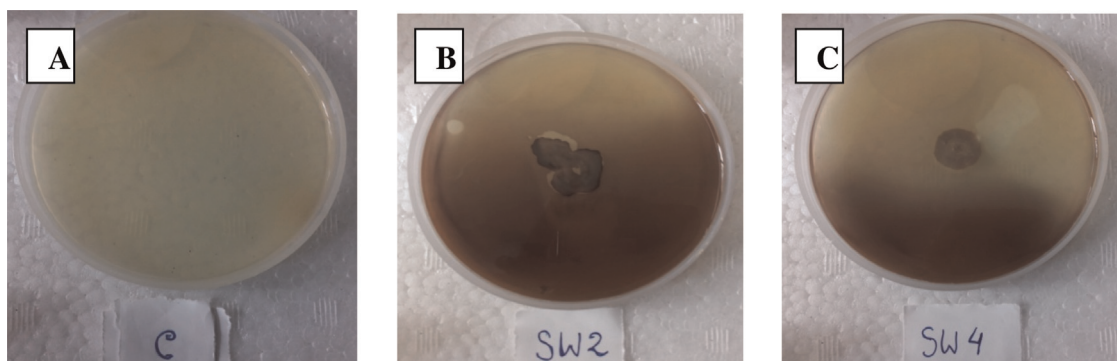


Fig. 5. Arsenite oxidation (A) arsenite plate without bacteria, (B) arsenite plate inoculated with isolate SW2 and (C) arsenite plate inoculated with isolate SW4.

and with increasing concentration of the toxic metals they gain more energy and their growth also enhanced [37,38]. These two bacteria were arsenic oxidizing bacteria. The increased growth pattern of the bacteria with increasing arsenite concentration suggested that the bacteria get additional energy from the oxidation process [39]. Once the bacteria converted the arsenite to arsenate, they easily resist its effect as they are already resistant to higher concentration of arsenate.

Both the isolates were Gram-positive in nature. The thicker cell wall of the Gram-positive cells might resist the toxic arsenic to enter inside the cell which may cause damage to the internal cell organelles and DNA [40]. Although Na^+ ions are not essential for the growth of the two isolated bacteria, their growth was optimized in the media containing 4–6% of sodium chloride. Similar results were reported by Brettar et al. [41] and Pal et al. [42] for *Rheinheimera baltica* and *Bacillus flexus*, respectively.

The phylogenetic analysis of the isolate SW2 revealed that the strain *Bacillus* sp. (KT462575) branched with *Bacillus cereus* strain DC3 (GQ344805) and *Bacillus* sp. NIOT-3 (AM981260) with 76% bootstrap value and 67% with *Bacillus* sp. KM02 (KJ948672). From the phylogenetic analysis of isolate SW4, it can be observed that the strain branched with *Aneurinibacillus* sp. AT8 (FJ821593), *Aneurinibacillus migulanus* 2012BaDB21 (JX041916), *Aneurinibacillus migulanus* A72 (GU3386), *Aneurinibacillus migulanus* NBRC 15520 (AB680889), *Aneurinibacillus migulanus* DSM 2895 (AB112723), *Aneurinibacillus migulanus* G1 (JQ337949), *Aneurinibacillus migulanus* ABRI1 (JN252029), *Aneurinibacillus migulanus* BJ-44 (GQ280054), *Aneurinibacillus migulanus* U603 (DQ350838), *Bacillus migulanus* (X94195) and *Aneurinibacillus migulanus* HCB4 (KF534472) with 100% bootstrap value. It also made branch with *Aneurinibacillus aneurinilyticus* DM-TSB-1 (JX290554) and *Aneurinibacillus aneurinilyticus* RPS2 (HQ659710) with bootstrap value of 97%.

The isolate SW2 and SW4 can remove 51.45% and 51.99% arsenite and 53.29% and 50.37% arsenate, respectively from the media after 72 h of incubation. Arsenic resistant microbes can reduce the arsenic concentration from the media by developing a number of detoxifying mechanisms including metal reduction, metal efflux, bacterial cell membrane binding, adsorption of heavy metals on to cell surface and complexation of the metal with exopolysaccharides [34]. Complexation of heavy metal with carboxyl and phosphate group in the inner portion of metal resistant bacteria has also been reported as a mechanism of bioremediation [43,44]. In addition, arsenic resistant bacteria sometimes use both arsenite and arsenate as their natural primary substrate by some specific genes [41].

From SEM study it was observed that both SW2 and SW4 formed chain like arrangement when treated with arsenic and the isolate SW2 also reduced in size in comparison to that of control cells. These structural changes might be the response of the bacterial cells to heavy metal and as a result of accumulation of the heavy metals into the cell. Similar strategy of defense against arsenic was also reported by Banerjee et al. [7].

Both the bacteria were able to oxidize arsenite to less toxic form arsenate but none of them has the ability to reduce arsenate to arsenite. The arsenic resistant bacteria have some specific enzymes, arsenic reductase and arsenic oxidase by which they can oxidize or reduce arsenic. Some bacteria have been reported for containing both these enzymes and can oxidize as well as reduce arsenic [7]. But these two bacteria contain only arsenite oxidase, so that they are only able to oxidize it. *Brevibacillus brevis*, an arsenic resistant bacterium, isolated from arsenic contaminated regions of Chakdah, West Bengal [42] was also able to oxidize arsenite to arsenate.

5. Conclusions

Thus from this entire study, it can be concluded that the two bacterial isolates SW2 and SW4, which were identified as *Bacillus* sp. KM02 and *Aneurinibacillus aneurinilyticus* strain BS-1, can tolerate arsenate concentration up to 4500 ppm and 550 ppm of arsenite concentration. The isolates were halo tolerant and can withstand up to 10 and 8% of sodium chloride. The isolate SW2 and SW4 can remove 51.45% and 51.99% of arsenite and 53.29% and 50.37% of arsenate respectively from arsenic amended media at 72 h. Both of the isolates have the unique ability to oxidize arsenite to less toxic arsenate but none of them were able to reduce arsenate. These two arsenic resistant bacteria can be used as a novel pathway for the bioremediation of arsenic.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.btre.2016.02.002>.

References

- [1] D. Elangovan, M.L. Chalkh, Arsenic pollution in West Bengal, *Tech. Dig.* 9 (2006) 31–35.
- [2] B.K. Mandal, K.T. Suzuki, Arsenic around the world: a review, *Talanta* 58 (2002) 201–235.
- [3] P.L. Smedley, D.G. Kinniburgh, A review of the source, behaviour and distribution of arsenic in natural waters, *Appl. Geochem.* 17 (2002) 517–568.
- [4] A.R. Achour, P. Bauda, P. Billard, Diversity of arsenite transporter genes from arsenic-resistant soil bacteria, *Res. Microbiol.* 158 (2007) 128–137.
- [5] R.A. Shrestha, B. Lama, J. Joshi, M. Sillanpaa, Effect of Mn(II) and Fe(II) on microbial removal of arsenic(III), *Environ. Sci. Pollut. Res.* 15 (2008) 303–307.
- [6] A.H. Khan, S.B. Rasul, A. Munir, M. Habibuddowla, M. Alauddin, S.S. Newaz, A. Hussan, Appraisal of a simple arsenic removal method for groundwater of Bangladesh, *J. Environ. Sci. Health* 35 (2000) 1021–1041.
- [7] S. Banerjee, S. Datta, D. Chattopadhyay, P. Sarkar, Arsenic accumulating and transforming bacteria isolated from contaminated soil for potential use in bioremediation, *J. Environ. Sci. Health Part A* 46 (2011) 1736–1747.
- [8] E.D. Rhine, C.D. Phelps, L.Y. Young, Anaerobic arsenic oxidation by novel denitrifying isolates, *Environ. Microbiol.* 8 (2006) 899–908.
- [9] M.M. Bahar, M. Megharaj, R. Naidu, Arsenic bioremediation potential of a new arsenite-oxidizing bacterium *Stenotrophomonas* sp. MM-7 isolated from soil, *Biodegradation* 23 (2012) 803–812.
- [10] D.D. Simeonova, K. Mischeva, D.A.E. Muller, F. Lagarde, M.C. Lett, V.I. Groudeva, D. Lievreumont, Arsenite oxidation in batch reactors with alginate-immobilized ULPA1 strain, *Biotechnol. Bioeng.* 91 (2005) 441–446.
- [11] S.P. Kamaluddin, K.R. Arunkumar, S. Avudainayagam, K. Ramasamy, Bioremediation of chromium contaminated environments, *Ind. J. Exp. Biol.* 41 (2003) 972–985.
- [12] J. Qin, B.P. Rosen, Y. Zhang, G. Wang, S. Franke, C. Rensing, Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite-S-adenosyl-methionine methyltransferase, *Proc. Natl. Acad. Sci. U. S. A.* 103 (2006) 2075–2080.
- [13] V. Andreoni, R. Zanchi, L. Cavalca, A. Corsini, C. Romagnoli, E. Canzi, Arsenite oxidation in ancylobacter dichloromethanicus As3-1b strain: detection of genes involved in arsenite oxidation and CO₂ fixation, *Curr. Microbiol.* 65 (2012) 212–218.
- [14] R. Chowdhury, A.K. Sen, P. Karak, R. Chatterjee, A.K. Giri, K. Chaudhuri, Isolation and characterization of an arsenic-resistant bacterium from a bore-well in West Bengal, India, *Ann. Microbiol.* 59 (2009) 253–258.
- [15] S. Shivaji, K. Suresh, P. Chaturvedi, S. Dube, S. Sengupta, *Bacillus arsenicus* sp. nov., an arsenic-resistant bacterium isolated from a siderite concretion in West Bengal, India, *Int. J. Syst. Evol. Microbiol.* 55 (2005) 1123–1127.
- [16] P. Roy, N.K. Mondal, B. Das, K. Das, Arsenic contamination in groundwater: a statistical modeling, *J. Urban Environ. Eng.* 7 (2013) 24–29.
- [17] American Public Health Association (APHA), Standard Methods for the Examination of Water and Wastewater, 21st ed., American Public Health Association, Washington DC, 2005.
- [18] M.J. Pelczar, R.C. Bard, G.W. Burnett, H.J. Conn, R.D. Demoss, E.E. Euans, F.A. Weiss, M.W. Jennison, A.P. Meckee, A.J. Riker, J. Warren, O.B. Weeks, Manual of Microbiological Methods, Society of American Bacteriology, McGraw Hill Book Company, New York, 1957, pp. 315.
- [19] A.E. Brown, Benson's Microbiological Applications, Laboratory Manual in General Microbiology, Short Version, tenth ed., The McGraw Hill Companies, 2007, pp. 50.
- [20] T.H. Jukes, C.R. Cantor, Evolution of protein molecules, in: H.N. Munro (Ed.), Mammalian Protein Metabolism, Academic Press, New York, 1969.
- [21] N. Saitou, M. Nei, The neighbor-joining method: a new method for reconstructing phylogenetic trees, *Mol. Biol.* 4 (1987) 406–425.
- [22] U. Dey, K. Das, P. Roy, S.N. Chatterjee, N.K. Mondal, Searching of microbial agent for bioremediation of arsenic, *Int. J. Ext. Res.* 5 (2015) 60–64.
- [23] World Health Organization (WHO), Guidelines for drinking water quality, second ed., Surveillance and Control of Community Supplies, vol. 3, World Health Organization, Geneva, Switzerland, 1997, pp. 113.
- [24] T.G. Kazi, M.B. Arain, M.K. Jamali, N. Jalbani, H.I. Afridi, R.A. Sarfraz, J.A. Baig, A. Q. Shah, Assessment of water quality of polluted lake using multivariate statistical techniques: a case study, *Ecotoxicol. Environ. Saf.* 72 (2009) 301–309.
- [25] United States Public Health Service (USPHS), Drinking water standards, *J. Am. Water Works Assoc.* 53 (1961) 935–945.
- [26] S. Honschopp, N. Brunken, A. Nehr Kornl, H.J. Breunig, Isolation and characterization of a new arsenic methylating bacterium from soil, *Microbiol. Res.* 151 (1996) 37–41.
- [27] A. Huang, M. Teplitski, B. Rathinasabapathi, L. Ma, Characterization of arsenic-resistant bacteria from the rhizosphere of arsenic hyperaccumulator *Pteris vittata*, *Can. J. Microbiol.* 56 (2010) 236–246.
- [28] T.F.C. Mah, G.A. O'Toole, Mechanisms of biofilm resistance to antimicrobial agents, *Trends Microbiol.* 9 (2001) 34–39.
- [29] K.J. Tsai, C.M. Hsu, B.P. Rosen, Efflux mechanisms of resistance to cadmium, arsenic and antimony in prokaryotes and eukaryotes, *Zool. Stud.* 36 (1997) 1–16.
- [30] A. Carlin, W. Shi, S. Dey, B.P. Rosen, The ars operon of *Escherichia coli* confers arsenical and antimomial resistance, *J. Bacteriol.* 177 (1995) 981–986.
- [31] B.P. Rosen, Biochemistry of arsenic detoxification, *FEBS Lett.* 529 (2002) 86–92.
- [32] C.R. Anderson, G.M. Cook, Isolation and characterization of arsenate-reducing bacteria from arsenic contaminated sites in New Zealand, *Curr. Microbiol.* 48 (2004) 341–347.
- [33] J.L.J. Zelibor, M.W. Doughten, D.J. Grimes, R.R. Colwell, Testing for bacterial resistance to arsenic in monitoring well water by the direct viable counting method, *Appl. Environ. Microbiol.* 53 (1987) 2929–2934.
- [34] C.U. Anyanwu, C.E. Ugwu, Incidence of arsenic resistant bacteria isolated from a sewage treatment plant, *Int. J. Basic Appl. Sci.* 10 (2010) 64–78.
- [35] S.P. Bachate, L. Cavalca, V. Andreoni, Arsenic-resistant bacteria isolated from agricultural soils of Bangladesh and characterization of arsenate-reducing strains, *J. Appl. Microbiol.* 107 (2009) 145–156.
- [36] S. Banerjee, A. Majumdar, A.K. Samal, P. Bhattachariya, S.C. Santra, Biotransformation and bioaccumulation of arsenic by *Brevibacillus brevis* isolated from arsenic contaminated region of West Bengal, *IOSR J. Environ. Sci. Toxicol. Food Technol.* 3 (2013) 1–10.
- [37] R.S. Oremland, J.F. Stolz, The ecology of arsenic, *Science* 300 (2003) 939–944.
- [38] J.F. Stolz, P. Basu, J.M. Santini, R.S. Oremland, Arsenic and selenium in microbial metabolism, *Annu. Rev. Microbiol.* 60 (2006) 107–130.
- [39] D. Mullar, D. Livermort, D.D. Simeonova, J.C. Hubert, M.C. Lett, Arsenite oxidase aox genes from a metal resistant beta-proteobacterium, *J. Bacteriol.* 185 (2009) 135–141.
- [40] G.A. Berlanga, M.W. Persans, T.M. Eubanks, K.L. Lowe, Characterization of arsenic-tolerant bacterial cultures from the Lower Laguna Madre of south Texas, *Tex. J. Sci.* 61 (2009) 1–24.
- [41] I. Brettar, R. Christen, M.G. Hofle, *Rheinheimera baltica* gen. nov., sp. nov., a blue-coloured bacterium isolated from the central Baltic Sea, *Int. J. Syst. Evol. Microbiol.* 52 (2002) 1851–1857.
- [42] K.C. Pal, N.K. Mondal, S.N. Chatterjee, T.S. Ghosh, J.K. Datta, Characterization of fluoride-tolerant halophilic *Bacillus flexus* NM25 (HQ875778) isolated from fluoride-affected soil in Birbhum District, West Bengal, India, *Environ. Monit. Assess.* 186 (2013) 699–709.
- [43] L. Fang, X. Wei, P. Cai, Q. Huang, H. Chen, W. Liang, X. Rong, Role of extracellular polymeric substances in Cu(II) adsorption on *Bacillus subtilis* and *Pseudomonas putida*, *Bioresour. Technol.* 102 (2011) 137–141.
- [44] M. Rajkumar, S. Sandhya, M.N.V. Prasad, H. Freitas, Perspectives of plant associated microbes in heavy metal phytoremediation, *Biotechnol. Adv.* 30 (2012) 1562–1574.