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Isolation and Characterization of an Arsenic-Resistant Bacterial Strain from Changki, Nagaland

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ABSTRACT

The present study focused on isolating arsenic (As)-resistant bacterium from acid mine tailings of Changki, Nagaland, and evaluating its bioremediation potential. Isolation was performed using an enrichment culture approach and further characterized using standard procedures. The obtained bacterial strain AS3 was found to be resistant to As^{3+} and As^{5+} ions up to 1562 $\mu g.mL^{-1}$ and 125000 $\mu g.mL^{-1}$, respectively. The 16S rRNA gene sequence of the strain was found to be identical to that of *Lysinibacillus* sp. The growth behavior of the strain in the presence of selected heavy metals (HMs) showed a prolonged lag phase, especially in As^{5+}. Moreover, the strain was found to be resistant to several antibiotics. SEM and EDX analyses revealed the presence of HM ions on the outer surface of the AS3 strain. The available functional groups on the surface of the AS3 strain cells engaged in the metal-binding process were identified using FTIR, suggesting their active participation in adsorption. AAS showed that the strain had the potential to remove As^{3+} and As^{5+} ions with removal efficiencies of 99.94% and 99.49%, respectively. Based on the findings, the strain exhibits intriguing biotechnological potential for HM bioremediation.

INTRODUCTION

Numerous technological developments and industrial growth have resulted in the substantial deposition of hazardous waste in the environment, including HMs, which are reported to have several toxic effects on the environment and pose a threat to living beings (Elahi et al. 2019). The HM, metalloid arsenic (As), is known to be one of the most lethal toxic elements that poses a significant public health risk (Bermanec et al. 2021, Haroon et al. 2023). As usually occurs in different environmental matrices, including water, soil, and air, in the form of trivalent arsenite (As^{3+}) or pentavalent arsenate (As^{5+}) (Goswami et al. 2015). According to previous reports, it controls a variety of redox processes between the oxidation states of As^{3+} and As^{5+}, which can have harmful and dispersed effects on the environment (Maizel et al. 2016). This toxic metalloid, As, is extensively spread in the environment, originating from both natural and anthropogenic sources (Takeuchi et al. 2007). As usually occurs in numerous minerals and can also be present in sulfide minerals of mine waste that can be easily oxidized with redox conditions, pH, and microbial activity, resulting in very high As levels in mine tailings (Matlakowska et al. 2008). Microorganisms are crucial to the mineral cycle and they may even perpetuate the As cycle (Goswami et al. 2015).

The presence of As in groundwater has become a major cause of concern, as humans depend on groundwater as a drinking water source, thereby increasing the chances of exposure to As-contaminated water worldwide (Maizel et al. 2016). The Indo-Bangladesh Gangetic Basin, also known as the Bengal Basin, has reported very high As contamination in soil sediments (Goswami et al. 2015). There have been reports of serious health impacts from groundwater arsenic pollution for residents of developing nations, such as Bangladesh and India, including skin cancer, lung cancer, arsenicosis, and Bowen's disease (Ahmad et al. 2018). Millions of individuals in several nations, including Argentina, Cambodia, China, Hungary, Nepal, Mexico, and Romania, have been also claimed to be affected by As exposure. According to previous studies, the nations with the highest levels of As pollution in drinking water are Bangladesh and India, followed by Vietnam and Cambodia (McCarty et al. 2011).

Microorganisms that dwell in HM-rich environments frequently evolve diverse HM resistance and detoxification systems (Chien et al. 2013). Various As-resistant bacteria have been isolated from As-contaminated soils (Turpeinen et al. 2004) and hydrothermal vents (Jeanthon & Prieur 1990). Bacterial species can become resistant to arsenic due to the presence of phosphate-specific transport systems that prevent the uptake of arsenate (Willsky & Malamy 1980) and efflux pathways mediated by the ars operon (Takeuchi et al. 2007). The ars operon is an integration of various genes, such as *arsH* and *arsM*, responsible for arsenic resistance in bacterial systems. This operon is also responsible for the detoxification of arsenic (Gogoi et al. 2023). Moreover, bacteria are known to produce siderophores, biofilms, and EPS, which facilitate the effective removal of HM ions (Gogoi et al. 2023). Several researchers have isolated As-resistant bacterial species from diverse environments, such as *Corynebacterium glutamicum*, which was isolated from As-contaminated soil and was able to remove 60 mM As^{3+} (Mateos et al. 2006), *Marinomonas communis* IAM 12914, which was isolated from a marine environment in Japan, was able to accumulate 2290 µg As per gram dry weight of arsenic in the presence of 5 mg $As.L^{-1}$ of arsenate (45.8%) (Takeuchi et al. 2007), *Stenotrophomonas* sp. and *Microbacterium* sp., which were isolated from Crven Dol mine of North Macedonia, exhibited hyper-resistance to As^{3+} (209 mM) together with extremely high resistance to arsenate (564 mM) (Bermanec et al. 2021).

Although various physical and chemical remediation approaches are available, most are either expensive or ineffective (Khalid et al. 2017). Moreover, the toxic chemical sludge produced by these treatment processes is not ecologically sustainable (Zakaria et al. 2024), requires high disposal or treatment costs, and cannot entirely reduce the quantities of HMs to acceptable levels (Khalid et al. 2017). Thus, it is imperative to economically and sustainably reduce the concentration of such pollutants to a level that does not disturb the environment. The recent trend in developing newer environment-friendly technologies has drawn attention from the scientific community and technologists for the remediation of HMs (Takeuchi et al. 2007). Bioremediation of HMs has several major advantages, including lower chemical involvement throughout the treatment process, lower overhead expenses, being a greener and more affordable alternative to current methods, and being efficacious at decreasing contamination levels (Khalid et al. 2017).

In this perspective, the current study attempted to isolate and identify arsenic-resistant bacteria from the mine tailings area of the Changki Hills in the Mokokchung district of Nagaland. The main objectives of this study were: (i) isolation, characterization, and identification of the bacterial strain, (ii) heavy metal accumulation study, and (iii) determining the strain's susceptibility to antibiotics.

MATERIALS AND METHODS

Chemicals and Reagents

Chemicals, reagents, and salts, including Na_3AsO_4, $NaAsO_2$, NaCl, Na_2HPO_4, NaH_2PO_4, KCl, Glutaraldehyde (2%, $C_5H_8O_2$), ethanol, and acetone, were of analytical grade (AR) and purchased from Qualigens Fine Chemicals and Thermo Electron LLS India Private Limited, Mumbai. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], Luria-Bertani broth (LBB), agar, and H_2O_2 were purchased from HiMedia Laboratories Private Limited, Mumbai, India. Whatman Filter paper (Grade 1) was purchased from Cytiva, Global Life Science Solutions Operations UK Limited.

Study Area and Sample Collection

Six sampling locations were used, with three upstream and three downstream locations. Subsurface soil was collected and stored at 4°C in sterile containers.

Isolation of As-Resistant Bacteria

Culturable As-resistant bacteria from soil samples using an enrichment culture technique were isoloated (Bowman et al. 2018). The soil samples were mixed and sieved, and then introduced to the sterilized As^{3+} (300 $\mu g.mL^{-1}$ $NaAsO_2$) and As^{5+} (300 $\mu g.mL^{-1}$ Na_3AsO_4) loaded Luria Bertani broth (LBB) medium (Sanders 2012). The culture medium was incubated for three cycles at 37°C, followed by serial dilution and the spread plate method. After 48 h, AS-resistant

bacterial colonies were documented based on their distinct morphological characteristics.

Screening of Potential As-Resistant Bacteria

The study involved loading a 96-well microtiter plate with serially diluted As^{3+} and As^{5+} solutions and adding fresh bacterial inoculum. Sterile LBB media with fresh bacterial inoculum and without HMs served as the control. The plate was incubated for 24 h at 37°C, then MTT reagent [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] was added, and absorbance was measured at 600 nm using a Multiskan SkyHigh Microplate Spectrophotometer. The growth pattern of the selected bacterial strain was observed based on optical density (OD) and color changes from bluish to pale yellowish upon the administration of MTT, and the Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Maximum Tolerable Concentration (MTC) were determined (Agarwal et al. 2020).

Morphological, Biochemical and Molecular Characterization of Bacterial Isolate

The study examined bacterial colony characteristics using Bergey's Manual of Determinative Bacteriology, Gram staining, spore staining, and motility tests (Bergey 1994). Biochemical characterization was performed using standard procedures (Banerjee et al. 2011). The Sanger dideoxy sequencing method was used for molecular identification of the 16S rRNA gene of the selected bacterial strains. BLAST analysis was performed using the NCBI Gene Bank database, and multiple sequence alignments were performed using the muscle alignment. Molecular Evolutionary Genetics Analysis (MEGA) software (version 4.0) was used to construct a consensus maximum likelihood tree (Tamura et al. 2021).

Growth Kinetics

This study used the modified approach of Ka-ot and Joshi (2000) to study the growth behavior of a selected bacterial strain. Two sets of conical flasks were used, each containing specific HM salts (Na_3AsO_4 and $NaAsO_2$) along with LBB at their MTC values. The culture broth devoid of HMs served as the control. The cultures were kept in a shaker incubator at 160 rpm and 37°C throughout the experiment, and the OD was measured using a UV-VIS spectrophotometer at 600 nm wavelength. The growth curves of each treatment and the control were compared.

Antibiotic Susceptibility Test

The Kirby-Bauer disc diffusion method (Bauer et al. 1966) and well diffusion method (Balouiri et al. 2017) were used to determine the antibiotic sensitivity of the chosen As-resistant strain against 14 distinct antibiotics. Inhibition zones, whose diameters are indicated in the standard antibiotic disc chart, have been recorded to classifying the bacterium as either susceptible or resistant to antibiotics (Dey et al. 2016).

Cellular Study of the Selected Strain

Scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX): The influence of As on the surface properties of the cells and changes in cell morphology under stress were examined using SEM. With a few modifications, the sample was prepared as stated by Pandey and Bhatt (2015). In separate Erlenmeyer flasks, the cultures were grown in 50 mL LB broth supplemented with 100 $\mu g.mL^{-1}$ Na_3AsO_4 and 100 $\mu g.mL^{-1}$ $NaAsO_2$ and incubated at 37°C for 48 h. Subsequently, the cultures were centrifuged at 6000 rpm, and the bacterial pellets were collected and rinsed three times with phosphate-buffered saline (PBS) (pH 7). The bacterial pellets were then stored at 4 °C overnight in a fixative solution containing 2% (v/v) glutaraldehyde in Na-phosphate with a pH of 7.2. The bacterial pellets were then centrifuged and rinsed three times with Na-phosphate buffer. Subsequently, the cells underwent a succession of ethanol dehydration (30–100%) and drying. To study the morphology and microstructure of the bacterial strain, the samples were prepared for SEM examination using a 3 kV accelerating voltage. The SEM Microscope (Carl Zeiss NTS GMBH, Germany, JSM 6390LV, Japan) was used for mapping and point ID of the samples for EDX analysis to determine the elemental composition.

Fourier transform infrared (FTIR) spectroscopy of bacterial biomass: With minor adjustments, the approach of Singh et al. (2016) was used for FTIR analysis. The bacterial strain was cultured with HM salts in the same way as previously described. A bacterial biomass pellet was obtained by centrifuging the fully grown culture for 10 min at 6000 rpm. The pellets underwent two rounds of washing in sterile distilled water before being dried for 48 h at 40°C. A Perkin Elmer FTIR spectrophotometer (Spectrum Two, Perkin Elmer) was used to analyse the dehydrated samples further. After mixing potassium bromide with the powdered bacterial sample, a manual hydraulic press was used to press the mixture into a translucent pellet disc for analysis. The 4000-400 cm^{-1} scan range was used to produce the FTIR spectrum.

Bioremediation Potential of the Selected Strains

To assess the bioremediation potential of the selected As-resistant strain, the method described by Pandey and Bhatt

(2015) was followed with modifications (Fig. 1). First, the strain was cultured in Erlenmeyer flasks with 100 ml of LBB medium supplemented with selected HMs at their MTC values individually. The cultures were incubated for 72 h with agitation at 160 rpm at 37 °C. After incubation, the cultures were centrifuged at 6000 rpm and pelleted. The supernatant was passed through a membrane filter (0.22 µm) and further subjected to microwave digestion (Xing 2022) for the quantification of available HM in the test sample using an inductively coupled plasma mass spectrometer (Agilent 7850 ICP-MS). The removal percentage was calculated using the following formula:

$$\text{Removal } \% = (C_i - C_f)/C_i \times 100$$

Where C_i is the initial concentration, and C_f is the final concentration/concentration of the sample.

Statistical Analysis

All experiments were conducted in triplicate and statistically analyzed using the SPSS software. The obtained values were expressed as mean ± standard deviation (SD). The experimental data were checked using one-way analysis of variance (ANOVA) at a $P \leq 0.005$ confidence level.

RESULTS

Study Area and Sample Sites

The collected soil samples were wet and exhibited yellowish AMD precipitation. The six sampling sites (26º26'4.10"N-94º25'18.23"E, 26º26'3.28"N-94º25'16.09"E, 26º25'59.38"N-94º25'18.04"E, 26º24'57.87"N-94º22'52.17"E, 26º24'55.95"N-94º22'51.80"E, and 26º24'53.03"N-94º22'47.61"E) were mapped with the help of Google Earth and the same is shown in Fig. 2A and Fig. 2B.

Isolation of Arsenic-Resistant Bacteria

After completing the third cycle, followed by serial dilution (Fig. 3) and the spread plate technique, ten bacterial isolates appeared from Sites 1, 2, and 3 that could grow in an As-amended medium. These ten isolates were further screened for their tolerance to the selected HMs. Based on the MTC values obtained for As, three bacterial isolates, AS3, AS4, and AS11, were screened. In the present study, bacterial isolate AS3 was selected for further investigation.

MIC, MBC and MTC of Selected Bacterial Strain

According to the results, the bacterial isolate AS3 was least tolerant of As^{3+} HM ions up to a concentration of 781.25 µg.mL^{-1} and extremely tolerant of As^{5+} HM ions up to a concentration of 62500 µg.mL^{-1}. Table 1 shows the MIC, MBC, and MTC values of the selected isolates against the selected HMs.

Morphology and Biochemical Characterization of Selected Bacterial Isolates

Table 1: MIC, MTC and MBC of selected isolate AS3 against selected HMs.

Type of HMs tested	MIC [µg.mL^{-1}]	MBC [µg. mL^{-1}]	MTC [µg.mL^{-1}]
Na_3AsO_4	125000	125000	62500
$NaAsO_2$	1562.50	1562.50	781.25

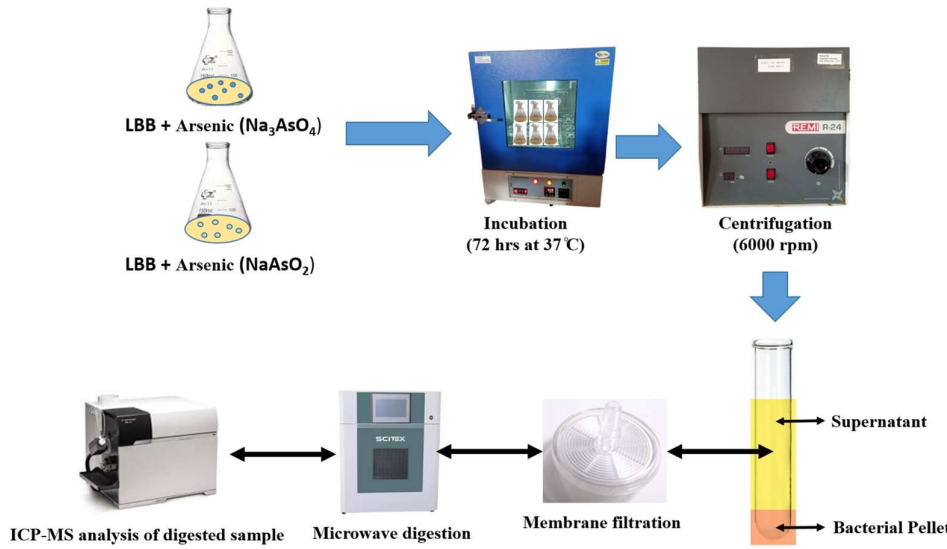

Fig. 1: Bioremediation study of AS3 in presence of Na_3AsO_4 and $NaAsO_2$.

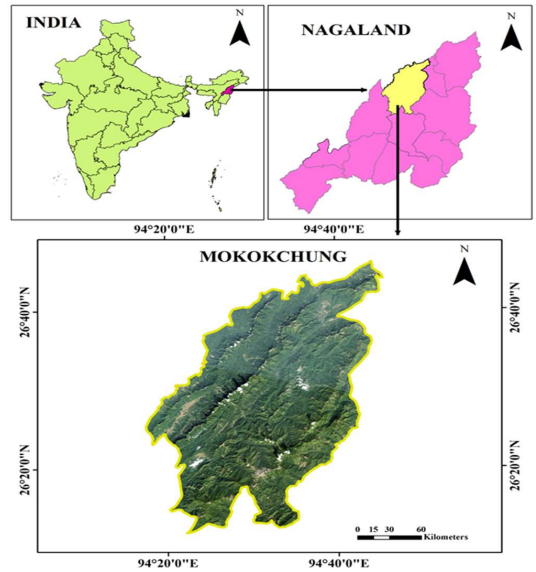

Fig. 2A: Map of Mokokchung.

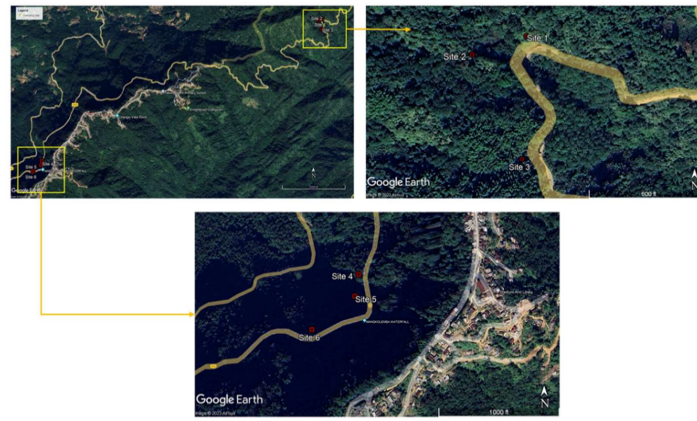

Fig. 2B: Sampling sites of the study area.

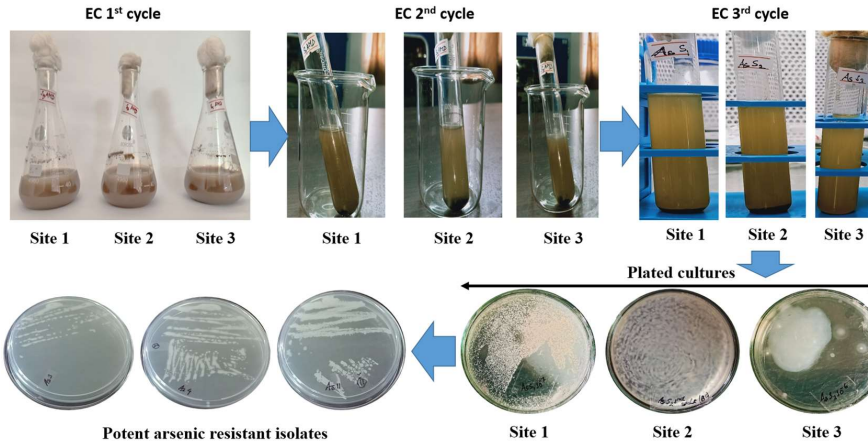

Fig. 3: Enrichment culture method for isolation of arsenic-resistant isolates.

The morphological characteristics of the selected bacterial isolate AS3 and its colony, including shape, and surface texture, are listed in Table 2. The strain was motile, spore-forming, rod-shaped, and Gram-positive. Moreover, it was found to be positive for Catalase and Oxidase and negative for H_2S production, Indole, Citrate utilization, Methyl Red, Voges–Proskauer, Nitrate reduction, glucose and lactose fermentation.

The bacterial colonies were irregular in shape, with undulated margins, raised, shiny, creamy-colored texture, and appeared optically opaque. Through 16S ribosomal RNA gene sequencing, the chosen bacterial strain was further identified as *Lysinibacillus* sp. strain AS3 (Fig. 4). The obtained sequence was submitted to the NCBI database under accession number OQ202230.

Growth Characteristics of the Selected Bacterial Strain

The growth curves of the AS3 strain indicated that in the presence of As^{3+} and As^{5+} HM ions, the lag phase was prolonged, or a late exponential phase was observed (Fig. 5). Moreover, the lag phase in As^{5+} HM ions was more prolonged (12 h) than that in As^{3+} HM ions (6 h). Additionally, the stationary phase in both As^{5+} and As^{3+} was achieved after 72 h, with a gradual decline in growth after 120 h.

Antibiogram Study

Based on the antibiotic susceptibility testing, AS3 was found to be resistant to 8 major antibiotics, while it was found to be susceptible to other 6 major antibiotics. The diameter of the zone of inhibition around the antibiotic-loaded wells/discs was measured and is presented in Table 3 and Fig. 6.

Intracellular and Extracellular Study of the Selected Strain

SEM-EDX analysis of selected strain under different treatments: The SEM-EDX results confirmed the presence of As^{5+} ions on the surface of AS3 bacterial cells, which were previously grown in Na_3AsO_4 supplemented medium (Fig. 8). However, the strains did not show adsorption of As^{3+} metal ions on their surfaces (Fig. 8). Furthermore, distinct changes in morphological features were observed in both As^{5+} and As^{3+} treated samples (Fig. 8) as compared to the control (Fig. 7), such as a decrease in population size and an increase in cell volume. In As^{3+} treated samples, the bacterial surface was rougher than that of the control.

IR spectroscopic analysis of the selected bacterial strain: The IR spectra of the dry bacterial biomass of strain AS3 in the presence of various HMs at their MTC, compared to a

Table 2: Morphological characterization of the arsenic-resistant isolates.

Sl No	Name of isolates	Shape	Margin	Elevation	Texture	Colour	Opacity
1	As 3	Irregular	Undulated	Raised	Shiny	Creamy	Opaque

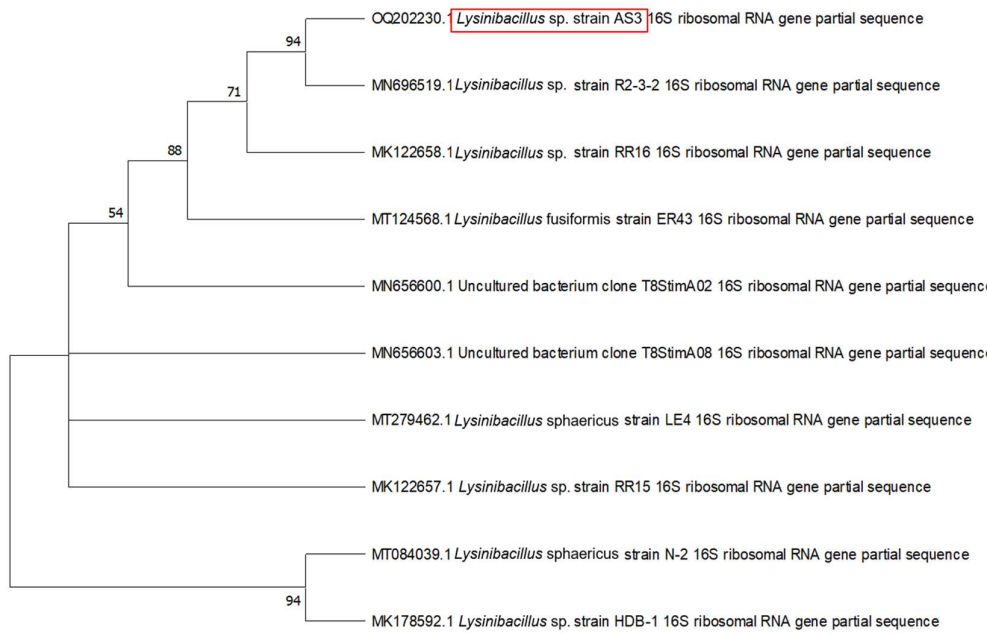

Fig. 4: 16s rRNA-based phylogenetic analysis of the bacterial strain AS3.

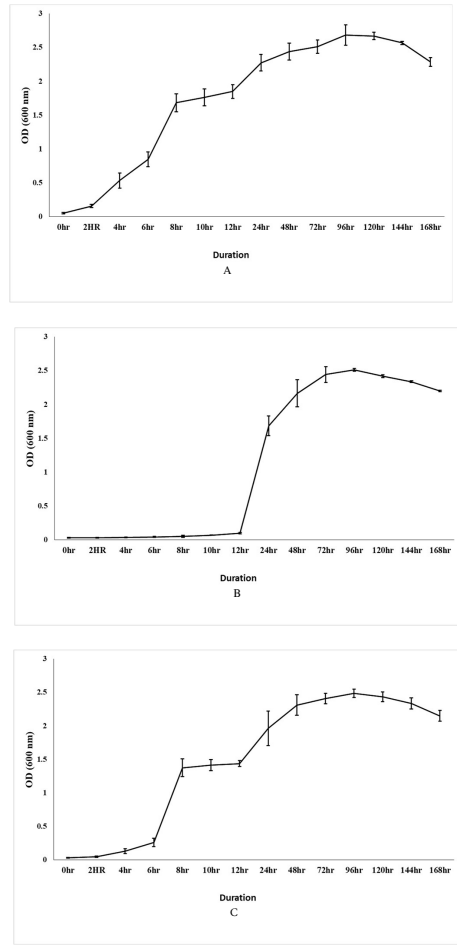

Fig. 5: Growth curve of AS3 strain in (A) Control, (B) Treated with As^{5+} and (C) Treatment with As^{3+}.

Table 3: Antibiotic susceptibility of the AS3 strain.

Sl No.	Antibiotics	Class of antibiotics	Spectrum of activity	Zone of inhibition	Effect on isolates
1.	Tetracycline [30 mcg]	Tetracycline	Broad	0	Resistant
2.	Ampicillin [10 mcg]	β-lactam	Broad	0	Resistant
3.	Penicillin [1 unit]	β-lactam	Narrow	0	Resistant
4.	Cefaloridine [30 mcg]	β-lactam	Broad	0	Resistant
5.	Kanamycin [5 mcg]	Aminoglycoside	Broad	0	Resistant
6.	Cloramphenicol [30 mcg]	Chloramphenicol	Broad	20	Sensitive
7.	Streptomycin [0 mcg]	Aminoglycoside	Broad	0	Resistant
8.	Cephalexin [30 mcg]	Cephalosporin	Broad	0	Resistant
9.	Ciprofloxacin [5 mcg]	Quinolone	Broad	25.67±0.57	Sensitive
10.	Azithromycin [15 mcg]	Macrolide	Broad	0	Resistant
11.	Norfloxacin [10 mcg]	Quinolone	Broad	14.67±0.58	Sensitive
12.	Clarithromycin [15 mcg]	Macrolides	Broad	30.67±0.57	Sensitive
13.	Erythromycin [15 mcg]	Macrolides	Broad	19	Sensitive
14.	Amoxicillin [10 mcg]	Penicillin-type	Broad	34.33±0.58	Sensitive

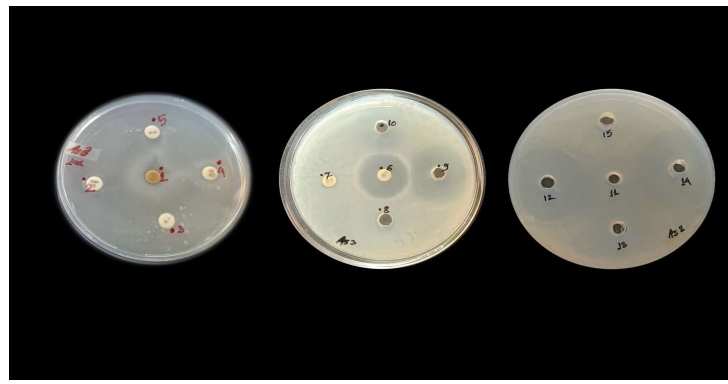

Fig. 6: Inhibition zones produced in the antibiotic susceptibility test.

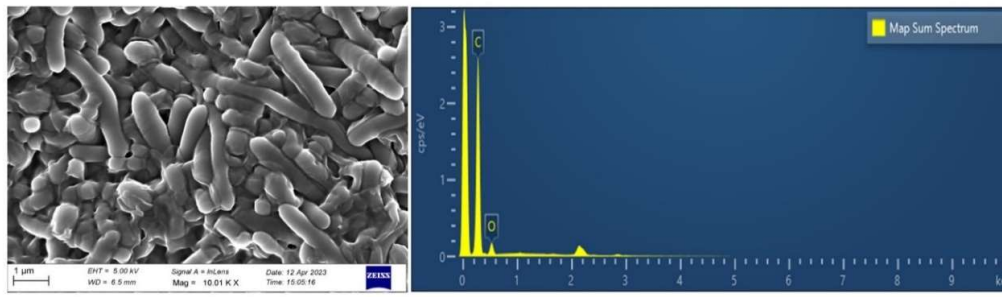

Fig. 7: (A) SEM micrograph of AS3 control and (B) EDX graph of AS3 Control.

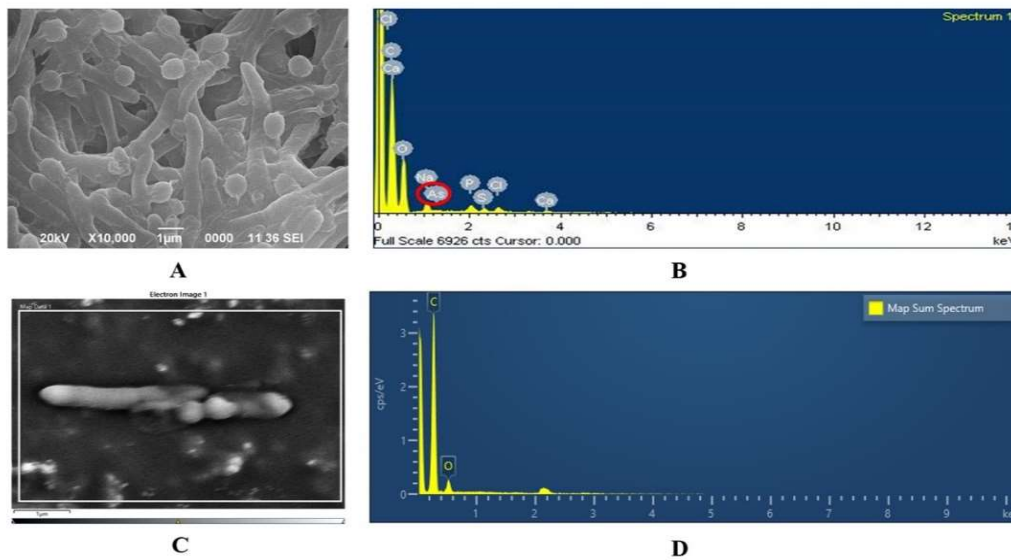

Fig. 8: (A) SEM micrograph of AS3 treated with As^{5+}, (B) EDX graph of AS3 treated with As^{5+}, (C) SEM micrograph of AS3 treated with As^{3+}, and (D) EDX graph of AS3 treated with As^{3+}.

control without HM, are presented in Fig. 9. The resulting spectra varied noticeably depending on the presence of HMs, compared to the control. In this study, it was observed that the peak values shifted from 3462 cm^{-1} (control) to higher wavenumbers in both the As treatments in the range of 3,477-3,478 cm^{-1}, corresponding to O-H stretching vibrations. In the range between 3000-2800 cm^{-1}, corresponding to methyl, methylene, and –CH stretching vibrations, the peaks were

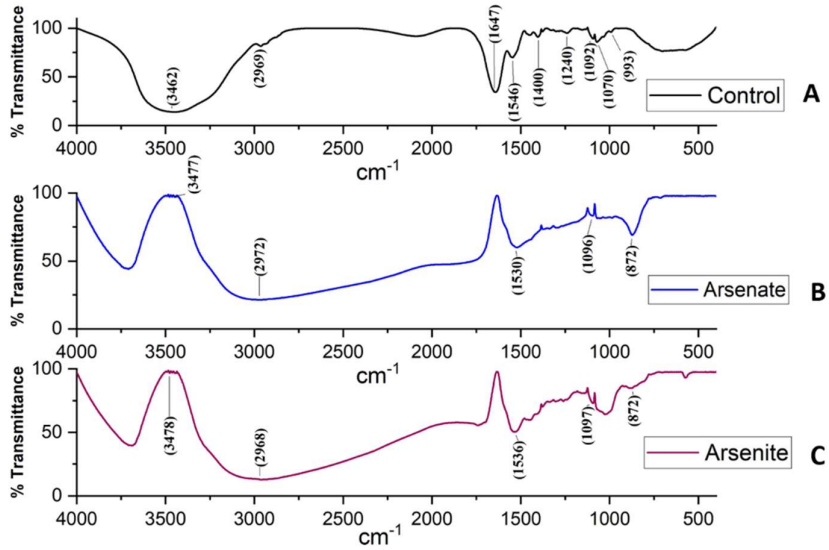

Fig. 9: FTIR study of AS3 strain (A) without HMs (control), (B) in the presence of As^{5+}, and (C) in the presence of As^{3+}.

observed around 2969 cm^{-1} in the control, along with the As treatments. In the range of 1,550–1,700 cm^{-1}, the 1647 cm^{-1} peak was recorded in the control, corresponding to N-H bending vibrations. However, no peak was observed in the As treatments. A significant peak was observed at 1546 cm^{-1} in the control, which was attributed to N-H in-plane bending or the CN stretching vibration. The peak value was found to shift to a lower wavenumber in the As treatments. Another significant peak at 1400 cm^{-1} was observed, which was attributed to COO– stretching vibrations. The peak was absent in the As-amended treatments. Moreover, the peaks around 1236-1240 cm^{-1}, which were attributed to P=O asymmetric vibrations, were observed in the control but not in the As treatments. In the range of 1000–1200 cm^{-1}, the peak value in the control treatment was observed at 1092 cm^{-1}, corresponding to C–O–C and C–O stretching vibrations. This peak shifted to higher wavenumbers in the As^{5+} (1096) and As^{3+} (1097) HM treatments. Another significant peak at 872 cm^{-1} was found in both the As^{5+} and As^{3+} treatments.

Bioremediation Potential of the Selected Strain

The accumulating capacity of the selected HM ions by the AS3 strain was determined using ICP-MS, and the strain exhibited significantly high accumulating capacity for both As^{3+} and As^{5+} ions, with 99.94% ± 0.005 and 99.49% ± 0.59, respectively.

DISCUSSION

Study Area and Sample Sites

Due to its proximity to human activity, a coal mining area, and a passing highway, the Changki study location is vulnerable to naturally occurring acid mine drainage (NOAMD). Prior records of Changki indicate the presence of HMs, such as zinc (Zn), copper (Cu) and cadmium (Cd), beyond the permissible limit (Semy & Singh 2021). Reports have revealed the presence of harmful HMs, such as arsenic, in NOAMD (Morgante et al. 2015). Furthermore, bacteria have persistently evolved in response to HM exposure, thereby acquiring resistance to various HMs through several mechanisms including metal resistance systems (Diba et al. 2021), extracellular metal sequestration (Voica et al. 2016), biosorption (Sevak et al. 2021), reduction of heavy metal ions (Ukkund et al. 2021), and morphological alterations (Mathivanan et al. 2021). Furthermore, HM-resistant rhizobacteria that demonstrate resistance against Cu, Cr, Zn, Cd, Ni, Sb, and As were reported from Nagaland in a study conducted by Tatung and Deb (2024).

Isolation of Arsenic-Resistant Bacterial Strain

The key objective of the current study was to identify bacterial species resistant to hazardous HM ions such as As. During the isolation and screening process, the enrichment culture method facilitates the growth and multiplication of certain microorganisms that possess the desired traits, thereby increasing the population of the target species (Gupta 2023). In addition, the enrichment culture method was performed not only to generate a population of bacteria resistant to HMs but also to eliminate the auxotroph (Roncero 1984).

Through a combination of ribotyping techniques and routine biochemical testing, the selected potential bacterial strain was identified as *Lysinibacillus* sp. The strain was found to be 94% identical to *Lysinibacillus* sp. strain R2-3-2 (Accession ID: MN696519) that had previously been

reported. To the best of our knowledge, this is the first report of *Lysinibacillus* species from North-East India and Nagaland that exhibit great tolerance against HMs, including As, Cd, and Pb ions. Several bacteria from the *Lysinibacillus* genus have been documented to exhibit resistance to HMs, including As, such as *L.* strain B1-CDA (Rahman et al. 2014) and *L.* sp. DMAB5 (Mandal et al. 2022) and *L. boronitolerans* P2IIIb (Aguilar et al. 2020). Moreover, *Lysinibacillus* species, including *L. fusiformis* L13 (Ma et al. 2023) and *L. varians* KUBM17 (Pal & Sengupta 2019), are resistant to Cd. In addition, L. varians strain KUBM17 has also been reported to exhibit resistance to Pb^{2+} HM ions (Pal & Sengupta 2019).

MIC, MBC and MTC of the Selected Bacterial Strain

The capacity of the bacterium to proliferate at elevated concentrations was utilized to ascertain its tolerance threshold to a particular HM in the medium. The limit of tolerance to the highest concentration of a particular HM in the medium was determined by measuring the growth of the bacteria at the resulting higher concentration. The frequency of resistant bacteria in metal-polluted environments increases as the quantity of HMs in these habitats increases (Piotrowska-Seget et al. 2005).

Due to its repeatability (Hasselmann 2003), speed, affordability, and suitability for several HM assays (Agarwal et al. 2020, Wiegand et al. 2008), the MIC for AS3 was determined using Luria Bertani Broth. The MIC values observed in this study (Table 4), exceeded those reported by previous researchers. Typically, elevated levels of HMs correlate with diminished bacterial proliferation, mostly due to compromised membrane function and the binding of metal ions to surfaces. Nevertheless, HM-resistant strains typically endure in HM-rich harsh environment owing to various intrinsic mechanisms, including the synthesis of exopolysaccharides (EPS), the presence of efflux and transporter proteins, metal adsorption on cellular envelopes, methylation, reduction to less toxic forms, the existence of multiple heavy metal resistance genes and operons, and several additional pathways (Haferburg & Kothe 2010, Gogoi et al. 2023).

Furthermore, compared to arsenite ions, arsenate exhibits greater minimum inhibitory concentrations (MIC) because of its lower toxicity. Table 4 shows that the findings are consistent with those of other studies. According to Abbas et al. (2014), this is due to the greater solubility of metal arsenites relative to that of metal arsenates. This implies that the isolated strains are highly suitable for the bioremediation of arsenic-contaminated environments.

Growth Characteristics of the Selected Bacterial Strain

A definite sign of metallic stress developed on the strain and the same can be seen in the growth curves of the AS3 strain, which showed a prolonged lag phase. According to Cristani et al. (2012), there may be a shift in the growth pattern of bacteria in the presence of metals due to a physico-chemical interaction that occurs between the bacteria and metals. Unambiguously, the protracted lag phase and late exponential phase of the growth curve show that the strain was under metallic stress or deliberately starts to oxidize As^{3+} as a defensive mechanism (Bachate et al. 2012, Bertrand 2019). Additionally, studies have revealed that As damages the bacterial cell wall, causing the bacteria to respond to As stress with an extended lag phase during which cells strive to adjust to their new surroundings (Abbas et al. 2014). The growth pattern of As^{3+} ions was comparable a certain extent with that of the control, suggesting that the bacteria were not under much stress. However, it required over 12 h to adjust to the stress in the presence of As^{5+} HM ions.

Antibiogram Study

Among the tested 14 different antibiotics, bacterial strain AS3 acquired resistance against 8 that include tetracycline (30 mcg) that belongs to the class of tetracycline, ampicillin (10 mcg), penicillin (0.6 mcg), and cefaloridine (30 mcg) that belongs to the class of β-lactam, kanamycin (5 mcg)

Table 4: MICs of bacteria against arsenate (As^{5+}) and arsenite (As^{3+}) HM ions.

Sl. No.	Bacterial strains	MIC against arsenate ions	MIC against arsenite ions	Type of media	Reference
1.	*Serratia marcescens*	2500 μg mL^{-1}	500 μg mL^{-1}	Minimal salts Medium (MSM) broth	Roy et al. (2024)
2.	*Alcaligenes faecalis*	1800 μg mL^{-1}	600 μg mL^{-1}		
3.	*Enterobacter* sp.	–	300 μg mL^{-1}	Acetate minimal medium broth	Abbas et al. (2014)
4.	*Klebsiella pneumoniae*	–	300 μg mL^{-1}		
5.	*Klebsiella pneumoniae*	–	370 μg mL^{-1}		
6.	*Sporosarcina luteola* M10	70000 μg mL^{-1}	1300 μg mL^{-1}	Minimal Medium broth	Salam et al. (2020)
7.	*Microbacterium paraoxydans*	36428.58 μg mL^{-1}	4800.78 μg mL^{-1}	Luria Bertani Agar Medium	Mandal et al. (2024)

and streptomycin (10 mcg) that belongs to the class of aminoglycoside, cephalexin (30 mcg) that belongs to the class of cephalosporin, and azithromycin (15 mcg) that belongs to the class of macrolide. Except for penicillin, all other tested antibiotics were broad-spectrum. However, the strain was also found to be sensitive to six broad-spectrum antibiotics, including chloramphenicol (30 mcg) that belonging to the class of chloramphenicol, ciprofloxacin (5 mcg) that belonging to the class of quinolone, clarithromycin (15 mcg) and erythromycin (15 mcg) that belonging to the class of macrolides, and amoxicillin (10 mcg) that belonging to the class of penicillin-type.

The present study employed environmental samples collected from the immediate vicinity of an open coal mine located in the rugged landscape of Changki. A substantial number of human populations near the top of the hill inhabit this location. Hence, pharmaceutical antimicrobial medications can be spread from human-populated areas to lower sections of hills by the washing of sewage water *via* rain or natural waterways (Pan et al. 2023). According to Spain and Alm (2003), exposure to metal-contaminated environments appears to be the source of microorganisms that are both tolerant to metals and resistant to antibiotics, leading to coincidental selection for resistance characteristics in both. A recent study by Chen et al. (2020) demonstrated that bacteria in ecosystems polluted with HMs can develop resistance to both HMs and antibiotics. This phenomenon has also been observed in habitats affected by other types of pollutants (Cen et al. 2020). These studies clearly highlight the necessity of avoiding the build-up of toxic metals in soil and water bodies.

SEM-EDX Analysis of Selected Strain Under Different Treatments

An attribute common to HM-resistant bacterial strains is their ability to adsorb metal ions on their surfaces, as demonstrated by the SEM-EDX investigation of multi-metal-resistant bacterial strains. The ions included arsenate, lead, and cadmium. Pandey and Bhatt (2015) obtained similar results for arsenate adsorption on bacterial surfaces. However, absence of arsenite ion adsorption in the present study suggests that, the bacteria may have evolved an adaptation mechanism, such as the extrusion of arsenite metal ions or the presence of efflux channels that allow for the clearance of arsenite metal ions (Gogoi et al. 2023). The alterations in surface and shape, as shown by several researchers (Rani et al. 2009, Zolgharnein et al. 2010, Banerjee et al. 2011, Shakya et al. 2012, Pandey & Bhatt 2015), clearly demonstrate an adaptive characteristic to accumulate more HM ions or as a site of reaction in a hazardous metallic environment.

IR Spectroscopic Analysis of the Selected Bacterial Strain

Peak shifting to higher wavenumbers of approximately 3200–3600 cm^{-1} was noted in the IR spectra, which might be the result of the conjugation effect (Dai et al. 2023). Such changes were observed in samples treated with arsenic, suggesting that the AS3 strain metabolized arsenic (Watanabe & Hirano 2013). The stretching vibrations of $–CH_3$, $–CH_2$, and –CH in membrane amphiphiles, including amphiphilic lipids in bacterial membranes, may be represented by the peaks seen in the range of 3000–2800 cm^{-1} (Shi et al. 2020, Sohlenkamp & Geiger 2016). The amides N-H bending vibrations around 1647 cm^{-1} are indicative of protein structure (Xu et al. 2021, Usoltsev et al. 2019, Kassem et al. 2023). Proteins are also responsible for the peak at 1546 cm^{-1} (Gupta et al. 2022). Peptidoglycans in the bacterial capsule are indicated by the peak at 1400 cm^{-1} (Saraeva et al. 2023). The absence of a peak in this range in the As treatments indicates peptidoglycan degradation. According to Parikh and Chorover (2006) and Quilès et al. (2010), the peaks at 1236–1240 cm^{-1} are often associated with phosphodiester, phospholipids, lipopolysaccharide (LPS), and ribose, which are involved bacterial membranes, nucleoid, and ribosomes, were not found in As treatments.

According to Fan and Zhang (2019), the peak at 872 cm^{-1} may be the result of As-O bending vibrations, which suggests that arsenic was present in both treatments. It could additionally be caused by the aromatic rings of particular nucleotides and amino acids vibrations (Kassem et al. 2023). The mechanism by which metals bind to ligands on the surface of bacteria is known as metal chelation, and it is most likely the reason for the changes seen as compared to the control. Functional groups are implicated in metal binding, as demonstrated by their interactions with metal ions (Singh et al. 2016, Bueno et al. 2008). When metal ions interact with negatively charged groups on the cell wall through mechanisms such as electrostatic interactions, van der Waals forces, and covalent bonding, fluctuations in the peak regions imply biosorption activity. It is commonly known that carboxyl, hydroxyl, and amino groups are functional groups that interact with metal ions (Singh et al. 2016).

Bioremediation Potential of the Selected Bacterial Strains

Several species of Lysinibacillus have been reported to exhibit HM resistance. For example, *L.* strain B1-CDA, isolated from cultivated land in Chuadanga district, Bangladesh, demonstrated 50% remediation of As^{5+} HM ions (Rahman et al. 2014), *L.* sp. DMAB5, isolated from Asanpara village (Bhagobangola I block) of Murshidabad district,

demonstrated 32.33 %, 31.29 %, and 31.20 % bioremediation in the presence of 2 $\mu g.mL^{-1}$, 10 $\mu g.mL^{-1}$, and 50 $\mu g.mL^{-1}$ of As^{3+} HM ions , respectively (Mandal et al. 2022), and *L. boronitolerans* P2IIIb, isolated from soil of a gold mining area in Paracatu, Brazil, demonstrated 69.38% and 85.72% bioremediation of As^{3+} and As^{5+} HM ions (Aguilar et al. 2020). Results of the present investigation clearly suggest that the selected strain is an effective bioremediating agent against As^{3+} and As^{5+} HM ions.

Limitations and Future Perspectives

In our study, we have found a bacterial strain capable of tolerating high levels of As HMs. Moreover, it was also resistant to a number of antibiotics with high bioremediation potential for removing As HMs. Thus, the strain shows promising results to be utilized in bioremediation. However, the efficacy of the strain was not evaluated under field conditions, hence this could be a future goal of the present study for its possible application as a bioremediating agent as well as beneficial bacteria for the plant systems.

CONCLUSIONS

The ongoing unsustainable levels of human exploitation of natural resources have led to the search for alternative methods for treating natural systems tainted with HMs. The goal of the present study was to screen bacterial isolates from natural streamlines of the Changki range of Nagaland, India, using an *in vitro* setting enriched with As HMs. The potential isolates based on the obtained MTC and MIC values against the selected HMs, i.e., As^{3+} and As^{5+}, was selected and identified as *Lysinibacillus* sp. AS3. The strain was resistant to As^{3+} and As^{5+} up to 1562.50 $\mu g.mL^{-1}$ and 125000 $\mu g.mL^{-1}$, respectively. The bacteria demonstrated resistance to commercial antibiotics, particularly to Streptomycin, Cephalexin, and Azithromycin, along with five other antibiotics. The cell surface adsorption of As^{3+} and HM As particularly was verified using SEM-EDX and FT-IR analyses of the biomass. Moreover, the strain demonstrated the ability to remove 99.94% and 99.49% of As^{3+} and As^{5+} under *in vitro* conditions, respectively, indicating its potential for bioremediation. With such notable traits, the potential As-resistant native strain exhibits potential for wide-scale usage in both basic research and real-world applications, especially in bioremediation technology. In this study, strain AS3 has exhibited enormous potential as a bioremediating agent in wastewater treatment plants loaded with HMs. Moreover, the strain can be used for functional gene analysis, which can be helpful in identifying the mechanisms and genes responsible for HM resistance. Another important aspect of future research could be checking its ability in the development of a novel broad-spectrum drug, and for plant growth-promoting rhizobacteria (PGPR) activity.

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REFERENCES

Abbas, S.Z., Riaz, M., Ramzan, N., Zahid, M., Tahir, S. and Iqbal, M., 2014. Isolation and characterization of arsenic-resistant bacteria from wastewater. *Brazilian Journal of Microbiology*, 45, pp.1309–1315. [DOI]

Agarwal, M., Rathore, R.S. and Chauhan, A., 2020. A rapid and high-throughput MIC determination method to screen uranium-resistant microorganisms. *Methods and Protocols*, 3, p.21. [DOI]

Aguilar, N.C., Faria, M.C., Pedron, T., Batista, B.L., Mesquita, J.P., Bomfeti, C.A. and Rodrigues, J.L., 2020. Isolation and characterization of bacteria from a Brazilian gold mining area with a capacity of arsenic bioaccumulation. *Chemosphere*, 240, p.124871. [DOI]

Ahmad, S.A., Khan, M.H. and Haque, M., 2018. Arsenic contamination in groundwater in Bangladesh: Implications and challenges for healthcare policy. *Risk Management and Healthcare Policy*, pp.251–261. [DOI]

Bachate, S.P., Khapare, R.M. and Kodam, K.M., 2012. Oxidation of arsenite by two β-proteobacteria isolated from soil. *Applied Microbiology and Biotechnology*, 93, pp.2135–2145. [DOI]

Balouiri, M., Sadiki, M. and Ibnsouda, S.K., 2017. Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), pp.71–79. [DOI]

Banerjee, S., Datta, S., Chattyopadhyay, D. and Sarkar, P., 2011. Arsenic-accumulating and transforming bacteria isolated from contaminated soil for potential use in bioremediation. *Journal of Environmental Science and Health Part A*, 46(14), pp.1736–1747. [DOI]

Bauer, A.W., 1966. Antibiotic susceptibility testing by a standardized single diffusion method. *American Journal of Clinical Pathology*, 45(4), pp.493–496.

Bergey, D.H., 1994. *Bergey's manual of determinative bacteriology*. Lippincott Williams & Wilkins, Baltimore.

Bermanec, V., Paradžik, T., Kazazić, S.P., Venter, C., Hrenović, J., Vujaklija, D., Duran, R., Boev, I. and Boev, B., 2021. Novel arsenic hyper-resistant bacteria from an extreme environment, Crven Dol mine, Allchar, North Macedonia. *Journal of Hazardous Materials*, 402, p.123437. [DOI]

Bertrand, R.L., 2019. The lag phase is a dynamic, organized, adaptive, and evolvable period that prepares bacteria for cell division. *Journal of Bacteriology*, 201(7), p.e00697-18. [DOI]

Bowman, N., Patel, D., Sanchez, A., Xu, W., Alsaffar, A. and Tiquia-Arashiro, S.M., 2018. Lead-resistant bacteria from Saint Clair River sediments and Pb removal in aqueous solutions. *Applied Microbiology and Biotechnology*, 102(5), pp.2391–2398. [DOI]

Bueno, B.Y.M., Torem, M.L., Molina, F.A.L.M.S. and De Mesquita, L.M.S., 2008. Biosorption of lead (II), chromium (III) and copper (II) by R. opacus: Equilibrium and kinetic studies. *Minerals Engineering*, 21(1), pp.65–75. [DOI]

Cen, T., Zhang, X., Xie, S. and Li, D., 2020. Preservatives accelerate the horizontal transfer of plasmid-mediated antimicrobial resistance genes via differential mechanisms. *Environment International*, 138, p.105544. [DOI]

Chen, J., Li, J., Zhang, H., Shi, W. and Liu, Y., 2019. Bacterial heavy-metal and antibiotic resistance genes in a copper tailing dam area in northern China. *Frontiers in Microbiology*, 10, p.1916. [DOI]

Chien, C.C., Lin, B.C. and Wu, C.H., 2013. Biofilm formation and heavy metal resistance by an environmental Pseudomonas sp. *Biochemical Engineering Journal*, 78, pp.132–137. [DOI]

Cristani, M., Naccari, C., Nostro, A., Pizzimenti, A., Trombetta, D. and Pizzimenti, F., 2012. Possible use of Serratia marcescens in toxic metal biosorption (removal). *Environmental Science and Pollution Research*, 19, pp.161–168. [DOI]

Dai, F., Zhuang, Q., Huang, G., Deng, H. and Zhang, X., 2023. Infrared spectrum characteristics and quantification of OH groups in coal. *ACS Omega*, 8(19), pp.17064–17076. [DOI]

Dey, U., Chatterjee, S. and Mondal, N.K., 2016. Isolation and characterization of arsenic-resistant bacteria and possible application in bioremediation. *Biotechnology Reports*, 10, pp.1–7. [DOI]

Diba, F., Khan, M.Z.H., Uddin, S.Z., Istiaq, A., Shuvo, M.S.R., Ul Alam, A.R., Hossain, M.A. and Sultana, M., 2021. Bioaccumulation and detoxification of trivalent arsenic by Achromobacter xylosoxidans BHW-15 and electrochemical detection of its transformation efficiency. *Scientific Reports*, 11(1), p.21312. [DOI]

Elahi, A., Ajaz, M., Rehman, A., Vuilleumier, S., Khan, Z. and Hussain, S.Z., 2019. Isolation, characterization, and multiple heavy metal-resistant and hexavalent chromium-reducing Microbacterium testaceum B-HS2 from tannery effluent. *Journal of King Saud University – Science*, 31(4), pp.1437–1444. [DOI]

Fan, L. and Zhang, X., 2019. ATR-FTIR spectroscopic characterization of ferric arsenic-containing colloid and gypsum. *Desalination and Water Treatment*, 153, pp.130–135. [DOI]

Gogoi, B., Bharali, P., Sorhie, V. and Rudithongru, L., 2023. An understanding of the underlying mechanisms involved in the bacterial remediation of arsenic, cadmium, and lead. In: S. Dey (ed.) *Biohydrometallurgical Processes*. 1st edn. CRC Press, Boca Raton, pp.130–154. [DOI]

Goswami, R., Mukherjee, S., Rana, V.S., Saha, D.R., Raman, R., Padhy, P.K. and Mazumder, S., 2015. Isolation and characterization of arsenic-resistant bacteria from contaminated water-bodies in West Bengal, India. *Geomicrobiology Journal*, 32(1), pp.17–26. [DOI]

Gupta, B.S., Jelle, B.P. and Gao, T., 2022. In vitro cell composition identification of wood decay fungi by Fourier transform infrared spectroscopy. *Royal Society Open Science*, 9(2), p.201935. [DOI]

Gupta, M.K., 2023. Isolation of microorganisms. In: A.K. Bhatt, R.K. Bhatia and T.C. Bhalla (eds.) *Basic Biotechniques for Bioprocess and Bioentrepreneurship*. 1st edn. Academic Press, Cambridge, pp.3–21. [DOI]

Haferburg, G. and Kothe, E., 2010. Metallomics: Lessons for metalliferous soil remediation. *Applied Microbiology and Biotechnology*, 87, pp.1271–1280. [DOI]

Haroon, K., Kherb, J., Jeyaseelan, C. and Sen, M., 2023. Recent advances and sustainable approaches towards efficient wastewater treatment using natural waste-derived nanocomposites: A review. *Nature Environment and Pollution Technology*, 22(3). [DOI]

Hasselmann, C., 2003. Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution. *Clinical Microbiology and Infection*, 9(8), pp.9–15.

Jeanthon, C. and Prieur, D., 1990. Susceptibility to heavy metals and characterization of heterotrophic bacteria isolated from two hydrothermal vent polychaete annelids, Alvinella pompejana and Alvinella caudata. *Applied and Environmental Microbiology*, 56(11), pp.3308–3314. [DOI]

Ka-ot, A.L. and Joshi, S.R., 2022. Application of acid and heavy metal-resistant bacteria from rat-hole coal mines in a bioremediation strategy. *Journal of Basic Microbiology*, 62(3-4), pp.480–488. [DOI]

Kassem, A., Abbas, L., Coutinho, O., Opara, S., Najaf, H., Kasperek, D., Pokhrel, K., Li, X. and Tiquia-Arashiro, S., 2023. Applications of Fourier transform-infrared spectroscopy in microbial cell biology and environmental microbiology: Advances, challenges, and future perspectives. *Frontiers in Microbiology*, 14, pp.1304081. [DOI]

Khalid, S., Shahid, M., Niazi, N.K., Murtaza, B., Bibi, I. and Dumat, C., 2017. A comparison of technologies for remediation of heavy metal contaminated soils. *Journal of Geochemical Exploration*, 182, pp.247-268. [DOI]

Ma, B., Song, W., Zhang, X., Chen, M., Li, J., Yang, X. and Zhang, L., 2023. Potential application of novel cadmium-tolerant bacteria in bioremediation of Cd-contaminated soil. *Ecotoxicology and Environmental Safety*, 255, pp.114766. [DOI]

Maizel, D., Blum, J.S., Ferrero, M.A., Utturkar, S.M., Brown, S.D., Rosen, B.P. and Oremland, R.S., 2016. Characterization of the extremely arsenic-resistant Brevibacterium linens strain AE038-8 isolated from contaminated groundwater in Tucumán, Argentina. *International Biodeterioration & Biodegradation*, 107, pp.147-153. [DOI]

Mandal, D., Das, S.K., Adhikari, J., Singh, R. and Choudhury, A., 2024. Genome sequencing, annotation and application of a strain of Microbacterium paraoxydans – A heavy metal hypertolerant and plant growth promoting bacterium. *Research Square*, 12(2), pp.55-70. [DOI]

Mandal, D., Sonar, R., Saha, I., Ahmed, S. and Basu, A., 2022. Isolation and identification of arsenic resistant bacteria: A tool for bioremediation of arsenic toxicity. *International Journal of Environmental Science and Technology*, 19(10), pp.9883-9900. [DOI]

Mateos, L.M., Ordóñez, E., Letek, M. and Gil, J.A., 2006. Corynebacterium glutamicum as a model bacterium for the bioremediation of arsenic. *International Microbiology*, 9(3), pp.207-215.

Mathivanan, K., Chandirika, J.U., Vinothkanna, A., Yin, H., Liu, X. and Meng, D., 2021. Bacterial adaptive strategies to cope with metal toxicity in the contaminated environment – A review. *Ecotoxicology and Environmental Safety*, 226, pp.112863. [DOI]

Matlakowska, R., Drewniak, L. and Sklodowska, A., 2008. Arsenic-hypertolerant Pseudomonads isolated from ancient gold and copper-bearing black shale deposits. *Geomicrobiology Journal*, 25(7-8), pp.357-362. [DOI]

McCarty, K.M., Hanh, H.T. and Kim, K.W., 2011. Arsenic geochemistry and human health in South East Asia. *Reviews on Environmental Health*, 26(1), pp.71. [DOI]

Morgante, V., Mirete, S., de Figueras, C.G., Postigo Cacho, M. and González-Pastor, J.E., 2015. Exploring the diversity of arsenic resistance genes from acid mine drainage microorganisms. *Environmental Microbiology*, 17(6), pp.1910-1925. [DOI]

Pal, A.K. and Sengupta, C., 2019. Isolation of cadmium and lead tolerant plant growth promoting rhizobacteria: Lysinibacillus varians and Pseudomonas putida from Indian agricultural soil. *Soil and Sediment Contamination*, 28(7), pp.601-629. [DOI]

Pan, X.R., Chen, L., Zhang, L.P. and Zuo, J.E., 2023. Characteristics of antibiotic resistance gene distribution in rainfall runoff and combined sewer overflow. *Environmental Science and Pollution Research*, 30(11), pp.30766-30778. [DOI]

Pandey, N. and Bhatt, R., 2015. Arsenic resistance and accumulation by two bacteria isolated from a natural arsenic contaminated site. *Journal of Basic Microbiology*, 55(11), pp.1275-1286. [DOI]

Parikh, S.J. and Chorover, J., 2006. ATR-FTIR spectroscopy reveals bond formation during bacterial adhesion to iron oxide. *Langmuir*, 22(20), pp.8492-8500. [DOI]

Piotrowska-Seget, Z., Cycoń, M. and Kozdroj, J., 2005. Metal-tolerant bacteria occur in heavily polluted soil and mine spoil. *Applied Soil Ecology*, 28(3), pp.237-246. [DOI]

Quilès, F., Humbert, F. and Delille, A., 2010. Analysis of changes in attenuated total reflection FTIR fingerprints of Pseudomonas fluorescens from planktonic state to nascent biofilm state. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 75(2), pp.610-616. [DOI]

Rahman, A., Nahar, N., Nawani, N.N., Jass, J., Desale, P., Kapadnis, B.P., Hossain, K., Saha, A.K., Ghosh, S., Olsson, B. and Mandal, A., 2014. Isolation and characterization of a Lysinibacillus strain B1-CDA showing potential for bioremediation of arsenics from contaminated water. *Journal of Environmental Science and Health, Part A*, 49(12), pp.1349-1360. [DOI]

Rani, A., Souche, Y.S. and Goel, R., 2009. Comparative assessment of in situ bioremediation potential of cadmium resistant acidophilic Pseudomonas putida 62BN and alkalophilic Pseudomonas monteilli 97AN strains on soybean. *International Biodeterioration & Biodegradation*, 63(1), pp.62-66. [DOI]

Roncero, M.I.G., 1984. Enrichment method for the isolation of auxotrophic mutants of Mucor using the polyene antibiotic N-glycosyl-polifungin. *Carlsberg Research Communications*, 49, pp.685-690. [DOI]

Roy, V., Saha, B.K., Adhikary, S., Chaki, M.G., Sarkar, M. and Pal, A., 2024. Isolation, characterization, identification, genomics and analyses of bioaccumulation and biosorption potential of two arsenic-resistant bacteria obtained from natural environments. *Scientific Reports*, 14(1), pp.5716. [DOI]

Salam, M., Varma, A., Chaudhary, D. and Aggarwal, H., 2020. Novel arsenic-resistant bacterium Sporosarcina luteola M10 has potential bioremediation properties. *Journal of Microbiology and Experimentation*, 8, pp.213-218. [DOI]

Sanders, E.R., 2012. Aseptic laboratory techniques: Plating methods. *Journal of Visualized Experiments*, 63, e3064. [DOI]

Saraeva, I., Tolordava, E., Sheligyna, S., Nastulyavichus, A., Khmelnitskii, R., Pokryshkin, N., Khmelenin, D., Kudryashov, S., Ionin, A. and Akhmatkhanov, A., 2023. FT-IR analysis of Pseudomonas aeruginosa bacteria inactivation by femtosecond IR laser radiation. *International Journal of Molecular Sciences*, 24(6), pp.5119. [DOI]

Semy, K. and Singh, M.R., 2021. Assessment of soil physico-chemical properties and heavy metals bioaccumulation on plants at a coal mining affected forest of Changki, Nagaland. *Environment and Ecology*, 39(1A), pp.192-199.

Sevak, P.I., Pushkar, B.K. and Kapadne, P.N., 2021. Lead pollution and bacterial bioremediation: A review. *Environmental Chemistry Letters*, 19(6), pp.4463-4488. [DOI]

Shakya, S., Pradhan, B., Smith, L., Shrestha, J. and Tuladhar, S., 2012. Isolation and characterization of aerobic culturable arsenic-resistant bacteria from surface water and groundwater of Rautahat District, Nepal. *Journal of Environmental Management*, 95, pp.S250-S255. [DOI]

Shi, H., Sun, J., Han, R., Ding, C., Hu, F. and Yu, S., 2020. The strategy for correcting interference from water in the Fourier transform infrared spectrum based bacterial typing. *Talanta*, 208, p.120347. [DOI]

Singh, N., Gupta, S., Marwa, N., Pandey, V., Verma, P.C., Rathaur, S. and Singh, N., 2016. Arsenic-mediated modifications in Bacillus aryabhattai and their biotechnological applications for arsenic bioremediation. *Chemosphere*, 164, pp.524-534. [DOI]

Sohlenkamp, C. and Geiger, O., 2016. Bacterial membrane lipids: Diversity in structures and pathways. *FEMS Microbiology Reviews*, 40(1), pp.133-159. [DOI]

Spain, A. and Alm, E., 2003. Implications of microbial heavy metal tolerance in the environment. *Reviews in Undergraduate Research*, 2, pp.1-6.

Takeuchi, M., Kawahata, H., Gupta, L.P., Kita, N., Morishita, Y., Ono, Y. and Komai, T., 2007. Arsenic resistance and removal by marine and non-marine bacteria. *Journal of Biotechnology*, 127(3), pp.434-442. [DOI]

Tamura, K., Stecher, G. and Kumar, S., 2021. MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38(7), pp.3022-3027. [DOI]

Tatung, M. and Deb, C.R., 2024. Screening and characterization of heavy metal tolerant rhizobacteria from wild Musa rhizosphere from coal mining area of Changki, Nagaland, India and assessment of their growth promoting potential under Cd/Cu contaminated conditions. *South African Journal of Botany*, 165, pp.217-227. [DOI]

Turpeinen, R., Kairesalo, T. and Häggblom, M.M., 2004. Microbial community structure and activity in arsenic-, chromium- and copper-contaminated soils. *FEMS Microbiology Ecology*, 47(1), pp.39-50. [DOI]

Ukkund, S.J., Adarsh, D.P., Nair, H., Manasa, J., Krishna, S., Naveen, R., Jacob, S., Shettar, A.B., Badiuddin, A.F. and Nellerichale, A., 2021. Antimicrobial coating of fabric by biosynthesized silver nanoparticles from Panchakavya. *Nano Express*, 2(1), p.010033. [DOI]

Usoltsev, D., Sitnikova, V., Kajava, A. and Uspenskaya, M., 2019. Systematic FTIR spectroscopy study of the secondary structure changes in human serum albumin under various denaturation conditions. *Biomolecules*, 9(8), p.359. [DOI]

Voica, D.M., Bartha, L., Banciu, H.L. and Oren, A., 2016. Heavy metal resistance in halophilic bacteria and archaea. *FEMS Microbiology Letters*, 363(14), p.146. [DOI]

Watanabe, T. and Hirano, S., 2013. Metabolism of arsenic and its toxicological relevance. *Archives of Toxicology*, 87, pp.969-979. [DOI]

Wiegand, I., Hilpert, K. and Hancock, R.E., 2008. Agar and broth dilution methods are used to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*, 3(2), pp.163-175. [DOI]

Willsky, G.R. and Malamy, M.H., 1980. Effect of arsenate on inorganic phosphate transport in Escherichia coli. *Journal of Bacteriology*, 144(1), pp.366-374. [DOI]

Xing, L., 2022. Determination of toxic elements in food by ICP-MS using the AOAC method 2015.01. *Spectroscopy*, 37(10), pp.7-12. [DOI]

Xu, J.L., Herrero-Langreo, A., Lamba, S., Ferone, M., Scannell, A.G., Caponigro, V. and Gowen, A.A., 2021. Characterisation and classification of foodborne bacteria using reflectance FTIR microscopic imaging. *Molecules*, 26(20), p.6318. [DOI]

Zakaria, S.N.F., Aziz, H.A., Hung, Y.T., Wang, M.H.S. and Wang, L.K., 2024. Treatment of hazardous sludge from water and wastewater treatment plants. In: L.K. Wang, M.S. Wang and Y. Hung (eds.) *Industrial Waste Engineering*. 1st edn. Springer International Publishing, Cham, Switzerland, pp.1-41.

Zolgharnein, H., Karami, K., Assadi, M.M. and Sohrab, A.D., 2010. Investigation of heavy metals biosorption on Pseudomonas aeruginosa strain MCCB 102 isolated from the Persian Gulf. *Asian Journal of Biotechnology*, 2(2), pp.99-109. [DOI]