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Utilising the Potential of *Bacillus stratosphericus* MKS for Managing Arsenic Contamination of Soil: A Feasible and Sustainable Approach

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Abstract: In the soil of Bihar (latitudes N25-40.997' and E84-52.288'), a new type of bacteria resistant to arsenic was found. *Bacillus stratosphericus* was identified as the isolate using phylogenetic analysis and 16S rRNA sequencing. The isolated strain was also registered as *Bacillus stratosphericus* MKS under accession numbers OM841514, according to IMTECH, Chandigarh. The isolated strain can eliminate 99.9% of arsenate at 50 mM As(V) concentrations and 90% of Arsenite at 2 mM As (III) concentrations, according to the batch experiment's impressive results. The strain's ability to withstand high arsenite and arsenate concentrations, with minimum inhibitory values of 10 mM and 250 mM, respectively, is also noteworthy. A bacterium species called *Bacillus stratosphericus* is well known for its capacity to endure harsh conditions like the stratosphere. It may have evolved special adaptations to deal with this deadly metalloid given that it was discovered in soil that was contaminated with arsenic. In conclusion, this work offers insightful information about the possible application of *Bacillus stratosphericus* MKS for arsenic remediation in contaminated areas. As well as examining the underlying processes of this strain's tolerance to arsenic, future studies might concentrate on improving the circumstances for its growth and elimination of arsenic.

Keywords: Arsenate (AsV), Arsenite (AsIII), *Bacillus stratosphericus*, Arsenic

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Introduction

Arsenic contamination in groundwater is a serious health concern for people globally. Continuous exposure to toxic arsenic present in ground drinking water can cause various health problems for humans such as skin lesions, cancer of the skin, lung, bladder, kidney, cardiovascular disease, and neurological disorders. In India, the state of Bihar is particularly affected by arsenic contamination. This is because the region has many shallow aquifers with high levels of naturally occurring

arsenic, coupled with a high dependency on groundwater as a source of drinking water. It is estimated that more than 80% of drinking water sources in Bihar are based on groundwater, which means that many people are exposed to the severe effects of arsenic toxicity. This has led to a high incidence of arsenic-related health problems in the region, with many people suffering from skin lesions and various forms of cancer (Ahamed *et al*., 2006; Argos *et al*., 2010, 2011; IARC, 2012; Kumar *et al*., 2023). Arsenic enters the body through contaminated drinking water and food, and it can affect different organs of the body by interfering with the normal functioning of cellular metabolism. Arsenic is known to accumulate in the liver, kidneys, lungs, and other organs, leading to organ toxicity and a range of associated health problems (Smith *et al*., 2000; Jarup *et al*., 2003; Anwar *et al*., 2005; Rodriques *et al*., 2008; Sela *et al*., 2013).

The high number of cancer cases reported in Bihar, India is indeed a big challenge for the people and government of Bihar. Arsenic contamination in water and soil is a serious environmental issue in many parts of the world, including Bihar. There are several approaches to removing toxic arsenic from environmental components such as water and soil. One common method is to use adsorbents, such as activated alumina, activated carbon, and iron oxide, which can bind with arsenic and remove it from water (Linlin Hao *et al*., 2018; Yadav *et al*., 2022). Another method is to use plants for removal of toxic arsenic from environmental components, this process is known as phytoremediation (An Yan *et al*., 2020). In addition to these methods, it is also important to understand how arsenic is taken up by bacterial cells and how it can be mobilized and made more bioavailable. There are two inorganic forms of toxic arsenic mainly found in the environment arsenite As (III) more toxic and reduced form and arsenate As(V) predominately present in oxidized conditions (Escalante *et al*., 2009; Bachate *et al*., 2009).

Arsenate (AsO_4^{3-}) has structural similarity with phosphate (PO_4^{3-}), so it can be taken up by bacterial cells through phosphate transporters (Silver *et al*., 2005). Arsenite, on the other hand, can be transported into bacterial cells through aquaglyceroporins (Rosen *et al*., 2009). Arsenate can also be reduced to arsenite in both aerobic and anaerobic conditions, which can increase its mobility and bioavailability of toxic arsenic (Macur *et al*., 2001; Harvey *et al*., 2002). Therefore, understanding the mechanisms of arsenic uptake and mobilization is crucial for developing effective strategies to remove arsenic from the environment and mitigate its harmful effects on human health.

Toxic arsenic tolerating microbes especially bacteria are involved in conversion of toxic arsenic species to non-toxic forms by different strategies such as reduction, oxidation etc. and can act as arsenic remediating agent (Hussein *et al*., 2005). In this study, isolation and characterization of an arsenic tolerating/resistant bacterium was done with species name *Bacillus stratosphericus* MKS (accession number OM841514) from soil (724 ug/l arsenic concentration) taken from Naya tola, Maner, Patna, Bihar (N25•40.997′, E84•52.288′) (Ghosh *et al*., 2009). The similar type of bacteria species has been previously isolated from air samples at altitudes of 41 km (from stratosphere) with strain type 41KF2aT, which suggests that it is capable of surviving in extreme environments. It is also noteworthy that *Bacillus stratosphericus* MKS can tolerate a wide range of temperatures (8 to 37^0C) and pH levels (pH 6 and pH 10) and is resistant to UV radiation (Shivaji *et al*., 2006). These characteristics make it a promising candidate for bioremediation applications. The present study aimed to investigate new approaches for arsenic remediation using *Bacillus stratosphericus* MKS. The outcomes of this study may provide an eco-friendly approach to arsenic remediation, which is an area that has not been extensively investigated using *Bacillus stratosphericus*.

Materials and Methods

Isolation of *Bacillus stratosphericus* MKS was done by the spread plate method (Prescott, 2002), detailed methods are given below. Amplification of 16S RNA gene was carried out by using a universal primer (Grifoni *et al*., 1995). Sequencing of PCR products done at Barcode Biosciences (An ISO9001:2015 Certified company). 16S-rRNA sequence of bacterial strain is compared with nucleotide sequences present at database NCBI (National Center for Biotechnology Information) (Altschul *et al*., 1997). Further 16S r RNA sequence of isolate has been deposited to Gene Bank with species name *Bacillus stratosphericus* MKS under accession number OM841514.

Growth profile and MIC (Minimum inhibitory concentration) of isolated strain was studied under different stress conditions [Arsenite (AsIII), Arsenate (AsV) and pH] by growing them in LB growth media at 150 rpm and 37°C. The optical density (OD) is measured at 600 nm using a double beam spectrophotometer (Systronics) during different growth phases from 0 to 35 h. Bacterial biomass digested by the nitric acid method to measure the concentrations of arsenite and arsenate in biomass. The arsenate concentration was measured in residual broth media and bacterial biomass by using the azure B method (APHA, 2005; Cherian *et al*., 2005). The concentration of arsenate was obtained by subtracting the arsenite concentration from the total arsenic concentration measured in the residual growth medium.

Study of growth phases and MIC under different stresses:

Overnight grown 100 ml LB broth bacterial culture was prepared and taken for subculturing into test and control. 1ml grown bacterial culture was transferred to 9 ml of LB broth medium containing test tubes, which was previously added with control-without arsenic. Then all the culture tubes were incubated at 150 rpm and 37 °C in incubator. Culture was taken for turbidity measurement at different time points 0 to 35 h.Turbidity of bacterial broth medium were measured at 600 nm by double beam spectrophotometer. Bacterial culture tubes were amended by a particular concentration of arsenate or arsenite for one set of experiment and same process were repeated for different concentration until MIC obtained.

Arsenate As(V) and Arsenite As (III) concentration measurement in left broth culture medium and Arsenic present in bacterial biomass:

Previously grown bacterial cultures that utilized for study of growth phases and MIC, was also taken for measurement of concentration of arsenite and arsenate in left broth medium and total arsenic in bacterial biomass. Finally bacterial broth culture was taken in sterile Eppendorf (1.5 ml) tube and centrifuged at 10000 rpm for 2-4 min. 1 ml supernatant i.e. cell free bacterial culture(suspension) was taken into culture tube with 9 ml distilled water and pellet was digested by nitric acid method for estimating arsenic in bacterial biomass (APHA, 2005).

Before digestion of bacterial biomass, supernatant was taken for measuring arsenite concentration in bacterial broth medium with Azure B method (Cherian *et al*., 2005). After digestion, bacterial medium was further used to measure arsenate in left bacterial medium by utilizing Azure B method. Briefly, 1 ml non-digested and 1ml digested bacterial broth culture was taken two separate 10 ml flasks and some drops of 10% potassium iodide (KI) was mixed only in digested bacterial culture for reduction of arsenate to arsenite and excess amount of KI can be removed by some amount of ascorbic acid (10.8%).

Further, 1 ml 2% KIO3 with 1 ml of HCl (0.4 M) were added and shaken gently. 1 ml azure B (0.1%) was added followed by 2 ml (2 M) CH_3COONa (as buffer solution) and left for few minutes and after that the amount of test tube made up to 10 ml by adding DW (distilled water) and turbidity were measured at 644 nm. Obtained pellets were washed twice with distilled water and left in oven for drying of bacterial broth culture and further bacterial broth culture was digested

Table 1: Significant alignments generated by sequences

Description	Max Score	Total Score	Query cover	E-Value	Per. Ident	Accession
Bacillus stratosphericus strain 41KF2a	2689	2689	100%	0.0	100.00%	NR_118441.1
Bacillus aerius strain 24K	2684	2684	100%	0.0	99.93%	NR_118439.1
Bacillus altitudinis 41KF2b	2684	2684	100%	0.0	99.93%	NR_042337.1
Bacillus xiamenensis MCCC 1A00008	2678	2678	100%	0.0	99.86%	NR_148244.1
Bacillus aerophilus strain 28K	2678	2678	100%	0.0	99.86%	NR_042339.1
Bacillus stratosphericus strain 41KF2a	2678	2678	100%	0.0	99.86%	NR_042336.1
Bacillus pumilus strain NBRC 12092	2658	2658	99%	0.0	99.66%	NR_112637.1
Bacillus zhangzhouensis MCCC 1A08372	2656	2656	100%	0.0	99.59%	NR_148786.1
Bacillus australimaris strain MCCC 1A05787	2651	2651	100%	0.0	99.52%	NR_148787.1
Bacillus safensis strain NBRC 100820	2647	2647	99%	0.0	99.52%	NR_113945.1

by utilizing nitric acid method and after that arsenic present in bacterial biomass was measured by azure B method.

Maximum Likelihood method utilized for Evolutionary analysis:

Maximum Likelihood method and Tamura-Nei model are utilized for conclude the evolutionary history of isolated bacterial strain (Kimura *et al*., 1980). Automatically by applying Neighbor-Join and BioNJ algorithms Initial tree(s) for the heuristic search were obtained to a matrix of pairwise distances between bacillus species and then by selecting the topology with superior log likelihood value tree was constructed. Evolutionary analyses were conducted in MEGA11 (Kumar *et al*., 2018).

Data analysis:

In all the given figures were obtained by measuring bacterial growth, reduction, and removal under different stress conditions, and the mean values and standard errors of three replicates were calculated. ANOVA was conducted to assess the significance for all differences between the experimental results, with P-value of 0.05 or less than 0.05 can be considered significant. Microsoft Excel 2019 was using for creating all figures given in this study.

Results

Isolation and characterization of arsenic tolerating bacterial strain:

A bacterial strain was isolated by using the agar plating method (LB media) and further allowed them to grow on various concentrations of As(V) such as 200 mM, 150 mM, 100 mM and 50 mM As(V) added in different LB agar plates. Colonies of bacteria that grown on 50 mM As(V) containing LB media culture plates was considered as more tolerant concentration for bacteria because of bacteria rapidly grown on this culture plate and one isolate was randomly taken from this and further identified as *Bacillus stratosphericus* MKS with the help of 16S-rRNA sequencing. The phylogenetic tree shown in Figure 1 shows the relationship of *Bacillus stratosphericus* MKS

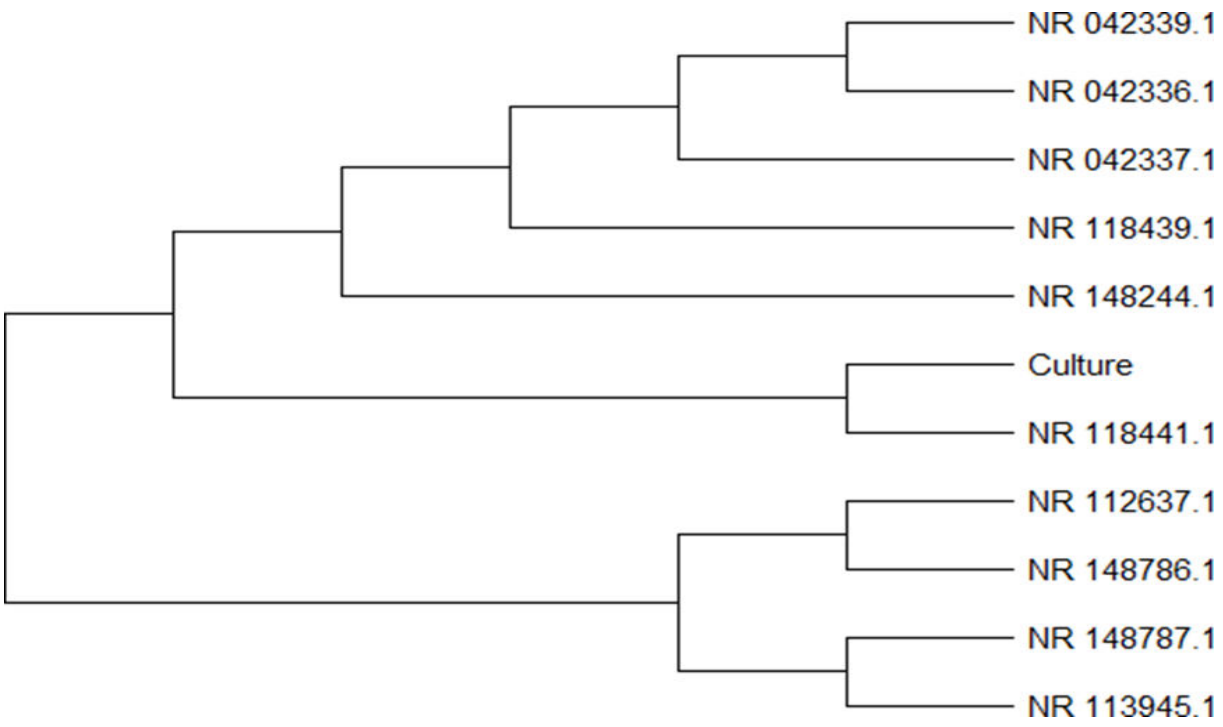

Fig. 1: Phylogenetic tree constructed by neighbor-joining method, showing relationship of *Bacillus stratosphericus* MKS (OM841514) and another member of *Bacillus* sp. present in NCBI database.

(Accession number OM841514 with other members of the *Bacillus* species) (Table 1).

Growth profile and MIC (Minimal inhibitory concentration) of Bacillus stratosphericus MKS under Arsenate (AsV) and Arsenite (AsIII) stress:

Newly isolated *Bacillus stratosphericus* MKS was further used for studies; from the bacterial colonies grown on LB media plate amended with 2 mM arsenate. Bacteria were grown in LB broth medium containing different concentrations of arsenate (control to 250 mM) and arsenite (control to 10 mM). In control broth media (no arsenic added) bacteria spent their life cycle in three phases, lag phase (2 h), log phase (28 h) and ultimately go to stationary phase with little number of bacteria. Bacterial growth dependent on arsenic concentration (AsV and As III) supplemented in growth media as concentrations increases, growth of bacteria reduced. At 50 mM As(V), 10% increased cell growth was observed in comparison to control which suggests that *Bacillus stratosphericus* MKS may has an arsenic-utilizing system that is activated at lower concentrations of arsenic. However, as the concentration of arsenic is further increased, the growth of the bacteria was significantly reduced, with the highest concentration of arsenic (250 mM As(V)MIC) leading to an 86% reduction in cell growth (Fig. 2A). This indicates that while *Bacillus stratosphericus* MKS can utilize arsenic at lower concentrations, higher concentrations of arsenic become toxic to the bacteria.

It appears that *Bacillus stratosphericus* MKS is more sensitive to arsenite (AsIII) than arsenate (AsV) based on the observed reduction in growth rate under low concentrations of arsenite stress conditions as compared to arsenate stress. The reduction in growth rate was more pronounced with increasing concentrations of arsenite, with an 87.5% reduction in growth observed at 10mM arsenite concentration, that result as the Minimum Inhibitory Concentration (MIC) for

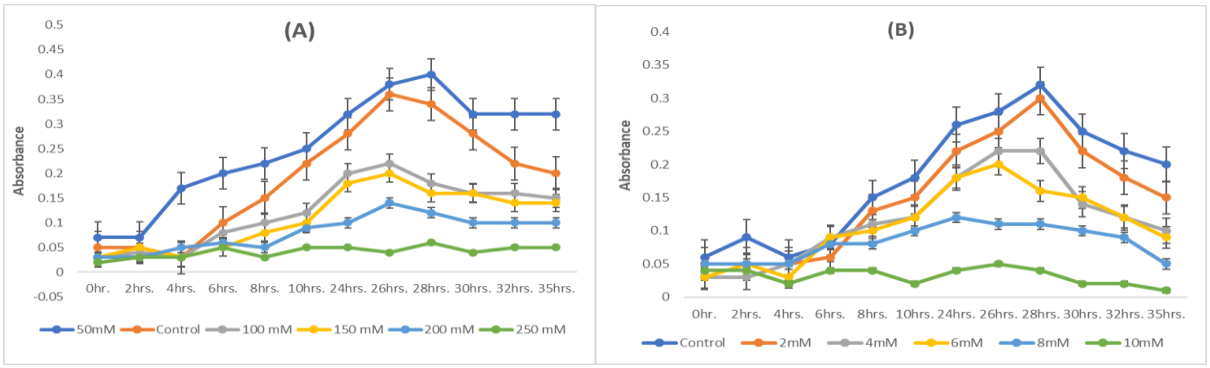

Fig. 2: *Bacillus stratosphericus* MKS growth profile in LB broth medium supplemented with various amount of arsenic. (A) Arsenate (50 mM, 100 mM,150 mM 200 mM and 250 mM) (B) Arsenite (2 mM, 4 mM, 6 mM,8mM and 10 mM). Control with no added arsenic [As(V) or As (III)] i.e. (0 mM). Change in Absorbance of the broth culture was measured over 35 h at 600nm. standard error of the mean of three experiment are shown by Error bars (±).

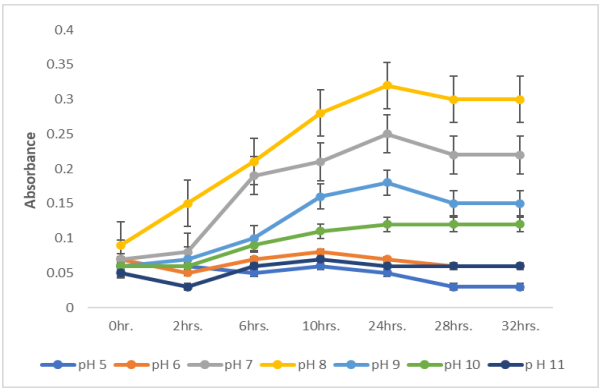

Fig. 3: *Bacillus stratosphericus* MKS growth profile in LB broth medium, over the different pH (pH 5 to pH 11). Control (neutral pH). Change in Absorbance of the bacterial culture was determined over 35 h, standard error of the mean of three experiments indicated by Error bars (±).

Bacillus stratosphericus MKS (Fig. 2B). Additionally, significant reciprocal correlation between bacterial growth and different As(V) concentration with r value -0.81469 and As (IIII) concentration with r value -0.98606 in the LB broth medium, suggesting that increasing concentrations of these compounds may have a negative impact on the growth of *Bacillus stratosphericus* MKS.

pH effects on the growth profile of Bacillus stratosphericus MKS:

The optimal pH for the growth of the above strain of bacteria was observed at pH 8, and growth decreases as the pH moves towards more acidic or basic conditions. However, it was found that growth at alkaline pH was better than at acidic pH. Therefore, it appears that the bacterium's optimal growth occurs at a slightly alkaline pH. To confirm this observation, pH change in the media (from pH 5 to pH 11) was done by adding either acid or base solution that depends on present pH, and monitored bacterium's growth. Maximum growth of bacteria was observed at pH 8, while growth was retarded at acidic pH (5 and 6) and basic pH (11) (Fig. 3).

Removal/uptake potential of arsenate (AsV) and its reduction to arsenite (AsIII):

Left arsenate concentrations in residual LB broth medium, total arsenic in bacterial biomass and arsenite present in arsenate containing media

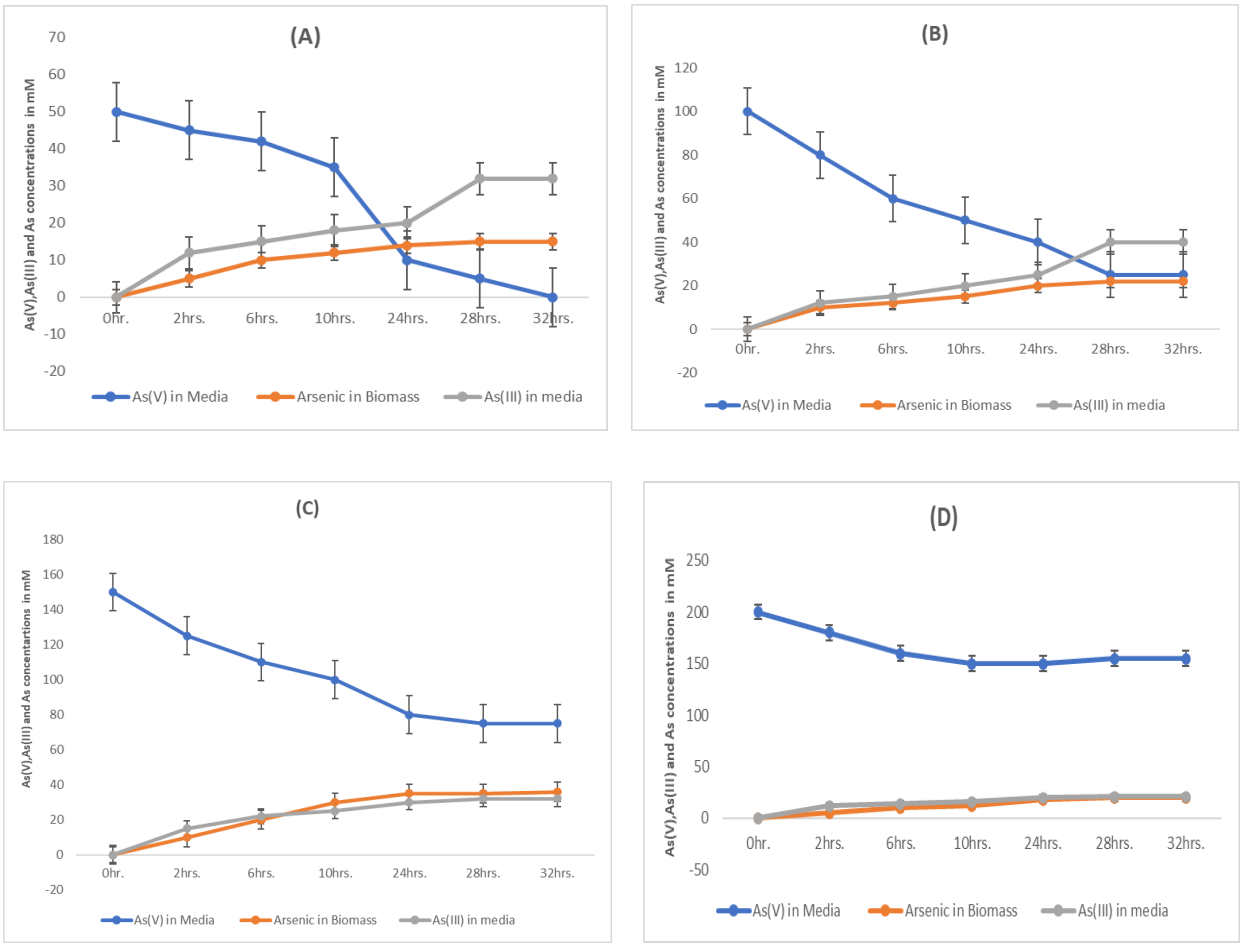

Fig. 4: Y-axis represents the Arsenate concentration left in residual bacterial growth media, concentration of Arsenite As (III) [uptaken As(V) reduced to As (III)] formed in media and Arsenic (As) in bacterial biomass, at different time periods (X-axis) of growth phase (0 to 32 h). (A) 50 mM (B) 100 mM (C) 150 mM (D) 200 mM As(V) enriched media. All values are mean of three experiments and standard errors (SE) are presented as error bars (±) with $p < 0.001$.

(due to arsenate reduced to arsenite) measured at different time intervals of bacterial growth (Fig. 4). 99.9% arsenate removal was examined at 50 mM arsenate As(V) containing LB media and 30% arsenic measured in bacterial biomass at the end of experiment. Removal percentage was gradually decreased with increasing arsenate concentrations at 100 mM,150 mM and 200 mM (removal percentages were 75%, 50% and 22.5%, respectively). But percentage accumulation in biomass was almost same at 100 mM and 150 mM arsenate concentrations, with approximately 20% accumulation.

In this study, arsenate (uptaken by bacteria) may be reduced to arsenite and further extrusion of arsenite occurs in LB broth media. Reduction potential of bacteria was also depending on amount of arsenate supplied to the LB broth medium. As the concentration of arsenate increased, the percentage of arsenate that was reduced to arsenite decreased gradually. For example, at an arsenate concentration of 50 mM, 64% of the arsenate was reduced to arsenite, but at higher concentrations of 100, 150, and 200 mM, the percentages reduced arsenite were 40%, 21%, and 10.5%, respectively.

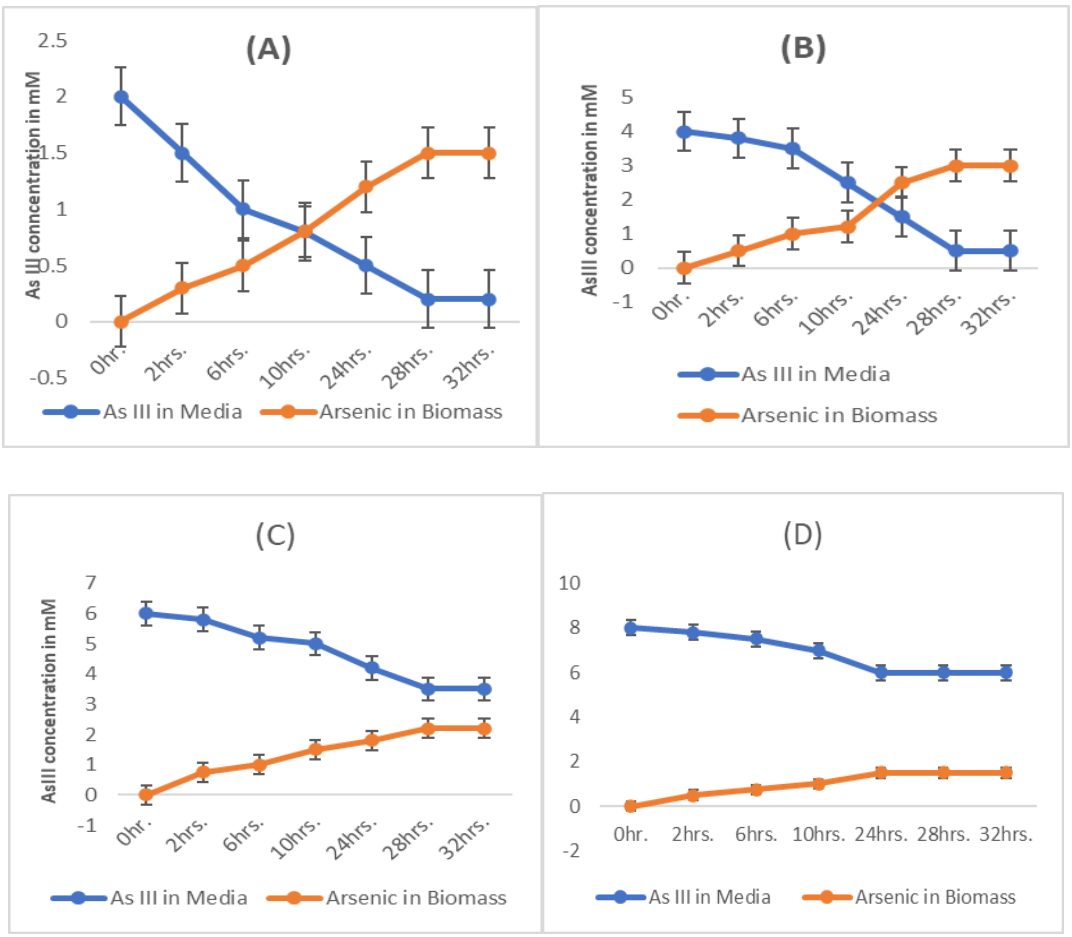

Fig. 5: Y-axis represents Arsenite concentration left in residual bacterial broth media and total arsenic uptaken in bacterial biomass at different time periods (X-axis) of bacterial growth phase.(A) 2 mM (B) 4 mM (C) 6 mM (D) 8 mM As(III) enriched media. Concentration of arsenite gradually decreased in media showing Arsenite removal from the medium. All values are mean of three replicates and standard errors (SE) are presented as error bars (±).

Percentage removal/ uptake potential of arsenite (AsIII):

It appears that *Bacillus stratosphericus* MKS can remove arsenite and the percentage removal and uptake of arsenite depends on supplied concentration of arsenite in the medium. 10 mM arsenite concentration was measured as MIC for *Bacillus stratosphericus* MKS. To determine the percentage removal/uptake of arsenite, the left concentration of arsenite in residual media determined at various time periods of the growth phases of bacteria depicted in (Fig. 5). At a concentration of 2 mM arsenite, the bacteria removed 90% of the arsenite, and 75% of the arsenic was found in the bacterial biomass. At 4 mM arsenite, the bacteria removed 87.5% of the arsenite, and again 75% of the arsenic was found in the bacterial biomass. At higher concentrations of arsenite (6 mM and 8 mM), the percentage of arsenite removed by the bacteria decreased significantly, with only 41.6% removal at 6 mM and 25% removal at 8 mM. Moreover, the percentage of arsenic found in the bacterial biomass decreased as well, with only 36.6% and 18.75% at 6 mM and 8 mM, respectively.

Discussion

Previous studies have suggested that changes in microbial metabolism and geochemical cycles may be responsible for the high levels of toxic arsenic

in certain regions, such as the Bengal Delta region in India (Islam *et al.*, 2004;Tsai *et al.*, 2009). Both the above-mentioned mechanisms may be responsible for arsenic release into the water used for drinking purposes by millions of people. In the present study, the selected site for the study is in the State Bihar (India), which is a part of the Bengal Delta region (85° 32′E longitude and 25° 11′N latitude), that was previously reported as arsenic contaminated region (Chakrabarti *et al.*, 2004; Chowdhury *et al.*, 2009). Bacteria can utilize arsenic as an energy source that results in the detoxification of toxic arsenic from environmental components (Macy *et al.*, 1996; Santini *et al.*, 2000; Anderson and Cook 2004).

So, one potential solution to remove arsenic toxicity is to identify and study native bacterial species that can utilize arsenic as an energy source, leading to the detoxification of the environment. In this study, we isolated and identified a specific bacterial species, *Bacillus stratosphericus* MKS with accession number OM841514, which showed promise in this regard. This species could potentially be used in future efforts to mitigate the effects of arsenic contamination in affected areas.

Arsenic-resistant bacteria isolated from arsenic-contaminated soil:

Anthropogenic activities can alter the geochemical cycles of arsenic, which can result in higher amounts of arsenic in groundwater (Tsai *et al.*, 2009). These activities include the use of fertilizers and pesticides, mining, and industrial operations. Arsenic poisoning of groundwater can have major health repercussions, including skin lesions, cancer, and other ailments, in Bihar (Kumar *et al.*, 2021). In naturally contaminated environments, it crucial to look at the potential uptake and removal of arsenic species by native bacterial species. The arsenic toxicity and mobility in the environmental components can be significantly impacted by the biotransformation of arsenic by bacteria. Understanding the arsenic biogeochemistry and its bad impacts on health of human being and the environment requires research on the possible uptake and removal of arsenic species by native bacterial species in naturally contaminated places.

Toxicity of arsenic revealed by Growth profile of MKS:

Bacterial growth gradually slowed down as a result of the cell toxicity that was demonstrated by raising the arsenic concentration in the LB growth medium. The minimum inhibitory concentration (MIC) for the above-mentioned strain of bacteria was determined to be 86% at 250 mM arsenate and 87.5% at 10 mM arsenite. This supports the findings published by Kaltreider *et al.* (2001) that arsenite was more harmful than arsenate. Because of its high affinity for thiols, arsenite has a higher toxicity than arsenate because it can more readily connect to proteins found inside cells.

According to Liu *et al*. (2001), this could cause toxicity and direct damage to the cell's biomolecules. Given its capacity to resist both arsenate and arsenite, *Bacillus stratosphericus* MKS is a viable option for the cleanup of areas contaminated by arsenic. According to earlier reports, the species Bacillus is arsenate and arsenite-tolerant (Bachate *et al.*, 2009; Tripti *et al.*, 2014, 2016). However, very few reports have been made about organisms that can withstand both arsenate and arsenite (Banerjee and Santra, 2010).

The fact that there are few reports of species that can tolerate both forms of arsenic (arsenate and arsenite) make this finding particularly interesting. *Bacillus stratosphericus* isolate 14N has been identified as a highly lead-resistant rhizosphere bacteria which can tolerate 50 mg/l lead concentration (Kamaruzzaman *et al.*, 2020). This suggests that this species may have a broad range of tolerance to various environmental toxins, making it a valuable tool for bioremediation efforts.

According to Shivaji *et al.* (2006), *Bacillus stratosphericus* MKS grows atoptimum at pH 6 to pH 10, but not at pH 5 and pH 11. The most optimum pH for bacterial development was 8.

Most of the bacillus species showed optimum growth at pH range 5.4 to 8.5, which may be why changes in pH had a negative impact on bacterial growth (Combet-Blanc *et al.*, 1995; Tripti *et al.*, 2016).

Removal and uptake of arsenate and arsenite by Bacillus stratosphericus MKS:

The ability of *Bacillus stratosphericus* MKS to

remove arsenate and arsenite depends on the concentration of arsenate and arsenite provided in the growth medium. Maximum removal of arsenate (99.9%) was observed at 50 mM, while 90% of arsenite removal was observed at a concentration of 10 mM. It is noteworthy that this bacterial strain can tolerate high levels of arsenate and arsenite, up to 250 mM As(V) and 10 mM As (III), respectively. The toxicity of arsenate (AsO_4^{3-}) is attributed to its structural similarity to phosphate (Po_4^{3-}), which makes it a substrate for the phosphate transport system, leading to its entry into the cell and inhibition of oxidative phosphorylation (Sanders *et al.*; 1997, Wolfe-Simon *et al.*, 2011).

Arsenite, on the other hand, occurs at neutral pH in from of $As(OH)_3$, which is commonly available to bacteria (Tu and Ma, 2003) and can use the glycerol transport system to cross the cell membrane (Mukhopadhyay *et al.*, 2002; Ramirez-Solis *et al.*, 2004). The MIC of *Bacillus stratosphericus* MKS, which is greater than that of the majority of native soil bacterial species that are arsenite-tolerant (1 to 7 mM), is believed to be hyper-tolerant to arsenite (Huang *et al.*, 2010; Liao *et al.*, 2011; Tripti *et al.*, 2014, 2016, 2021). However, as the concentration of arsenite rose from 2 mM to 8 mM, the removal efficiency fell from 90% to 25%, indicating that very high quantities of arsenite may be difficult for the strain to remove effectively.

Bacillus stratosphericus MKS reduced Arsenate to Arsenite:

Bacteria can adapt to and resist harmful quantities of arsenic in their surroundings. These mechanisms include the immobilization of arsenic within the cell as well as its transformation, transportation, reduction, and extrusion from the cell (Rosen *et al.*, 2007; Banerjee and Santra, 2010; Tripti *et al.*, 2014, 2016).

Arsenic is more bioavailable in the environment, which is the microbial reduction of arsenate to arsenite (Harvey *et al.*, 2002). In this study, the reduction of arsenate to arsenite was investigated using *Bacillus stratosphericus* MKS, and the presence of arsenite in residual media enriched with arsenate demonstrated that arsenate was converted into arsenite. It can be explained in the way as follows. The reduction of arsenate, which was extruded in the broth medium, occurs after arsenate first reaches the bacterial cell. This is explained by the residual medium's gradually declining arsenate concentration and rising arsenite concentration. The ars B gene, which codes for an antiporter protein channel that extrudes accumulated arsenite out of the bacterial cell, and the ars C gene, which codes for arsenate reductase, are both involved in this reduction process (Mukhopadhyay and Rosen, 2002, Meng *et al.*, 2004). Overall, these systems enable bacteria to withstand and adjust to environments that include toxic levels of arsenic.

Conclusion

An arsenic-contaminated area in Bihar has led to the isolation of the natural soil bacteria *Bacillus stratosphericus* MKS (N25•40.997′, E84•52.288′). This bacterium stands out because it has proven to be able to withstand hazardous concentrations of both arsenate and arsenite. The minimal inhibitory concentration (MIC) has been demonstrated to be 250 mM for arsenate and 10 mM for arsenite. Additionally, it has been demonstrated that *Bacillus stratosphericus* MKS can remove up to 99.9% of arsenate at 50 mM As(V) and 90% of arsenite at 2 mM As (III) concentrations. Intriguingly, one of the ways the bacterium can endure in an arsenic-contaminated environment may be by the conversion of arsenate to arsenite within the bacterial cell. *Bacillus stratosphericus* MKS may hold significant potential

for the cleanup of arsenic-contaminated soils due to its exceptional capacity to withstand and extract arsenic. The improvement of the health and safety of those residing in locations with high levels of arsenic contamination may result from this in major ways. Undoubtedly, more study in this area is necessary.

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References

Ahamed S, Kumar Sengupta M, Mukherjee A, Amir Hossain M, Das B, Nayak B, Pal A, Chandra Mukherjee S, Pati S, Nath Dutta R, Chatterjee G, Mukherjee A, Srivastava R and Chakraborti D. (2006) Arsenic groundwater contamination and its health effects in the state of Uttar Pradesh (UP) in upper and middle Ganga plain, India: A severe danger. Sci Total Environ. 370(2–3): 310-322.

Altschul S. (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res. 25(17): 3389-3402.

Anwar M. (2006) Arsenic, cadmium and lead levels in hair and toenail samples in Pakistan. Environ Sci. 12(2): 71-86.

Anderson CR and Cook GM. (2004) Isolation and characterization of arsenate-reducing bacteria from arsenic-contaminated sites in New Zealand. Curr Microbiol. 48(5): 341-347.

APHA, AWWA, WEF. (2005) In: Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenburg, A.E. (eds.) Standards methods for the examination of water and waste water. American public health association, American Water Works Association. Water Environment Federation Joint Publication, USA, 3: 1-11.

Argos M, Kalra T, Rathouz PJ, Chen Y, Pierce B, Parvez F, Islam T, Ahmed A, Rakibuz-Zaman M, Hasan R, Sarwar G, Slavkovich V, van Geen A, Graziano J and Ahsan H. (2010) Arsenic exposure from drinking water, and all-cause and chronic-disease mortalities in Bangladesh (heals): A prospective cohort study. Lancet 376(9737): 252-258.

Ghosh Ashok K, Singh SK, Bose N, Roy NP, Singh SK, Upadhaya Ajay K, Kumar S and Singh A. (2009) Arsenic hot spots detected in Patna, Bihar (India)- A serious health hazard for estimated human population of 5.5 lakhs. In: Assessment of groundwater resources and management, (eds.) Ramanathan A.L., Bhattacharya P., Keshari A.K., Bundschuh J., Cgandrashekharam D. and Singh S.K., ISGSD Special Publication, 62-70.

Bachate SP, Cavalca L and Andreoni V. (2009) Arsenic-resistant bacteria isolated from agricultural soils of Bangladesh and characterization of arsenate-reducing strains. J Appl Microbiol. 107(1): 145-156.

Banerjee S and Santra SC. (2010) Studies on the role of aerobic soil bacteria in arsenic transformation and mobilization. Int Q J Life Sci. 2: 587-594.

Cherian T and Narayana B. (2005) A new spectrophotometric method for the determination of arsenic in environmental and biological samples. Analytical Lett. 38(13): 2207-2216.

Chakraborti D, Sengupta MK, Rahman MM and Ahamed S. (2004) Groundwater arsenic contamination and its health effects in the Ganga-Meghna-Brahmaputra plain. J Environ Monit. 6: 74N-83N.

Chowdhury R, Sen AK, Karak P, Chatterjee R, Giri AK and Chaudhuri K. (2009) Isolation and characterization of an arsenic-resistant bacterium from a bore-well in West Bengal, India. Annals Microbiol. 59(2): 253-258.

Combet-Blanc Y, Kalamba KK and Kergoat PY. (1995) Effect of pH on *Bacillus thermoamylovorans* growth and glucose fermentation. Appl Environ Microbiol. 61(2): 656-659.

Escalante G, Campos VL, Valenzuela C, Yañez J, Zaror C and Mondaca MA. (2009) Arsenic resistant bacteria isolated from arsenic contaminated river in the Atacama Desert (chile). Bull Environ Contam Toxicol. 83(5): 657-661.

Grifoni A, Bazzicalupo M, Di Serio C, Fancelli S and Fani R. (1995) Identification of azospirillum strains by restriction fragment length polymorphism of the 16S rdna and of the histidine operon. FEMS Microbiol Lett. 127(1–2): 85-91.

Hao L, Liu M, Wang N and Li G. (2018) A critical review on arsenic removal from water using iron-based adsorbents. RSC Adv. 8(69): 39545-39560.

Harvey CF, Swartz CH, Badruzzaman AB, Keon-Blute N, Yu W, Ali MA, Jay J, Beckie R, Niedan V, Brabander D, Oates PM, Ashfaque KN, Islam S, Hemond HF and Ahmed MF. (2002) Arsenic mobility and groundwater extraction in Bangladesh. Science 298(5598): 1602–1606.

Huang A, Teplitski M, Rathinasabapathi B and Ma L. (2010) Characterization of arsenic-resistant bacteria from the rhizosphere of arsenic hyperaccumulator *Pteris vittata*. Canadian J Microbiol. 56(3): 236-246.

Hussein H, Farag S, Kandil K and Moawad H. (2005)

Tolerance and uptake of heavy metals by pseudomonads. Process Biochem. 40(2): 955-961.

IARC Working Group on the Evaluation of Carcinogenic Risk to Humans (2012) Arsenic, metals, fbres and dusts (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 100C, Lyon, 2012).

Islam FS, Gault AG, Boothman C, Polya DA, Charnock JM, Chatterjee D and Lloyd JR. (2004) Role of metal-reducing bacteria in arsenic release from Bengal Delta sediments. Nature 430(6995): 68-71.

Järup L. (2003) Hazards of heavy metal contamination. British Med Bull. 68(1): 167-182.

Kaltreider RC, Davis AM, Lariviere JP and Hamilton JW. (2001) Arsenic alters the function of the glucocorticoid receptor as a transcription factor. Environ Hth Perspect. 109(3): 245-251.

Kamaruzzaman MA, Abdullah SRS, Hasan HA, Hassan M, Othman AR and Idris M. (2020) Characterisation of PB-resistant plant growth-promoting rhizobacteria (PGPR) from *Scirpus grossus*. Biocatalysis Agricult Biotechnol. 23: 101456.

Kimura M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Molec Evol. 16:111-120.

Kumar S, Stecher G, Li M, KnyazC and Tamura K. (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molec Biol Evol. 35: 1547-1549.

Kumar A, Ali M, Kumar R, Kumar M, Sagar P, Pandey RK, Akhouri V, Kumar V, Anand G, Niraj PK, Rani R, Kumar S, Kumar D, Bishwapriya A and Ghosh AK. (2021) Arsenic exposure in Indo Gangetic plains of Bihar causing increased cancer risk. Sci Rep. 11(1):2376.

Kumar A, Ali M, Raj V, Kumari A, Rachamalla M, Niyogi S, Kumar D, Sharma A, Saxena A, Panjawani G, Jain P, Vidyarthi A, Kumar N, Kumar M, Niraj PK, Rahman MS, Bishwapriya A, Kumar R, Sakamoto M, Kumar S, Singh M and Ghosh AK. (2023) Arsenic causing gallbladder cancer disease in Bihar. Sci Rep. 13(1): 4259.

Liu SX, Athar M, Lippai I, Waldren C and Hei TK. (2001) Induction of oxyradicals by arsenic: Implication for mechanism of genotoxicity. Proc Nat Acad Sci. 98(4): 1643-1648.

Liao VHC, Chu YJ, Su YC, Hsiao SY, Wei CC, Liu CW, Liao CM, Shen WC and Chang FJ. (2011) Arsenite-oxidizing and arsenate-reducing bacteria associated with arsenic-rich groundwater in Taiwan. J Contaminant Hydrol. 123(1–2): 20–29.

Macur RE, Wheeler JT, McDermott TR and Inskeep WP. (2001) Microbial populations associated with the reduction and enhanced mobilization of arsenic in mine tailings. Environ Sci Technol. 35(18): 3676-3682.

Macy JM, Nunan K, Hagen KD, Dixon DR, Harbour PJ, Cahill M and Sly LI. (1996) *Chrysiogenes arsenatis* gen. nov., sp. nov., a new arsenate-respiring bacterium isolated from gold mine wastewater. Int J Systematic Bacteriol. 46(4): 1153-1157.

Meng YL, Liu Z and Rosen BP. (2004) As(III) and SB(III) uptake by GLPF and efflux by ARSB in *Escherichia coli.* J Biol Chem. 279(18): 18334-18341.

Mukhopadhyay R and Rosen BP. (2002) Arsenate reductases in prokaryotes and eukaryotes. Environ Hlth Perspect. 110(suppl 5): 745-748.

Ramírez-Solís A, Mukopadhyay R, Rosen BP and Stemmler TL. (2004) Experimental and theoretical characterization of arsenite in water: insights into the coordination environment of AS–O. Inorganic Chemistry 43(9): 2954-2959.

Prescott H. (2002) Laboratory exercise in microbiology. The Mcgraw-Hill, United States.

Rodrigues JL, Batista BL, Nunes JA, Passos CJS and Barbosa F. (2008) Evaluation of the use of human hair for biomonitoring the deficiency of essential and exposure to toxic elements. Sci Total Environ. 405(1–3): 370-376.

Rosen BP and Liu Z. (2009) Transport pathways for arsenic and selenium: A minireview. Environ Int. 35(3): 512-515.

Santini JM, Sly LI, Schnagl RD and Macy JM. (2000) A new chemolithoautotrophic arsenite-oxidizing bacterium isolated from a gold mine: Phylogenetic, physiological, and preliminary biochemical studies. Appl Environ Microbiol. 66(1): 92-97.

Sanders OI, Rensing C, Kuroda M, Mitra B and Rosen BP. (1997) Antimonite is accumulated by the glycerol facilitator glpf in *Escherichia coli*. J Bacteriol, 179(10): 3365-3367.

Sela H, Karpas Z, Cohen H, Tal A and Zeiri Y. (2013) Trace element concentration in hair samples as an indicator of exposure of population in the Negev, Israel. Biol Trace Element Res. 155(2): 209-220.

Shivaji S, Chaturvedi P, Suresh K, Reddy G, Dutt C, Wainwright M, Narlikar J and Bhargava P. (2006) *Bacillus aerius* sp. nov., *Bacillus aerophilus* sp. nov., *Bacillus stratosphericus* sp. nov. and *Bacillus altitudinis* sp. nov., isolated from cryogenic tubes used for collecting air samples from high altitudes. Int J Syst Evol Microbiol. 56(Pt 7): 1465-1473.

Silver S and Phung LT. (2005) Genes and enzymes

involved in bacterial oxidation and reduction of inorganic arsenic. Appl Environ Microbiol. 71(2): 599-608.

Smith AH, Lingas EO and Rahman M. (2000) Contamination of drinking-water by arsenic in Bangladesh: A public health emergency. Bull World Hlth Organ. 78(9): 1093-1103.

Tripti Kumari, Sayantan D, Shardendu S, Singh DN and Tripathi AK. (2014) Potential for the uptake and removal of arsenic [as (V) and as (iii)] and the reduction of as (V) to as (III) by *Bacillus licheniformis* (DAS1) under different stresses. Korean J Microbiol Biotechnol. 42(3): 238-248.

Tripti Kumari and Shardendu S. (2016) pH modulates arsenic toxicity in *Bacillus licheniformis* DAS-2. Ecotoxicol Environ Saf. 130: 240-247.

Tripti Kumari and Shardendu S. (2021) Efficiency of arsenic remediation from growth medium through *Bacillus licheniformis* modulated by phosphate $(PO_4)^{3-}$ and nitrate $(NO_3)^-$ enrichment. Arch Microbiol. 203(7): 4081-4089.

Tsai SL, Singh S and Chen W. (2009) Arsenic metabolism by microbes in nature and the impact on arsenic remediation. Curr Opinion Biotechnol. 20(6): 659-667.

Tu S and Ma LQ. (2003) Interactive effects of pH, arsenic and phosphorus on uptake of AS and P and growth of the arsenic hyperaccumulator *Pteris vittata* L. under hydroponic conditions. Environ Exp Botany 50(3): 243-251.

Wolfe-Simon F, Blum JS, Kulp TR, Gordon GW, Hoeft SE, Pett-Ridge J, Stolz JF, Webb SM, Weber PK, Davies PC, Anbar AD and Oremland RS. (2011) A bacterium that can grow by using arsenic instead of phosphorus. Science 332(6034): 1163-1166.

Yadav MK, Duduku S , Ghosal Partha S, Mukherjee A and Gupta AK. (2022) A review on the management of arsenic-laden spent adsorbent: Insights of global practices, process criticality, and sustainable solutions. Environ Technol Innov. 27: 102500.

Yan A, Wang Y, Tan SN, Mohd Yusof ML, Ghosh S and Chen Z. (2020) Phytoremediation: A promising approach for revegetation of heavy metal-polluted land. Front Plant Sci. 11: 359.