Possible Involvement of Siderophore Producing Staphylococcus sp. TA6 in arsenic geocycle of Brahmaputra delta

Saurav Das[1], Madhumita Barooah*[1]

[1]Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat, Assam, India

[2]Centre for Studies in Biotechnology, Dibrugarh University, Dibrugarh, Assam, India

Authors Name and Affiliations

1. **Saurav Das –** Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat – 785013, Assam. Email Id: saurav12das@gmail.com

Corresponding Author

2. **Madhumita Barooah –**Professor, Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat – 785013, Assam. Email id. – m17barooah@yahoo.co.in

Abstract:

Increased concentration of arsenic in the groundwater of Brahmaputra valley has been a matter of serious concern for the region. Although the presence of Arsenic in sediments and carbonaceous rocks are geogenic, its entry into the groundwater is mediated by several processes including microorganisms. It is well known that microorganisms play a crucial role in the biogeochemical cycle of elements. However, the precise role of bacteria in regulating the concentration of arsenic in Brahmaputra valley has not been investigated in detail. In this paper, we report the isolation of bacterium *Staphylococcus sp*. (TA6) that can reduce arsenate to arsenite. The isolate was able to grow in arsenate concentration exceeding ≥250 mM and arsenite up to ≤ 30 mM. It also showed cross-tolerance to other heavy metals like Hg^{+2}, Cd^{+2}, Co^{+2}, Ni^{+2}, Cr^{+2}. The bacterium also had a high siderophore activity (78.7 ± 0.004 µmol), which is positively correlated with increased arsenate reduction efficiency. The bacterium could significantly reduce ~88.2% of initial 2mM As(V) into As(III). The arsenate reductase enzyme showed the highest activity at pH 5.5 and temperature 50^0C. This bacterium can actively control the concentration of arsenate and arsenite through a reduction reaction.

Keywords: Arsenic, Arsenate reductase, Siderophore, *Staphylococcus sp*., Titabor

1.0 INTRODUCTION

Brahmaputra river basin is long known for its high geogenic arsenic (As) concentration. However, during the recent times, the problem has much aggravated as 23 out of the 32 districts in Assam have been reported to be affected by high Arsenic concentration [1,2]. Titabor subdivision of Jorhat district is considered as one of the most As affected areas of Assam with arsenic concentration 194-657 µg/l, which is far above the permissible standard of BSI (50 µg/l) and WHO (10 µg/l)

[3].Arsenic is a metalloid widely distributed in the earth's crust and its concentration can exist from traces to up to hundreds of mg/kg or mg/l in both soil and in water. In groundwater, the element is predominantly found in two states *viz.*, arsenate (AsV) and arsenite (AsIII). Arsenate is dominant in the oxic environment and gets strongly absorbed by chemicals like ferric-oxyhydroxide, ferrihydrite, apatite, and alumina. The arsenite form is dominant in an anoxic environment and is more mobile and toxic than arsenate [4].The geochemical cycling of arsenic is composite in nature; in fact, in addition to various physical and chemical factors, it also involves biological agents. Microorganisms play a critical role in mobilization and speciation of arsenical compounds in aquifer systems [5]. Microorganisms in the course of evolution have developed the necessary genetic makeup which confers them with resistance to high concentration of arsenic as well other toxic metalloids [6]. Microorganism arbitrated mechanisms of arsenic mobilization are still poorly understood and therefore, needs further studies to reveal their role in sediment-bound arsenic mobilization. They can either reduce, oxidize or can methylate the element to other organic compounds to generate energy [7]. Arsenate reducing bacteria are able to reduce As (V) to As (III) and use the same as an electron acceptor in a respiratory pathway or efflux the same as a mean of resistance mechanisms [8]. Arsenic resistant bacteria are frequently detected with siderophore activity. Siderophore are high-affinity iron chelating compounds produced and secreted by few microorganisms to forage the environmental iron from inorganic phase by formation of soluble Fe^{3+} complex, which can be taken up by active transport mechanisms [9]. The Fe sequestering ability of bacteria through siderophore production confers them with added advantages over the non-producers in mobilizing arsenic. The previous study has shown that the rate of arsenic uptake and reduction efficiency of a bacteria significantly varies with varied siderophore concentration [10].

In this paper, we report the isolation of a gram-positive bacterium TA6, which is resistant to both arsenate and arsenite. Based on its morphological, molecular and chemotaxonomic characterization the isolate was identified as a species of the genus *Staphylococcus* that had facilitated arsenate reduction along with siderophore activity.

2.0 MATERIALS AND METHODS

2.1 Sample Collection

Contaminated groundwater sample was collected from Tanti-Gaon (GPS: 26.58.101, 94.16.391) (**Fig. 1**), a village in Titabor subdivision of Jorhat district, Assam, India. The concentration of arsenic in the water was measured by atomic absorption spectrophotometer (AAS) following the standard protocol as described by Behari and Prakash [11].

2.2 Isolation of arsenic-tolerant bacteria

The collected sample was subjected to serial dilution and cultured in arsenate amended Lauria-Bertani (LB) agar plates (10mM Arsenate) and incubated at 30^0C for 48 hrs. Individual colonies were picked up based on the morphological identities and sub-cultured to obtain the pure isolates**.**

2.3 Minimum Inhibitory Concentration Test

The minimum inhibitory concentration (MIC) of arsenate [As (V)] and arsenite [As (III)] was evaluated to determine the resistance capacity of the isolated bacteria. The bacterial isolates were cultured in freshly prepared LB broth at 30^0C for 48 hours and then 100 µl of the freshly cultured bacterial suspension was inoculated in minimal salt media (MSM) supplemented with different concentration of Arsenite (0.5-30 mM) added as sodium meta-arsenite (m-Na-AsO_2) and Arsenate (10-300mM) added as disodium hydrogen arsenate ($Na_2HAsO_4.7H_2O$) and incubated for 72 hours at 30^0 C and 142 rpm. The microbial growth was recorded with a UV-Visible spectrophotometer at 600nm.

2.4 Growth of the bacterial isolate in the presence and the absence of arsenite/arsenate

Among all the isolates, TA6 showed the highest MIC and as such, was taken for studying the growth kinetics in presence and absence of arsenite and arsenate. The isolate was cultured in Luria-Bertani broth containing arsenate in a concentration of 1mM to 30mM and arsenite from 0.5 mM to 10 mM respectively. The growth of the isolate was monitored through measurement of the optical density (OD) with a spectrophotometer (Thermo-Scientific, India) at 600 nm (OD600) at a specified interval of time (4hr, 8hr, 12hr, 24hr, 48hr, and 72hr).

2.5 Biotransformation assay

The ability of the bacteria to reduce As (V) or to oxidize As (III) was evaluated using the silver nitrate ($AgNO_3$) method as described by Simeonova *et al.*,[12]. Freshly cultured bacterium grown in minimal salt medium supplemented with 5mM glucose was cultured on two different LB agar plates supplemented with 2mM of Sodium Meta-Arsenite and Sodium Arsenate respectively and

incubated for 48 hours at 30^0C. The streaked plates were then flooded with 0.1M Silver Nitrate ($AgNO_3$) solution. Formation of light yellow color will indicate the precipitation of silver ortho-arsenite (Ag3AsO3) due to As (III) and light brown-red color for precipitation of silver-ortho-arsenate (Ag3AsO4) due to As(V).

Quantitative assay of arsenate reduction was analyzed by growing the bacteria in arsenic amended LB broth (2mM of Arsenate). In a time interval of 6, 12, 24, 48, 72 hours the bacterial cells were collected at a time by centrifugation and arsenite content of the supernatant was determined by AAS following standard protocols as described by Aggett and Aspell [13].

2.6 Arsenate reductase enzyme assay

The enzyme assay was done using NADPH coupled assay as described by Gladysheva *et al.*, [14]. Cell-free crude extracts of Escherichia sp. SD23 was used as positive control. Effect of pH and temperature on enzyme activity was also measured using this method.

2.7 Cross tolerance

The isolate was tested for its cross-tolerance efficiency with other heavy metals like Hg^{+2} added as $HgCl_2$, Cd^{+2} added as $CdCl_2$, Co^{+2} added as $CoCl_2$, Ni^{+2} added as $NiCl_2$ and Al^{+3} added as $AlCl_3$ in a concentration ranging from 0.5 to 10mM in MSM broth culture and OD (OD600) was recorded after 48 hours to evaluate the bacterial growth.

2.8 Biochemical Tests

Biochemical tests for starch hydrolysis, catalase, oxidase, casein production, nitrate reduction, urease, malate, citrate, indole, and motility were done according to the standard protocol described by Krieg [15].

2.9 Identification based on 16S rRNA

Genomic DNA was extracted from approximately 100 mg of the cell as per standard phenol-chloroform method. The 1500bp region of the 16S rRNA gene was amplified from the extracted genomic DNA using the universal primer 5' TACGGYTACCTTGTTACGACTT 3' (1492R), 5' AGAGTTTGATCMTGGCTCAG 3' (27F). The amplification was carried out in a reaction with a final volume of 25 µl containing 1.5 µl of template DNA, 1µl (20pM) of the forward primer, 1 µl (20 pM) of

the reverse primer, 2.5 µl (2.5 mM of each) dNTP mix, 2.5 µl of 10x PCR buffer, 1 µl (1U) of Taq DNA polymerase. A negative control (PCR mix without DNA) was included in all PCR experiments. The PCR reaction conditions were set for 94 ºC for 3min, followed by 30 cycles of denaturation at 94 ºC for 30s, annealing at 58 ºC for 1min and extension at 72 ºC for 2 min, before a final extension at 72 ºC for 7min. The PCR products were sequenced and the forward and reverse sequences obtained were assembled using the Codon-Code Aligner software (version: 5.1). Nucleotide sequence identities were determined using the BLAST tool from the National Center for Biotechnology Information (NCBI) and Similarity index value from EzTaxon Server. Then the partial sequence data for the 16SrRNA genes have been submitted to GeneBank.

2.10 FAME analysis

The fatty acid methyl ester (FAME) profile was analyzed using Sherlock-Midi system and compared with few reference strains of *Staphylococcus* genus for taxonomical validation.

2.11 Siderophore production

Production of siderophore was studied using Chrome Azurol S (CAS) agar media as described by Schwyn and Neilands [16]. CAS agar was prepared from four solutions which were sterilized separately before mixing. The **solution I**: Blue dye was prepared by mixing 10 ml of 1 mM $FeCl_3$, $6H_2O$ in 10mM HCl then with 50 ml of an aqueous solution of 2mM CAS. The resulting dark purple mixture was added slowly with constant stirring to 40 ml of an aqueous solution of 5mM Hex- Decyl Tri-Methyl Ammonium [HDTMA]. The dark blue solution was produced which was autoclaved and then cooled to 50^0 C. All reagents in the indicator solution were freshly prepared for each batch of CAS agar. Solution II: CAS agar was prepared by dissolving 30.24 g of Piperazine-N, N'-bis ethane sulfonic acid (PIPES) in 750 ml of a salt solution containing 0.3 gKH_2PO_4, 0.5g NaCI, and 1.0g NH_4Cl. The pH adjusted to 6.8 with 50 % KOH, and water was added to bring the volume to 800 ml and autoclaved after adding 15g of agar, and then cooled to 50^0 C. **Solution III**: Mix Solution containing the followings: 2g glucose, 2g mannitol, 493 mg $MgSO_4$,$7H_2O$, l mg $CaCl_2$, 1.17 mg $MnSO_4H_2O$, 1.4 mg H_3BO_3, 0.04 mg $CuSO_4.5H_2O$, 1.2 mg $ZnSO_4$,$7H_2O$, and 1.0mg $Na_2MoO_4.2H_2O$, was autoclaved, cooled to 50^0C then added to the buffer solution along with 30 ml filter-sterilized 10 % (w/v) casamino acid (Solution IV). The indicator solution was added last, with sufficient stirring to mix the ingredients without forming bubbles. CAS agar plates were inoculated with bacterial isolate and incubated at 30^0 C

for 7 days. Colonies showing orange hollow zone following incubation were recognized as siderophore positive [17].

2.12 Siderophore quantification

The method of Alexander *et al.* [18] was used to measure siderophore production in vitro. The bacterial cells were grown at 30 ^{0}C for 24 h in 50 ml of Chrome Azurol S (CAS) medium with 5 mM MES (2-(N-morpholino ethanesulfonic acid)– KOH buffer at pH 6.8. After the culture growth attains exponential phase at OD-600, the cells were pelleted by centrifugation at 10,000g for 10 min and the supernatant was filtered through 0.25 µm filter. Siderophore concentration in the filtrate was measured by mixing 500 µl of modified CAS assay solution with 500 µl filtrates. The standard solution of deferoxamine mesylate was used for siderophore quantification. The sterile CAS-MES-KOH solution was used as a reference solution, which did not contain siderophores. A standard curve was prepared by analyzing the absorbance (630 nm) of the reference solution (A/Aref) as a function of the siderophore concentration.

2.13 Resistance to Arsenic in comparison to siderophore mutant

The role of siderophore in arsenic tolerance was determined following the protocol described by Ghosh *et al*., [10] using one siderophore mutant (non-producer) *Pseudomonas putida* (Lp10L02M) and one control *Acinetobacter guillourie* (S02Ar2) with low siderophore production ability (10.8µmol). Arsenic tolerance of the isolate was measured as a percentage of growth rate and As(V) reduction at 5 and 10 mM of As(V) modified LB medium incubated at 30 ^{0}C for 24 h shaking at 142 rpm and compared with the TA6. Growth was measured as OD at 600nm on UV–Vis spectrophotometer. All the data were taken in triplicates.

3.0 RESULTS

3.1 Groundwater Sample

The contaminated groundwater sample collected from Titabor subdivision had pH 6.2 and arsenic concentration of 356µg/l.

3.2 Isolation of arsenic-resistant bacteria and MIC

The collected groundwater sample was inoculated in arsenate amended LB medium and morphologically different bacterial colonies were picked up and tested for minimum inhibitory concentration of arsenate and arsenite. Among the isolates, TA6 showed highest MIC and was able to grow in medium with ≥250mM of arsenate and ≤30mM of arsenite.

3.3 Bacterial growth in presence of arsenate and arsenite

Growth curve analysis showed the effect of arsenate and arsenite in the bacterial growth pattern. The isolate TA6 was cultured in fresh LB broth with a concentration of arsenate varying from 1mM – 30mM and arsenite from 0.5mM – 10mM respectively. Bacterial growth was not much affected in the presence of arsenate as compared with control. However, the presence of arsenite in the medium greatly affected the rate of growth. In the presence of arsenate, TA6 started doubling at the lowest time of 4hrs but in the presence of arsenite, it took approximately 24hrs to start multiplying. At the highest concentration of arsenate (~30mM) taken for the test and at 72 hours of incubation time, OD was measured as 1.474 ± 0.067, which is statistically at par with the OD 1.962 ± 0.058 for control, at the same time of incubation. But in comparison, at 72hrs of incubation in the presence of 10mM of arsenite growth was rigidly reduced when compared to the control. For control, OD was recorded as 1.962 ± 0.058, whereas in the 10mM of arsenite, the growth was recorded as OD 0.1036 ± 0.043. At lowest concentration of arsenite 0.5mM, the bacterial cell (TA6) approximately took 8 ± 2 hrs of incubation to multiply. Arsenite can strongly cease the bacterial growth even at a minimal concentration or as low as 0.5mM when compared to arsenate (**Fig. 2**). Both arsenate and arsenite are toxic to bacteria but arsenite is more toxic as evident from the present growth kinetics studies.

3.4 Cross tolerance

Other heavy metal tolerance test also showed the resistive capacity of the isolate to various heavy metals like Hg^{+2}, Cd^{+2}, Co^{+2}, Ni^{+2}, Cr^{+2}. MIC was found as 0.8mM, 1.0mM, 4mM, and 6mM respectively.

3.5 Biotransformation assay

TA6 was found to be an arsenate reducer. Reduction of arsenate in the petri dish formed a yellow precipitation of silver ortho-arsenite (Ag_3AsO_3) which indicates the presence of arsenite. In the quantitative assay, it was also found that with a gradual increase in time and with the increased

bacterial cell count, the concentration of As(V) gradually decreased with increased concentration of AS(III). In the duration of 72 hours, nearly 88.2% of the initial 2mM As(V) is reduced to As(III) (**Fig. 3**).

3.6 Arsenate reductase enzyme activity

Arsenate reductase activity was measured using NADPH coupled oxidation method. A Km of 0.44 mM arsenate and Vmax of 6395 U/mg were measured (**Fig. 4)**. There was no change in activity for 500µM and 1mM of arsenate. Temperature and pH are some critical factors for enzyme activity. Temperature-dependent activity assay revealed that 50^0C was the optimal temperature for highest enzymatic activity and in pH-dependent activity assay, pH 5.5 was measured as optimal for highest enzymatic activity (**Fig. 5**). Graphical representation of both the data formed a characteristic bell-shaped curved, where initial increased pH and temperature raised the activity till it reaches the optimal point of maximum activity and then the activity was found to gradually cease after the respective optimal value of pH 5.5 and temperature 50^0 C.

3.7 Biochemical test

The bacterium (TA6) was a gram-positive, nonmotile, coccus shaped bacterium. It was able to hydrolyze starch, casein and utilize citrate, reduce catalase and showed high siderophore activity (78.7 ± 0.004 µmol) but tested negative for oxidase, nitrate, urease and indole.

3.8 Chemotaxonomic and Molecular Identification

16S rRNA sequence similarity search identified the isolate as one of the species of the genus *Staphylococcus* having 97.6% pairwise similarity with *Staphylococcus saprophyticus subsp. bovis* (AB233327) and 97.5% with *Staphylococcus saprophyticus subsp. Saprophyticus* (**Table 1)**; Fatty acid methyl ester profile showed most of the fatty acids are branched chain like anteiso-C15, anteiso-C17 and iso-C15, when compared with the fatty acid profile of *S. xylosus, S. cohnii and S. saprophyticus* showed considerable differences of C17:0, iso C17:0, iso C18:0 **(Table 2)**. Therefore, based on both molecular and chemotaxonomic data the bacterium was identified as *Staphylococcus sp.* and the sequence was submitted under the GeneBank accession: KF134542.1 for further references.

3.9 Siderophore associated arsenate reduction

Microorganisms are the primary chelator of iron which dissociates Fe^{+3} ions with their siderophore activity. Siderophore associated arsenic resistance assay revealed that bacteria with high siderophore TA6 (78.7 ± 0.004 µmol) was significantly a strong As(V) reducer than the mutant strain Lp10L02M (non-producer). The growth of TA6 was also found reflective in comparison to the control and the mutant implying the added resistance ability of the strain to arsenate. In 5mM and 10mM arsenate broth, the TA6 showed higher growth as compared to the control strain S02Ar2. However, the mutant strain (Lp10L02M) had slower growth rate as compared to control and showed lesser reduction efficiency (**Fig. 6**).

4.0 Discussion

Increased arsenic concentration in groundwater is a matter of serious concern due to its carcinogenic effects on human health. The Brahmaputra river basin is considered as one of the severely affected basins [19]. Flood-line areas of the river have been detected with higher arsenic concentration than the standard permissible limit. which has become a major health risk for the people residing within these vicinities as they solely depend on the natural streams and groundwater for potable water. Titabor subdivision of Jorhat district, Assam has been severely affected by a alarming concentration of arsenic (194-657 µg/l)[3]. Although, several studies on arsenic poisoning and geogenic distribution in this region has been documented, the role of microbes in the geocycle is unknown. The study of contaminated aquifers is still a long way to go. Bacteria play an important role in the biogeochemical cycle of arsenic and they are contemplated as one of the major factors that have a pivotal role in the mobilization of sediment-bound arsenic [20]. They can change the bioavailability, solubility and mobility status of arsenical species by inter-conversion through redox reactions and thus, control toxicity level. Bacteria can resist the toxic arsenic using an array of cellular and metabolic mechanisms which basically includes active cellular transport of the toxic material out of the cellular environment, entrapment by cellular capsules or by precipitation, oxidation-reduction reaction [5,21]. We isolated a bacterium TA6 from groundwater sample containing 356µg/l of arsenic. The bacterium was identified as *Staphylococcus* sp. based on phylogenetic analysis of 16S rDNA sequence and FAME analysis. Both 16S rDNA and FAME profile showed significant differences with the reference strains of *Staphylococcus*. Similarity search with the NCBI nr/nt database and EzTaxon server showed an average of 97% similarity with the different species of *Staphylococcus* genus. Straight chain fatty acids like c16:0, c17:0 and branched chain fatty acids like iso c14:0, iso c17:0, anteiso c15:0 showed

considerable difference when compared with the FAME profile of *S. xylosus*, *S. cohnii* and *S. saprophyticus* (**Table. 2**). The bacterium was found to tolerate arsenate (MIC: ≥250 mM), arsenite (MTC: ≤30 mM). Resistance to arsenite concentration greater than 10 mM and arsenate greater than 100 mM has been considered as very high, whereas resistance to 200 mM As^{5+} and 30 mM $As3^{+}$ is a hyper-tolerance property [22]. The bacterium was also showed resistance to other heavy metals like Hg^{+2}, Cd^{+2}, Co^{+2}, Ni^{+2}, Cr^{+2} that ranged from ≥0.5 -10.0 mM and produced siderophore (78.7 ± 0.004 µmol). Microorganisms are known for their ability to produce different biogenic chelating agents like siderophore in iron-limiting environment. Siderophore solubilizes the ferric iron in the iron-starved environment and transports the Fe^{+3} into the cell [23]. It determines the growth of microorganisms in an environment where iron is the limiting factor [24]. Above and beyond being a limiting factor for growth in iron-starved environment, siderophore confers an added advantage of higher arsenic resistance as compared to the non-producers. In this study, screening of comparative resistance efficiency of TA6 (78.7 ± 0.004 µmol) revealed a higher siderophore activity against the control strain *Acinetobacter guillourie* S02Ar2 (10.8 ± 0.003 µmol) and a mutant strain *Pseudomonas putida* (Lp10L02M). The isolate TA6 not only had a higher siderophore activity in comparison to the mutant strain but also displayed a significantly higher resistance to arsenate and higher growth rate in an incubation period of 24h at 30 ^{0}C. Siderophore assisted resistance to arsenical compounds has been reported earlier [10]. High siderophore concentration confers higher resistance to arsenite and that the reduction efficiency of bacteria is significantly influenced by varied siderophore concentration [10].

Biotransformation assay indicated the isolate as one of the members of the arsenate-reducing genera that can actively catalyze the reduction of As(V) to As(III) using an enzyme arsenate reductase encoded by *arsC* gene of the ars operon. Aerobic arsenate reduction is the most distributed detoxification mechanism among the microorganisms and the ars operon has been detected in more than 50 organisms within the domains of bacteria, yeast, and protist. The first recognized arsenate reductase gene was identified on a gram-positive *Staphylococcus* plasmid [21]. Since then there have been several reports of this gene in different bacterial species *viz. Staphylococcus sp., Thermus thermophiles* [25] *Bacillus sp., Shewanella sp.,* [21] etc. Analysis of NADPH coupled assay showed that the enzyme is slightly acidic in nature with optimal pH 5.5 and optimal temperature 50^{0}C for its highest activity. Michaelis Menten kinetic constant, km was found to be 0.44 mM arsenate and V_{max} of 6395 U/mg protein. A similar kinetics of this enzyme was reported from *Chrysiogenes arsenatis* with a

Km value of 0.3mM arsenate and Vmax of 7013 U/mg protein [26]. Higher reduction efficiency of the isolate can be ascertained from the fact that it can actively reduce 88.2% of the initial 2mM arsenate [As(V)] to arsenite [As(III)] over a period of 72 hours. High activity of the enzyme leads to the conversion of arsenate to more mobile arsenite in the shallow aquifers that leads to its accumulation over a time period and could be one of the major reason for the increasing carcinogenic development in the northeastern region. In a transitory, the production of siderophore by bacteria helps in the mobilization of the sedimentary arsenate by displacing iron from the iron-arseno compounds forming a soluble Fe^{+3} ion. Increase concentration of arsenate in surrounding milieu competes with the phosphate ion and structural homology of the arsenate with phosphate gives an added advantage to enter the cellular system through *pit/pst* phosphate transporter channel. Cellular arsenate is then converted to arsenite by arsenate reductase enzyme and soon effluxes out of the system through arsenite transporter channel to maintain the cellular homeostasis. This essentially increases the concentration of both arsenite and arsenate in the aquifer system and eventually increases the arsenic contamination in the aquifers.

Acknowledgment

Authors are grateful towards UGC for the Rajiv Gandhi National Fellowship grant to carry out the research work and all the faculty members of Department of Agricultural Biotechnology, Assam Agricultural University and Centre for Studies in Biotechnology, Dibrugarh University for their continuous support and guidance during the work.

Conflict of Interest

The authors have no conflict of Interests.

Reference

[1] Devi, N.L., Chandra, I., Shihua, Q., 2009. Recent Status of Arsenic Contamination in Groundwater of Northeastern India – A Review. Rep. Opin., 2009, **1**, 22–32.

[2] Today, N., 2017. Water Sources in 23 Districts of Assam Contaminated with Arsenic and Fluoride□» Northeast Today 2017.

[3] Das, S., Bora, S., Lahan, J., Barooah, M., et al., 2014. Groundwater arsenic contamination in northeastern states of india. J. Environ. Res. Dev. J. Environ. Res. Dev., 2014, **9**.

[4] Oremland, R.S., Stolz, J.F., 2005. Arsenic, microbes and contaminated aquifers. Trends Microbiol., 2005, **13**, 45–49.

[5] Mukhopadhyay, R., Rosen, B.P., Phung, L.T., Silver, S., 2002. Microbial arsenic: From geocycles to genes and enzymes. FEMS Microbiol. Rev., 2002, **26**, 311–325.

[6] Sarkar, A., Kazy, S.K., Sar, P., 2013. Characterization of arsenic resistant bacteria from arsenic rich groundwater of West Bengal, India. Ecotoxicology, 2013, **22**, 363–376.

[7] Wang, G., Huang, Y., Li, J., 2011. [Bacteria live on arsenic analysis of microbial arsenic metabolism--a review]. Wei Sheng Wu Xue Bao, 2011, **51**, 154–60.

[8] Lloyd, J.R., Oremland, R.S., 2006. Microbial transformations of arsenic in the environment: From soda lakes to aquifers. Elements, 2006, **2**, 85–90.

[9] Kraemer, S.M., 2004. Iron oxide dissolution and solubility in the presence of siderophores. Aquat. Sci. - Res. Across Boundaries, 2004, **66**, 3–18.

[10] Ghosh, P., Rathinasabapathi, B., Teplitski, M., Ma, L.Q., 2015. Bacterial ability in AsIII oxidation and AsV reduction: Relation to arsenic tolerance, P uptake, and siderophore production. Chemosphere, 2015, **138**, 995–1000.

[11] Behari, J.R., Prakash, R., 2006. Determination of total arsenic content in water by atomic absorption spectroscopy (AAS) using vapour generation assembly (VGA). Chemosphere, 2006, **63**, 17–21.

[12] Simeonova, D.D., LiÃ¨vremont, D., Lagarde, F., Muller, D.A.E., et al., 2004. Microplate screening assay for the detection of arsenite-oxidizing and arsenate-reducing bacteria. FEMS Microbiol. Lett., 2004, **237**, 249–253.

[13] Aggett, J., Aspell, A.C., 1976. The determination of arsenic(III) and total arsenic by atomic-absorption spectroscopy. Analyst, 1976, **101**, 341.

[14] Gladysheva, T.B., Oden, K.L., Rosen, B.P., 1994. Properties of the Arsenate Reductase of Plasmid R773. Biochemistry, 1994, **33**, 7288–7293.

[15] Krieg, N.R., Krieg, R., N., 2015. *Bergey's Man. Syst. Archaea Bact.*, John Wiley & Sons, Ltd, Chichester, UK, pp. 1–14.

[16] Schwyn, B., Neilands, J.B., 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem., 1987, **160**, 47–56.

[17] Sarkar, A., Kazy, S.K., Sar, P., 2013. Characterization of arsenic resistant bacteria from arsenic rich groundwater of West Bengal, India. Ecotoxicology, 2013, **22**, 363–376.

[18] Alexander, D.B., Zuberer, D.A., 1991. Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biol. Fertil. Soils, 1991, **12**, 39–45.

[19] Chetia, M., Chatterjee, S., Banerjee, S., Nath, M.J., et al., 2011. Groundwater arsenic contamination in Brahmaputra river basin: a water quality assessment in Golaghat (Assam), India. Environ. Monit. Assess., 2011, **173**, 371–385.

[20] Islam, F., Gault, G., Bootham, C., Polya, D., et al., 2004. Role of metal-reducing bacteria in arsenic release from Bengal delta sediments. Nature, 2004, **430**, 68.

[21] Silver, S., Phung, L.T., 2005. Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. Appl. Environ. Microbiol., 2005, **71**, 599–608.

[22] Jackson, C., Jackson, E., Dugas, S., Gamble, K., William, S., 2003. Microbial transformations of arsenite and arsenate in natural environments. Recent Res Dev Microbiol, 2003, **7**, 103–118.

[23] Hammer, N.D., Skaar, E.P., 2011. Molecular Mechanisms of Staphylococcus aureus Iron Acquisition. Annu. Rev. Microbiol., 2011, **65**, 129–147.

[24] Basavraj, N., Deepak, V., 2011. Medical applications of siderophores. Eur. J. Gen. Med., 2011, **8**, 229–235.

[25] Del Giudice, I., Limauro, D., Pedone, E., Bartolucci, S., Fiorentino, G., 2013. A novel arsenate reductase from the bacterium Thermus thermophilus HB27: Its role in arsenic detoxification. Biochim. Biophys. Acta - Proteins Proteomics, 2013, **1834**, 2071–2079.

[26] Krafft, T., Macy, J., 1998. Purification and characterization of the respiratory arsenate reductase of Chrysiogenes arsenatis. FEBS J., 1998, **255**, 647–653.

Tables

Table 1: Sequence similarity (SIM) table showing the percentage (%) sequence similarity of strain TA6 (GenBank Accession No: KF134542.1) with other strains of *Staphylococcus* genus. (SIM table was generated using EZtaxon Server)

Rank	Names/Title	Accession	Pair-wise Similarity
1.	*Staphylococcus saprophyticus subsp. bovis*	AB233327	97.602
2.	*Staphylococcus saprophyticus subsp. saprophyticus*	AP008934	97.525
3.	*Staphylococcus xylosus*	D83374	97.370
4.	*Staphylococcus succinus subsp. succinus*	AF004220	96.674
5.	*Staphylococcus cohnii subsp. urealyticus*	AB009936	96.597
6.	*Staphylococcus equorum subsp. equorum*	AB009936	96.520
7.	*Staphylococcus gallinarum*	D83366	96.520
8.	*Staphylococcus succinussubspcasei*	AJ320272	96.520
9.	*Staphylococcus haemolyticus*	L37600	96.442
10.	*Staphylococcus equorum subsp. linens*	AF527483	96.442

Table 2: Cellular Fatty acid Profile of isolateTA6 (1) and *S. xylosus* (2) *S. cohnii* (3) and *S. saprophyticus* (4).

Straight chain fatty acids	1	2	3	4
C15:0	--	--	--	--
C16:0	1.61	3.44	3.21	3.57

C17:0	0.07	--	--	--
Branched chain fatty acids				
iso C14:0	0.96	2.40	2.48	1.88
iso C15:0	14.04	23.73	15.01	21.09
anteiso- C15:0	50.58	39.24	43.28	41.55
iso C16:0	2.06	0.97	3.14	1.94
iso C17:0	6.80	--	--	--
anteiso - C17:0	13.13	4.48	10.18	4.66
iso C18:0	0.47	--	0.62	--
Unsaturated fatty acids				
C16:1ω7c	--	--	--	--
C16:1ω11c	--	--	--	--

FIGURE CAPTIONS

Figure 1: Site map of the study area.

Figure 2: Effect of arsenic on bacterial growth rate (A) Arsenate (B) Arsenite.

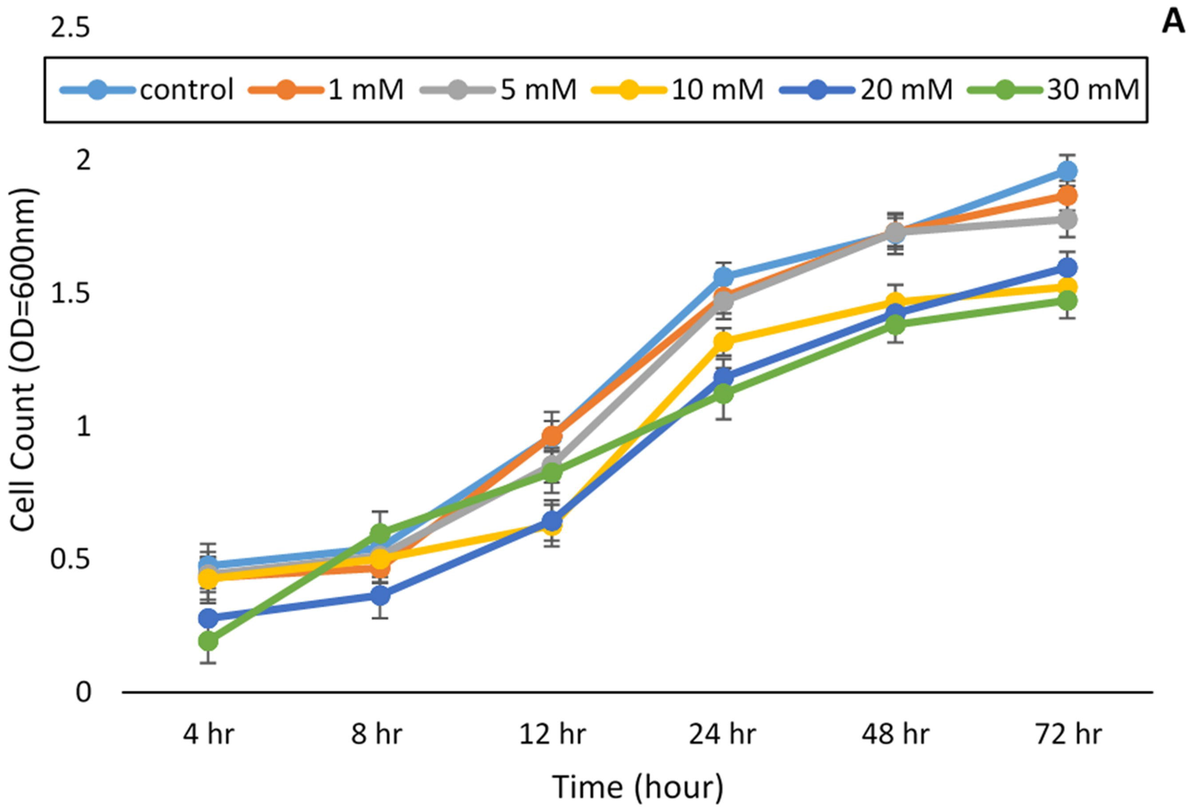
Figure 3: (A) Bioconversion of arsenate [As (V)] to arsenite [As (III)]. **(B)** Rate of Biotransformation over an incubation period of 72 hours.

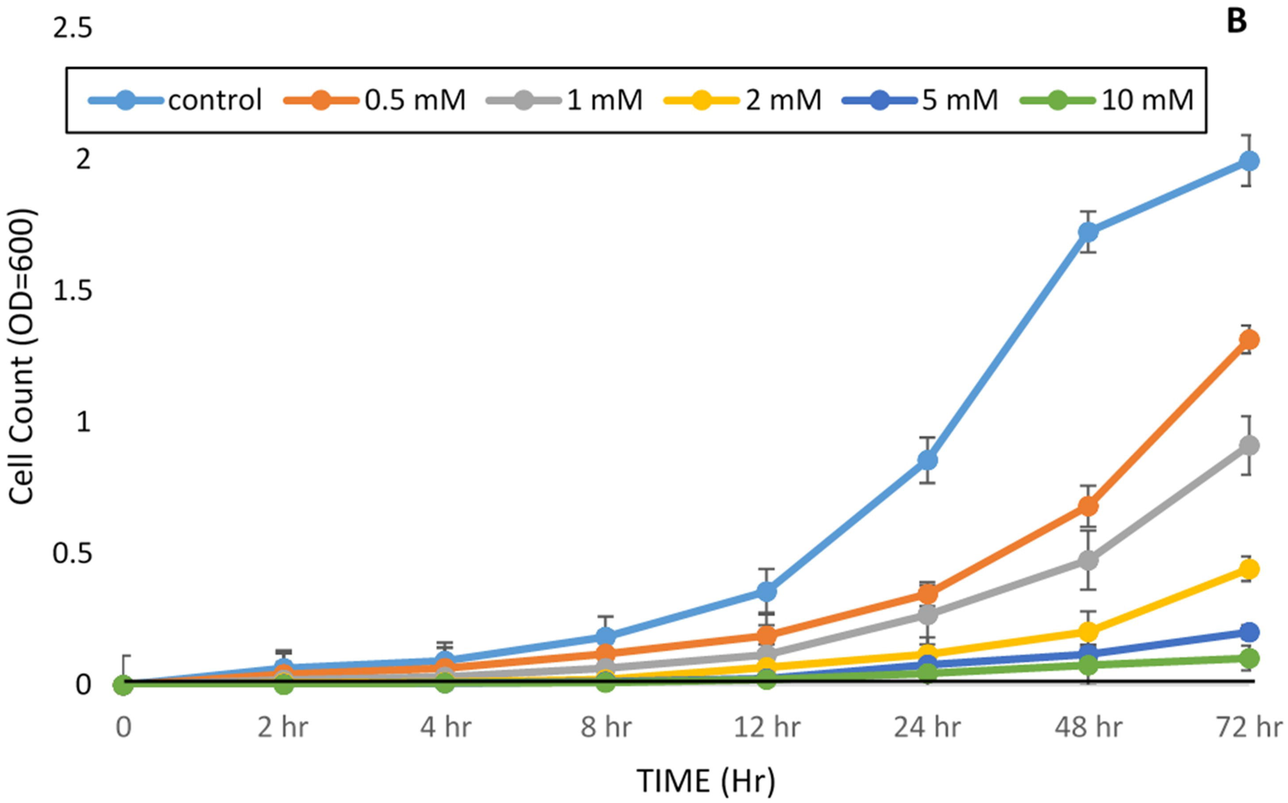
Figure 4: Kinetic Profile of enzyme activity (Lineweaver Burk Plot).

Figure 5: Arsenate Reductase enzyme activity **(A)** At different pH (pH 5.5 was found to be optimal for maximum activity) **(B)** At different Temperature (50 ^{0}C was found as the optimal temperature for maximum activity).

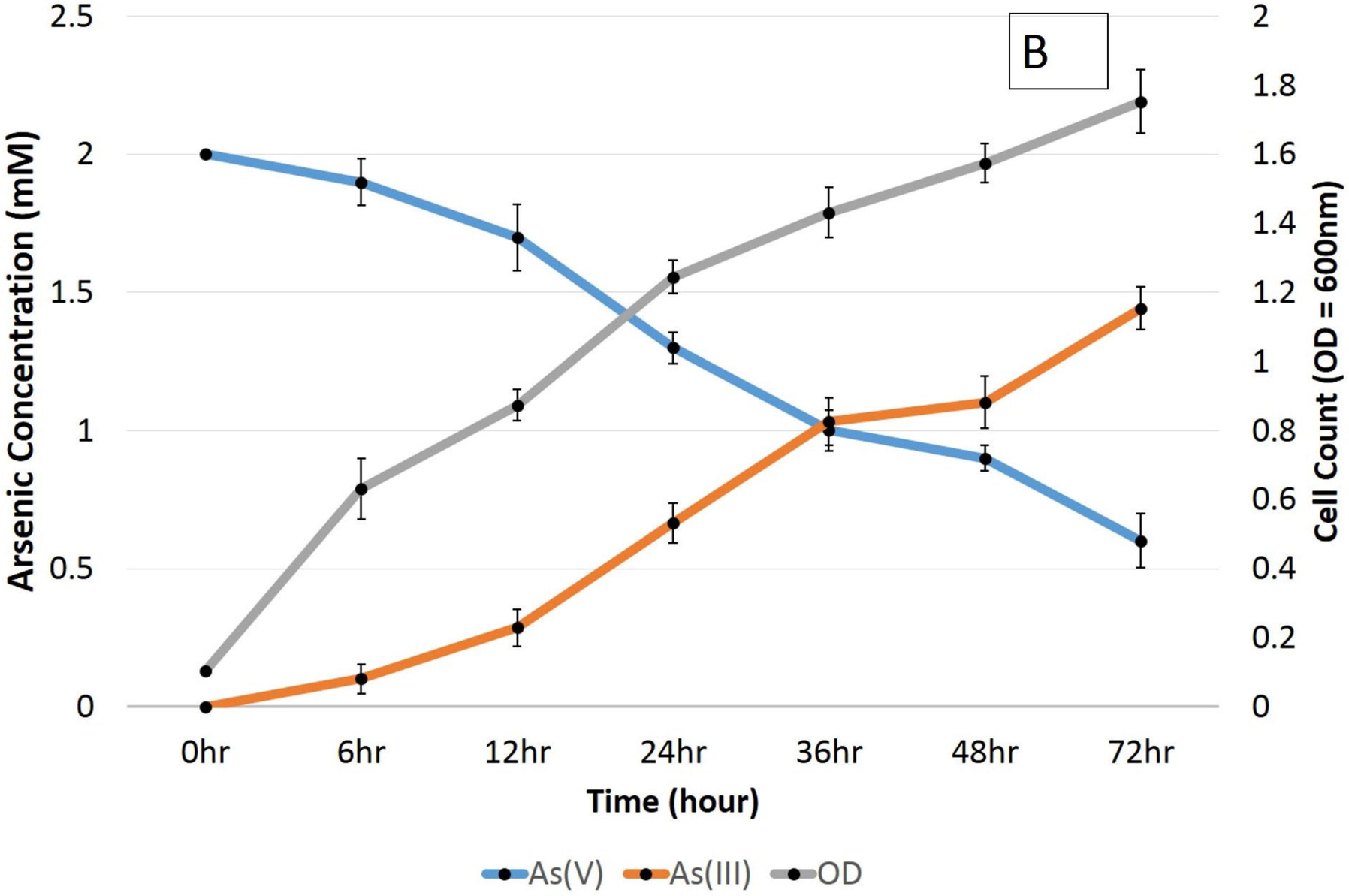
Figure 6: Effect of siderophore on growth percentage and arsenate reduction in the modified LB medium containing (A) 5mM arsenate (B) 10mM arsenate.

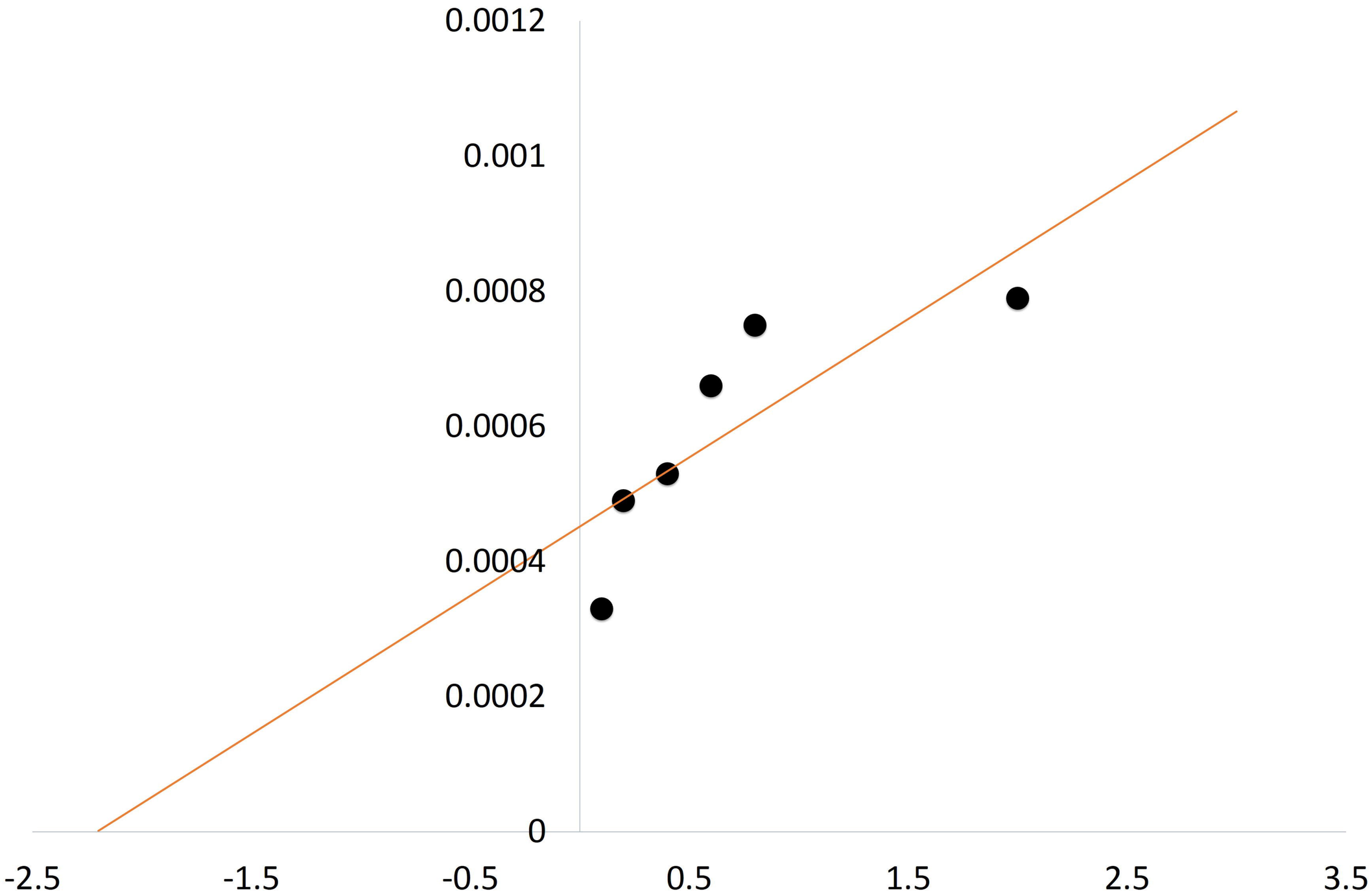
Chenijaan
Jorhat
Bodhboria
Lahing
Tilikiam
Mariani
Molungkimon
Nagajangka
Titabor
Tiniali
Waromung
Madhapur
Golaghat
Rajabari
Rangajan
Khar
Changki
Naambor Habi
Merapani
Akuk
Tanti Gaon
Titabor Subdivision
Jorhat District,Assam
26.58.101/94.16.391

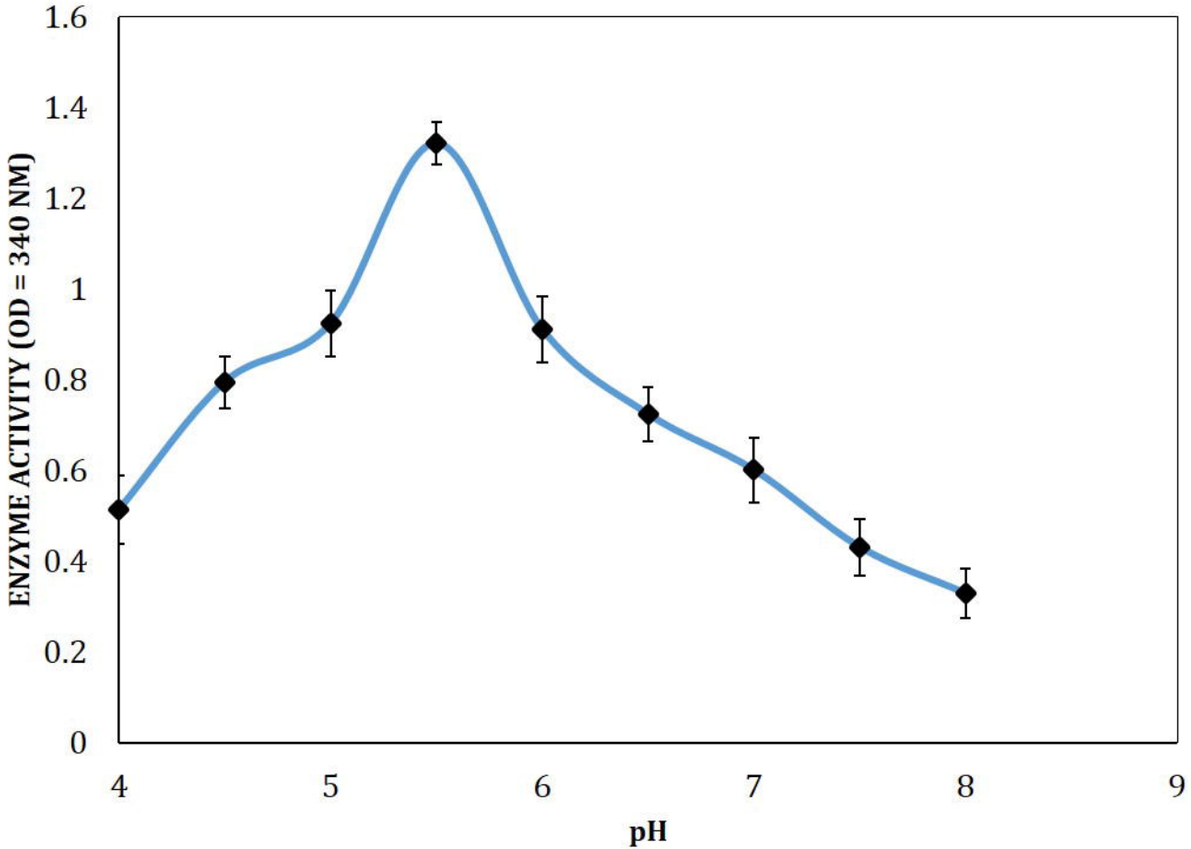

A
control
1 mM
5 mM
10 mM
20 mM
30 mM
Cell Count (OD=600nm)
2.5
2
1.5
1
0.5
0
4 hr
8 hr
12 hr
24 hr
48 hr
72 hr
Time (hour)

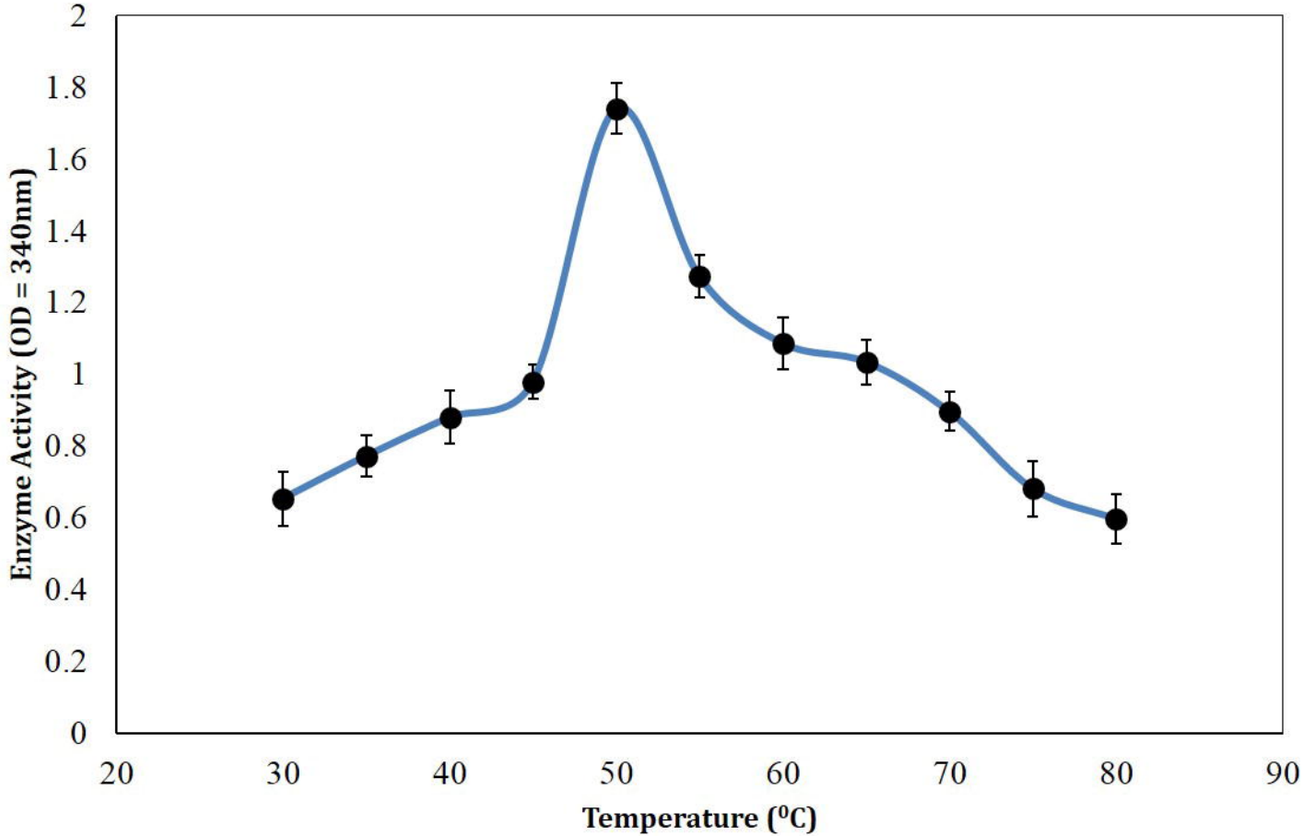

B
control
0.5 mM
1 mM
2 mM
5 mM
10 mM
Cell Count (OD=600)
2.5
2
1.5
1
0.5
0
0
2 hr
4 hr
8 hr
12 hr
24 hr
48 hr
72 hr
TIME (Hr)

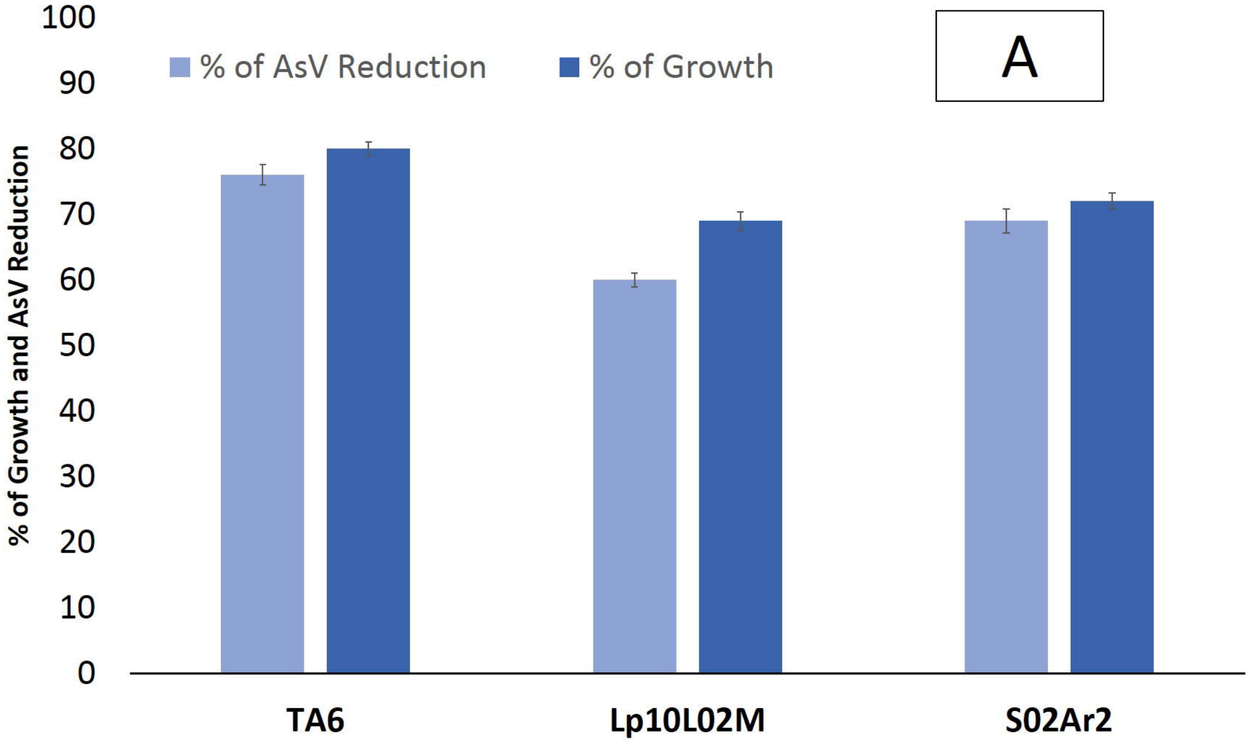
A

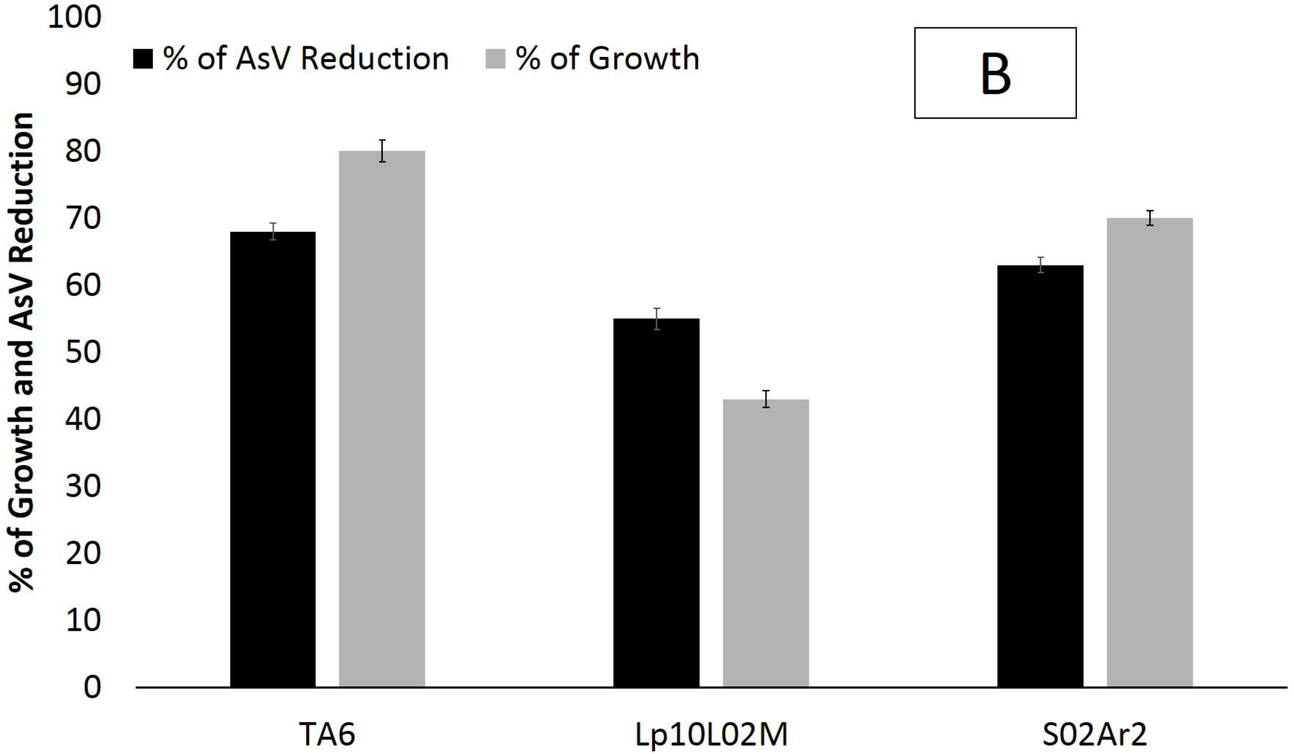

B
Arsenic Concentration (mM)
Cell Count (OD = 600nm)
2.5
2
1.5
1
0.5
0
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0hr
6hr
12hr
24hr
36hr
48hr
72hr
Time (hour)
As(V)
As(III)
OD

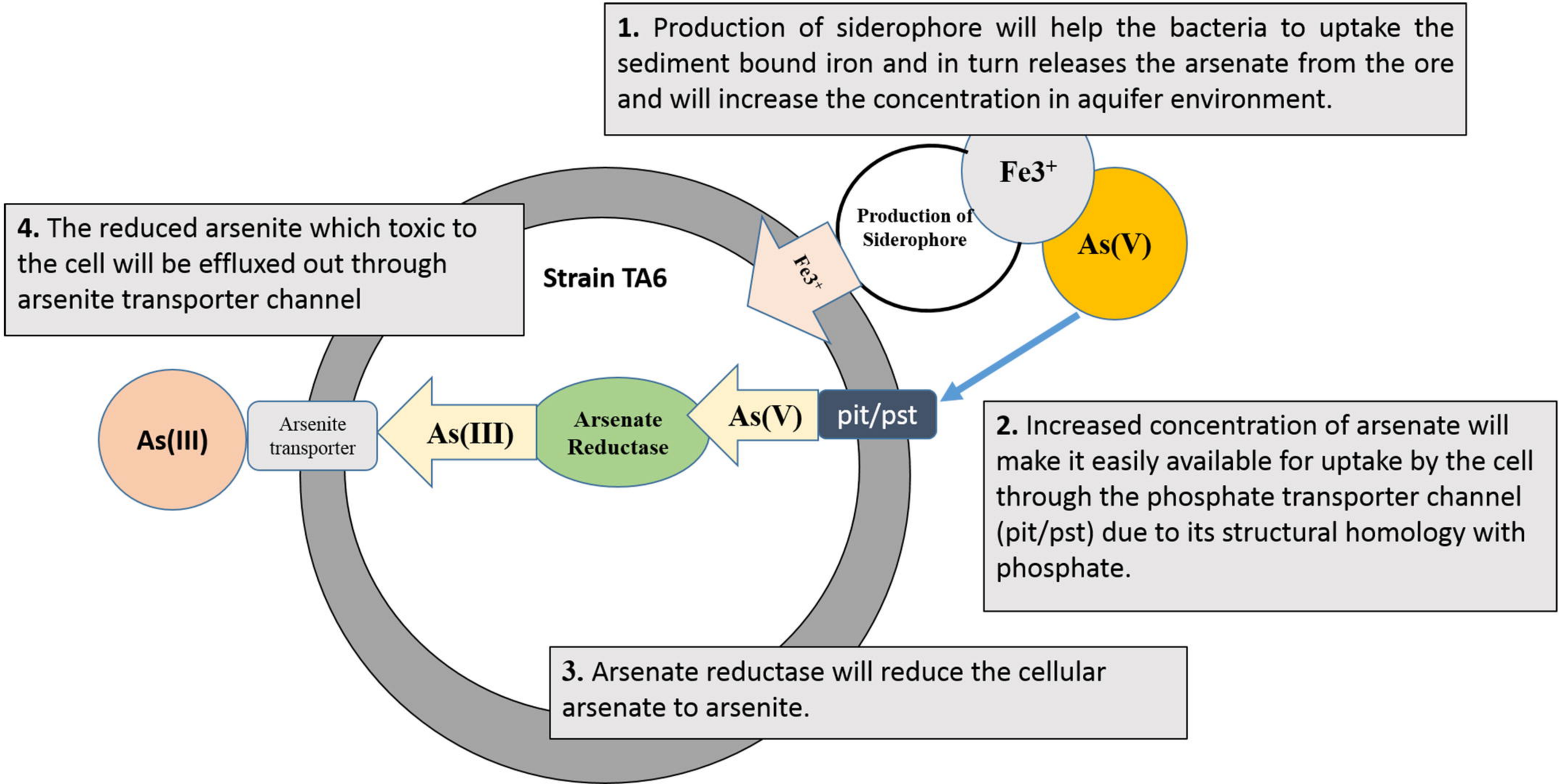

0.0012
0.001
0.0008
0.0006
0.0004
0.0002
0
-2.5
-1.5
-0.5
0.5
1.5
2.5
3.5


ENZYME ACTIVITY (OD = 340 NM)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
4
5
6
7
8
9
pH


Enzyme Activity (OD = 340nm)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
20
30
40
50
60
70
80
90
Temperature (⁰C)


A
% of AsV Reduction
% of Growth
% of Growth and AsV Reduction
100
90
80
70
60
50
40
30
20
10
0
TA6
Lp10L02M
S02Ar2


B
% of AsV Reduction
% of Growth
% of Growth and AsV Reduction
100
90
80
70
60
50
40
30
20
10
0
TA6
Lp10L02M
S02Ar2


1. Production of siderophore will help the bacteria to uptake the sediment bound iron and in turn releases the arsenate from the ore and will increase the concentration in aquifer environment.
Fe3+
Production of Siderophore
As(V)
Fe3+
Strain TA6
4. The reduced arsenite which toxic to the cell will be effluxed out through arsenite transporter channel
As(III)
Arsenite transporter
As(III)
Arsenate Reductase
As(V)
pit/pst
2. Increased concentration of arsenate will make it easily available for uptake by the cell through the phosphate transporter channel (pit/pst) due to its structural homology with phosphate.
3. Arsenate reductase will reduce the cellular arsenate to arsenite.