Environment and Ecology 43 (2) : 475—488, April—June 2025
Article DOI: https://doi.org/10.60151/envec/NUBN8247
ISSN 0970-0420

Unveiling the Plant Growth-Promoting Potential of an Arsenic-Resistant Isolate: A Natural Ally for Sustainable Agriculture

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Received 16 March 2025, Accepted 25 April 2025, Published on 30 May 2025

ABSTRACT

This study utilizes a bacterial strain, *Lysinibacillus* sp. AS3 which was previously found resistant to arsenic (As). The strain was studied for exopolysaccharides (EPS), biofilm, siderophore, Indole-3-acetic acid (IAA) producing ability in presence of arsenate (As^{5+}) and arsenite (As^{3+}) heavy metal (HM) ions, which would help to determine its plant growth promoting activity. The study was done through both qualitative and quantitative assessment to observe the significant changes in exposure to As HMs. Moreover, phytotoxicity assay was performed on *Vigna radiata* seeds in presence of the both the HM ions. The results indicated enhanced production of biofilm, IAA and EPS in presence of both HM ions. While, reduced production of siderophore was recorded in presence of As^{3+} HM ions. Further, it was observed that employing AS3 strain on *V. radiata* seeds showed stimulating effect on the growth of the seedlings albeit arsenic stress, along with enhanced growth of the seedlings as compared to control. Thus, present study shows that this AS3 strain could be a potent strain in agriculture applications.

Keywords Heavy metal, Arsenic, Bacterium, Siderophore, Bioremediation, Exopolysaccharides (EPS), Biofilm.

INTRODUCTION

Arsenic (As) contamination is a persistent environmental issue affecting globally humans, plants animals and microbes. This metalloid, once if it enters into the ecosystems through human activity or natural origins can cause significant damages to biological functions in different living organisms (Fatoki and Badmus 2022). In plants, it is often reported to hinder useful biochemical pathways (Martínez-Castillo *et al.* 2022), reduced growth and development (Emamverdian *et al.* 2023) or induce oxidative stress (Sharma 2012). While, in humans chronic exposure to arsenic can been to cause severe health issues such as cancer and damages to the organs (Speer *et al.* 2023). Addressing arsenic pollution, requires human interventions and among many techniques to tackle as pollution, utilizing arsenic-resistant bacterial isolates

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integrated with plant growth-promoting rhizobacteria (PGPR) capability has emerged as a favorable approach (Kumar *et al.* 2022).

A global rise in arsenic groundwater contamination in almost 106 countries has been reported and around 230 millions of people are known to be affected by arsenic exposures inducing severe health issues. Arsenic is undoubtedly toxic metalloid and is thus ranked first in Substance Priority List (SPL) of ATSDR (Agency for Toxic Substances and Disease Registry) (Bhat *et al.* 2024). Even though a number of remediation techniques are available, certain limitations are associated with conventional remediation techniques such as being expensive, time-consuming, and environmentally damaging (Khalid *et al.* 2017). Moreover, reports indicates that HM contamination in soil has a noteworthy global economic impact, estimated over $10 billion annually (Azhar *et al.* 2022). Furthermore, these methods do not state the ecological disturbances and environmental problems which occurs due to these metals. This led to a broader awareness on bioremediation, which is sustainable and uses the intrinsic properties of microorganisms to eradicate the toxins (Gadd 2009, Pande *et al.* 2022).

Bioremediation can be regarded as a potent tool for utilizing microbial potential. Recent studies suggest that bioremediation might be a successful strategy for heavy metal contaminated soils (Zheng *et al.* 2024). Microorganisms are known to contribute in this interaction thanks to the fact that they may evolve and employ a diverse strategy to deal with metals (Mathivanan *et al*. 2021). The expression includes systems that are actually encapsulation, intracellular sequestration and enzyme secretion or pump action as well as metal detoxification (Pande *et al.* 2022, Gogoi *et al.* 2023).

When iron is in limited supply, microorganisms generate siderophores, which are low-molecular-weight, high-affinity iron-chelating compounds (Park *et al*. 2023). Siderophore production stimulates microbial inhabitability in heavy metal-contaminated soils and helps halt the metals to stop them from seeping into groundwater (Rajkumar *et al.* 2010). In addition, the formation of chlorophyll and the healthy functioning of enzymes depend on the amount of iron that siderophores make available to plants (Bera *et al.* 2022, Radzki *et al.* 2013).

Biofilms, as microbial communities, progress on an exopolymeric matrix that guards them from environmental stresses, including HMs (Yin *et al.* 2019). The formation of a microenvironment that safeguards cells from toxic metal augments microbial resistance, a phenomenon known as biofilm formation, which is common in soils that are polluted with metals (Prinzi and Rohde 2023). The exopolysaccharide (EPS) biosource material, through ionic and complexation interactions, enhances heavy metal binding significantly by reducing its mobility and bioavailability in wastewater (Flemming and Wingender 2010). To efficiently bioremediate such complex substrates, biofilms are indispensable for sustaining commensalisms among bacteria (Sharma *et al.* 2023) and broadening the metabolic diversity of the bioremediators (Mangwani *et al.* 2016, Mishra *et al*. 2022).

Exopolysaccharides are high molecular weight biopolymers secreted by microorganisms that help in the immobilization of hazardous metals and the accumulation of soil particles (Zhang *et al.* 2024). The functional groups of EPS such as -OH, -COOH, and -NH_2 can interact with HM ions (Li *et al.* 2021). These groups are responsible for reducing the toxicity imposed by the HMs which takes place with reduction in the metal's bioavailability (Gupta and Diwan 2017). Further, EPS can change soil structure as well as enhance the microbial diversity in soil through biofilm formation, cell-cell cohesion or induced adaptive capability (Flemming *et al.* 2016, Costa *et al.* 2018). Moreover, it can upsurge the water retention capacity of soil, which is vital for soil health (Morcillo and Manzanera 2021).

Current research shows the beneficial role played by plant growth-promoting strains, or PGPs, which reside near plant roots and actively participate in upliftment of the plant health in innumerable ways - breaking nitrogen, phosphate or secreting hormones which enhance plant growth such as indole-3-acetic acid (IAA), or fight plant pathogens (de Andrade *et al.* 2023, Lugtenberg and Kamilova 2009). Further, they are also capable in reducing HM stress in plants by secreting organic acids or compounds which hold the

metals (Qin *et al.* 2024, Khanna *et al.* 2019, Mushtaq *et al.* 2022). They are also seen in generating antioxidants which combat with the oxidative stress by the HMs in plants (Iqbal *et al.* 2024).

When important biomolecules such as siderophore, biofilm, EPS and IAA combine together, they are capable of enhancing the bioremediation efficiency (Roskova *et al.* 2022, Sharma 2022, Wei *et al.* 2024). Such intrinsic capabilities have been previously reported in a number of bacterial strains such as *Burkholderia* sp. EIKU24 (Basak *et al.* 2024), *Pseudomonas fluorescens* (Upadhyay *et al.* 2018); *Variovorax* sp. S12S4 (Zouagui *et al.* 2024) as well as IAA producing *Sphingobium* sp. strain AEW4 which was reported to be a potent PGPs which demonstrated effective bioremediation (Ganesh *et al.* 2024, Minari *et al.* 2020). Thus, these inherent capabilities by PGPs such as HMs retention in soil, reduced bioavailability and interactive interactions among microorganisms and plants help in building a symbiotic relationship with plants, thereby demonstrating the positive impacts of mixed microbial communities in soil which is crucial for effective remediation (Timofeeva *et al.* 2022, Kramer *et al.* 2020).

The current study assessed the ability of a previously isolated arsenic resistant strain, *Lysinibacillus* sp. strain AS3 in synthesizing IAA, siderophore, EPS and biofilm which is vital for effective bioremediation and stimulating growth in plants. In this study, the production of these biomolecules was screened and quantified, indicating their effective role in metal-microbe interactions whereby these biomolecules were generated in absence or presence of As^{3+} and As^{5+}. Furthermore, this potent strain was amended with mung bean seeds in phytotoxicity experiments, in presence or absence of As HMs, thereby demonstrating an integrated bioremediation method for combatting As stress in plants. Thus, this study was aimed in developing sustainable and innovative ways to accelerate crop development albeit As stress in plants exposed to As pollution.

MATERIALS AND METHODS

Heavy metal tolerant bacterial strain

The bacterial strain, *Lysinibacillus* sp. strain AS3 was obtained by previous isolation from Changki, Nagaland (Accession id: OQ202230). It was found to be an arsenic resistant strain (MIC: 1562 µg/mL for As^{3+} and 125000 µg/mL for As^{5+} HM ions) as was used in this paper to check its application as potent PGP traits.

Chemicals and reagents

Chemicals, reagents and salts were of analytical grade (AR) purchased from Qualigens fine chemicals, Thermo electron LLS India Private Limited, Mumbai. Microbiological media were purchased Hi-Media Laboratories Private Limited, Mumbai, India. Whatman Filter paper (Grade 1) was purchased from Cytiva, Global Life Science Solutions Operations UK Limited.

Estimation of PGP traits

The qualitative and quantitative study was performed for biofilm, EPS, siderophore and IAA synthesis using standard procedures. Except IAA synthesis, all the tests were checked for their changes in presence of both As^{5+} (500 µg/mL) and As^{3+} (500 µg/mL) HM ions. Culture grown in absence of HMs was taken as control.

Evaluation of biofilm producing capacity

The biofilm-forming ability of the bacterial strain was ascertained using the tube technique, a qualitative biofilm detection test (Kırmusaoğlu 2019). The isolate after forming linings on the walls of the polystyrene test tubes, containing Tryptic Soy Broth (TSB), was rinsed with phosphate buffer saline (PBS) and further stained with crystal violet (0.1 %) for about an hour. Post staining, cells was washed with PBS, air dried and observed for visible film lining on the walls of test tube.

To quantify the generation of biofilm quantitatively, the procedure described by Kırmusaoğlu (2019) was slightly altered. Initially, freshly prepared bacterial suspensions of the selected strain were cultured in Mueller-Hinton Broth (MHB) supplemented with 1% glucose and the density of the bacterial cells was adjusted to 0.5 McFarland (1×10^8 cfu/ml). The bacterial cells suspension density of 5×10^6 cfu/ml was

achieved by diluting the freshly grown bacterial in MHB for 20 times. Subsequently, a 96-well flat-bottomed sterile polystyrene microplate was then filled with 20 µl of the aforementioned bacterial suspension and 180 µl of MHB modified with 1% glucose. The final bacterial cells suspension density was kept constant at 5×10^5 cfu/ml. The microplate plate later was incubated at 37 ºC for 24 h. Following a 24 h, the wells that revealed biofilm linings on their walls were washed using phosphate-buffered saline (PBS) with a pH of 7.2. The samples were then dried for approximately an hour at 60°C to fix the biofilm (Christensen *et al.* 1985). Next, 100 µl of 0.1% crystal violet solution were added to each well that contained dried biofilms (Lade *et al.* 2019). The wells took around five minutes to dry after being dyed. To remove the unbounded crystal violet stain, the stained wells were further rinsed twice using PBS. After 30 min, 150 µL of 33% glacial acetic acid (v/v) was added to each well in order to dissolve the stained adhering biofilm. Finally, a 96-well microplate reader was used to measure the absorbance at 595 nm in order to quantify the resulting biofilm development (Lade *et al.* 2019). Serving as a negative control were inoculation wells with MHB medium enhanced with 1% glucose. Using the blank absorbance readings, it was possible to assess if a strain had developed a biofilm. Cut off value (ODc) may be used to categorize strain as either producer of biofilm or not (Kırmusaoğlu 2019). Using Eqn. 1 and 2, ODc and OD values of the strain were determined. A zero denotes the presence of biofilm development, whereas a negative number indicates the absence of biofilm growth. The data may be analyzed and categorized using the ODc values as follows: (i) no biofilm; (ii) weak biofilm (+ or 1); (iii) moderate biofilm (++ or 2), and (iv) strong biofilm (+++ or 3) (Kırmusaoğlu 2019).

$$OD_c = \text{Average OD of negative control} + (3 \times \text{standard deviation of negative control}) \quad (1)$$

$$OD_{strain} = \text{Average OD of strain} - OD_c \quad (2)$$

Evaluation of siderophore producing capacity

The universal CAS test was used to screen the chosen As-resistant strain for siderophore production (Schwyn and Neilands 1987). With certain modifications, the procedure outlined by Arora and Verma (2017) was used for the qualitative estimation. Initially 90 ml of autoclaved LB agar medium were combined with 10 ml of CAS reagent to create CAS agar plates. The CAS plate was spot-inoculated with the strain, while a control plate was kept uninoculated. For about six days, the inoculation plates were eventually incubated at 30 ºC. According to Louden *et al.* (2011), the formation of an orange zone around the bacterial colony suggested the existence of a siderophore.

Using a 96-well microtiter plate, quantitative estimation was carried out with minor adjustments in accordance with the technique described by Arora and Verma (2017). After centrifuging the cultured broth of the chosen strain for six minutes at 6000 rpm, the supernatant was collected. Then, each well of a 96-well microtiter plate was filled with 100 µl of the collected cell-free supernatant and 100 µl of CAS reagent. Using a microplate reader, the OD of every sample was measured at 630 nm. The following formula (Eqn. 3), provided by Payne (1993), was used to quantify siderophore production as percent siderophore units (psu).

$$\text{Siderophore production (psu)} = \frac{\{(A_r - A_s) \times 100\}}{A_r} \quad (3)$$

Where, A_s represents sample absorbance (CAS solution and cell free supernatant), A_r represents absorbance of reference (CAS solution and uninoculated broth).

Evaluation of EPS producing capacity

The dry weight of the EPS generated by the chosen As-resistant strain was measured as part of a gravimetric test for EPS production screening. To achieve this, a modified version of the Nwosu *et al.* (2019) approach was used. First, 50 ml of nutrient broth supplemented with 2% glucose was infused with a fresh cell suspension of an overnight grown culture containing 1.5×10^8 cfu/ml of bacteria. The resulting mixture was then incubated at 30 ºC for three days at 160 rpm and eventually centrifuged at 12000 rpm. Exopolysaccharides were precipitated after being further extracted from the cell-free supernatant using equal amounts of cold ethanol and kept in a refrigerator for an overnight period at 4 °C. Centrifugation

was done for 20 min at 12,000 rpm for collecting the precipitates. To get a consistent weight, the pellets underwent further drying at 60 ºC, and their total dry weight was determined.

As part of the quantification process, the total carbohydrates in the dried EPS were calculated using the standard anthrone technique (Ludwig and Goldberg 1956). The sample's total carbohydrate content was determined by measuring its optical density (OD) at 630 nm, with glucose serving as a reference.

Evaluation of IAA producing capability

The production of IAA was measured following the methodology by Widawati (2020) with some modifications. TSB media (100 ml) including L Tryptophan (100 mg/mL) served as the media for growing the bacterial cultures. Post inoculation, the cultures were incubated in an orbital incubator shaker at 37ºC for 48 hrs until turbidity was observed in the media, at 160 rpm. Post incubation, cultures were harvested at 8000 rpm for about 10 minutes for obtaining the supernatant. After that 1 ml of supernatant was mixed slowly with 2 ml of Salkowski reagent (0.5M ferric chloride+ 35% perchloric acid), and incubated in room temperature in a dark area for about 20 minutes. The qualitative indication of IAA presence was indicated by pinkish color. The quantification of IAA was done with the help of UV-Vis Spectrophotometer at a wavelength of 530 nm. IAA in the sample was estimated with the help of IAA standard curve (20 µg/mL-100 µg/mL). Uninoculated TSB media (1ml) with Salkowski reagent (2 ml) served as control.

Phytotoxicity assay of treated bacterial supernatant

The phytotoxicity of the treated bacterial supernatant was evaluated in a static test following the protocol stated in Gidudu and Chirwa (2022) with some modifications. During this experiment, Root elongation and seed germination served as the basis for the static test. Seeds of mung bean (*Vigna radiata*) were used, and they were of good quality. Firstly, 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 hrs with an agitation of 160 rpm at 37 ºC. Selected HMs at MTC without AS3 strain were maintained as blank. Post incubation period, the cultures were centrifuged at 6000 rpm and pelleted. The supernatant was passed through a membrane filter (0.22 µm) and used for phytotoxicity assay.

Toxicity was determined in sterilized petri dishes (90 × 15 mm) containing Whatman Filter Paper (Grade No.1 Size 110 mm). Twelve seeds were pre-treated with surface sterilizer 70% ethanol for 1 min and were transferred in each Petri dish with 1 mL of the cell-free bacterial supernatant test solutions at room temperature. After five days of dark incubation at room temperature, seed germination and root elongation (≥5 mm) were evaluated. The germination index (GI), relative root length (RRL), and relative seed germination (RSG) and germination vigour index (GVI) were then calculated (Eqns. 4-7), as may be seen below (Gidudu and Chirwa 2022, Sencan *et al.* 2024).

$$\text{RSG (\%)} = \frac{\text{Number of seeds germinated in the supernatant extract}}{\text{Number of seed germinated in the control}} \times 100\% \quad \text{(eq 4)}$$

$$\text{RRL (\%)} = \frac{\text{Mean root length in the supernatant extract}}{\text{Mean root length in the control}} \times 100\% \quad \text{(eq 5)}$$

$$\text{GI} = \frac{\text{\% of root growth} \times \text{\% of seed germination}}{100\%} \quad \text{(eq 6)}$$

$$\text{GVI} = \frac{\text{Seedling length (mm)} \times \text{Germination percentage (\%)}}{100} \quad \text{(eq 7)}$$

Statistical analysis

All the experiments were conducted in triplicate and analyzed statistically using SPSS software. The obtained values were expressed as Mean values ± Standard Deviation (SD). The experimental data were checked for one-way analysis (ANOVA) at $P \leq 0.005$ confidence level.

RESULTS AND DISCUSSION

Biofilm producing capacity of the AS3 strain

The qualitative assay for biofilm production against

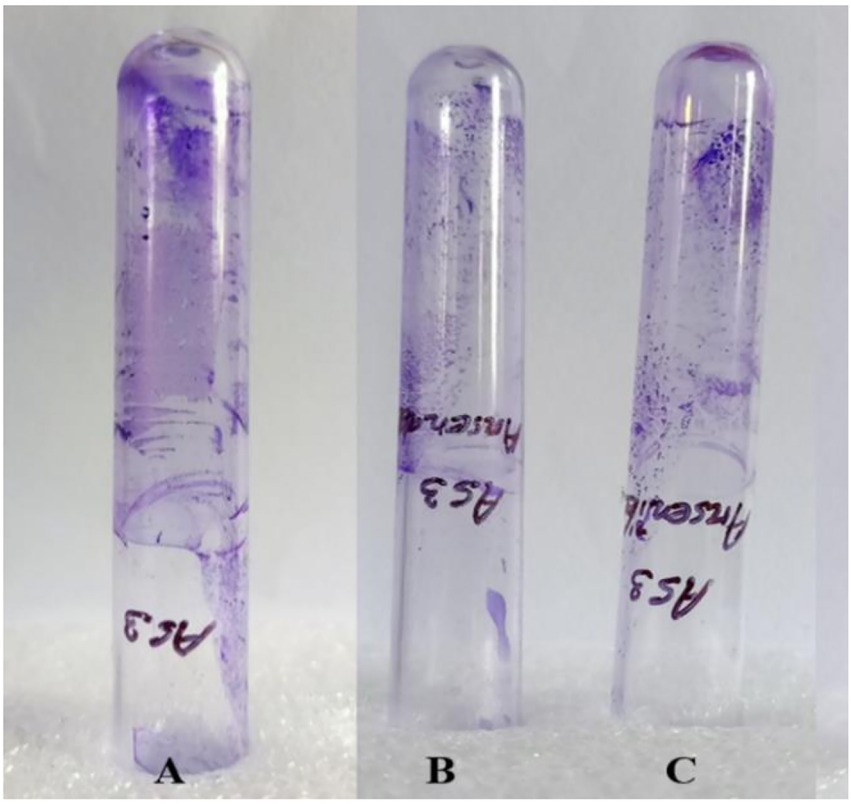

Fig. 1. Tube method for the screening of biofilm production in the presence (A) control, (B) As^{5+}, (C) As^{3+}.

different As^{5+} and As^{3+} HM treatments indicated the presence of biofilms due to presence of lining on the tubes. AS3 strain showed weak lining formation on the tube (Fig. 1). According to Table 1, the microtiter plate test findings showed that the AS3 strain had a limited ability to produce biofilm (0.49 ± 0.12) without the presence of HMs. On the other hand, biofilm development was boosted in the presence of HMs, with As^{5+} (1.09 ± 0.16) surpassing As^{3+} (0.87 ± 0.28).

Table 1. Biofilm production capacity of the selected strain under different treatments.

Test samples	Observed OD value	Biofilm forming potential
Bacterial strain AS3 without HM	0.49 ± 0.12	Weak biofilm (+ or 1) production since, ODc < OD < 2 × ODc
Bacterial strain AS3 with AS^{5+} ions	1.09 ± 0.16	Weak biofilm (+ or 1) production since, ODc < OD < 2 × ODc
Bacterial strain AS3 with AS^{3+} ions	0.87 ± 0.28	Weak biofilm (+ or 1) production since, ODc < OD < 2 × ODc

Within active bacterial biofilms, it has been reported that both biosorption and bioaccumulation processes occur concurrently (Kumar *et al*. 2007). By employing the biofilm as a protective coating, bacterial cells may proliferate effectively in a variety of heavy metal-stressed environments. EPS provides the protective layer of the biofilm and improves the immobilization and subsequent sequestration of the HMs (Ianeva 2009, Priyadarshanee and Das 2021). By means of electrostatic attraction, the positively charged cations readily adsorb on the negatively charged functional groups of the biofilm in the surrounding environment. According to Gupta and Diwan (2017), this idea has been verified and biofilms produced by a variety of bacteria have been identified as possible adsorbents for various HMs. Apart from the removal of HMs from the environment

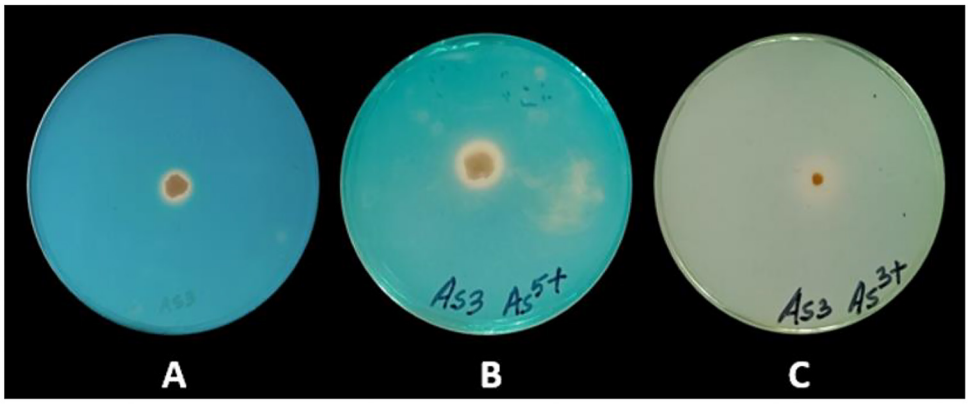

Fig. 2. Halo formation by AS strain in CAS agar assay in the presence (A) control, (B) As^{5+} and (C) As^{3+}.

and biofilm have also been valued for a number of bacterial-dominated processes (Balan *et al.* 2021, Haque *et al.* 2021).When exposed to arsenic, Pavez *et al.* (2023) observed that *Exiguobacterium* strains, a gram-positive heavy metal resistant bacterium isolated from a highly arsenic-contaminated environment in Chile, formed biofilms more readily. This suggests that biofilm plays a role in the bacterium's resistance to arsenic. Teitzel and Parsek (2003) and Koechler *et al.* (2015), reported that bacteria in biofilms are 2-600 times more resistant to HMs than individual cells. According to Van Houdt and Michiels (2010), interactions among bacterial cells, substrates, and the environment are generally necessary for the development of bacterial biofilms. Active biofilms have been shown to be successful in removing HMs from both actual industrial and municipal effluents and continuous treatment effluents (Gadd 2009, Kotrba *et al.* 2011). Moreover, under the stress of HMs, plants grew more when biofilm-forming microorganisms were present (Sharma 2022). Thus, AS3 strain has also intriguing biofilm producing ability which would help in plants affected by As stress as well as inducer of plant growth.

Table 2. Siderophore production capacity of the selected strain under different treatments.

Tested HMs	Qualitative analysis	Quantitative analysis (psu)
Control	++	35.71 ± 0.96
As^{5+}	+++	84.33 ± 1.09
As^{3+}	+	29.78 ± 0.76

Siderophore production by AS3 strain

AS3 strain clearly showed production of siderophore in presence of the As^{3+} and As^{5+} HMs ions. The production of siderophore was qualitatively estimated by the formation of an orange-colored halo around the bacterial colonies on CAS agar in the presence and absence of selected HMs at their MTC values and the same is presented in Fig. 2. The concentration of siderophore production varied from 29.78 ± 0.76 psu to 84.33 ± 1.09 psu (Table 2).

Generally, siderophores primarily serve to transform iron that is attached to proteins or water-soluble substances into a form that bacteria may access (Timofeeva *et al.* 2022). Bacteria have been seen to synthesize siderophores, which aid in the remediation of HM contaminated environments through many mechanisms, including enhancing the bioavailability of HMs and metalloids (Gaonkar and Bhosle 2013, Roskova *et al.* 2022). Additionally, it can reduce the toxicity of HMs, serve as protective agents for plants against microbial pathogens, and stimulate the synthesis of indole-3-acetic acid (IAA) in the presence of HMs to promote plant growth (Rajkumar *et al.* 2010, Zhang *et al.* 2023). It also acts as metal-chelators by producing soluble metallophores capable of solubilizing or mobilizing the associated HMs in soil (Gaonkar and Bhosle 2013, Gomes *et al.* 2024).

In the present study, the increased siderophore production in the presence of both As^{5+} and As^{3+} ions could be due to the high resistance showed by

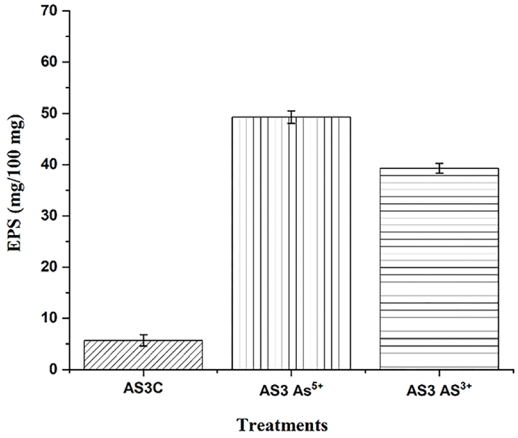

Fig. 3. EPS production of AS3 strain under As treatments.

the bacterial strain against arsenic which is also evident in another study by Drewniak *et al.* (2008) and Ghosh *et al.* (2015), where they have established a correlation between increased arsenic resistance with siderophore generation.

EPS production by AS3 strain

EPS was initially quantified by weighing the samples, which were produced under identical conditions to those used in the siderophore production experiment. Figure 3 illustrates the results, which indicated that the AS3 strain without HM treatment had an EPS yield (dry weight) of 96 mg > with AS^{3+} (16 mg), > with As^{5+} (8 mg). The results revealed that the dried EPS had a considerably higher carbohydrate content when HMs were present. The bacterial samples that were exposed to As^{5+} (49.34±1.22 mg/100 mg of EPS) and AS^{3+}(39.23±0.99 mg/100 mg of EPS) had the higher carbohydrate contents as compared to control sample which was only 5.69±1.07 mg/100 mg of EPS.

Strain AS3 demonstrated a significant production of EPS in absence of As HM ions, which escalated in presence of both As HM ions. An increment of 43.65% and 33.54% production was recorded in presence of As^{5+} and As^{3+} HM ions as compared to control. The observed stimulating effect on EPS production by arsenic is coincidence with the previous reports of Tournay *et al.* (2023), where they reported an enhancement in EPS production by *Rahnella laticis* PD12R, an endophytic bacterium, in response to As^{5+} and As^{3+} exposure. Bacterial EPS synthesis serves as a shield for survival in harsh environments, notably heavy metal poisoning (Poli *et al.* 2011). When present in excessive quantity, the metal ions penetrate the cells where their toxicity can be prevented by intracellular complexing or reduction to a less hazardous oxidation state (Nies 1999).

The primary components of EPSs released by bacteria include polysaccharides, proteins, and nucleic acids. The EPS matrix contains negatively charged functional groups like carboxyl, hydroxyl, sulphate, phosphate, and amine groups. Therefore, chelating metal cations and avoiding direct cell-to-toxic metal contact are made possible by the total negative charge of bacterial EPS (Wu *et al.* 2019, Sheng *et al.* 2010). Moreover, bioleaching may be facilitated by EPS generated by bacterial cells, comprising polysaccharides, lipids, lipopeptides, glycolipids, and neutral lipids, which demonstrate significant surface activity to engage with HMs (Yang *et al.* 2018). Due to its binding capability, EPS has been proposed as a viable adsorbent for metal pollutants.

IAA producing capability of AS3 strain

Qualitative analysis indicated AS3 strain had IAA producing ability (Fig. 4). Further, on quantification, IAA produced by AS3 was determined to be 69.32 ± 7.04 µg/mL

The current strain showed distinct presence of IAA which is an indicator of its utility as growth booster in mung bean plants. Previous reports in *Lysinibacillus* species such as- *L. fusiformis* B-CM18 showed beneficial role as PGPR in chickpea plants (Singh *et al.* 2013), *L. fusiformis* EI20 showed several PGPR traits in ginseng plant (Vendan *et al.* 2010). This study indicates a first arsenic resistant bacterial strain from Nagaland which links the beneficial role of *L.* species in leguminous plants such as mung beans, by enhancing their plant growth through secretion of IAA which is a vital hormone for plant growth and development.

Phytotoxicity assay

The impact of different treatments, specifically the

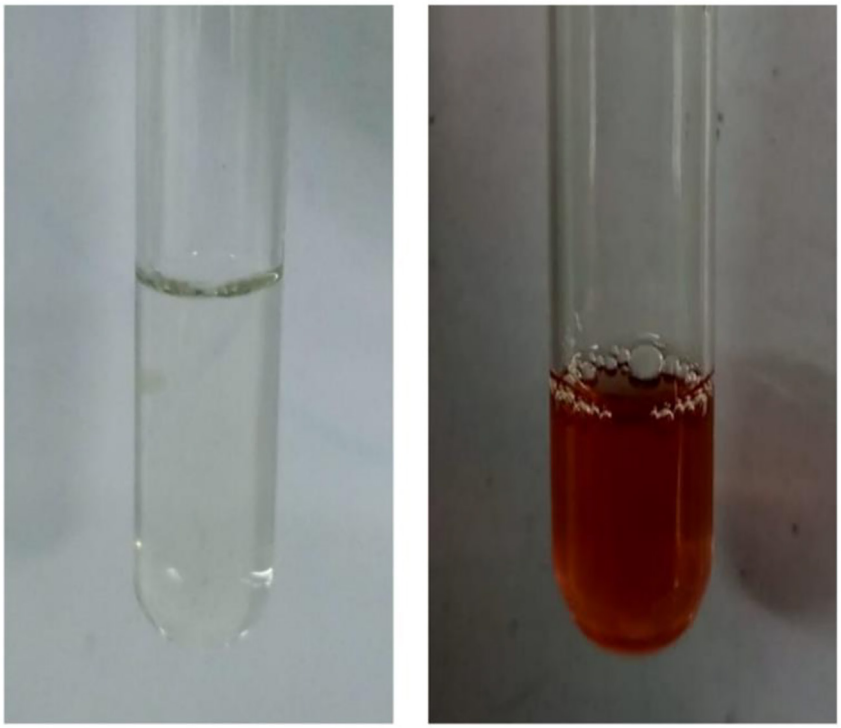

Fig. 4. IAA production in (A) control, (B) AS3 strain.

use of HMs alone, HMs combined with BI (Bacterial inoculum) AS3 strain, and BI alone, on the germination of *Vigna radiata* seeds was investigated (Fig. 5). This was done by analysing the germination indices (GI) data, which involved assessing the results of RSG (%) and RRL (%) at a significance level of $p<0.05$. The RSG for samples treated with BI, BI with arsenate, BI with As^{3+} was determined to be 100%. However, the RSG (%) reached 50% for both As^{5+} treated samples and As^{3+} treated samples. The highest RRL (%) was observed in seeds treated with BI (349.16%), followed by BI + As^{3+} (99.20 %) and BI + As^{5+} (96.44%), As^{5+} (41.32 %), and finally As^{3+} (30.22 %).

The GI and GVI of the AS3 strain were highest in seeds treated with BI alone, without any metallic treatments. The GI was 349.16% and the GVI was 13.84%. Seeds treated with BI and As^{3+} had a GI of 99.20% and a GVI of 2.43%. While, seeds treated with BI and arsenate had a GI of 96.44% and a GVI of 2.38%. When metals were used for treatments, the GI value was found to be greatest for As^{5+} (20.66%), followed by As^{3+} (15.11%). The GVI was shown to be greatest in As^{3+} (0.76%) followed by As^{5+} (0.72%).

Seed phytotoxicity tests are bioassays used to assess the toxicity of chemicals, waste, or industrial effluents by analysing the germination and development of plant seeds. This approach is often employed in ecotoxicology research (Luo *et al.* 2019, Martínez-Cruz and Rojas-Valencia 2024). The current study evaluated the toxicity of selected HMs on mung bean seeds using phytotoxicity assays. The results of this study indicated stimulating effect by incorporation of *Lysinibacillus* sp. strain AS3 on the growth of mung bean seeds. While, in seeds treated with HMs showed inhibition in growth. Moreover, the highest recorded GI and GVI were observed in BI amended seeds, followed by control seeds and seeds amended with BI and HMs. This clearly indicates that upon incorporation of AS3 BI, it stimulated the growth in

Fig. 5. Phytotoxicity assay of *Vigna radiata* in the presence of (A) distilled water only, (B) AS3 strain and As^{5+}, (C) AS3 strain and As^{3+}, (D) AS3 strain only, (E) As^{5+} only, (F) As^{3+} only.

mung beans seedlings, capable of withstanding As stress. This stimulating effect could be attributed to hydrolytic enzymes such as proteases, cellulases or chitinases which mitigate the As stress. This is also evident in a study by Naureen *et al.* (2017), whereby *L. sphaericus* ZA9 exhibited hydrolytic enzymes that increased the shoot length in tomato and cucumber seeds, along with increased seed germination and vigor.

In the current study, all the biomolecules, viz. siderophores, EPS, biofilm and IAA demonstrated stimulated activity in mung bean seedlings' growth. This result is also in accordance with other reports such as *L. xylanilyticus* Strain GIC41 (Ahsan *et al.* 2021) and *L. sphaericus* ZA9 (Naureen *et al*. 2017) which showed stimulated growth in plants, having inherent capabilities of producing siderophores. Likewise, biofilm producing strains also showed similar results as biostimulant in the growth of mung bean plants such as *L. macroides* BF15 and *L. xylanilyticus* strain GIC41 as reported by Rafique *et al.* (2024) and Ahsan *et al.* (2021). Additionally, the IAA and EPS producing strains showed stimulated growth of mung beans such as *L. pakistanensis* PCPSMR15 and *Lysinibacillus fusiformis* strain S4C11 (Lelapalli *et al.* 2021, Passera *et al.* 2021).

CONCLUSION

The research capitalizes on the significance of this particular bacterial strain in promoting plant growth by synthesizing biomolecules such as siderophores, biofilm, exopolysaccharides (EPS), and indole-3-ace-

tic acid (IAA). Interestingly, the strain was capable of producing substantial amounts of biomolecules in the absence or presence of arsenic. These compounds enable the plants to capture resources more effectively and construct robust root systems while providing protection from environmental extremes. When the mung bean seedlings were supplemented with AS3 bacterial inoculum, they exhibited exceptional growth even in the face of arsenic exposure showing that this particular strain is capable of Arsenic oxidative stress mitigation. Being a potential tool for sustainable agriculture, this strain has the capacity to naturally improve soil quality and increase crop yield without the application of chemical fertilizers. Also, this strain's ability to withstand arsenic introduces an opportunity to engineer crops that can grow in contaminated soils to resolve both environmental and agricultural problems. This strain holds great potential for not only improving the plant growth and agricultural production but also leveraging biotechnological advancements for environmental remediation.

ACKNOWLEDGMENT

The first author acknowledges Nagaland University for providing financial assistance through the Non-Net fellowship program. The authors would like to express their gratitude to Professor Upasana Bora Sinha, Head of the Chemistry Department at Nagaland University, for providing instrumentation support during the entire work. The first author would also like to thank Late Purna Kanta Gogoi, and people who supported him and guided him during the accomplishment of this study.

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