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Biotransformation of arsenic by bacterial strains mediated by oxido-reductase enzyme system

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Abstract

The present study deals with the enzyme mediated biotransformation of arsenic in five arsenic tolerant strains (*Bacillus subtilis*, *Bacillus megaterium*, *Bacillus pumilus*, *Paenibacillus macerans* and *Escherichia coli*). Biotransformation ability of these isolates was evaluated by monitoring arsenite oxidase and arsenate reductase activity. Results showed that arsenic oxidase activity was exclusively present in *P. macerans* and *B. pumilus* while *B. subtilis*, *B. megaterium* and *E. coli* strains showed presence of Arsenic oxido-reductase enzyme. The reversible nature of arsenic oxido- reductase suggested that same enzyme can carry out oxidation and reduction of arsenic depending upon the relative concentration of arsenic species. Lineweaver-Burk plot of the arsenite oxidase activity in *P. macerans* showed highest K_m value (K_m- 200 µM) and lower V_{max} (0.012 µmol mg^{-1} protein min^{-1}) indicating lowest affinity of the enzyme for arsenite. On the contrary, *E. coli* showed the lower K_m value (K_m- 38.46 µM) and higher V_{max} (0.044 µmol mg^{-1} protein min^{-1}) suggesting for higher affinity for the arsenite. Lineweaver-Burk plot of arsenate reductase activity showed the presence of this enzyme in *B. subtilis*, *B. megaterium* and *E. coli* which were in the range of 200-360 µM K_m and V_{max} value between 0.256- 0.129 mmol mg^{-1} protein min^{-1}. These results suggested that affinity of the as reductase enzyme is lowest for arsenate than that for the arsenite. Thus, arsenite oxidase system appears to be a predominant mechanism of cellular defense in these bacterial strains.

Key words: Arsenic, Bacteria, Detoxfication, Enzyme activity, Lineweaver-Burk plot.

Introduction

Arsenic is a toxic metalloid of global concern. It is usually originated geogenically but can be intensified by human activities suchas applications of pesticides and wood preservatives, mining and smelting operations, and coal combustion (1). Consequentially, elevated levels of arsenic have been reported in soils and groundwater worldwide. The permissible Arsenic concentration recommended by WHO for drinking water is 10 mg L^{-1}. Elevated levels of arsenic in drinking water can seriously impact human health and have been implicated in human diseases and mortality (2, 3, 4, 5). The worst arsenic calamity in groundwater has been reported in West Bengal and Bangladesh, where groundwater is the main source for drinking water. Around 6 million people in West Bengal and more than 46 million people in Bangladesh are estimated to be at risk from drinking water with arsenic above 50 mg L^{-1} (6, 7).

Arsenic is lethal to most of the microorganisms, yet certain bacteria can use arsenic compounds as electron donors or electron acceptors, and that they can possess arsenic detoxification mechanisms (8,9,10).Microorganisms have evolved a variety of mechanisms for coping with arsenic toxicity, including minimizing the amount of arsenic that enters the cell such as through increased specificity of phosphate uptake system (11, 12, 13) transformation of arsenic species through (14, 15, 16) reduction(15, 17) and methylation (18).Certain microorganism have evolved the necessary genetic components which confer resistance against arsenic and allows them to survive and grow in environments containing high levels of arsenic usually toxic to the most of other organisms(19, 20).This physiologically diverse groups of microbes include both heterotrophic and chemolitho autotrophic arsenite oxidizers (14). Bacterial oxidation of arsenite to arsenate represents a potential partial detoxification mechanism (21) where it generates less toxic and less mobile form of arsenic. Microorganisms combat the collateral importation of arsenate by a two-step mechanism in which this compound is first reduced to arsenite. Although arsenite is a more potent toxicant than arsenate, the former can be selectively banished from the cell's interior through the intervention of a dedicated, inducible transporter (22).

The present study is an attempt to study the biotransformation potential of five bacterial isolates (i.e. *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus pumilus*, *Paenibacillus macerans* and *Escherichia coli*) selected on the basis of their tolerance to high level of arsenic. Arsenite oxidase and reductase activities were monitored in these strains to evaluate their arsenic biotransformation ability.

Materials and methods

Isolation and Screening of Bacterial isolates

The Bacterial strains selected for this study were isolated from arsenic contaminated soil of Lakhimpur kheri (27˚57'0"N and 80˚46'0"E) and Unnao district (26˚32'0"N and 80˚30'0"E), Uttar Pradesh (India). The soil samples were collected from a depth of 15 cm in sterile plastic bags. Arsenic tolerant bacteria were isolated from collected soil samples through enrichment technique. In this technique 2 g of each collected soil sample was dissolved in 20 ml of 0.9 % NaCl and shaken for

3 minutes. Then 5 ml of soil suspension was inoculated into 50 ml BSMY I (23) containing 5 mM sodium arsenite and incubated at room temperature on rotary shaker at 120 rpm for 3 days. Five ml culture was transferred to a fresh BSMY I medium containing 10 mM sodium arsenite, and then transferred twice into new medium that was supplemented with 20 and 40 mM sodium-arsenite, respectively. For isolation of arsenic tolerant bacteria, 0.1 ml of culture was spreaded on BSMY agar plates that contained 40 mM sodium-arsenite and incubated at 30°C for 3 days.

Identification of Bacterial Isolates

The bacterial isolates were identified first by using standard morphological and biochemical tests (24) and then send for 16S rDNA sequencing at Genetech, Biotech Park Lucknow (U.P., India). The gene sequences of these strains were submitted at NCBI for obtaining Accession number.

Arsenic transformation assay

Qualitative and quantitative test was carried out for determining the presence of enzyme oxidase/reductase in these fivearsenite tolerant and 1 control bacteria isolated from control (garden soil) site.Arsenic transformation ability of these strains was evaluated by monitoring arsenite oxidase and arsenate reductase activity. $AgNO_3$ method and $KMnO_4$ method are the two qualitative tests employed for determining the arsenic transformation ability of bacterial isolates. Positive results of these tests showed the presence of enzyme arsenite oxidase.

Qualitative test for arsenite oxidase activity by $AgNO_3$ method

The transforming ability of five isolates along with control was carried out by using $AgNO_3$ method (25). Silver nitrate test was based on the quality reaction between $AgNO_3$ and arsenite or arsenate ions. Bacterial colonies on agar plates supplemented with Arsenite were flooded with a solution of 0.1 M $AgNO_3$. A brownish precipitate revealed the presence of arsenite oxidase activity, while colonies without arsenite oxidase activity were detected by a bright yellow precipitate (26). The arsenite transforming ability was further monitored also in liquid medium. The bacterial suspensions were incubated at room temperature for 72 h in presence of arsenite (100 $\mu g ml^{-1}$). Subsequently, the bacterial cultures were centrifuged, and 100 µl of the liquid phase was mixed with 100 µl of a 0.1 M $AgNO_3$ solution. The resulting precipitates containing arsenic were from light yellow due to unoxidized As (III) to light brown red colour due to oxidized form As (V) (27).

Qualitative test for arsenite oxidase activity by $KMnO_4$ method

Each arsenite- resistant bacteria and a control bacteria was inoculated in broth with a final concentration of 800 µM $NaAsO_2$ and then shaken at 160 rpm for 5 days at 28°C. For each isolate 1 ml culture was added to a 1.5 ml centrifuge tube containing 30 µL of 0.01 M $KMnO_4$ and the colour change of $KMnO_4$ was monitored. A pink colour of the mixture indicated a positive arsenite oxidation reaction and formation of As (V) (28).

Quantitative estimation of Arsenite oxidase activity

Bacteria grown in the presence of As (III) (100 mg L^{-1}) were centrifuged, resuspended in 20 mMTris- HCl, 0.1 mM Phenylmethylsulfonyl fluoride, 10 mM EDTA (pH 8.4) containing 20% sucrose, and treated with 0.4 mg of lysozyme ml^{-1} for 40 min at 25°C. Arsenite oxidase activity was determined based on the transfer of reducing equivalents from arsenite to 2,4- dichlorophenolindophenol (DCIP). Reduction of DCIP (60 µM) was monitored at 600 nm in presence of 200 µM sodium arsenite in 50 mM morpholine ethane sulfonic acid (MES buffer), pH 6.0, at 25°C. Specific activity was defined as µmol DCIP reduced mg^{-1} protein min^{-1} as described by (29).

Quantitative estimation of Arsenate reductase activity

In order to determine the ability of bacterial isolates to reduce As (V) to As (III), the NADPH oxidation method was used (30). Bacteria were grown to mid-log phase in 200 ml of NB medium supplemented with 50 mM of arsenate, harvested by centrifugation for 10 min at 5,000 rpm, and washed twice in 25 ml reaction buffer (10 mMTris, pH 7.5, with 1 mM Na_2EDTA and 1 mM $MgCl_2$).The cells were resuspended in 5 ml of reaction buffer, disrupted by sonication and cell free extract was prepared by centrifugation at 5,000 rpm for 10 min at 4°C. Arsenate reductase activity was measured using a method based on NADPH oxidation. The reaction was initiated at 37°C by mixing 50 µl of cell-free crude extract in 820 µl of reaction buffer, 20 µl of 10 mM DTT (Dithiothreitol,final concentration 300 µl), and 50 µl of 3 mM NADPH (Nicotinamide adenine dinucleotide phosphate,final concentration 0.15 mM). Arsenate concentrations from 100-1000µM were assayed, using control set without arsenic. A decrease in absorbance at 340 nm was recorded as the NADPH oxidation was coupled to the reduction of arsenate to arsenite. Enzyme activity was calculated using molar extinction coefficient of 6.2 X 10^3 for $NADP^+$. The auto oxidation of NADPH occurring in the control set was subtracted from the arsenate-induced NADPH oxidation.

Enzyme kinetics

Kinetics parameters like K_m and V_{max} value of the enzyme were calculated using Lineweaver- Burk plot (31). This plot was constructed between specific activity of oxidase/reductase vs. arsenite/arsenate concentration. The data were derived from Michaelis–Menten kinetics of enzyme activity. K_m and V_{max} were calculated from the Lineweaver- Burk plots using 1/intercept on 'x' axis and 'y' axis.

Chemicals and Equipment

All chemicals used are of analytical reagent (AR) grade and were product of Loba Chemicals Pvt Ltd. All aqueous solutions were prepared in double distilled water. Stock of As (III) and As (V) was prepared by dissolving sodium arsenite and sodium arsenate in sterile deionized Milli Q water, stored at 4°C in dark. Spectrophotometric readings were taken by a Double beam UV-visible spectrophotometer (Shimadzu, 1601, Japan).

Statistical Analysis

All the experiments were conducted in triplicates

Table 1. Qualitative Test for Arsenite Oxidation.

Bacterial Isolates	Arsenite Oxidation in cell	
	By $AgNO_3$ method	By $KMnO_4$ method
Control	-	-
Bacillus subtilis	+	++
Paenibacillusmacerans	+++	+++
Bacillus megaterium	++	-
Bacillus pumilus	+++	++
Escherichia coli	++	++

and the reported value for each studied parameter are means ±SD. Data were analyzed by one way analysis of variance (ANOVA) at $p < 0.05$. Statistically significant data are shown by different alphabets by using Duncan multiple range test (DMRT) using the SPSS software (Version 7).

Results

Isolation and Screening of arsenic tolerant bacteria

The soil enrichment technique yielded 28 bacterial isolates selected on the basis of different colony characteristics. These 28 bacterial cultures were further screened on the basis of spot test. Among 28 isolates, 10 bacterial isolates showed growth in the presence of 100 mM Arsenite, tested by using gradient plates assay. These 10 strains were further screened for their arsenite tolerance at a fix concentration of 40 mM and finally 5 bacteria were selected on the basis of luxuriant growth at 40 mM concentration in the presence of arsenite. One bacteria was also isolated from the garden soil of university campus, which was not able to tolerate even the low doses of Arsenic. This bacterium was assigned as control bacteria for further study.

Identification of Isolated Bacterial Strains

The fivearsenic tolerant bacterial strains along with their accession no. are as follows:*Bacillus subtilis* (Accession no. KC625596) and *Paenibacillus macerans* (Accession no. KC633280) isolated from Lakhimpur Kheri district (U.P.), *Bacillus megaterium* (Accession no.KC633281), *Bacillus pumilus* (Accession no. KC633283) and *Escerichia coli* (Accession no. KC633282) isolated from Unnao district (U.P.).

Qualitative screening of bacterial strains for arsenite oxidation

The arsenite-tolerant bacterial strains *B. subtilis*, *P. macerans*, *B.megaterium*, *B. pumilus, E. coli* and control bacteria were tested for their abilities to oxidize As (III) using $AgNO_3$ and $KMnO_4$ as qualitative screening method. In the $AgNO_3$ method, a brownish precipitate formed by bacterial colony indicated a positive arsenite oxidation reaction. In $KMnO_4$ screening method, a dark pink colour of the mixture indicated a positive reaction. The intensity of colour change is indicated by + sign and no change in colour is indicated by – sign.

In the presence of $AgNO_3$ all the bacteria showed colour change (except control bacteria) which indicates the presence of oxidase activity in these strains but the intensity of change in colour was different. *P.macerans* showed dark brown precipitate whereas *B.megaterium*, *B.pumilus* and *E.coli* showed almost equal intensity of colour changed however, there was little yellowish colour shown by *B. subtilis* (Table 1).

When arsenite oxidation activity of bacterial strains was checked by $KMnO_4$ method, there was no colour change in the case of *B. megaterium.* Bacterial strains *B. pumilus, B. subtilis*and *E.coli* showed almost equal intensity of colour change but *P.macerans* showed maximum colour change, suggesting for higher arsenite oxidase activity. Bacteria isolated from the control site did not show colour change in the presence of $AgNO_3$ and $KMnO_4$ which depicted that this bacterium does not oxidizes arsenite to arsenate.

Arsenite Oxidase Activity

The specific arsenite oxidase activity of five bacterial strains (*B. subtilis, P. macerans, B. megaterium, B. pumilus* and *E. coli*) was measured at different concentration (50-600 µM) of arsenite. As control bacteria doesn't showed positive results in qualitative test it was not further analyzed for specific arsenite oxidase activity. The results (Fig.1) showed highest arsenite oxidase activity in *P. macerans* and *B. pumilus.* The *B. subtilis*, *E. coli* and *B. megaterium* exhibited a declining pattern in the As oxidase activity with increasing concentration of arsenite. The decreasing order of arsenite oxidase activity was recorded as *P. macerans>B. pumilus>B. subtilis>B. megaterium> E. coli* at 500 µM concentration of arsenite. At low concentration of arsenite (<200 µM), it was observed that oxidase activity was almost equal in *B. megaterium*, *E. coli* and *B. subtilis.* But at 600 µM concentration of arsenite, the oxidase activity decreased by 2, 4 and 1.33 folds in *B. megaterium*, *E. coli* and *B. subtilis*, respectively whereas there was almost no effect of arsenite concentration on oxidase activity in case of *P. macerans* and *B. pumilus.*

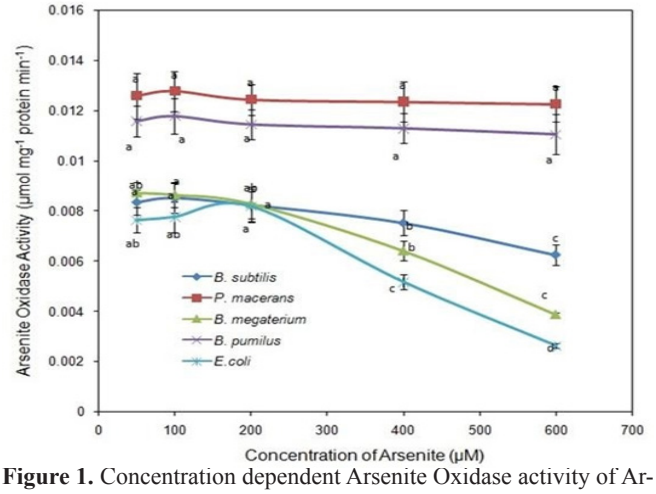

Figure 1. Concentration dependent Arsenite Oxidase activity of Arsenic tolerant bacterial strains. Data are the mean of three replicates ± SD. Data was analyzed by one way analysis of variance (Duncan Multiple Range Test) at $p<0.05$. Different alphabets show significant differences between the treatments.

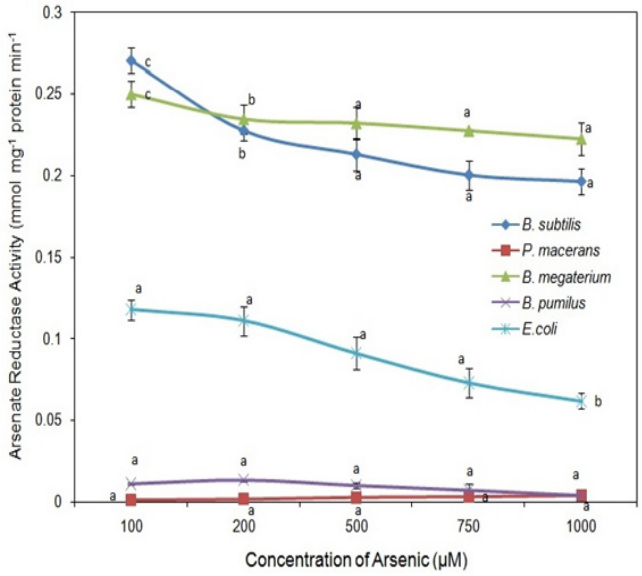

Figure 2. Concentration dependent Arsenate Reductase activity of Arsenic tolerant bacterial strains. Data are the mean of three replicates ± SD. Data was analyzed by one way analysis of variance (Duncan Multiple Range Test) at $p<0.05$. Different alphabets show significant differences between the treatments.

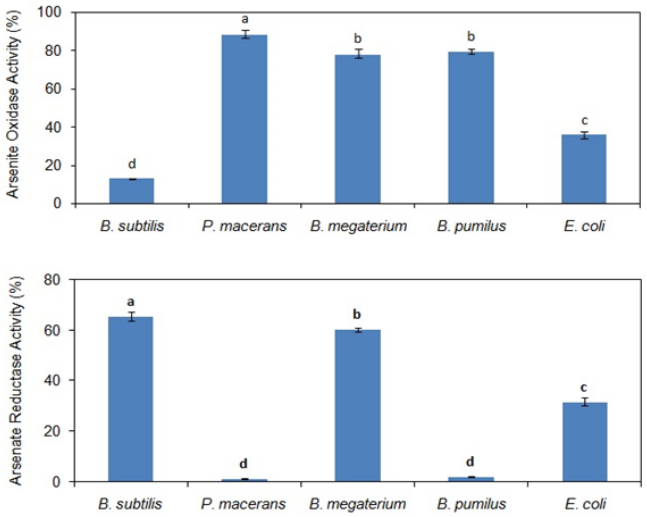

Figure 3. Oxidase and Reductase Activity of Bacterial Strains at 200 µM Arsenic. Data are the mean of three replicates ± SD. Data was analyzed by one way analysis of variance (Duncan Multiple Range Test) at $p<0.05$. Different alphabets show significant differences between the treatments.

Arsenate reductase activity

Arsenate reductase activityof five bacterial strains (*B. subtilis, P. macerans, B. megaterium, B. pumilus* and *E. coli*) was measured at different concentration (100-1000 µM) of arsenate. The results (Fig.2) showed that the reductase activity was highest in the bacterial strains *B. megaterium* and *B. subtilis*. The bacterial strain *E. coli* showed lower rate of arsenate reductase activity, than that recorded in *B. megaterium* and *B. subtilis* at 500 µM of arsenate. Further, *P. macerans* and *B. pumilus* showed little or no Arsenate reductase activity when compared with other strains.

Comparison of arsenic Oxidase/ Reductase activity

The percent oxidase and reductase activity of five bacterial strains (*B. subtilis*, *P. macerans*, *B. megaterium*, *B. pumilus* and *E. coli*) was compared at a fix concentration of 200 µM arsenic. The result (Fig.3) showed that percent oxidase activity was highest in *P.macerans* (88.69 %) and was found to be lowest in *E. coli* (35.71 %). The decreasing pattern of oxidase activity was observed as *P. macerans* (88.69 %) >*B. pumilus* (79.61 %) >*B. megaterium* (78.20 %) >*E. coli* (35.71 %)>*B. subtilis* (12.76 %). Percent reductase activity was found to be the highest in *B. megaterium* (60.25 %) as compared to other bacterial strains whereas *P. macerans* (1.14 %) and *B. pumilus* (2.15 %) did not show significant arsenate reductase activity. The decreasing order of reductase activity was observed as *B. subtilis* (65.57 %) >*B. megaterium* (60.25 %)>*E. coli* (31.72 %)> *B. pumilus* (2.15 %) > *P. macerans* (1.14 %).

Oxidase/Reductase Activity at equimolar concentration (100 µM) of As(III) & As(V)

As evident from the above results, some of the bacterial strains like *P. macerans* and *B. pumilus* showed predominantly As oxidase activity. On the contrary *B. subtilis* exhibited an As reductase activity but *B. megaterium* and *E. coli* showed almost same level of As oxidase or As reductase activities. There might be possibility that both these bacterial strains have one enzyme which alternatively works as oxidase or reductase enzyme.

To see the reversibility of the enzyme depending upon the level of As (III) and As(V), the Arsenite oxidase and Arsenate reductase activities were monitored using equimolar concentration (100 µM,each) of both forms of As (Fig. 4). It was observed that arsenite oxidase and arsenate reductase activity of *B. megaterium* and *E. coli* were supressed by about 50 %,whereas in case of *B. subtilis*an equimolar concentration of As III and As V suppressed the activity by about 15 % only when compared with their respective control (arsenite/ arsenate, alone).In case of *P. macerans* and *B. pumilus*, no significant effect was observed on oxidase/reductase activity in the presence of equimolar conc. of As III and As V. The predominantly Arsenite oxidase activity in *P. macerans* and *B. pumilus* was also relatively unaffected by the presence of equimolar concentration of As (III) and As (V). But the arsenite oxidase and reductase activities in *B. megaterium* and *E. coli* with increase in molar concentration of their respective products resulted into feed back inhibition of enzyme activity. These results suggested a reversible nature of As oxidase/reductase in *B. megaterium*, *B. subtilis* and *E. coli*.

Enzyme Kinetics

When plot was constructed between arsenite concentration *vs* specific activity of oxidase enzyme and arsenate concentration *vs* reductase activity of enzyme, linear graphs were obtained in case of oxidase activity of all the five bacterial strains as shown in (Fig.5) while in case of reductase activity, linear graphs were obtained in only *B.subtilis*, *B.megaterium* and *E.coli* bacterial strains as shown in (Fig. 6). The values of K_{max} and V_{max} for both As oxidase and reductase activities were calculated from the Lineweaver- Burk plot. The results showed that V_{max} value for arsenite oxidase activity was the highest in *E. coli* (0.044 µmol mg^{-1} protein min^{-1}) whereas K_m value was found maximum for *P. macerans* (200 µM) and minimum in *E. coli* (38.66 µM) as shown in (Table2)**.** The results of arsenate reductase activity showed that out of five bacterial strains three bacterial

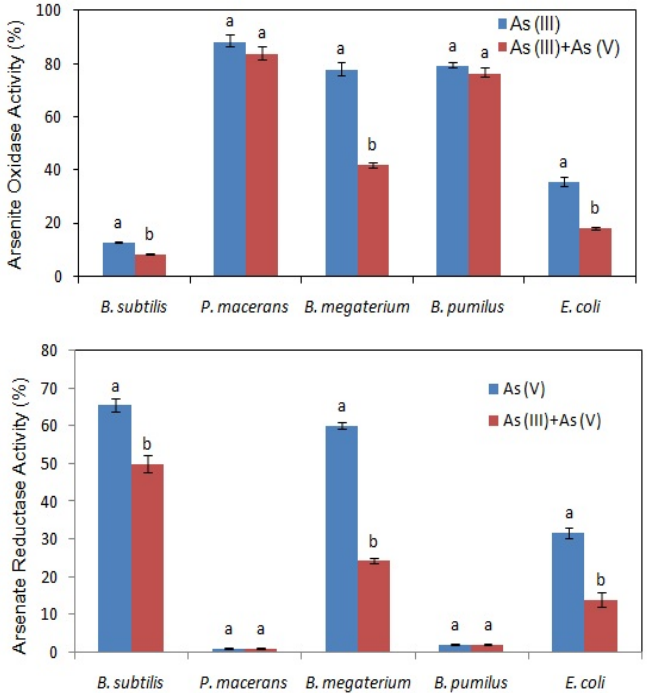

Figure 4. Percent Oxidase and Reductase Activity of Bacterial Strains at equimolar concentration (100 µM) of As (III) and As (V). Data are the mean of three replicates ± SD. Data was analyzed by one way analysis of variance (Duncan Multiple Range Test) at $p<0.05$. Different alphabets show significant differences between the treatments.

strains showed predominantly arsenate reductase activity. The V_{max} values of strains *B. subtilis*, *B. megaterium* and *E. coli* were found to be 0.238, 0.256 and 0.129 mmol (arsenate reduced) mg^{-1} protein min^{-1}, respectively. The K_m constant was found to be the maximum in *B. subtilis* (360 µM) and minimum in *E. coli* (200 µM).

Discussion

It is well known that microbes are able to develop tolerance against various toxic metals in order to protect themselves from toxic effect of these metals. Many gram positive and gram negative bacteria are known to employ different tolerance mechanism against arsenic toxicity (26). The two necessary components of arsenic tolerance include the membrane level barrier and intracellular defense (32, 27, 33, 34, 35, 36, 37).

In the present study five As tolerance bacterial strains (*Paenibacillus macerans, Bacillus pumilus, Bacillus megaterium, Bacillus subtilis* and *Escherichia coli*) were selected and screened for As oxidase/reductase enzyme activities. Out of thesefive bacterial strains, *P. macerans* and *B. pumilus* showed oxidation of As (III) to As (V). The arsenite oxidase system in bacteria represents a potential detoxification process that allow microorgamisms to tolerate higher level of arsenite. Other findings (38, 39, 32, 40, 34, 41) also revealed that arsenic resistance in microbes is usually due to the transformation of toxic arsenite into less toxic arsenate by As oxidase activity in bacteria. At higher concentration (600 µM) of As (III) only *P. macerans* and *B. pumilus* showed the oxidation of arsenite, but at lower concentrations of arsenite (200 µM), all the bacterial isolates showed arsenite oxidase activity to a varying extent. Percent oxidase activity of all the five bacterial strains at 200 µM concentration of arsenite, in decreasing order is given as *P.macerans* (88.69 %) > *B.pumilus* (79.61 %) >*B.megaterium* (78.20 %) >*E.coli* (35.71 %)>*B.subtilis* (12.76 %). It suggested that arsenite oxidase system is a general defense mechanism in arsenite tolerant bacteria

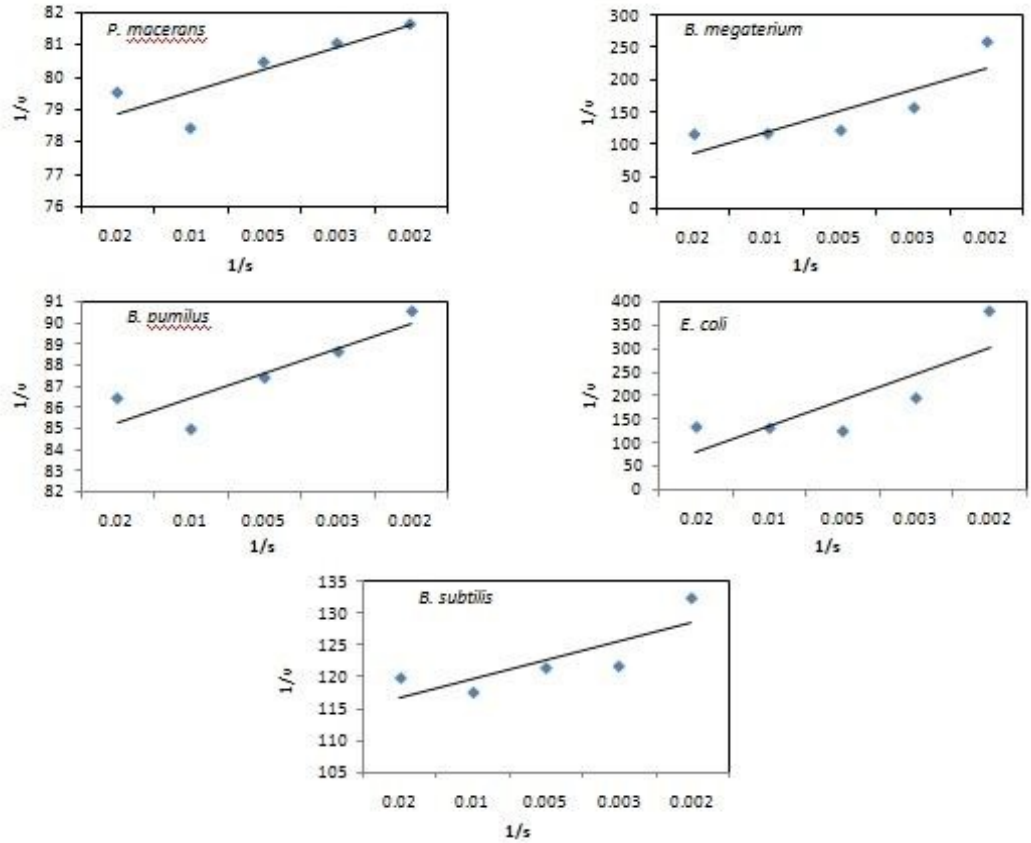

Figure 5. Lineweaver- Burk Plot for Arsenite Oxidase Activity.

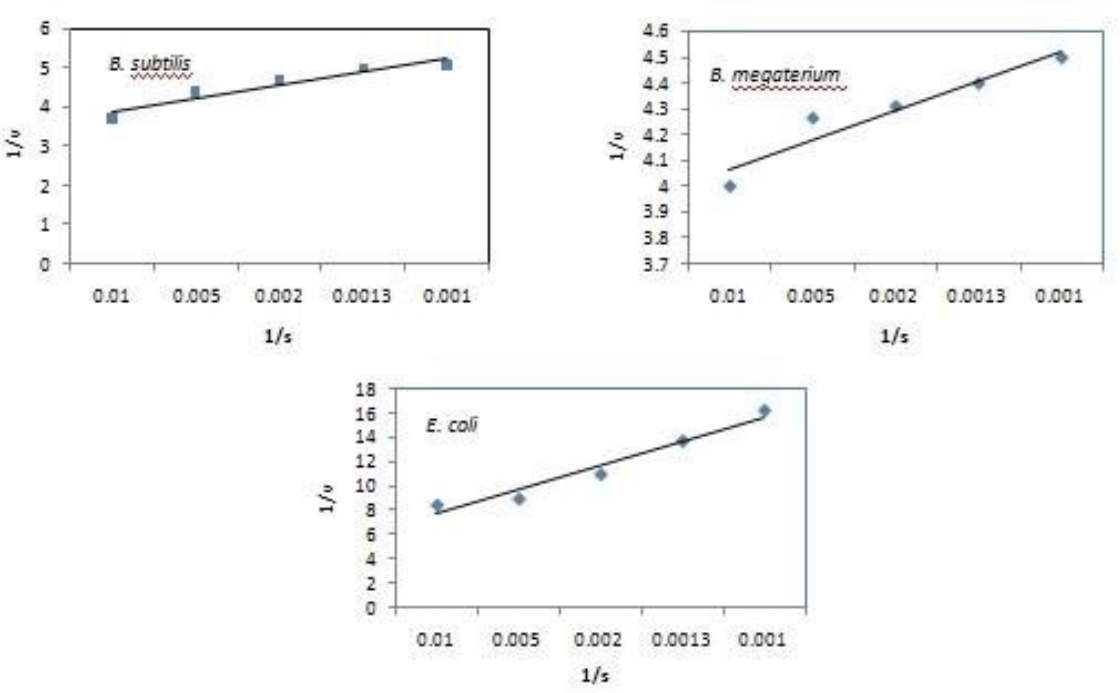

Figure 6. Lineweaver- Burk Plot for Arsenate Reductase Activity.

Table 2. $V_{max,}$ K_m value obtained from Lineweaver–Burk plot for As Oxidase/Reductase Enzymes.

Bacteria	Oxidase Activity		Reductase Activity	
	V_{max} (µmol mg^{-1}protein min^{-1})	K_m (µM)	V_{max} (mmol mg^{-1}protein min^{-1})	K_m (µM)
B. subtilis	0.008	66.66	0.238	360
P. macerans	0.012	200	-	-
B. pumilus	0.0119	83.33	-	-
B. megaterium	0.018	100	0.256	272
E. coli	0.044	38.46	0.129	200

against arsenite toxicity.

Arsenate (As V) reductase activity observed in all the five bacterial strains showed that only three strains *B. subtilis, B. megaterium* and *E. coli* are endowed with arsenate reductase activity. But *P. macerans* and *B. pumilus* did not exhibit arsenate reductase activity. When concentration dependent arsenate reductase activity was tested then it was found that at higher concentration (1000 µM) of As (V) only *B. megaterium* and *B. subtilis* showed reductase activity, whereasstrain *E.coli* showed reductase activity upto concentration of 500 µM of arsenate only. Many other workers (27, 30, 42, 43) have also demonstrated the arsenate reductase activity in bacteria and suggested that this activity was indicative of the conversion of arsenate to arsenite, which may be coupled with intracellular efflux mechanism for removal of arsenite (22, 44). Microbial reduction of As (V) is described to be an intracellular reductive mechanism coupled to detoxification of As (22, 43, 45, 46) or to dissimilatory reduction (respiration) (15, 47).

A Line weaver- Burk of the arsenite oxidase activity showed highest K_m value (K_m - 200 µM) and lower V_{max} (0.012 µmol (DCIP reduced) mg^{-1} protein min^{-1}) for *P. macerans*, indicating lowest affinity of the enzyme for arsenite. On the contrary, *E. coli* strain showed the lowest $K_m$value (K_m- 38.46 µM) and higherV_{max} (0.044 µmol (DCIP reduced)mg^{-1}protein min^{-1}) suggesting for higher affinity of the enzyme for the arsenite as compared to other strains. The K_m and V_{max} values obtained from Lineweaver- Burk plot of arsenate reductase activity showed that reductase activity was completely absent in *P. macerans* and *B. pumilus,*whereas other three bacterial strains exhibited Km forreductase enzyme in the range of0.238-0.129 mmol (Arsenate reduced) mg^{-1}proteinmin^{-1} with a K_m values in the range of 200- 360 µM. From these results, it may be deduced that affinity of the As reductase enzyme is the lowest for arsenate. Thus, arsenite oxidase appears to be a predominant mechanism of cellular defense in all the bacterial cells. The reversible nature of the oxido- reductase in *B. megaterium, B. subtilis* and *E. coli* suggested change inthe dyanamics of enzyme when the concentration of arsenite is equal to or higher than arsenate.Whereas *B. pumilus* and *P. macerans* did not show significant reversibility in their activities in presence of equimolar concentration of both As III and As V. They contain only Arsenite oxidase enzyme.

From the foregoing results, it was deduced that Arsenite tolerant bacteria survive very well on the arsenic contaminated sites as they have developed varying

intrinsic mechanisms to overcome the toxic effect of arsenic. Detoxification of Arsenic metal by the bacterial strains is found to be predominantly through arsenite oxidase activity. A variable anti-arsenic defense system in different bacteria might be an added advantage with this microbial world to cope with the environmental adversities. This study needs to be investigated further at molecular level.

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Other articles in this theme issue include references (48-63).

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