
APST

Asia-Pacific Journal of Science and Technology<https://www.tci-thaijo.org/index.php/APST/index>Published by the Research and Graduate Studies,
Khon Kaen University, Thailand

Isolation, characterization and identification of an As(V)-resistant plant growth promoting bacteria for potential use in bioremediation

Puja Agnihotri^{1,*}, Sharanya Banerjee¹, Madhumita Maitra¹ and Arup K. Mitra¹¹Department of Microbiology, St .Xavier's College, West Bengal, India

*Corresponding author: puja.agnihotri001@gmail.com

Received 13 November 2020

Revised 19 January 2021

Accepted 21 January 2021

Abstract

Soil is a reservoir of various kinds of bacteria that have several constitutively expressed as well as inductively expressed functions. These bacteria not only have plant growth promoting activities but also often come with added features, such as heavy metal tolerance, nitrogen fixation, iron chelation and plant hormone production. Because arsenic (As) pollution has become a rising global threat, it is important to utilize eco-friendly strategies for As mitigation, such as bioremediation. In the present study, bacterial strains were obtained from the soil of North 24 Parganas, an arsenic polluted district in West Bengal and their As-tolerance was tested under in-vitro conditions. Several bacterial strains were isolated and their arsenate (As(V)) tolerance was studied. Out of these bacteria, the strain S3C2 was found to have the highest tolerance level. Furthermore, this strain was found to retain 40 % of As in the cell pellet from the medium, as revealed by inductively coupled plasma optical emission spectroscopy analysis data. This efficient As(V)-resistant strain was identified using 16S rRNA sequencing and was found to be a *Bacillus cereus* strain (GenBank Accession Number MW012261). It was found to be positive for siderophore production, with the ability to solubilise 19% of phosphate and produce 1.87±3.2 µg/ml indole acetic acid in Tryptophan supplemented medium in vitro. This highly As(V)-resistant plant growth promoting bacterial strain has potential for As mitigation from polluted natural sources and promote the growth of plants in arsenic polluted zones at the same time.

Keywords: As(V), Inductively coupled plasma optical emission spectroscopy, 16S rRNA, Siderophore, Indole acetic acid, *Bacillus cereus*

1. Introduction

Heavy metals, one of the major environmental contaminants, have been noted to cause long lasting health effects mostly as a result of their toxicity and tendency to bio accumulate, thereby increasing with every advancing level of the food chain, finally crossing the required threshold by the time it reaches human beings. They are soluble in water hence also pollute surface and groundwater systems. Thus, due to the harmful effect it has on the ecology, environment, general health and evolutionary aspect, heavy metal toxicity is an increasing problem [1,2].

The common heavy metal contaminants are arsenic, cadmium, chromium, copper, lead, zinc, aluminum, manganese, which are either present naturally in the environment as a result of pedology, seepage from rocks into the aquatic system, volcanic activities, atmospheric deposition, sediment resuspension in soil due to extraction of heavy metals from passing water by humus (high affinity for heavy metals) or due to anthropogenic activities. In the wake of industrialization, one of the major downsides observed was that of heavy metal contamination from mining activities, tanneries, smelting, electroplating and galvanization industries, paper mills etc. Excessive pesticide and fertilizer usage have also been linked with heavy metal toxicity in the environment [3].

One of the most common heavy metal is arsenic (As). It is a metalloid that exists in the atmosphere, lithosphere, hydrosphere as well as in biospheres. As either occurs naturally in the environment or arises due to anthropogenic causes. It can occur in both organic (As³⁻ & As⁵⁻) and inorganic forms (As³⁺ & As⁵⁺) but the latter

has greater toxicity. Mobilization of As has been observed to be greater through water and soil, thus they can easily be consumed, inhaled or be in contact with life forms, thereby causing a major risk to them. Major health concerns relating to As toxicity are: gastrointestinal issues, central and peripheral nerve damage, anemia, leucopenia, keratosis, cancer of the lung, liver, kidney etc, among other health issues [4].

As contamination has been recorded in many parts of the world but its severity has been noticed in countries of Bangladesh followed by India then Mongolia, China and finally Taiwan. In India out of the 8 places, namely Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura, screened it was seen that in Assam, Tripura, Arunachal Pradesh, Nagaland, Manipur the As exceeded the permissible level of 50 $\mu\text{g/L}$ every 2 L water consumed as stated by WHO. No case of arsenicosis has been recorded despite the high levels of As [5].

The most extensive effect of As contamination in India has been recorded in the aquifers of Indo-Gangetic delta plains due to multi-factorial reasons. In the state of West Bengal there are 9 districts affected by As pollution, out of which North 24 Parganas will be discussed here. As polluted water was used not just for consumption but also for domestic and agricultural purposes. The main source of the As polluted water are the shallow hand tubewells (deep tubewells did not show contamination). The estimate showed that about 29.2% individuals were using water having greater than 50 $\mu\text{g/L}$ As contamination (maximum permissible limit as stated by WHO) and 52.8% using water with As contamination above 10 $\mu\text{g/L}$ out of all the 22 examined blocks. Apart from this, water was also used for farming which resulted in As accumulation in the produce. A consumption of over 100 μg per day As from rice and vegetables was recorded. 16 of the total blocks under study showed presence of As in dermatological samples but not all suffered from As toxicity. However, even when the citizens were provided As free water to drink, presence of As never reduced in the dermatological samples. Those that suffered from it, showed symptoms like arsenic neuropathy, skin lesions and skin cancer [6-8].

This contaminated water is often used for irrigation purposes hence it is very likely for rice, like other plants, to come in contact with As. In a hydroponic study conducted, it was noticed that, the bioaccumulation of As occurs on root surface iron plaques in a phosphorus dependent manner. Lower phosphorus levels favour for an increased plaque formation and depending upon the variations in plaque formation from one variety to another, the As sequestration will also vary. More the As is sequestered in the plaques, the less likely is it to translocate to the upper regions of the rice plant (stem, leaf, grains, husk etc). In another study of various rice varieties grown on As contaminated soils, it was also seen that in addition to As mobilization being dependent on plaque formation, the speciation of As (organic and inorganic) also played a crucial role in bioaccumulation of it. Inorganic species (As^{3+} and As^{5+}) were present in grains while organic species (monomethylarsenate and dimethylarsenate) were absent suggesting that the rice cultivars could not metabolize the organic varieties through methylation. The overall As concentration was also higher than the permissible levels thus making it unsafe for consumption. This is especially a bigger threat in West Bengal since rice is the staple food of this region [9].

Thus, a requirement for As remediation of soil and groundwater is necessary. As compared to expensive ex-situ remediation methods, in-situ methods may prove to be more suitable. Some of the methods are due through sorption studies where As is immobilized through sorption to or chemical oxidation-reduction processes by iron, aluminum, manganese hydroxides and oxides, to clay minerals, cations and anions. Oxidation-reduction by sulfides is also performed. As can also be bio-transformed by chemical or microbial means as well as through phytoremediation using plants that bio-accumulate high doses of As among others. In general, bioremediation as a method is more effective over physico-chemical means and can be used for groundwater, surface water, soil and sediment remediation. The most appropriate method under bioremediation in case of reducing heavy metal pollution is microbial remediation, which makes the use of various fungi and bacteria. The main advantages served by this method are: fast reproduction time and small size making the tool an effective and portable one, compact genetic material with polycistronic DNA that produces a variety of proteins to help execute different functions despite small genome size, reduced production of waste or by products thus becoming eco-friendly, cost efficacy [10]. The present study aims on identifying and characterizing the soil-borne bacterial strain for its arsenate (As(V)) resistance as well as plant growth promoting activities. This can further be exploited to remediate As from naturally polluted sites and promote the growth of plants that grow in such As-polluted zones.

2. Materials and methods

2.1 Collection of soil samples and isolation of bacteria

Soil samples were collected from local fields in Barrackpore (22.767° N, 88.388° E) of North 24 Parganas district, West Bengal, in autoclaved falcon tubes. The arsenic content in the soil, as measured by inductively coupled plasma optical emission spectroscopy, was found to be 5.94 ± 0.02 mg/kg. Samples were stored at 4 °C till

further use. For isolation of bacteria, serial dilution and pour plating technique was used. Autoclaved nutrient agar media (HiMedia) was used to pour plate aliquots of serially diluted soil samples.

2.2 Screening of isolated bacteria for As(V) tolerance

The bacterial strains were cultured in autoclaved Nutrient broth media (HiMedia) for 16 h and thereafter inoculated in As(V) supplemented Nutrient broth media, with As(V) supplementation given in gradually increasing order. The concentrations of As(V) ranged from 0 mg/L to 100 mg/L, and sodium arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) (LobaChemie) was used as the source of As(V), while deionized water of an electrical conductivity of 0.2 μS was used to make all the preparations. The inoculum was given at a concentration of 1% (v/v) of the media volume (cell count 2.8×10^8 cfu/mL). A negative control was also kept, having no As(V) supplementation. Growth was assessed spectrophotometrically at OD₅₈₀ (Optical Density at 580 nm wavelength), after an incubation period of 24 h at 37 °C, with respect to the control [11,12].

2.3 Inductively coupled plasma optical emission spectroscopy (ICP-OES) analysis of the amount of arsenic retained in the cells of the most efficient As(V) tolerant strain

The bacterial strain (S3C2) was made to grow at its maximum tolerable As(V) dose for 24 h, followed by centrifuging the culture at 7.826 $\times g$ for 10 min. The cell pellet was collected, dried and acidified to a pH less than 2 using 2% HNO_3 , 16 h prior to ICP-OES analysis. For the analysis, Perkin Elmer model Avio 220 Max was used. The plasma power was optimized between 1300-1400 W, while the nebulizer flow was adjusted between 0.90 and 0.60 L/min for radial and axial plasmas respectively. As was measured spectrophotometrically at the UV wavelength of 193 nm. The As content of the sample was obtained with reference to an As standard calibration curve.

2.4 Morphological and biochemical characterization of the bacterial strain S3C2

Purified culture was grown on Nutrient Agar plates and the colony morphology was recorded. Gram staining was done and cellular morphology was studied under a total magnification of 400X, using Dewinter microscope digital camera, DIG1510, 5.1 MP 1/2.5'' CMOS sensor. Biochemical characterization was also performed, according to *Bergey's Manual of Systematic Bacteriology*, to test for carbohydrate utilization, nitrate reduction, H_2S production, citrate utilization and presence of enzymes such as oxidase, catalase, gelatinase and amylase [13].

2.5 Identification through 16S rRNA sequencing of S3C2 bacterial strain

gDNA, isolated from the pure culture of bacteria, was checked for its quality on 1% Agarose gel. The 16S rDNA gene was amplified using 27F and 1492R primers. A discrete PCR amplicon band corresponding to 1500bp was observed upon resolution on Agarose gel and was thereafter purified. This amplicon was then subjected to forward and reverse DNA sequencing reactions using the specific primers, by means of BDT v3.1 cycle sequencing kit, on ABI 3730xl Genetic Analyzer. Contigs were generated from the forward and reverse sequence data using the BioEdit software. Thereafter, it was used to carry out BLAST with the NCBI GenBank database. Maximum query coverage and % identity were used as parameters to estimate the species of the bacteria. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated and the phylogenetic tree was constructed using MEGA 7. While constructing the phylogenetic tree, the Maximum Likelihood method, based on Kimura 2 parameter model, was used to infer the evolutionary history. To represent the evolutionary history of the taxa analyzed, bootstrap consensus tree, inferred from 1000 replicates, was taken. Branches that correspond to partitions reproduced in less than 50% bootstrap replicates have been collapsed. The percent of replicate trees, in which the associated taxa are clustered together in the bootstrap test (1000 replicates) have been shown next to the branches. Initial tree(s) for the heuristic search have been obtained automatically using the Neighbour-Join and BioNJ algorithms. The algorithms were applied to a matrix of pair-wise distances that were estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. 11 nucleotide sequences have been used for analysis. The codon positions that have been included are: 1st+2nd+3rd+Non-coding. All positions containing gaps and missing data have been eliminated. There are a total of 1434 positions in the final dataset. MEGA7 has been used to conduct the evolutionary analyses.

2.6 Total protein content and arsenate reductase assay

Two sets of Nutrient Broth medium were used, wherein one was the negative control and the other was supplemented with As(V) at the maximum tolerable dose of S3C2 strain. Overnight grown cultures were taken to obtain the cell pellets, followed by washing twice in reaction buffer (10mM Tris, pH 7.5, with 1mM Na₂EDTA and 1mM MgCl₂) (HiMedia). The pellet was finally suspended in the reaction buffer and lysed by sonication, followed by cold centrifugation and collecting the supernatant. To measure the total protein content, Thermo Fisher Scientific- Qubit 3.0 Fluorometer (Invitrogen Ref.: Q33216) was used. To the Qubit working solution, prepared by using Qubit buffer and reagent, 10 µL of the crude protein extract was added. The mixture was used to measure the protein content in the already calibrated fluorometer.

Assay for the arsenate reductase enzyme was carried out using the NADPH oxidation method. To initiate the NADPH oxidation at 37 °C, 50 µL of the crude extract was mixed with 820 µL of reaction buffer, 30 µL 10 mM DTT, 50 µL 2 mM arsenate, and 50 µL 3 mM NADPH. OD₃₄₀ was obtained to estimate the percentage reduction of arsenate with reference to NADPH oxidation (NADPH has an absorbance of 1.0 at 340 nm) [14]. Enzyme activity was also calculated.

2.7 Qualitative detection of siderophore production

A petri-plate was divided into two halves. One half of the petri-plates contained Nutrient Agar, while the other half contained Chrome Azurol S- agar (HiMedia). Bacterial culture was streaked on the Nutrient Agar, and was incubated for a period of 7 days at 37 °C. The distance of discharge of the blue colour on Chrome Azurol S- agar was measured [15].

2.8 Quantification of indole acetic acid (IAA) production

Bacterial cultures grown in King's B bacterial media (Peptone: 20 g/L, K₂HPO₄: 1.15 g/L, MgSO₄.7H₂O: 1.15 g/L, Glycerol: 1.5 % v/v), with and without the supplementation of Tryptophan (0.5 g/L), were used to collect the supernatant. One ml of this sup was treated with one ml of the Salkowski reagent (4.5 g FeCl₃ in 1 L 10.8 M H₂SO₄), followed by incubation in dark for 30 min. OD₅₃₀ was measured, which was plotted against an IAA (HiMedia) standard curve to obtain the concentration of IAA produced [16].

2.9 Quantification of phosphate solubilisation

The amount of phosphate solubilised by the bacteria in the Pikovskaya medium was measured using the Barton's reagent method [17].

2.10 Antibiotic sensitivity test

Actively growing cultures of the bacterial strains were spread on sterile Mueller-Hinton Agar (HiMedia) plates. After plating, the plates were allowed to dry. Commercially available antibiotic discs were placed atop the plates having the bacterial cultures and then gently pressed using sterile forceps. Plates were incubated at 37 °C for 18 h [18]. Thereafter the Zone of Inhibition (Z.O.I.) was measured and the response classified as Resistant, Sensitive and Intermediate based on comparing with standard reference table.

2.11 Statistical analysis

All the experiments were run in triplicates and their mean with the Standard Error of the Mean (S.E.M.) is provided. The software GraphPad Prism 5 was used for graphical representation of the data.

3. Results and discussion

3.1 Screening of isolated bacteria for As(V) tolerance and most efficient As(V) tolerant bacterial strain

Out of the several bacteria isolated through pour plating of serially diluted soil samples, one particular strain was found to have the highest As(V) tolerable value at 30 mg/L. Its growth was completely ceased at an As(V) concentration of 50 mg/L. The effect of As(V) on growth (OD₅₈₀) of this bacterial strain has been shown in Figure 1. This strain was labelled as S3C2 for further purposes. When grown at 30 mg/L of As(V), the bacteria could retain 12 ±0.32 mg/L As in its dried cell pellet as revealed by the ICP-OES analysis data. Thus the strain S3C2 can effectively retain 40% (w/v) of As in its dry cell pellet under in vitro conditions.

3.2 Morphological and biochemical characterization of S3C2 strain

The result of biochemical tests has been shown in Table 1. Table 2 shows the response of the bacteria to the various antibiotics. Colony morphology was whitish, slimy, circular, entire margin, flat, moderate in size. The cells were Gram positive, rods as seen in Figure 2.

The bacteria could utilize all the three types of sugars for growth after 48 h of incubation, however only the use of glucose and sucrose resulted in fermentation of acids, as revealed by the red colour in the medium, with no bubble formation in the Durham's tube.

Motility was observed under the microscope which could be attributed to flagella. Possible presence of amylase resulted in starch hydrolysis, while there was a lack of gelatinase activity. An important enzymatic factor which is involved in oxidative stress tolerance, namely catalase, was also present. Among the antibiotics tested, strain S3C2 showed strong resistance against the beta-lactam antibiotics piperacillin, penicillin, oxacillin, cephalothin (1st generation cephalosporin), amoxycylav, cephotaxime and ceftazidime (3rd generation cephalosporins) and aztreonam (which is similar in action to penicillin), indicating strong β -lactamase activity. However, it was found to be sensitive toward the protein synthesis inhibitors such as amikacin, gentamycin and erythromycin. Being a Gram positive bacteria strain S3C2 also showed sensitivity toward the glycopeptide antibiotic teicoplanin. The highly β -lactamase resistant carbapenem antibiotic imipenem also had sensitive response from the bacteria.

3.3 Identification of S3C2 strain

The 16S rRNA sequence was subjected to nucleotide BLAST in NCBI and the strain was classified according to its similarity to sequences in GenBank database. Based on NCBI BLAST analysis, bacterium S3C2 was found to be a strain of the species *Bacillus cereus*. The 16S rDNA sequence was submitted at the NCBI Genbank database under the accession number MW012261. The phylogenetic tree, highlighting the reference species has been shown in Figure 3. The closest similarity was found with *Bacillus cereus* (accession: NR_115714.1).

3.4 Total protein content and arsenate reductase assay

For the strain S3C2, there was 7% increase in the total protein content on treating with As(V), with respect to the control. There was 8% increase in the arsenate reductase enzyme activity under As(V) treatment, with respect to the control. The results have been shown in Figures 4 and 5 respectively. There was 100% oxidation of NADPH under in-vitro conditions, indicating complete reduction of arsenate to arsenite under the As(V) stress, with respect to the control, during the redox reaction. Both the protein content study and arsenate reductase assay indicate that the genes for reducing and detoxifying arsenic may not be constitutively expressed and that, the associated functions are induced in presence of As(V) stress.

3.5 Siderophore production

Siderophores are low molecular weight (<1000 Da) iron-uptake systems found in microorganisms. Because iron (III) is highly insoluble at biological pH and because iron is bound to its carrier protein transferrin in many biological systems, microorganisms have evolved these highly efficient iron-chelating tools. The method of siderophore detection described here uses the principle that an iron (III) dye (Chrome Azurol S-HDTMA) complex changes its blue color when the iron (III) is taken out by the competing siderophore molecule, which has greater affinity for the metal. The blue color on the Chrome Azurol S agar got discharged when siderophores produced by the bacteria diffused freely in the Chrome Azurol S agar half of the plate from the Nutrient agar half. The discharge of the blue color was higher in the region of dye front where the growth of microorganism in the corresponding nutrient agar front was denser (Figure 6). This clearly demonstrates the iron (III) chelating efficacy of the siderophores produced by the bacteria. However, the typical orange halo associated with iron-chelation by siderophore was not observed [15].

3.6 Indole acetic acid (IAA) production

IAA is the most abundant and effective auxin, a class of plant hormones that regulate plant growth and elongation. Production of IAA by bacteria is thus of special interest to plant biologists as well as microbiologists. S3C2 bacterial strain could produce IAA only in the medium supplemented with precursor molecule Tryptophan as seen by the development of rosy pink colour upon addition of Salkowski reagent. There was 1.87 $\mu\text{g/mL}$ IAA produced by the bacteria when 0.5 g/L Tryptophan was added in the culture medium.

Development of pink coloration upon the addition of Salkowski reagent lacked in the cultures which were grown in absence of Tryptophan, indicating the inducible expression of IAA biosynthetic pathway.

3.7 Phosphate solubilisation

Bacterial strain S3C2 was also found to solubilise calcium phosphate into soluble phosphate, as indicated by increase in OD₄₃₀ values of treatments containing the culture vs. the control (i.e. the one lacking bacterial culture). There was also a simultaneous drop in the pH values of the culture, indicating that the production of organic acids through utilization of various sugars assists in the process of phosphate solubilisation (Figure 7).

Table 1 Biochemical tests of S3C2 bacterial strain.

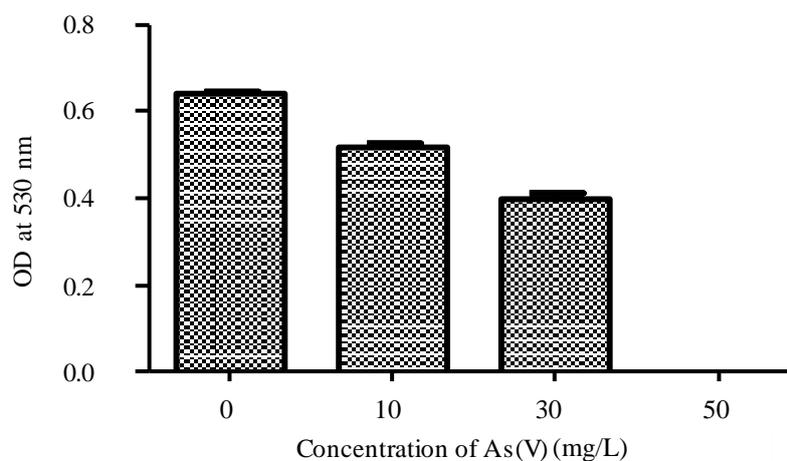
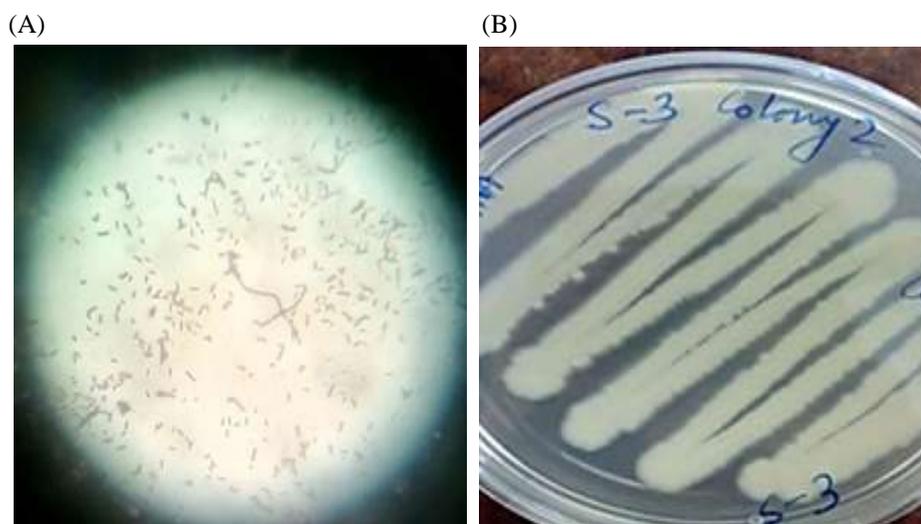
Test			Response
Motility test			+
Nitrate reduction test			+
H ₂ S production test			-
Indole test			-
Methyl red test			-
Voges-Proskauer test			+
Citrate test			+
Starch hydrolysis test			+
Gelatin liquefaction test			-
Catalase test			+
Oxidase test			-
Oxidation-Fermentation and Acid production from different carbohydrates	Glucose	Growth (Turbidity)	+
		Gas production	-
		Acid production	+
	Lactose	Growth (Turbidity)	+
		Gas production	-
		Acid production	-
	Sucrose	Growth (Turbidity)	+
		Gas production	-
		Acid production	+

Table 2 Antibiotic sensitivity test of S3C2 bacteria strain.

Antibiotic	Zone of Inhibition (mm)	Response
Piperacillin (100 µg)	None	Resistant
Linezolid (30 µg)	21±0.09	Intermediate
Ciprofloxacin (5 µg)	24±0.12	Intermediate
Teicoplanin (30 µg)	17±0.07	Sensitive
Vancomycin (30 µg)	16±0.06	Intermediate
Gentamicin (10 µg)	24±0.22	Sensitive
Penicillin G (10 units)	None	Resistant
Oxacillin (1 µg)	None	Resistant
Cephalothin (30 µg)	None	Resistant
Clindamycin (30 µg)	20±0.19	Intermediate
Erythromycin (15 µg)	22±0.26	Sensitive
Amoxyclav (30 µg)	None	Resistant
Cephotaxime (30 µg)	10±0.08	Resistant
Levofloxacin (5 µg)	26±0.09	Sensitive
Aztreonam (30 µg)	15±0.13	Resistant
Imipenem (10 µg)	30±0.36	Sensitive
Amikacin (30 µg)	28±0.41	Sensitive
Ceftazidime (30 µg)	10±0.02	Resistant

Table 3 Details of the isolates considered in phylogenetic analysis.

Accession	Species	Strain	% Identity
NR_115714.1	<i>B. cereus</i>	CCM 2010	99.93%
NR_157735.1	<i>B. proteolyticus</i>	MCCC 1A00365	99.87%
NR_157729.1	<i>B. albus</i>	MCCC 1A02146	99.87%
NR_074540.1	<i>B. cereus</i>	ATCC 14579	99.93%
NR_152692.1	<i>B. wiedmannii</i>	FSL W8-0169	99.80%
NR_157734.1	<i>B. paramycoides</i>	MCCC 1A04098	99.80%
NR_157728.1	<i>B. paranthracis</i>	MCCC 1A00395	99.73%
NR_157733.1	<i>B. pacificus</i>	MCCC 1A06182	99.67%
NR_157731.1	<i>B. mobilis</i>	MCCC 1A05942	99.54%
NR_121761.1	<i>B. toyonensis</i>	BCT 7112	99.60%

**Figure 1** Effect of As(V) on growth of S3C2 bacterial strain. Optical Density at 530 nm (OD₅₃₀) is plotted as a function of As(V) concentration (expressed as parts per million or mg/L).**Figure 2** Gram staining of strain S3C2 under a total magnification of 400X (A) and Colony morphology of S3C2 bacterial strain (B).

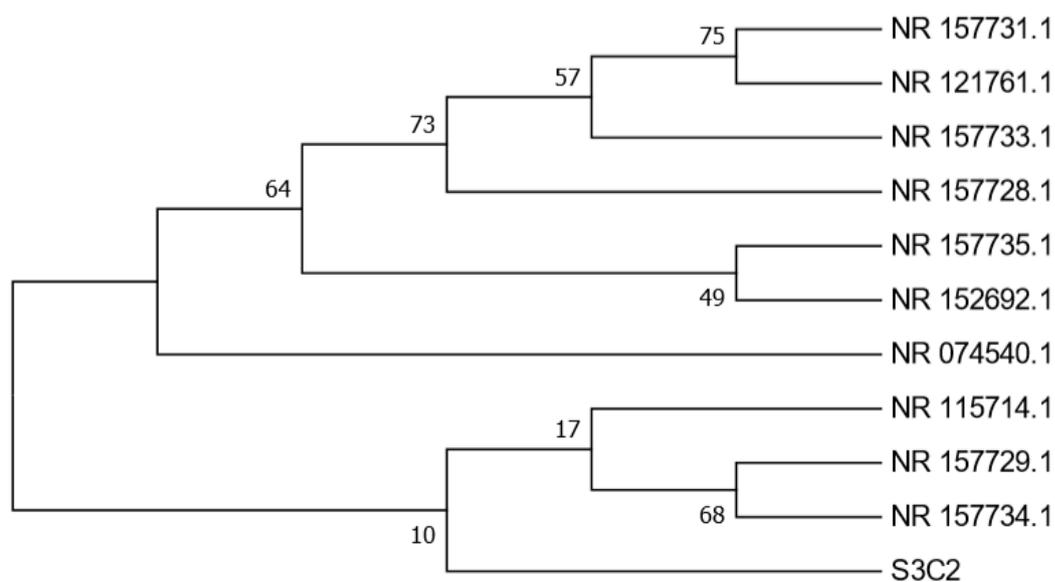


Figure 3 Molecular Phylogenetic analysis of bacterial strain S3C2 by Maximum Likelihood method.

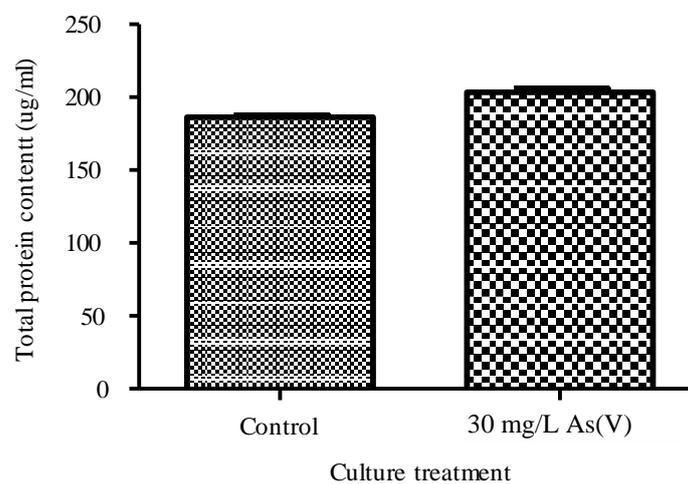


Figure 4 Total protein content of S3C2 bacterial strain under As(V) treated and non-treated conditions.

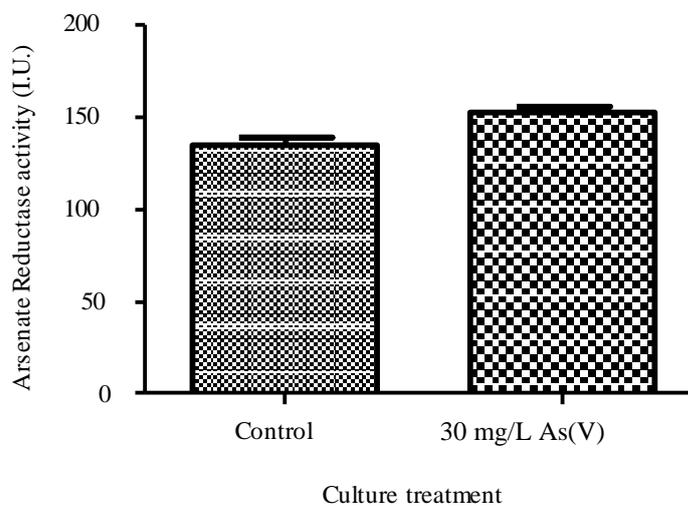


Figure 5 Arsenate Reductase activity of S3C2 bacterial strain, expressed as International Unit or I.U., under As(V) treated and non-treated conditions.

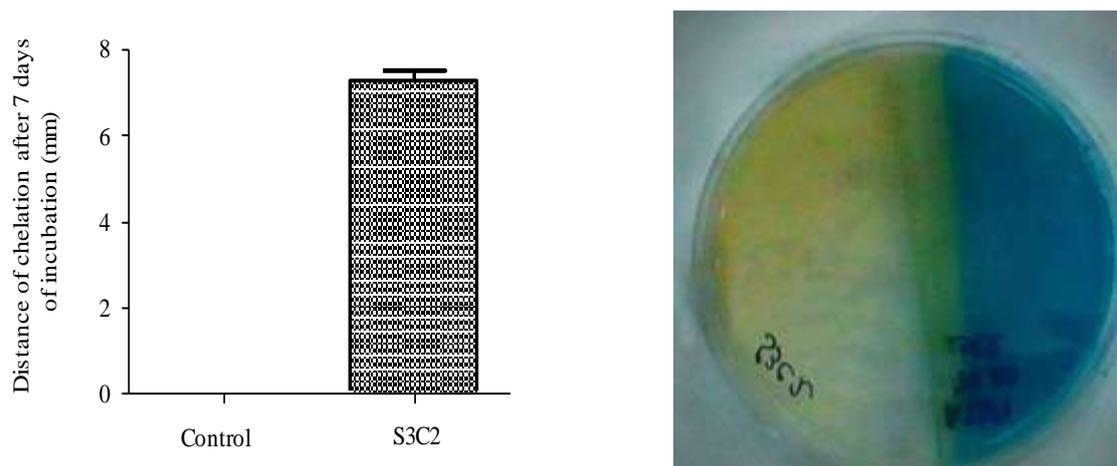


Figure 6 Siderophore detection in strain S3C2 by assessing the discharge of blue coloration on CAS-Agar plates.

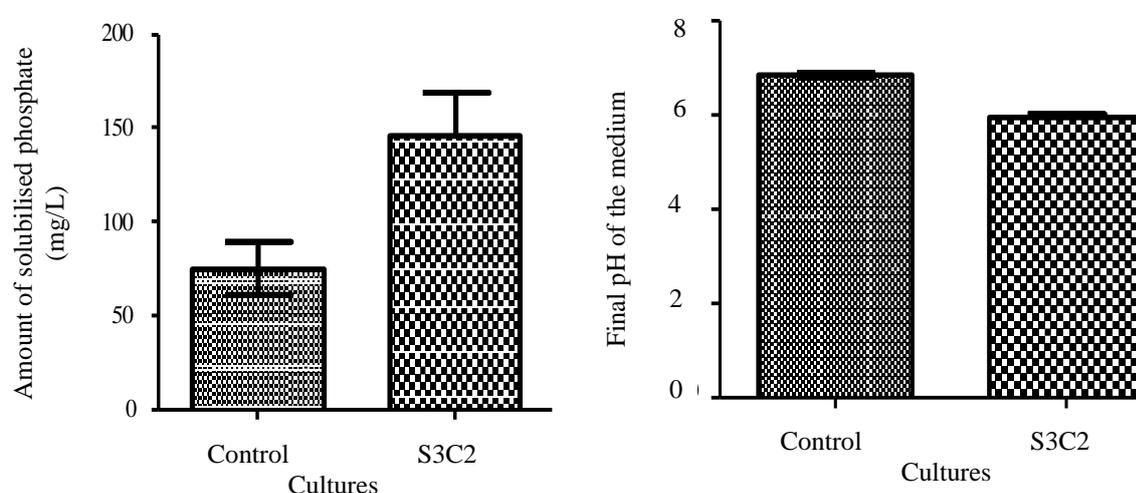


Figure 7 Phosphate solubilisation by strain S3C2, expressed in parts per million or mg/L, with a simultaneous drop in pH of the medium.

Earlier reports on As-resistant bacteria from heavily-polluted soil samples suggest high tolerance that goes up to 10,000 mg/kg. Such genera of bacteria as *Rhodococcus*, *Ochrobactrum*, *Pseudomonas*, *Brevibacterium* have been reported particularly from industrially polluted sites [19]. The development of tolerance could be linked with the amount and length of exposure to the heavy metal. These micro-organisms have potential for As bioremediation in-situ due to their ability to survive and grow under heavy metal stress [20].

A similar pattern of antibiotic-sensitivity response as reported in this study has also been reported earlier in *Bacillus cereus* isolated from raw vegetables [21].

Heavy metal stress in the environment positively selects and maintains microorganisms that possess the genes encoding resistance-conferring functions. It has been reported that many microbes, besides having the potential to uptake inorganic As as an alternative substrate to useful nutrients, also possess the genetic ability that renders them resistant to both As(V) and As(III) and allows its use as natural primary substrates [22,23]. It is well known that As(V)-resistance in bacteria is conferred by the *arsC* and *arsB* genes of the *ars* operon. The *arsC* gene encodes for an arsenate-reductase to reduce As(V) to As(III), while the *arsB* encodes for an arsenite efflux pump to throw the more toxic As(III) out of the cells. To avoid accumulation of As(III), the *ArsC* activity is almost always associated with *ArsB* activity [24,25]. Sometimes, a different class of arsenite efflux pump is encoded, by the *acr3* gene [26].

Arsenate reductase activity due to the *arsC* gene in *Bacillus cereus* has been reported earlier as well [27]. Polluted soil and other natural sources serve as a reservoir for this bacterial species to thrive and display its unique functions that help in the biochemical cycling of arsenic in nature.

Microbial siderophores help in the uptake of iron ions from insoluble minerals. During this process, arsenic ions often get mobilized into the aqueous phase from the minerals, thus making it available to the bacterial cells. Thus, production of siderophores often times exposes the inorganic As to microbial arsenic-cycling in the environment and can impact the dynamics of As-cycling at naturally polluted sites [28].

IAA is an important plant growth regulator. Thus, the plant growth promoting bacteria (PGPB) that can produce this molecule play a crucial role in plant's growth and their survival under varied conditions. Several PGPB that could produce IAA have been reported as potential bio-fertilizers [29,30]. Not only this, IAA has been reported to be an important signalling molecule for plant-microbe interaction.

4. Conclusion

Due to the rising global threat of As pollution, it has become necessary to implement techniques that are not only efficient in mitigating the heavy metal but also environment friendly at the same time. This study was designed with the aim of understanding the As(V) remediation potential of soil-borne bacterial species and its ability to show plant growth promoting traits. It successfully reports a soil-borne bacterial strain that shows such activities as siderophore production, phosphate solubilisation and inducible IAA production, along with As(V) tolerance and uptake. The strain has been identified as *Bacillus cereus* species. The bacteria can serve various purposes, such as use as a bio-fertilizer, use in As(V) bioremediation and plant-microbe interaction to help sustain those plants that survive under As polluted regions. This bacteria has a potential in all the aforesaid arenas and should be further tested.

5. References

- [1] Gautam PV, Gautam RV, Banerjee S, Chattopadhyaya MC, Pandey JD. Heavy metals in the environment: fate, transport, toxicity and remediation technologies. In: Pathania D, editor. Heavy metals: sources, toxicity and remediation techniques. 1st ed. New York: Nava Science Publishers, Inc. 2016. p.101-130.
- [2] Jaishankar M, Tseten T, Anbalagan N, Matthew BB, Beeregowda KN. Toxicity, mechanism and health effect of some heavy metals. *Interdiscip Toxicol.* 2014;7(2):60-72.
- [3] Jacob JM, Karthik C, Saratale RG, Kumar SS, Prabakar D, Kadirvelu K, et al. Biological approaches to tackle heavy metal pollution: a survey of literature. *J Environ Manage.* 2018;217:56-70.
- [4] Jang YC, Somanna Y, Kim H. Source, distribution, toxicity and remediation of arsenic in the environment-a review. *Int J Appl Environ Sci.* 2016;11(2):559-581.
- [5] Singh AK. Arsenic contamination in groundwater of North eastern India. In: Jain CK, Trivedi RC, Sharma KD, editors. National Seminar on Hydrology with focal theme on water quality; 2004 Nov 22-23; Roorkee, India. New Delhi: Allied Publishers; 2004. p. 255-262.
- [6] Bhattacharya P, Chatterjee D, Jacks G. Occurrence of arsenic contaminated groundwater in alluvial aquifers from delta plains, Eastern India: options for safe drinking water supply. *Int J Water Resour D.* 2010;13(1):79-92.
- [7] Rahman MM, Mandal BK, Chowdhury TR, Sengupta MK, Chowdhury UK, Lodh D, et al. Arsenic groundwater contamination and sufferings of people in north 24-Parganas, one of the nine arsenic affected districts of west Bengal, India. *J Environ Sci Health A Tox Hazard Subst Environ Eng.* 2011;38(1):25-59.
- [8] Pandey PK, Yadav S, Nair S, Bhui A. Arsenic contamination of the environment: a new perspective from central-east India. *Environ Int.* 2002;28(4):235-245.
- [9] Dwivedi S, Tripathi RD, Srivastava S, Singh R, Kumar A, Tripathi P, et al. Arsenic affects mineral nutrients in grains of various Indian rice (*Oryza sativa* L.) genotypes grown on arsenic-contaminated soils of west Bengal. *Protoplasma.* 2010;245(1-4):113-124.
- [10] Shukla A, Srivastava S. Emerging aspects of bioremediation of arsenic. In: Singh R., Kumar S, editors. *Green Technologies and Environmental Sustainability.* 1st ed. Cham: Springer International Publishing AG; 2017 p. 395-407.
- [11] Hussein H, Moawad H, Farag S. Isolation and characterization of pseudomonas resistant to heavy metals contaminants. *Arab J Biotech.* 2004;7(1):13-22.
- [12] Courvalin P, Goldstein F, Philippon A, Sirot J, editors. *L'Antibiogramme.* 1st ed. Paris:MPC-Videom; 1985.
- [13] Kreig NR. *Bergey's Manual of Systematic Bacteriology Vol I.* Baltimore MD: Williams and Wilkins; 1984.

- [14] Anderson CR, Cook GM. Isolation and characterization of arsenate-reducing bacteria from arsenic-contaminated sites in New Zealand. *Curr Microbiol.* 2004;48:341-347.
- [15] Milagres AM, Machuca A, Napoleão D. Detection of siderophore production from several fungi and bacteria by a modification of chrome azurol S (CAS) agar plate assay. *J Microbiol Methods.* 1999;37(1):1-6.
- [16] Glickmann E, Dessaux Y. A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. *Appl Env Microbiol.* 1995;61(2):793-796.
- [17] Jackson ML. *Soil chemical analysis - advanced course.* 2nd ed. Wisconsin: Parallel Press. 1985.
- [18] Barry AL, Garcia F, Thrupp LD. An improved single-disk method for testing the antibiotic susceptibility of rapidly-growing pathogens. *Amer J Clin Path.* 1969;53(2):149-158.
- [19] Wu D, Zhang Z, Gao Q, Ma Y. Isolation and characterization of aerobic, culturable, arsenic-tolerant bacteria from lead-zinc mine tailing in southern China. *World J Microbiol Biotechnol.* 2018;34:177.
- [20] Shagol CC, Krishnamoorthy R, Kim K, Sundaram S, Sa T. Arsenic-tolerant plant-growth-promoting bacteria isolated from arsenic-polluted soils in South Korea. *Environ Sci Pollut Res.* 2014;21(15):9356-9365.
- [21] Park KM, Jeong M, Park K, Koo M. Prevalence, enterotoxin genes and antibiotic resistance of *Bacillus cereus* isolated from raw vegetables in Korea. *J Food Prot.* 2018;81(10):1590-1597.
- [22] Silver S, Phung LT. Bacterial heavy metal resistance: new surprises. *Annu Rev Microbiol.* 1996;50:753-789.
- [23] Rosen BP. Families of arsenic transporter. *Trends Microbiol.* 1999;7(5):207-212.
- [24] Cervantes C, Ji G, Ramírez JL, Silver S. Resistance to arsenic compounds in microorganisms. *FEMS Microbiol Rev.* 1994;15(4):355-367.
- [25] Gatti D, Mitra B, Rosen BP. *Escherichia coli* soft metal ion-translocating ATPases. *J Biol Chem.* 2000;275(44):34009-34012.
- [26] Cavalca L, Zanchi R, Corsini A, Colombo M, Romagnoli C, Canzi E, et al. Arsenic-resistant bacteria associated with roots of the wild *Cirsium arvense* (L.) plant from an arsenic polluted soil, and screening of potential plant growth-promoting characteristics. *Syst Appl Microbiol.* 2010;33(3):154-164.
- [27] Jain S, Saluja B, Gupta A, Marla SS, Goel R. Validation of arsenic resistance in *Bacillus cereus* strain AG27 by comparative protein modelling of *arsC* gene product. *Protein J.* 2011;30(2):91-101.
- [28] Banerjee S, Datta S, Chattopadhyay D, Sarkar P. Arsenic accumulating and transforming bacteria isolated from contaminated soil for potential use in bioremediation. *J Environ Sci Health A.* 2011;46(14):1736-1747.
- [29] Khalid A, Arshad M, Zahir ZA. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *J Appl Microbiol.* 2004;96(3):473-480.
- [30] Arkhipova TN, Veselov SU, Melentiev TI, Martynenko EV, Kudoyarova GR. Ability of bacterium *Bacillus subtilis* to produce cytokinins and to influence the growth and endogenous hormone content of lettuce plant. *Plant Soil.* 2005;272(1):201-209.