Isolation, investigation and identification of arsenic-tolerant bacteria derived from the natural environment

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Abstract

Arsenic contamination poses significant environmental and health risks. The current investigation aims to isolate, characterize and identify arsenic-resistant bacteria from naturally contaminated soil due to its dual role in enhancing soil fertility and bioremediation of soil pollution. Soil samples were systematically collected from arsenic-affected regions and processed using selective enrichment techniques to isolate resistant strains, which were further screened for tolerance to varying arsenic concentrations. The minimum inhibitory concentration (MIC) study determined the highest arsenic levels tolerated by each strain. Biochemical and morphological characterization assessed the physiological traits of the isolates, while 16S rRNA gene sequencing facilitated their molecular identification. Biochemical analyses highlighted metabolic variations in response to arsenic exposure. Fourier Transform Infrared (FTIR) spectroscopy was utilized for metabolic profiling, revealing functional groups associated with arsenic resistance.

Among the isolates, a rod-shaped and Gram-positive Bacillus sp. (in: firmicutes) was identified (NCBI accession number PQ061509), exhibiting the highest MIC value of 38 mM for As(III) and 440 mM for As(V). The identified strains demonstrated high arsenic tolerance and diverse metabolic capabilities, underscoring their potential for bioremediation applications. These findings enhance our understanding of microbial adaptations in arsenic-polluted environments and emphasize the role of arsenic-tolerant bacteria in environmental remediation and sustainable soil management.

Keywords: Bioremediation, pollutant, heavy-metal, arsenic-resistant bacteria, *Bacillus sp.*

Introduction

Over the last decades, heavy metal contamination of soil has tremendously increased due to anthropogenic and geogenic sources. Especially, arsenic contamination of soil and water is a significant environmental and health concern worldwide, particularly in regions with intensive agricultural practices and natural geogenic activities[19,28]. Although soil arsenic contamination is now a worldwide problem, it is more prominent around Asia, especially in the lower Gangetic plains of West Bengal[23]. Arsenic is a poisonous metalloid element. There are four different structural forms present in the environment i.e. elemental arsenic (As^0), arsenite (As^{+3}), arsine (As^{-3}) and arsenate (As^{+5}). Among the several forms of arsenic, arsenite [$HAsO_3$, $As(OH)_3$] and arsenate [$H_2AsO_4^-$, $HAsO_4^{2-}$] are the most prevalent in soils and water systems. These are seriously harmful to living organisms.

Inorganic phosphate and arsenate show a structural analogy between them. For this reason, arsenate can easily enter the cell through a cell membrane like phosphate[21]. As a result, phosphorylation reaction and adenosine triphosphate synthesis become disturbed due to the absence of phosphate[20]. When human beings are exposed to arsenic levels above the permissible limit (0.05 ppm), various toxic effects take place on the human body like enlargement of the liver and spleen, nausea, anorexia, skin lesions, skin cancer etc.[15]

It is very difficult to eliminate arsenic from the environment because it persists in the environment for a long time due to its high magnitude of solubility in water and recalcitrance. Conventional remediation strategies such as chemical precipitation and adsorption, are often costly and environmentally toxic[30]. On the other hand, arsenic-resistant bacteria have garnered substantial attention due to their ability to survive in high-arsenic environments by employing various adaptive mechanisms. These include transforming arsenic species, sequestration and efflux systems that mitigate arsenic toxicity. Therefore, bioremediation, using microorganisms capable of detoxifying arsenic, has emerged as a sustainable alternative.

Various compounds like polysaccharides, proteins, lipids, etc. are released by bacteria that limit the bioavailability of heavy metals by combining with their ions[26]. Bioremediation using bacteria is significantly beneficial because they can absorb arsenic efficiently due to their high withstand capacity in toxic conditions and convert it into less harmful forms. Moreover, bacteria also increase fertility and improve soil physicochemical properties. Understanding the mechanisms of arsenic resistance in bacteria can pave the way for developing innovative biotechnological approaches to mitigate arsenic pollution. Moreover, such bacteria could be used to bioaugment contaminated sites or enhance natural attenuation processes[16].

This study investigates the isolation, characterization and identification of indigenous arsenic-tolerant bacteria from arsenic-polluted soil in Mitrapur, Nadia, West Bengal, India.

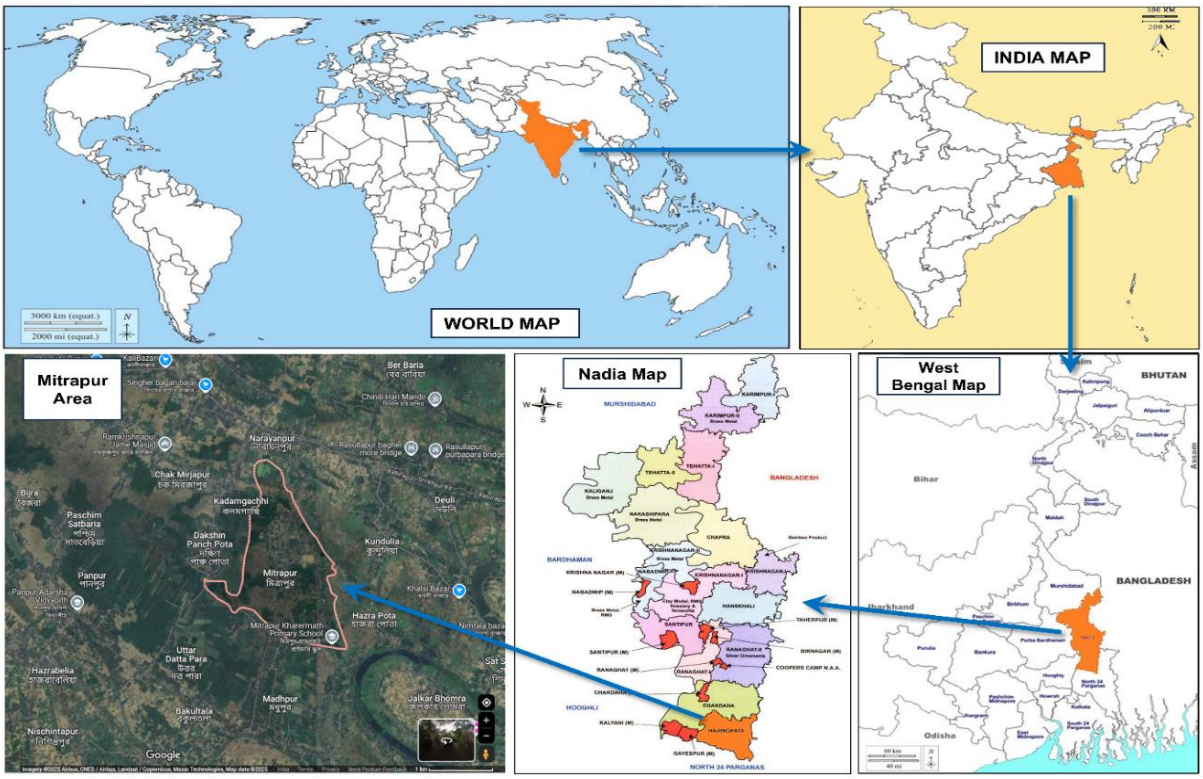

Figure 1: Soil collection area of Mitrapur village in Nadia district, West Bengal, India (22.9981° N, 88.6121° E).

The research focuses on isolating bacterial strains capable of tolerating high concentrations of arsenic, specifically As(III) and As(V). The determination of the minimal inhibitory concentration (MIC) for arsenic-tolerant bacteria and their growth kinetics was conducted using UV-Vis spectroscopy. Additionally, several biochemical tests were performed for characterization and Fourier-transform infrared (FTIR) spectroscopy was employed for metabolic profiling to recognize probable functional groups expressed by the bacterial cells under arsenic stress. Furthermore, 16S rRNA sequencing was used for bacterial identification. The findings of this investigation aim to enhance the understanding of microbial adaptations to arsenic toxicity and to identify promising candidates for potential bioremediation applications.

Material and Methods

Chemicals and Reagents: All the chemicals employed in this investigation were of analytical reagent grade and were purchased from Merck India Ltd. We purchased the media for the microbiological analysis from HiMedia Laboratories Pvt. Ltd. in India. All of the standards and reagents were made with Milli-Q water. Every glassware was thoroughly washed by soaking it in HNO_3 (15%) and finally rinsing it with Milli-Q water before being used for any experiments.

Site selection and sampling: Soil samples were collected for the isolation of arsenic-resistant bacteria from Mitrapur village, Nadia district, West Bengal, India (22.9981° N, 88.6121° E), an area with arsenic contamination exceeding safe levels of the Bureau of Indian Standards (BIS) as well as the World Health Organization (WHO)[24]. The samples (10 cm deep by 2 cm in diameter) were carefully collected in the summer season or pre-monsoon and preserved in a refrigerator (4°C) to prevent contamination.

Physicochemical analysis of the soil sample: Several physicochemical parameters of soil samples such as temperature, moisture content, pH, organic matter, organic carbon, phosphorus (P), nitrogen(N), potassium(K) and also arsenic (As) were determined using standard methods[5,17]. Determine the microbial populations (within 24h of soil collection), including the populations of bacteria in general and those resistant to arsenic and find the bacteria that have adapted to elevated arsenic levels[12].

Isolation of an arsenic-resistant bacterial strain: Arsenic trioxide (As_2O_3) and sodium arsenate ($Na_2HAsO_4{\cdot}7H_2O$) were used as sources of As(III) and As(V) respectively. Luria Bertani (LB) agar medium was prepared by mixing NaCl (10 g/L), yeast extract (5 g/L), tryptone (10 g/L) and agar (20 g/L) with adjusted pH (7.0) free from arsenic. After proper mixing, the medium was covered with a cotton plug and sterilized at 121°C (at 15 psi pressure) for 15-20 minutes. Two grams of collected soil were mixed with 2 mL Milli-Q water (autoclaved) and subjected to serial dilution up to 10^{-10}. From each diluted solution, 0.1 mL was plated onto an LB agar medium. Using a sterilized glass spreader, the samples were evenly distributed on the agar plates and incubated for 48 h at 30°C in an incubator (REMI RQV-300 plus).

The morphology of the colony was examined under a microscope and selected based on morphological

differences. The selected distinct colonies were further sub-cultured onto a fresh agar plate and incubated for another 48 hours to obtain the purified colonies. To select the As-resistant bacteria, selected purified strains were further cultured into LB agar medium [NaCl (10 g/L), yeast extract (5 g/L), tryptone (10 g/L), with adjusted pH (7.0) without agar] supplemented with either 1mM As(V) or As(III) followed by incubation at 30°C temperature for 48 h under shaking (120 rpm) conditions (REMI RQV-300 plus). Bacterial growth was assessed after 24 hours in terms of determining the optical density (UV–vis spectrophotometer, PerkinElmer Lambda 365+) at 600 nm[5,14]. Blank media (LB broth) without bacteria were used as a control to evaluate the growth of the isolated As-tolerant bacteria.

Determination of MIC study of arsenic-tolerant bacteria: The minimum inhibitory concentration (MIC) was determined as the lowest concentration of As(V) or As(III) that completely inhibited microbial growth and activity. The efficiency of As-tolerance [As(V) and As(III)] bacterial (isolated) strains has been further studied using the MIC test process. To conduct the MIC test, all the selected isolates (1 mL of an overnight culture), bacterial strain were individually inoculated into LB broth with different concentrations of either As(V) (100 to 500 mM; $Na_2HAsO_4 \cdot 7H_2O$) or As(III) (10 to 50 mM; As_2O_3).

The samples were incubated at 30°C for 48 h with shaking (120 rpm) conditions in an incubator (REMI RQV-300 plus) and microbial growth (by measuring optical density) was recorded by using a UV–vis spectrophotometer (PerkinElmer Lambda 365+) at 600 nm (λmax) wavelength. In addition, two control sets have been prepared without bacterial inoculation, one for As(III) and another for As(V). Finally, the isolated strain that had the highest MIC value was considered for subsequent analysis[5].

Characterization of As-resistant bacteria: For morphological identification, the selected bacterial strain (S-2) was confirmed by Gram staining and the shape was studied by binocular microscopy (LABOMED, Lx 300). Several biochemical tests like indole, methyl red, citrate, mannitol motility, eosin-methylene blue agar, catalase, urease and oxidase tests, were performed by standard protocols for biochemical characterization of isolates. The molecular identification of the bacteria (Arsenic-tolerant isolates) was conducted by 16S rRNA gene sequencing. The genomic DNA of bacteria was isolated and then the 16S rRNA gene (purified) was amplified using universal primers [27F (5′–AGAGTTTGATCCTGGCTCAG–3′) and 1492R (5′– CGGTTACCTTGTTACGACTT–3′)].

All the PCR products were cleaned using the gel purification method and DNA sequencing was carried out by Barcode Biosciences Private Limited. The 16S rRNA gene sequence (isolates) was subjected to comparison by nucleotide BLAST (Basic Local Alignment Search Tool). Finally, the 16S rRNA gene sequences have been submitted to the NCBI (National Center for Biotechnology Information) GeneBank database to retrieve accession numbers. After getting the accession number, phylogenetic tree construction was achieved using the neighbor-joining technique with MEGA 11 software[12].

Metabolic profiling using FTIR spectroscopy: The Fourier Transform Infrared Spectroscopy (FTIR) study was conducted to investigate changes in the expression patterns of several functional groups (associated biomolecules) on the bacterial cell surface before and after exposure to As(V) and As(III) heavy metals. The functional groups and probable corresponding biomolecules of bacterial cells with and without As(III) and As(V) were studied through an FTIR (PerkinElmer Spectrum Two FTIR) spectrometer. Fresh and overnight-grown bacterial cultures were centrifuged (Thermo Scientific Sorvall ST8R) at 12,000 rpm for 5 min to obtain sufficient biomass.

The resulting pellets were washed three times with NaCl (0.5 %) solution and dried in hot air (at 50 °C) oven for 8 h to eliminate the residual moisture and then the dried cell masses were ground into a fine powder by a mortar with pestle. The analysis utilized the potassium bromide (KBr) disk method which involves preparing sample disks by blending bacterial biomass (1 mg) with KBr (0.2 mg)[20]. Finally, the pellets were scanned in the 4000 to 500 cm^{-1} range spectral region with an applied resolution of 4 cm^{-1} and the obtained spectra were recorded using spectrum IR (version 10.7.2) software.

Statistical Analysis: All the experiments were performed thrice and data were shown as mean ± standard deviations (mean±SD). Duncan's multiple tests were performed using the SPSS (Statistical Package for Social Sciences, version 16.0) software to calculate the significance ($p < 0.05$) of values.

Results and Discussion

Biochemical analysis of the experimental soil: The physicochemical examination of the soil samples exposed the following properties including pH values ranging from 7.03 - 7.82, organic matter between 1.44 % to 2.03%, organic carbon 0.54 % and 0.73 % and moisture content ranging between 28 % and 43%. The soil samples were rich in available nitrogen (151.5 - 203.4 kg/ha) and potassium (237.3 - 275.4 kg/ha), but the phosphorus level was between 17.05 - 25.3 kg/ha. The soil samples (per gram) contained higher levels of As (10.37 - 16.32 mg/kg). As-resistance microbial (~ 3.6×10^3 in numbers) and total microbial count (~ 3.2×10^6 in numbers) present in the soil were also determined. The significantly high count of As-resistant microorganisms may indicate that low-cost microbial remediation is a more effective way to deal with the issue of As-contamination[18].

Isolation and determination of MIC value of arsenic-tolerant bacteria: Arsenic-resistant bacteria are those that

have evolved defenses against arsenic or systems to use it as a source of energy and allow them to survive in arsenic-contaminated environments. Primarily, 12 bacterial colonies were picked up (with different colony morphologies) to examine As-resistance potential from collecting soil samples. Among these, five bacterial isolates (marked as S-1 to S-5) exhibited varying levels of arsenic resistance, with three (S-2, S-4 and S-5) isolates demonstrating the highest resistance based on the tolerance of As (V) at 100 mM levels.

Further, the three (S-2, S-4 and S-5) isolates were selected for the next minimum inhibitory concentration (MIC) experiment. The isolate S-2, a bacterial strain, shows the highest level of MIC value (Figure 2) i.e. 440 mM for As(V) and 38.7 mM for AS (III). S-4 strain had a MIC level of 306 mM and 22 mM for arsenate and arsenite, respectively. S-5 had the lowest MIC level i.e. 226 mM for arsenate and 18 mM for arsenite. Overall, isolated S-2 bacteria exhibit the highest level of MIC value compared to the other two isolates, bacteria S-4 and S-5.

MIC is defined as the lowest concentration of arsenic at which no bacterial growth occurs after 48 hours of incubation at 30 °C. MIC studies are vital as they provide insights into the potential role of native bacteria in the biological treatment of arsenic. The MIC was determined using two different forms of inorganic arsenic: arsenite [As(III)] and arsenate [As(V)] to assess the susceptibility of the bacterial isolates to arsenic. These two forms are commonly found in nature and are considered more toxic compared to other arsenic species. The ability of bacteria to tolerate high arsenic concentrations results from their ecological adaptability to stressful conditions and prolonged exposure to arsenic-contaminated environments[25].

Arsenic-resistant bacteria have been previously reported in several locations worldwide, including uranium mines in India, spring water in an Iranian province, contaminated water in a village of Argentina, soils in Portugal, mines in Poland, as well as sediments along the Vietnam Sea coast[3,13,20]. These findings suggest that arsenic-tolerant bacteria are globally distributed in nature and their tolerance mechanisms may have evolved since prehistoric times. Various bacterial species have developed diverse protective strategies to survive in arsenic-stressed environments. The detoxification processes that are most frequently employed, are metal removal through efflux pumps, extracellular barriers, entrapment within the cell, enzymatic transformation of arsenic into a less harmful form and extracellular trapping via chelating agent secretion. The exposure of these bacterial strains to varying concentrations of arsenic during enrichment may have created selective pressure, prompting them to develop metal resistance mechanisms to safeguard their cellular components[4,22]. A higher MIC in arsenic-resistant isolates suggests greater adaptability to arsenic-contaminated soils, making them more suitable for bioremediation in agricultural fields. Utilizing bacteria in bioremediation offers benefits such as environmental sustainability and cost-effectiveness. Numerous arsenic-resistant bacterial strains have been documented for their significant role in remediating arsenic pollution.

Biochemical characterization and identification of Arsenic-tolerant bacteria: Gram staining is a popular morphological characterization method used to distinguish bacteria based on the physical and chemical characteristics of their cell walls by identifying the existence of peptidoglycan on the cell wall of Gram-positive bacteria. The colony morphology of the isolates S-2 strain appeared circular and smooth when cultured on plates and displayed white or slightly yellow colonies. Gram staining confirmed that the selected strain (isolates S-2) was rod-shaped and Gram-positive bacteria (identified by a binocular microscope). The morphological properties revealed that the isolated bacterium (S-2) was Gram-positive.

The strain S-2 showed a positive response for catalase enzyme production, but was negative in the oxidase enzyme production and urease enzyme test (Table 1). The catalase test confirmed resistance to oxidative stress by breaking down hydrogen peroxide into water and oxygen[5].

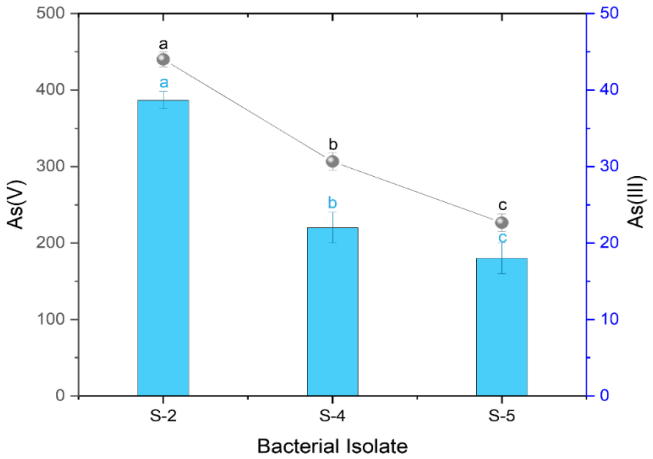

Figure 2: Minimum inhibitory concentration after exposure to As(V) [Spherical diagram] and As(III) [Bar diagram] of selected bacterial isolates. Results are represented as the mean with standard error (± SE).

Table 1
Biochemical and morphological identification of selected S-2 bacterial strain

Isolates	Gram stain	Cell shape	Colony colour	Catalase	Oxidase	Urease	Citrate	Mannitol Motility test	Methyl Red	Indole	Eosin-methylene blue agar (EMB) test
Strain S-2	Gram-positive	rod-shaped	White or slightly yellow colony	+	-	-	+	+	+	-	+

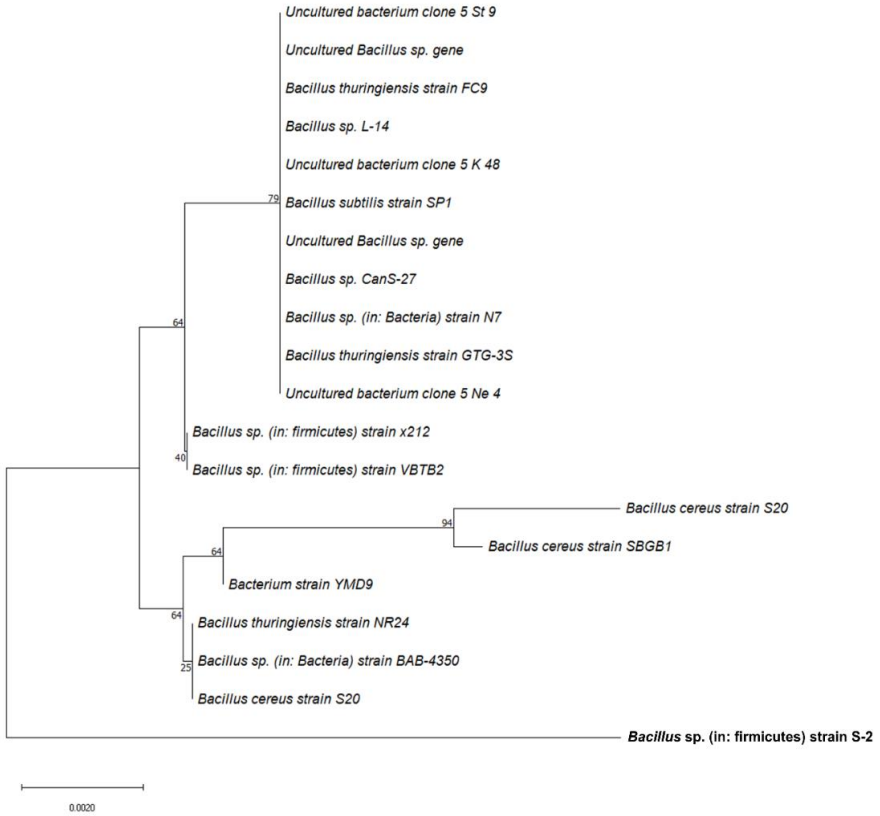

Figure 3: The phylogenetic tree of the bacterial (strain S-2) isolate was constructed by using 16S rDNA gene sequence, signifying its place among other members of the genus *Bacillus*.

Some other biochemical tests highlighted the metabolic (enzyme) adaptability strain including a positive citrate test which indicates the utilization of citrate as its sole carbon source (presence of enzyme citrate permease) and the mannitol motility test indicates S-2 is motile and able to ferment mannitol as a carbon source. In addition, the indole test shows negative, while the positive results of the eosin-methylene blue and methyl red tests indicate a strong ability to ferment glucose into stable acidic end products[12,23].

The molecular identification of bacterial strain S-2 was further validated through phylogenetic characterization of the 16S rDNA sequence. Phylogenetic analysis for As-resistant bacteria (strain S-2) revealed that it has a close linkage with members of the genus *Bacillus* (Figure 3). The As-resistant bacteria (strain S-2) was identified as *Bacillus* sp. (in: firmicutes) with a ~ 621 bp length of nucleotide. The sequence (strain S-2) is available in the NCBI GeneBank database with the accession number PQ061509. Previously, numerous arsenic-resistant bacteria have been described to play an important role in the bioremediation of arsenic contaminants. So, different biochemical and morphological characteristics of the As-resistant bacteria (strain S-2) highlight the enzymatic versatility and resilience of this S-2 strain, positioning it as a promising candidate for metal-contaminated water or soils.

Comparative metabolic profiling of arsenic-resistant bacteria through FTIR: The FTIR spectra were compared before and after exposure to As(III) and As(V) heavy metals. The analysis revealed that both exhibit a few specific changes in the spectra following heavy metal exposure compared to the control sample (Figure 4). The spectra of S_2 [As (V)] and S_2 [As (III)] samples displayed a diverse

combination of broad and sharp bands or peaks across different regions (Table 2). The absorbance bands were shifted from their control spectra 2927 cm^{-1}, 1718 cm^{-1}, 1647 cm^{-1}, 1541 cm^{-1}, 1455 cm^{-1}, 1380 cm^{-1}, 1278 cm^{-1}, 1230 cm^{-1}, 1131 cm^{-1}, 1050 cm^{-1} and 978 cm^{-1} to 2925 cm^{-1}, 1739 cm^{-1}, 1646 cm^{-1}, 1544 cm^{-1}, 1447 cm^{-1}, 1387 cm^{-1}, 1301 cm^{-1}, 1178 or 1261 cm^{-1}, 1131 cm^{-1}, 1053 cm^{-1}, 980 cm^{-1} respectively for post-exposure of As (V). For S_2 [As (III)], the absorbance bands were at 3410 cm^{-1}, 2928 cm^{-1}, 1635 cm^{-1}, 1549 cm^{-1}, 1460 cm^{-1}, 1398 cm^{-1}, 1235 cm^{-1} and 1065 cm^{-1} after post-exposure of As (III).

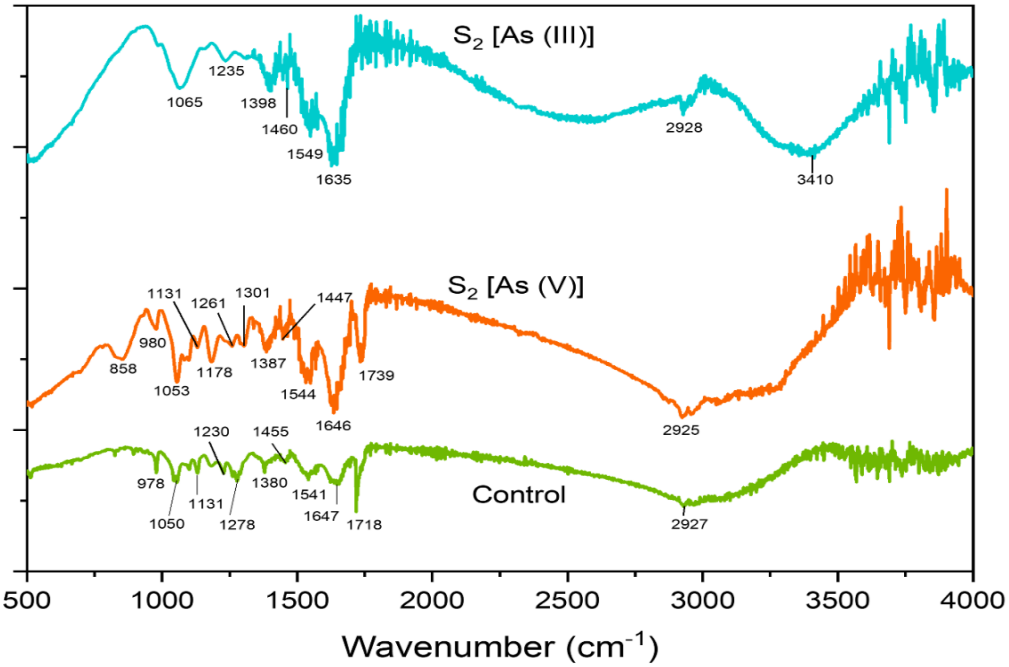

Figure 4: FTIR spectrum of control with comparative metabolic profiling of arsenic-resistant bacteria before and after exposure to As(III) and As(V) heavy metal.

Table 2
FTIR spectral data with probable corresponding functional groups existing in arsenic-resistant bacteria before and after exposure to As(III) and As(V) heavy metals

S.N.	Sample wavenumber (cm^{-1})			Probable functional bond	Corresponding functional groups
	Control	S_2 [As V]	S_2 [As III]		
1.			3410	O–H	alcohol/phenols
2.	2927	2925	2928	C–H	saturated alkanes/ lipids/polymers (hydrocarbons) long-chain
3.	1718	1739		C=O	ester/aromatic ester, aldehyde, ketones, carboxylic acids
4.	1647	1646	1635	C=C, C=N, C=O, N–H	alkanes, amides I /amines, amino acids/proteins
5.	1541	1544	1549	N–H, C=O	Amides II /amines, amino acids/protein secondary structure (α-helix and β-sheet)
6.	1455	1447	1460	C–H	Alkanes/aromatics
7.	1380	1387	1398	C–H, N–O, O=C–OH, O–H	alkanes, nitro, carboxylate, alcohol/phenols
8.	1278	1301		C–H, C–N, O–H, C–O	alkanes/aromatic, amides/amines, alcohol/phenols, ethers/esters/carboxylic acids
9.	1230	1178, 1261	1235	C–H, C–N, C–O, S=O	aromatic, amides/amines, ethers/esters/carboxylic acids, sulfones/sulfoxides
10.	1131	1131		C–H, C–N, C–O, S=O, P=O, or P–O	alkanes, amines, ethers/esters/carboxylic acids, sulfones/sulfoxides/sulfates, phosphate
11.	1050	1053	1065	C–N, C–O, S=O, P–O	amines, ethers/esters, sulfones/sulfoxides, phosphate/organophosphate
12.	978	980		C–H, C–N, C–O, C=O	alkanes, aromatic, ethers/esters, certain carbonyl-containing compounds

The symmetric and asymmetric C–H stretching vibrations at 2927 cm^{-1} shifted slightly to 2925 cm^{-1} and 2928 cm^{-1}, suggesting perturbations in the lipid membrane structure[9]. The amide I band at 1647 cm^{-1} shifted to 1646 cm^{-1} and 1635 cm^{-1}, however, the amide II band at 1541 cm^{-1} shifted to 1544 cm^{-1} and 1549 cm^{-1}. These changes suggest modifications in protein secondary structure (e.g. α-helices or β-sheets) due to arsenic interaction. The C–O stretching at 1050 cm^{-1} shifted to 1053 cm^{-1} and 1065 cm^{-1}, though the band at 1131 cm^{-1} remained unchanged[10].

These indicate minor changes in carbohydrate or nucleic acid components. The ester carbonyl (C=O) stretches at 1718 cm^{-1} shifted to 1739 cm^{-1}, suggesting modifications in ester-containing lipids or new ester linkages. A new broad peak at 3410 cm^{-1} and 3275 cm^{-1} suggests the presence of O–H or N–H stretching vibrations, indicating hydrogen bonding or stress-induced production of amines or hydroxylated compounds[6].

The FTIR results demonstrate significant biochemical changes in bacterial components upon exposure to arsenic. Bacteria naturally adapt by modifying their molecular fingerprint and employing various survival strategies when exposed to heavy metals. Some groups increase the synthesis and expression of specific proteins and enzymes that aid in detoxifying or blocking the entry of toxic metals. Others actively expel heavy metals that enter the cell or sequester them in specialized vesicles[9]. The observed spectral differences between the isolates indicate variations in their metabolic profiles when grown in an arsenic-polluted environment. Further examination using untargeted metabolomics is needed to identify differentially expressed metabolites that may play a crucial role in arsenic tolerance[20].

It is anticipated that any isolate with a higher MIC value will be more adaptable in As-contaminated soil and will be more useful for bioremediation in fields. According to previous reports, several microorganisms that are resistant to arsenic have the potential for the bioremediation of As-pollution[2,7]. So, *Bacillus sp* are naturally occurring bacteria that are resistant to arsenic and capable of utilizing arsenate and arsenite as alternate energy sources. This strain exhibits a unique ability to transform arsenic into a less toxic form through its metabolic processes, contributing to soil restoration[5]. These findings highlight the potential application of bacteria in the future as bioremediation process is advantageous because of its eco-friendliness, cost-effectiveness etc.

Probable mechanisms of microbial-assisted arsenic removal: The microbial detoxification of arsenic has been extensively studied over the past decades and plays a significant role in bioremediation strategies. The bacterial cell wall contains various functional groups such as ketones (–C=O–), carboxyl (–COOH) and aldehydes (R-CHO). Bacterial bio-blocks are commonly utilized as interactive materials for adsorption, facilitating the removal of heavy metals from liquid solutions[1]. Bacteria employ various mechanisms to detoxify and stabilize heavy metals (Figure 5), enabling their survival in arsenic-contaminated environments. These mechanisms include arsenic accumulation, enzymatic detoxification, efflux pumps, intracellular sequestration, methylation and volatilization[27].

Arsenic primarily exists in two forms in the environment: arsenate (AsV) and arsenite (AsIII). Arsenate, which structurally resembles phosphate, enters bacterial cells via the phosphate membrane transport system (Pit and Pst systems), but arsenite is transported through aquaglyceroporins (GlpF) [usually promotes glycerol transport while permitting arsenite to move through]. Once inside the cell, As(V) is enzymatically reduced to As(III) by arsenate reductase (ArsC) enzyme or eukaryotic arsenate reductase (Acr2p) enzyme[30].

This process relies on glutathione (GSH) or thioredoxin as electron donors. To prevent arsenic accumulation and toxicity, bacteria expel arsenite (AsIII) using efflux transporters, including ArsB and ArsA (which function as ATP-dependent pumps) or plasma membrane carrier proteins (Acr3p).

Additionally, some bacteria possess an alternative efflux system (ArsRDABC operon or ArsRBC) or ATP-binding cassette protein Ycf1p, which also contributes to arsenic resistance by actively removing arsenite from the cytoplasm. During arsenate reduction, the respiratory arsenate reductase (Arr) converts arsenate to arsenite, accompanied by the simultaneous oxidation of organic and inorganic matter. In contrast, arsenite oxidase (Aio), belonging to the DMSO (dimethyl sulfoxide) reductase family, facilitates the oxidation of As(III) to As(V) with As(III) serving as the electron donor[31].

Arsenic resistance in bacteria is a complex, multi-step process that allows survival in arsenic-contaminated environments. These mechanisms are crucial for bacterial adaptation and have potential applications in bioremediation for arsenic-contaminated water and soil. Additionally, their application in arsenic-contaminated soil would help to decrease arsenic levels and limit its uptake by plants[11]. However, the specific mechanisms underlying bioremediation still require further investigation. Conventional chemical and physical procedures for arsenic removal are costly and have a substantial environmental impact.

Unfortunately, many arsenic-affected areas in rural Bengal face both contamination and economic hardship. Developing bioremediation as a sustainable technology could play a crucial role in arsenic toxicity. Future research will optimize the bioremediation efficiency of these bacteria by evaluating key factors such as pH, incubation time and temperature.

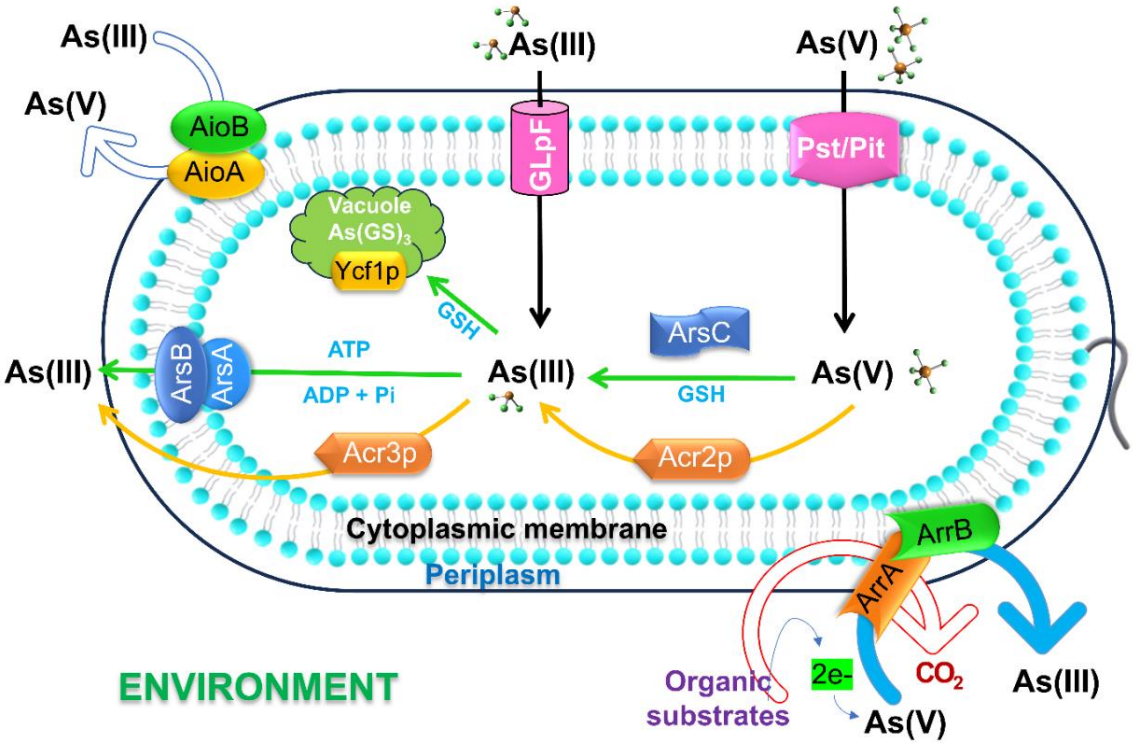

Figure 5: The schematic mechanisms for bacteria-assisted removal of Arsenic

Conclusion

This study successfully isolated, characterized and identified arsenic-resistant bacteria from naturally contaminated soil, demonstrating their potential for bioremediation and soil fertility enhancement. The identified strain is a rod-shaped and Gram-positive *Bacillus sp.* (*in: firmicutes*) with NCBI accession number PQ061509, exhibiting high arsenic tolerance and MIC values of 38 mM for As(III), also 440 mM for As(V). These findings highlight the ability of arsenic-resistant bacteria to survive and adapt in metal-contaminated environments, making them promising candidates for environmental remediation strategies. Biochemical characterization and FTIR metabolic profiling revealed significant variations in bacterial metabolism under arsenic exposure, providing deeper insights into their resistance mechanisms.

The study not only advances our understanding of microbial adaptation in arsenic-contaminated environments but also lays the groundwork for potential applications in bioremediation technologies. Future research should explore field-scale applications, genetic mechanisms of resistance and interactions with plant systems to fully harness their benefits for ecological restoration and agricultural sustainability. These findings contribute to develop cost-effective and eco-friendly solutions for managing arsenic pollution in affected regions.

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