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ORIGINAL PAPER

Investigation of arsenic-resistant, arsenite-oxidizing bacteria for plant growth promoting traits isolated from arsenic contaminated soils

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Abstract

The problem of arsenic (As) pollution being severe warrants opting for low-cost microbial remediation strategies. The present study of identifying suitable bacterial strains led to the isolation of eleven As-tolerant strains from the As-contaminated rhizosphere soils of West Bengal, India. They were found to oxidize/reduce 55–31.6% of 5 mM As(III) and 73–37.6% of 5 mM As(V) within 12 h. The four isolates (BcAl-1, JN 73, LAR-2, and AR-30) had a high level of As(III) oxidase activity along with a higher level of As(V) and As(III) resistance. The agar diffusion assay of the isolates further confirmed their ability to endure As stress. The presence of *aoxB* gene was observed in these four As(III) oxidizing isolates. Evaluation of plant growth-promoting characteristics revealed that BcAl-1 (*Burkholderia cepacia*), JN 73 (*Burkholderia metallica*), AR-30 (*Burkholderia cenocepacia*), and LAR-2 (*Burkholderia* sp.) had significant plant growth-promoting characteristics (PGP), including the ability to solubilize phosphate, siderophore production, indole acetic acid-like molecules production, ACC deaminase production, and nodule formation under As stressed condition. BcAl-1 and JN 73 emerged as the most promising traits in As removal as well as plant growth promotion.

Keywords Arsenic (As) · Plant-growth promotion · *Burkholderia* · As-oxidase activity · Minimum inhibitory concentration

Introduction

Eastern India (primarily West Bengal) and Bangladesh have a serious problem with arsenic (As) contaminated water and food (Chowdhury et al. 2001; Bhattacharya et al. 2010; Chakraborti et al. 2015). The use of As or heavy metal enriched water in agricultural fields is the reason for its significant build-up in the soil (Abedin et al. 2002; Meharg and Rahman 2003) and subsequent accumulation in standing crop (Matera et al. 2003; Bogdan and Schenk 2009). Generally, As residues are found in the top layer of soil because of their low volatility and low solubility, and further enable As entry in crops (Das et al. 2013). The As has both organic and inorganic forms (Matschullat 2000) and in the environment found as an oxyanion (Frankenberger and Arshad 2002). The significant health risk associated with As polluted soil and water (González and González-Chávez 2006; Xiong et al. 2019), along with the high cost of engineering-based remediation methods, argues for the use of bioremediation methods to remove As (Kumpiene et al. 2006; Ghosh et al. 2011).

The As build-up in the soil is gradually reduced through plant accumulation and other environmental processes such as leaching (Hartley et al. 2004), erosion, and methylation (Srivastava et al. 2013). In As-contaminated soil, the resident soil microorganisms (Farooq et al. 2016) will use various strategies to adapt and survive in the polluted soil, including utilizing As to support their growth (Das et al. 2013; Kumar et al. 2021). These microorganisms may help in metal decontamination (Bhattacharyya and Sengupta 2020) and promote plant growth simultaneously (Li and Ramakrishna 2011; Srivastava et al. 2013).

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Specific enzymes or respiratory chains in the bacteria are responsible for the redox transformation of As. These microbes can use As either as an electron donor or electron acceptor and thereby play a significant role in As detoxification mechanisms. Specific genes and/or operon systems are present in those bacteria to favor such processes. They may possess either As resistance gene or *ars* operon system; or As respiratory reduction gene or *arr*; or As(III) oxidation genes or *aox/aro/aso* system. During energy metabolisms, As(III) serves as an electron donor, and *aro* system encodes the gene encoding proteins (Santini and van den Hoven 2004). Apart from these, heterotrophic As(III) oxidizing bacteria also possess *aox* genes (Muller et al. 2003) and *aso* genes (Kashyap et al. 2006) that play a vital role in As detoxification. Phylogenetically As(III) oxidizing bacteria are diverse. As(III) oxidase enzyme is a protein that belongs to dimethyl sulphoxide reductase family of the molybdopterin-containing protein (*aro A/aso A/aox B*) and Fe–X Reiske protein (Ellis et al. 2001; Muller et al. 2003; Santini and van den Hoven 2004). Many of the betaproteobacteria such as *Burkholderia* sp., *Bosea* sp., *Alkaligens* sp. were found to carry the *aox* B subunit of As(III) oxidase gene (Quéméneur et al. 2008).

Further, some rhizospheric microbes also play the role through unique As resistance and plant growth-promoting (PGP) characters. These adopted indigenous soil microbes may manifest a bunch of PGP traits through secretion of 1–aminocyclopropane–1–carboxylate (ACC) deaminase, indole–3–acetic acid (IAA), being phosphate solubilizers, producing siderophores that reduce metal toxicity, encourage plant-assisted bioremediation and enhance nitrogenase activity (Ma et al. 2011; Rajkumar et al. 2012; de-Bashan et al. 2012; El-Meihy et al. 2019). Most remarkably, such PGP characters remain active even under intense As stress (Ghosh et al. 2018).

The objective of this study was to isolate, characterize and identify As resistant bacteria found in the rhizosphere of plants for their plant growth-promoting attributes and their ability to tolerate As. In this context, we have planned to select and identify efficient As-resistant bacteria from contaminated soil, precisely from the rhizospheric zone, to investigate the plant growth-promoting attributes of identified bacteria to harvest their capacity to develop plant's resistance to stress conditions, encourage plant growth, and give a path to contribute accelerated remediation of As-polluted soils. The ultimate goal would be to use these strains to remediate As-contaminated soils and enhance plant growth.

Materials and methods

Analysis of soil samples

Soil samples (2 cm diameter, 10 cm depth) were collected aseptically from the contaminated zone of Chakdaha, West Bengal (23° 05′ N and 88° 54′ E), India where As concentrations in the groundwater is above World Health Organization (WHO)-defined safe limit (Sarkar et al. 2012; Bhattacharyya et al. 2021; Sengupta et al. 2021). Atomic Absorption Spectrophotometer (AAS) coupled with hydride generator was used to assess the soil load of the total (Sparks et al. 2006) and available As (Johnston and Barnard 1979). Total bacterial population and As resistant bacterial population of the soil were also measured (Bachate et al. 2009). This soil was used to grow the plants from which the bacteria used in this study were isolated.

Enrichment of As tolerant bacteria

Two gram soil samples aseptically collected from the rhizosphere of 50-day old groundnut plants (*Arachis hypogaea* cv TG-51), and 45-day old lentil plants (*Lens culinaris* cv WBL 77, Moitree) were suspended in 2 mL of sterile distilled water. Yeast Extract Mannitol (YEM) broth and Yeast Extract Mannitol Agar (YEMA) was prepared using standard chemical constituents free from arsenic. One ml of each soil suspension was transferred to YEM broth (Mannitol 10.00 g, $MgSO_4{\cdot}7H_2O$ 0.20 g, NaCl 0.10 g, K_2HPO_4 0.50 g, $CaCl_2{\cdot}2H_2O$ 0.20 g, $FeCl_3{\cdot}6H_2O$ 0.01 g, yeast extract 1.00 g in 1000 ml distilled water at pH 6.8–7) supplemented with either 1 mM As(III) or 1 mM As(V) and incubated at 30 °C for 2 days (Kinegam et al. 2008). Subsequently, an enrichment culture was prepared by transferring 2 mL of the above culture in YEM broth supplemented with As and incubated at 30 °C for 2 days. The procedure was repeated twice. Around 0.1 mL of As spiked enriched culture was spread on a medium containing YEMA (Mannitol 10.00 g, $MgSO_4{\cdot}7H_2O$ 0.20 g, NaCl 0.10 g, K_2HPO_4 0.50 g, $CaCl_2{\cdot}2H_2O$ 0.20 g, $FeCl_3{\cdot}6H_2O$ 0.01 g, yeast extract 1.00 g, agar 20.00 g in 1000 mL distilled water at pH 6.8–7) supplemented with As and incubated at 30 °C. Selected distinct colonies of As tolerant bacteria were picked for isolation after 24 h incubation.

As oxidation and reduction activity by the strains

Silver nitrate ($AgNO_3$) method under the standard condition (Majumder et al. 2013a) was used to screen As-oxidizing bacterial isolates. The isolates were cultured on solidified chemically defined medium (CDM) (Weeger et al. 1999). CDM was prepared by mixing three solutions, as, Solution A comprising of 0.0812 M $MgSO_4{\cdot}7H_2O$, 0.187 M NH_4Cl, 0.07 M Na_2SO_4, 0.574 mM K_2HPO_4, 4.57 mM $CaCl_2{\cdot}2H_2O$, 0.446 M Na lactate; Solution B comprising of 4.8 mM $Fe_2SO_4{\cdot}7H_2O$; and Solution C comprising 0.95 M $NaHCO_3$. 100 mL of solution A, 2.5 mL of solution B, 10 mL of solution C were mixed and made up to 1000 mL with doubly deionized water previously sterilized

by autoclaving (121 °C, 15 min), and the final pH was adjusted to 7.2. Twenty gram of agar was added, along with 1 mM As(III) supplementation to solidify the medium and were incubated for 48 h at 30 °C. The plates were flooded with a 0.1 M $AgNO_3$ solution, and the resulting colony color change was recorded. $AgNO_3$, upon reaction with As(III), produces a bright yellow silver ortho As(III) (Ag_3AsO_3) precipitate, whereas ortho As(V) (Ag_3AsO_4) precipitate, produced by the reaction of $AgNO_3$ with As(V) is brownish silver in nature. The bacterial strains' As-oxidizing ability was further validated through the micro-plate technique (Simeonova et al. 2004), which was repeated three times. The formation of a brown color precipitate was used to indicate As oxidization.

As accumulation and oxidation/reduction

Freshly prepared culture (approximately 100 µL) of As resistant bacterial strains were inoculated in 50 mL YEM liquid culture medium previously spiked with 5 mM of As(V) and As(III) in 100 mL conical flask, with proper mercuric nitrate impregnated filter paper capping (Majumder et al. 2013b). The set-up was incubated at room temperature for 12 h in a shaker. First, the filter papers were removed followed by separation of cell pellet and liquid culture media by centrifugation at 10,000 rpm for 2 min. The As concentrations were measured with an Atomic Absorption Spectrophotometer by the standard method (Majumder et al. 2013b). Each experiment was repeated three times.

MIC (minimum inhibitory concentration) study of the bacteria

The MIC value is the lowest concentration of As(V) or As(III), which entirely hampers microbial activity (Majumder et al. 2013a). The MIC test has been used to isolate As(III) and As(V) resistant bacterial strains (Majumder et al. 2013b). In this study, the MIC test was performed by transferring 1 mL of overnight culture grown at 30 °C into YEM broth supplemented with either As(III) as $NaAsO_2$ at a concentration ranging from 1 to 50 mM or As(V) as $Na_2HAsO_4{\cdot}7H_2O$ at a concentration ranging from 1 to 500 mM and incubated at 30 °C for 48 h with shaking and aeration. The OD (optical density, measurement of microbial growth) of the bacterial cultures was measured using a microprocessor-based UV–Vis spectrophotometer at $\lambda_{max} \cong 600$ nm.

Agar diffusion assay of the bacterial isolates

The agar well-diffusion method was carried out to confirm and validate the MIC of arsenic-resistant bacteria (Hassen et al. 1998). Arsenic solutions were prepared in different concentrations (100, 200, 300, 390, 400, 408, 450, and 500 mM for arsenate; 10, 20, 30, 40, 41.2, 45, 46.2, and 50 mM for arsenite). Sterile Luria Broth (LB) agar plates were prepared, and each plate was spread with overnight cultures of the best strains (BcAl-1, JN 73) and a control bacterium (SAR-05). Wells were punched in the agar media by a sterile borer, 6 mm in diameter, and 100 µL of arsenate and arsenite solution of each concentration was added to each well and incubated at 37 °C for 24 h. After incubation, the inhibition zones were recorded by measuring the distance from the edge of the zone to the edge of the well.

As(III) oxidase assay

The As tolerant bacterial isolates were grown in a chemically defined medium (CDM) (recipe in "As oxidation and reduction activity by the strains") (Weeger et al. 1999) spiked with 30 mM of As(III). After centrifugation at 10,000 rpm for 2 min, late log-phase cells were collected. The cells were washed using 50 mM Tris–HCl buffer (pH 8.0) and suspended in 2 mL buffer with 0.5 mM phenylmethyl sulfonyl fluoride (PMSF) and lysozyme. Cell suspensions were thereafter incubated for 2 h and sonicated. After centrifugation at 10,000 rpm, for 30 min, cell debris was removed (Bachate et al. 2012). The protein concentration was measured using the Bradford assay (Bradford 1976) using bovine serum albumin (Sigma) as the standard. The As(III) oxidase assay was performed using a method previously described by Anderson et al. (1992).

Identification of the As tolerant bacteria

The selected As oxidizing bacteria were identified by 16S rRNA sequencing. Total genomic DNA of selected bacteria was extracted (Majumder et al. 2013a) and PCR amplification of 16S rRNA gene with forward primer 27F 5′–AGA GTT TGA TCM TGG CTC AG–3′ and the reverse primer 1492R 5′–GGY TAC CTT GTT ACG ACT–3′ (Chromous Biotech Private Limited, India) were performed.

The bacterial strains were studied for Gram reaction, colony morphology and characterized for catalase, urease, and oxidase activities by standard protocols (Holtz 1993). Phenotypic characterization can provide an indirect insight to plant growth promotion (Flores-Gallegos and Nava-Reyna 2019) and abiotic stress (like As) tolerance (Backer et al. 2018).

Scanning Electron Microscopic (SEM) Study

The SEM study of As-resistant bacteria was performed following Dey et al. (2016). For the SEM study, the harvested bacterial cells were first washed with sodium phosphate buffer (pH 7.4), followed by preparation of a bacterial

smear on a cover glass and heat fixing over a flame for 1–2 s, followed by fixation with 2.5% glutaraldehyde (aqueous) for 45 min. The slides were then dehydrated, passing through 50–90% of alcohol solutions, and finally through absolute alcohol for 5 min each. After that, the samples on the cover glass were gold coated and observed under a 15 kV scanning electron microscope (HITACHI, S-530 SEM, and ELKO Engineering).

Detection of aoxB gene

The selected As oxidizing bacterial genomic DNA was extracted, and PCR amplification of aoxB (As(III) oxidase) gene was carried out by using the forward primer 69F 5′–TGY ATYGTNGGNTGYGGNTAYMA–3′ and reverse primer 1374R 5′–TANCCYTCYTGRTGNCCNCC–3′ (Rhine et al. 2007). The reaction mixture contains 1X PCR buffer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 1 M of each primer, 25 ng of DNA Template, and 2 units of Taq DNA Polymerase. The remaining volume was filled with deionized water to maintain the final reaction volume of 25 µL. All the PCR products were gel eluted using Wizard SV gel and PCR clean-up system (Promega, Madison, WI). The sequencing of aoxB gene (BcAl-1, JN 73, AR-30, and LAR-2) was done with 69 F Primers (Chromous Biotech Private Limited). The aoxB gene sequences were compared by using a nucleotide BLAST algorithm (version: 2.11.0 +; *blast.ncbi.nlm.gov/blast*) with a public database (GenBank). The primary objective behind this study was to verify the gene sequencing that enables arsenite oxidase activity and also to compare and validate this with the genetic bases of existing strains of As(III) oxidizers as previously reported (Majumder et al. 2013a).

Phylogenetic tree

Phylogenetic tree of 16S rRNA gene and As(III) oxidase gene sequences of the As oxidizing bacteria were drawn through Maximum likelihood algorithms and bootstrapping procedure to statistically test branch support via Phylogeny.fr web service (Dereeper et al. 2008). Five hundred bootstraps were taken to construct the phylogenetic tree.

Plant growth-promoting (PGP) attributes of As tolerant bacteria

The PGP properties (IAA-production, ACC deaminase activity, phosphate solubilization, nodulation, and siderophore production) of the bacteria were assessed in the culture medium spiked with both As(V) and As(III) (spiking levels being 0 mg/L, 15 mg/L, and 30 mg/L As(III)/As(V)).

ACC deaminase activity

The ACC deaminase enzyme activity is attributed to the quantity of α-ketobutyric acid production by the breakdown of ACC (Penrose and Glick 2003) by the strains. For assessment, a minimal medium (Das et al. 2014) was prepared (KH_2PO_4 0.4 g/L, K_2HPO_4 2 g/L, $MgSO_4{\cdot}7H_2O$ 0.2 g/L, $FeSO_4{\cdot}7H_2O$ 0.1 g/L, $CaCl_2$ 0.1 g/L, NaCl 0.2 g/L, $NaMoO_4{\cdot}2H_2O$ 0.005 g/L, glucose 10 g/L) using 1–aminocyclopropane–1–carboxylic acid or ACC (3 g/L) as a source of nitrogen, spiked with three different concentrations of As(V) and As(III) (0, 15, 30 mg/L) separately and the bacterial cells were grown. The amount of ketobutyrate (KB) formed per mg of protein per hour is the total value of the specific enzyme activity (Penrose and Glick 2003).

Screening of Indole Acetic Acid (IAA)

The IAA production potential of the selected As resistant strains were determined by growing them in an L-tryptophan (0.5 mg/mL) supplemented minimal medium in different concentrations of As (0, 15, 30 mg/L) and incubated in the dark at 30 °C for 5 days. The experiment constitutes transferring 2 mL bacterial suspension in 100 µL of 10 mM orthophosphoric acid and 4 mL Salkowski's reagent (2% solution of 0.5 M $FeCl_3$ in 35% perchloric acid) in a test tube. The entire mixture was vigorously shaken before incubation for 45 min until a pink color develops. The absorbance of the resultant solution was measured at 530 nm for obtaining the content of IAA-like molecules in a liquid culture medium (Das et al. 2014).

Ability to solubilize phosphate

The phosphate-solubilizing potential was determined by growing the bacterial strains in Pikovskaya's medium (Sundararao 1963) (containing 0.5% of tri-calcium phosphate (TCP) spiked with three levels of As(V) and As (III) as 0, 15, 30 mg/L) at 30 °C for 5–6 days and 170 revs/min; followed by centrifugation at 6500 times gravity and supernatant collection. The phosphate solubilization in the culture medium's supernatant was estimated by the standard method (Zaidi et al. 2006).

Nodulation efficiency

The nodulation efficiency of bacterial strains (Reed and Glick 2013) was assessed through a pot study. The soils collected from the same site ("Analysis of soil samples") were sterilized by autoclaving at 121 °C and 15 psi of pressure

for 15 min. Groundnut seeds were sown into pots containing sterilized soil spiked with As (0, 15, 30 mg/L) and placed in a greenhouse. Thirty days later, the number of nodules on the root per plant was measured against the corresponding length of the root.

Screening for siderophore production

The ability of the As tolerant bacterial isolates to produce siderophores was qualitatively assayed using the Chrome Azural S method of Schwyn and Neilands, following Das et al. (2014). The bacterial strains were grown in MM9 [Tris buffer, casamino acids (0.3%), L-glutamic acid (0.05%), (+)-biotin (0.5 ppm), and sucrose (0.2%)] liquid medium without Fe and allowed to incubate for 5 days at 30 °C temperature at 175 revs/min. For control, 0.2 µM of Fe (freshly prepared, filter-sterilized $FeSO_4 \cdot 7H_2O$ stock solution) was also inoculated. The stationary phase bacterial culture were collected and pelleted by centrifugation (6500×*g* for 15 min). In supernatant solution, the qualitative confirmation of the presence of siderophore is simply the color change from blue to orange.

Statistical analysis

Statistical computations like Duncan's multiple range post hoc test, simple descriptive statistics, etc., were performed using *Microsoft Excel 2016* and *SPSS version 23.0.*

Results

Characterization of the experimental site

The assessment of the level of As contamination of the selected soil under study was chemically attributed in terms of total (tri-acid extracted) and Olsen-extractable available As. Results revealed a considerable load of As to the tune of 17.2 ± 1.72 and 1.50 ± 0.27 mg/kg, respectively. The total microbial count from the soil was 6.4 ± 0.07 $\log_{10}$ CFU/g (i.e., approximately 3.0×10^6 in number). The As resistant microbial count was 3.6 ± 0.09 $\log_{10}$ CFU/g soil (i.e., approximately 4.0×10^3 in number) (presented as a mean of three observations ± SD). The considerably high proportion of As resistant microbial count can be a clue to address the problem of As contamination more efficiently through low-cost microbial remediation.

Assessment of As resistant bacteria from enrichment culture

Employing the enrichment culture techniques for possible isolation of As resistant bacterial isolates in Yeast Extract Mannitol (YEMA) solid medium spiked with distinct As(V) and As(III) concentrations, few colonies were observed. Eleven separate colonies, namely, BcAl-1, JN 73, LAR-2, AR-30, GAR-1, GAR-2, LAR-7, GAR-11, LAR-20, LAR-3, and SAR-05, were picked from the plates and were selected for further study.

Arsenic accumulation and oxidation–reduction potential of bacterial isolates

The eleven bacterial strains were investigated for their potential of As accumulation and oxidation/reduction. Initially, a qualitative analysis of the bacterial strains' ability to form Ag_3AsO_3 or Ag_3AsO_4 from the $AgNO_3$ solution was determined by visualizing the intensity of color change to bright yellow and brownish silver (Fig. 1a). The qualitative visualization was further compared and validated through the microplate method (Fig. 1b). Four isolates were observed to have a distinguishably brighter color change. To confirm the test, the As accumulation and oxidation/reduction potential of the isolates were addressed through a quantitative estimation by incubating for 12 h in a liquid culture medium spiked with 5 mM As(V) and As(III) (Fig. 1c). The As content in cell pellet, liquid medium, and impregnated filter paper were separately analyzed to attain the quantity of As oxidized or reduced (filter paper) and accumulated (in cell pellet). The results interestingly revealed a similar pattern of microbial alteration of As as in the qualitative test. The strains BcAl-1, JN 73, LAR-2, and AR-30 had shown significant ability to oxidize/reduce and accumulate As and thus enunciated the reduction of the highest quantity of As from the initial concentration. As evident from the Table 1, the As recovery from the filter paper (a measure of As oxidization-reduction potential) followed the trend of BcAl-1 (1.63 ± 0.43 mM) > JN 73 (1.60 ± 0.69 mM) > LAR-2 (1.59 ± 0.66 mM) > AR-30 (1.56 ± 0.71 mM) for As (V) and BcAl-1 (1.08 ± 0.61 mM) > JN 73 (1.07 ± 0.80 mM) > LAR-2 (1.03 ± 0.50 mM) > AR-30 (1.01 ± 0.63 mM) for As (III). The bacterial strains BcAl-1 and JN 73 have shown maximum cellular absorption [40/39% for As(V) and 36% for As(III)], oxidation/reduction of As(III) (22/21%) and As(V) (33/32%) while left least residues [25/26% for As(V) and 40% for As(III)] (Table 2); in solution followed by LAR-2 > AR-30.

MIC, arsenite oxidase activity, and agar diffusion assay of the bacterial isolates

The four most efficient strains obtained from the previous section were tested for their minimum inhibitory concentration of As. The results in Table 3 revealed BcAl-1 had the highest MIC value [408 mM for As(V) and 46.2 mM for As(III)] followed by JN 73 [390 mM for As(V) and 41.2 mM

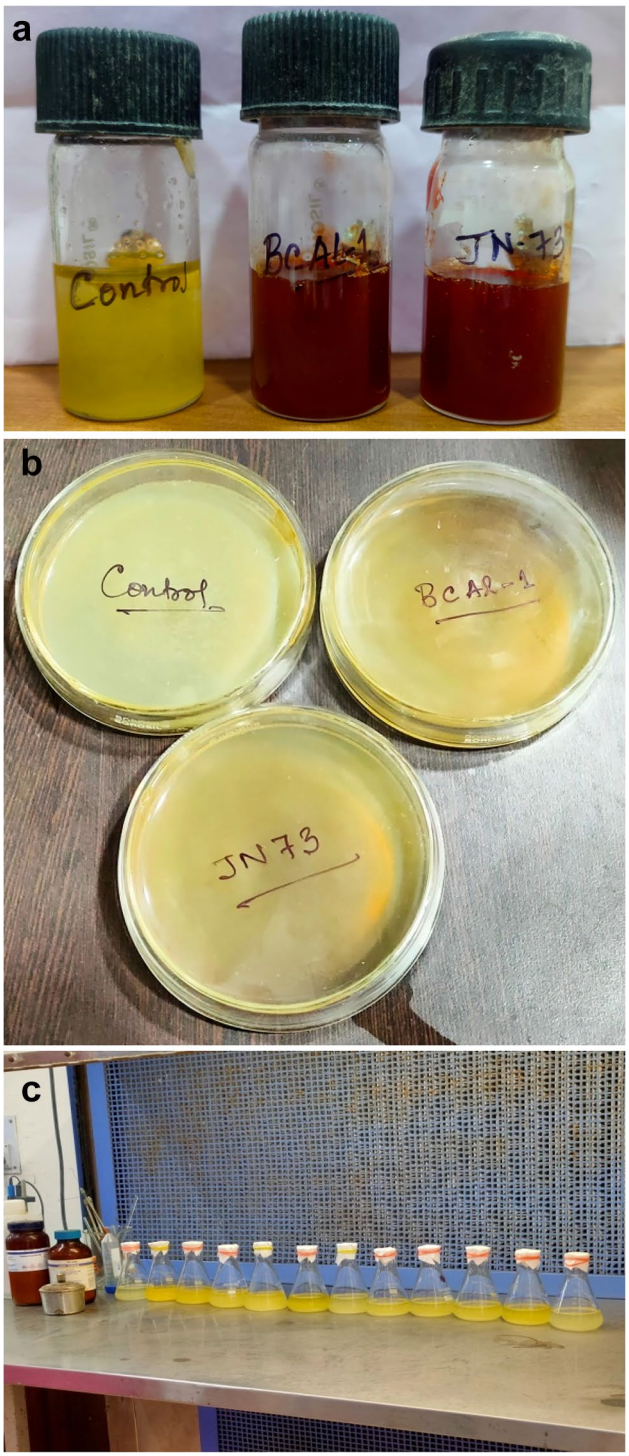

Fig. 1 **a** As oxidizing capability of the best two strains BcAl-1 and JN 73 compared with control (silver nitrate test). **b** Arsenite oxidation capability of the best two strains BcAl-1 and JN 73 compared with control (microplate method). **c** Quantitative estimation of the arsenite oxidation capability (As spiked liquid medium)

for As(III)]. The MIC values of the remaining two strains were considerably lower, as LAR-2 [300 mM, As(V) and 31.3 mM, As(III)] > AR-30 [275 mM, As(V) and 28.1 mM, As(III)]. The strain, SAR-05, as evident from Tables 1, 2 and 3, showing no resistance to As, was taken as control.

The highest MIC value carrying bacteria also had the highest arsenite enzyme activity. As in Table 3, the specific As(III) oxidase activity of these four bacterial isolates (BcAl-1, JN 73, LAR-2, and AR-30) were 5.82, 5.30, 4.97, 4.60 nM/min/mg of protein, respectively. Concurrently, the result brought about the synchrony that the bacterial strains having higher MIC value will have the higher As (III) oxidase activity.

Agar well diffusion experiment was performed to confirm the MIC of the selected bacterial isolates (Fig. 2). Upon applying varying concentrations of arsenate and arsenite solution, the bacterial isolates that either had no zone of growth inhibition or less than 1 mm zone of inhibition were considered as resistant strain (Yusof et al. 2020). Results obtained from three replicates of the three studied strains (Fig. 3a, b) revealed that control strain (SAR-05) is highly sensitive to both arsenate and arsenite as there is an increase in the zone of inhibition in all concentrations. Among the other two strains, the zone of inhibition is less for BcAl-1 than JN 73 for higher concentrations of both arsenate and arsenite, suggesting that the former strain is even superior to the later regarding As resistance.

Biochemical characterization and identification of the As-resistant PGP Bacteria

All the selected strains of bacteria were found to be Gram-negative and rod-shaped through SEM study (see Supplementary Fig. 1). The strains (BcAl-1, JN 73, LAR-2, and AR-30) screened based on phenotypic and biochemical tests have been represented in Table 4. All these strains were found to be oxidase, catalase, and urease positive. Further employing 16S rRNA gene sequencing, a phylogenetic tree was prepared (Fig. 4) through the Phylogeny.fr web service (Dereeper et al. 2008). These identified bacterial isolates are thus assumed to be *Burkholderia cepacia* (BcAl-1, accession number KJ461686), *Burkholderia metallica* (JN 73, accession number KJ507654), *Burkholderia* sp. (LAR-2, accession number MK634685), and *Burkholderia cenocepacia* (AR-30, accession number KY992359), as in Table 5.

Identification and comparison of arsenite oxidase gene in selected isolates

The As(III) oxidase gene was detected in our selected As(III) oxidizing bacteria. A fragment of 1200 bp was amplified via polymerase chain reaction (PCR) obtained from the genomic DNA of BcAl-1, JN 73, LAR-2, and

Table 1 Arsenic removal by selected bacterial isolates

Isolates	Arsenic (V) residue in liquid media (mM)	Arsenic (V) concentration in cell pellet (mM)	Arsenic (V) concentration in filter paper (mM)	Unaccounted part(mM)	Arsenic (III) residue in liquid media (mM)	Arsenic concentration(III) in cell pellet (mM)	Arsenic concentration (III) in filter paper (mM)	Unaccounted part (mM)
BcAl-1	1.25 ± 1.14^{b}	2.00 ± 0.85^{a}	1.63 ± 0.43^{a}	0.12 ± 0.01^{abc}	2.00 ± 1.28^{b}	1.80 ± 0.66^{a}	1.08 ± 0.61^{ab}	0.12 ± 0.03^{ab}
JN-73	1.32 ± 1.06^{b}	1.95 ± 0.71^{a}	1.60 ± 0.69^{ab}	0.13 ± 0.02^{ab}	2.00 ± 0.97^{b}	1.80 ± 1.04^{a}	1.07 ± 0.80^{ab}	0.13 ± 0.01^{ab}
LAR-2	1.33 ± 1.11^{b}	1.93 ± 0.55^{a}	1.59 ± 0.66^{ab}	0.15 ± 0.01^{a}	2.10 ± 1.15^{b}	1.72 ± 0.70^{a}	1.03 ± 0.50^{ab}	0.15 ± 0.01^{a}
AR-30	1.50 ± 1.28^{b}	1.82 ± 0.46^{a}	1.56 ± 0.71^{ab}	0.12 ± 0.02^{abc}	2.24 ± 1.37^{b}	1.62 ± 0.79^{ab}	1.01 ± 0.63^{c}	0.13 ± 0.02^{ab}
GAR-1	2.25 ± 1.74^{b}	1.39 ± 0.63^{a}	1.25 ± 1.09^{abc}	0.11 ± 0.02^{bc}	2.49 ± 1.04^{b}	1.45 ± 1.04^{ab}	0.93 ± 0.63^{ab}	0.13 ± 0.02^{a}
GAR-2	3.48 ± 1.06^{ab}	1.00 ± 0.75^{ab}	0.39 ± 0.29^{cd}	0.13 ± 0.02^{ab}	4.14 ± 1.13^{ab}	0.50 ± 0.39^{bc}	0.24 ± 0.18^{bc}	0.12 ± 0.02^{ab}
LAR-7	3.17 ± 1.39^{ab}	0.90 ± 0.71^{ab}	0.84 ± 0.79^{abcd}	0.09 ± 0.01^{c}	4.09 ± 1.30^{ab}	0.52 ± 0.34^{bc}	0.25 ± 0.18^{bc}	0.14 ± 0.02^{a}
GAR-11	3.47 ± 1.65^{ab}	0.92 ± 0.82^{ab}	0.48 ± 0.32^{bcd}	0.13 ± 0.02^{ab}	3.99 ± 1.25^{ab}	0.52 ± 0.37^{bc}	0.39 ± 0.12^{abc}	0.10 ± 0.02^{b}
LAR-20	2.99 ± 1.26^{ab}	1.03 ± 0.53^{ab}	0.84 ± 0.68^{abcd}	0.14 ± 0.02^{ab}	3.25 ± 1.10^{ab}	0.99 ± 0.43^{abc}	0.66 ± 0.26^{abc}	0.10 ± 0.02^{b}
LAR-3	3.00 ± 1.40^{ab}	1.00 ± 0.57^{ab}	0.87 ± 0.68^{abcd}	0.13 ± 0.02^{ab}	3.32 ± 1.27^{ab}	0.99 ± 0.39^{abc}	0.59 ± 0.20^{abc}	0.10 ± 0.01^{b}
SAR-05	4.87 ± 1.54^{a}	ND	ND	0.13 ± 0.03^{a}	4.88 ± 1.38^{a}	0.00 ± 0.00^{c}	ND	0.12 ± 0.02^{ab}
SEm (±)	0.70	0.34	0.33	0.01	0.63	0.34	0.25	0.01
CD ($p = 0.05$)	2.07	1.02	0.99	0.02	1.86	1.01	0.74	0.03

Values with different alphabets are significantly different from each other according to the DMRT test ($p < 0.05$). Each value is a mean of three replicates. ND represents not detectable range

Table 2 Percent arsenic removal by selected isolates

Isolates	As (V) residue in liquid media (%)	As (V) concentration in cell pellet (%)	As (V) concentration in filter paper (%)	Unaccounted part (%)	As (III) residue in liquid media (%)	As concentration (III) in cell pellet (%)	As concentration (III) in filter paper (%)	Unaccounted part (%)
BcAL-1	25.00	40.00	32.60	2.40	40.00	36.00	21.60	2.40
JN-73	26.40	39.00	32.00	2.60	40.00	36.00	21.40	2.60
LAR-2	26.60	38.60	31.80	3.00	42.00	34.40	20.60	3.00
AR-30	30.00	36.40	31.20	2.40	44.80	32.40	20.20	2.60
GAR-1	45.00	27.80	25.00	2.20	49.80	29.00	18.60	2.60
GAR-2	69.60	20.00	7.80	2.60	82.80	10.00	4.80	2.40
LAR-7	63.40	18.00	16.80	1.80	81.80	10.40	5.00	2.80
GAR-11	69.40	18.40	9.60	2.60	79.80	10.40	7.80	2.00
LAR-20	59.80	20.60	16.80	2.80	65.00	19.80	13.20	2.00
LAR-3	60.00	20.00	17.40	2.60	66.40	19.80	11.80	2.00
SAR-05	97.40	ND	ND	2.60	97.60	ND	ND	2.40

Percentage has been calculated based upon the mean content of three replicates (from Table 1). In all cases 5 mM of As(V) and As(III) have been applied and the residue retained in each case of liquid media, cell pellet and filter paper have been expressed as percentage. ND represents not detectable range

AR-30 (Fig. 5) following the protocol described in "Detection of aoxB gene". The sequences were submitted to GenBank, and the accession number was obtained. A phylogenetic tree of As(III) oxidase gene sequences of the As oxidizing bacteria were drawn (Fig. 6) through Maximum-likelihood algorithms and bootstrapping procedure. The percentage of replicate trees in which the associated taxa was clustered together is shown to the branches, as demonstrated by the bootstrap test (500 replicates). The tree was drawn to scale, with branch lengths shown in the same units as for evolutionary distances. Phylogenetic analyses suggest that *aox-b* gene of the four isolates AOX-1, AOX-2, AOX-3, and AOX-4 form a clade which implies that the four enzymes have a common ancestor. Further, there

Table 3 MIC and As(III) oxidase enzyme activity of selected bacterial isolates

Bacterial isolates	MIC of As(V) (mM)	MIC of As(III) (mM)	Enzyme activity (nM/min/mg protein)
BcAl-1	408 ± 63.81^{a}	46.2 ± 7.12^{a}	5.82 ± 2.28^{a}
JN-73	390 ± 58.47^{ab}	41.2 ± 7.06^{ab}	5.30 ± 2.67^{a}
LAR-2	300 ± 64.57^{bc}	31.3 ± 7.36^{bc}	4.97 ± 2.32^{a}
AR-30	275 ± 61.43^{c}	28.1 ± 7.76^{c}	4.60 ± 2.30^{a}
SAR-05	ND	ND	0.11 ± 0.06^{b}
SEm (±)	32.002	3.771	1.061
CD ($p = 0.05$)	98.608	11.620	3.181

Values with different alphabets are significantly different from each other according to the DMRT test ($p < 0.05$). Each value is a mean of three replicates. ND represents not detectable range

is also a synchrony regarding the arsenite oxidase gene behavior among the strains derived from earlier studies.

Potential plant growth-promoting attributes in screened As tolerant bacteria

The plant growth-promoting traits of the four most efficient As tolerant isolates and one control (SAR-05; *Escherichia coli*, previously isolated non As-tolerant strain) were categorized. All of these strains could solubilize phosphate, produce IAA and ACC deaminase under As(V) and As (III) stressed conditions. BcAl-1, JN 73, LAR-2, and AR-30 were observed to solubilize the highest amount of phosphate (570, 563, 553, 560 µg/L) under the As-free condition and even solubilized significant amount of phosphate when the culture medium is spiked with 15 and 30 mg/L of As(V) and As(III) (Tables 6 and 7).

BcAl-1 was the best performer in terms of nodulation, IAA production, and ACC production both in As free and As stressed condition. Stresses imposed by As(V) spiking failed to affect phosphate solubilization, IAA production, ACC deaminase activity, and nodulation. However, under As(III) stress, phosphate solubilization, IAA production, siderophore production, and ACC deaminase production were significantly impacted (Tables 6 and 7). Comparing all the aspects of PGP in the selected bacterial isolates, two isolates, namely BcAl-1 and JN 73 were able to produce siderophore under all conditions. The other two strains failed at higher As stress conditions.

Discussion

Identification and isolation of As-resistant bacterial strains from contaminated soils

In the course of identification and characterization of resistant PGP bacteria from the As-polluted area in the present investigation, BcAl-1 (*Burkholderia cepacia*) bacterial isolate had emerged with a high MIC towards As(V) (408 mM) and As(III) (46.2 mM) which is higher than previously reported *Geobacillus stearothermophilus, Bacillus megaterium, Rhodobacter sphaeroides* with MIC values of 380 mM, 400 mM, 400 mM of As(V) and 40 mM, 47 mM, 46.7 mM of As(III) in agricultural soils (Majumder et al. 2013a, b) and also greater than As-oxidizing, As-resistant bacteria in soil (183 mM As(V) and 6 mM of As(III); Srivastava et al. 2013), in ground water (200 mM As(V) and 5 mM As(III); Liao et al. 2011), in mines (10 mM As(V); Botes et al. 2007), and in estuaries (400 mM As(V) and 10 mM As(III); Jackson et al. 2005). Microbes can also bioaccumulate As (Garnaga et al. 2006). Here bacterial isolates BcAl-1 and JN 73 have also shown maximum cellular absorption [40/39% for As(V) and 36% for As(III)].

Arsenite oxidase activity of bacterial strains and their genetic base

The bacteria isolated from the contaminated soil having As resistance, develop a special type of tolerance mechanism to survive in the metal-contaminated environment. They contain *ars* genetic system, enabling the resistant mechanisms to endure in As-contaminated soil (Majumder et al. 2013a). Newer studies revealed that such toxicant-resistant bacteria contain a number of plant growth-promoting (PGP) characters (Ghosh et al. 2018). Our candidate isolates BcAl-1 (*Burkholderia cepacia*), and JN 73 (*Burkholderia metallica*) had As(III) oxidase enzyme activity of 5.82 and 5.30 nM/min/mg protein, respectively. Similar reports with *Arthrobacter* sp. (10 nM min/mg protein; Prasad et al. 2009), *β-proteobacteria* (12 nM/min/mg protein; Bachate et al. 2012) had shown high As(III) oxidase enzyme activity. The best performing isolates in the current experiment, BcAL-1 and JN 73 were found to remove more than 70% As(III) and 57% of As(V) from the liquid culture medium. Such efficiencies are formidably higher than previously reported bacteria like *Staphylococcus* sp. (volatilizing 24% As(V) and 26% As(III); Srivastava et al. 2012) and *Alcaligenes* sp. (oxidizing 1 mM As(III) within 40 h; Yoon et al. 2009). As(III) oxidase system was also reported in *Proteobacteria* (Lebrun et al. 2003). Different genera of proteobacteria such as *Burkholderia*, *Alcaligenes*, *Methylobacterium, Bradyrhizobium* and *Bosea* (Quéméneur et al. 2008) carry the As(III) oxidase

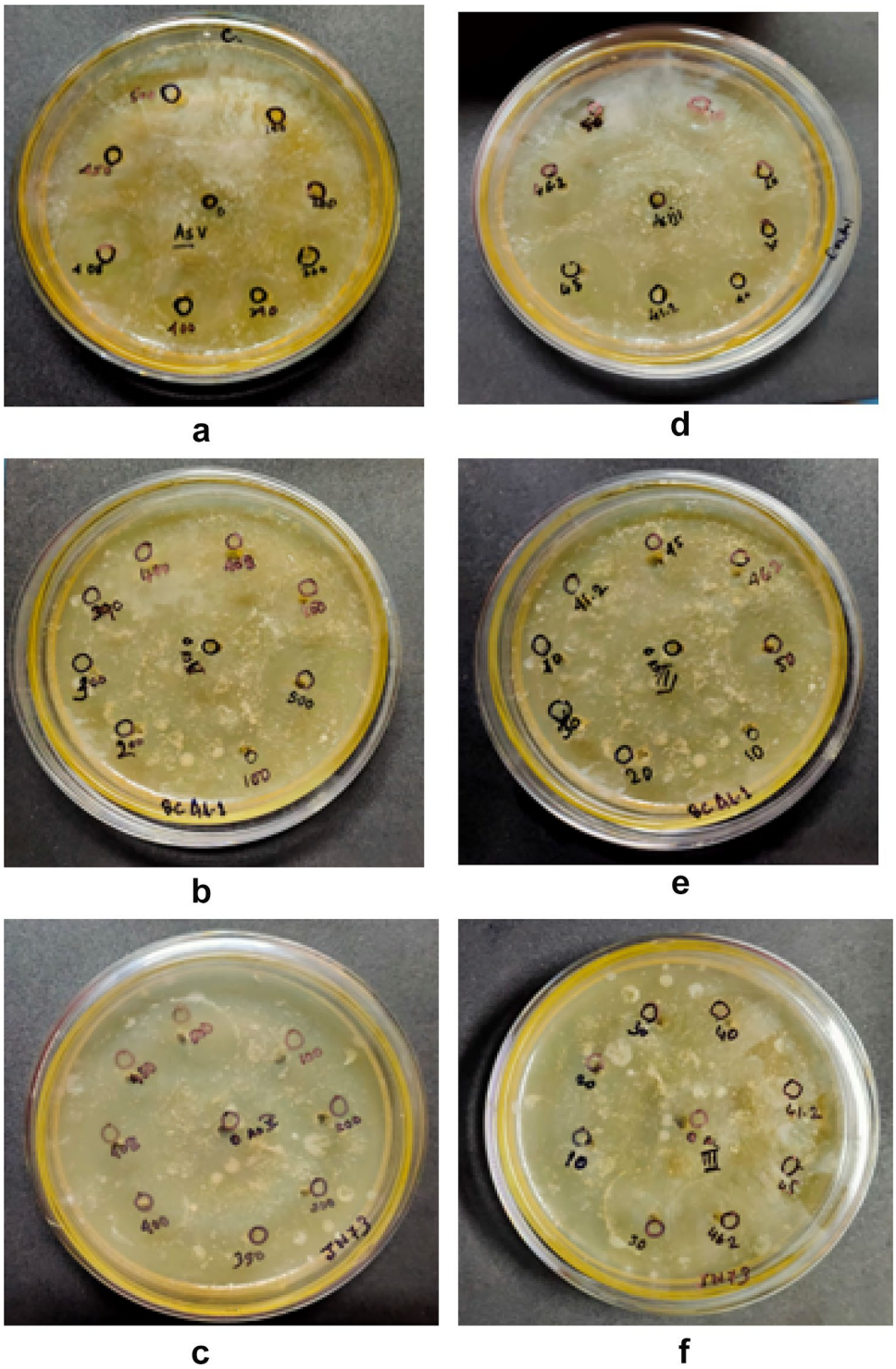

Fig. 2 Illustrations of agar diffusion assay experiment of isolates: **a** control (SAR-05) vs arsenate concentration (mM); **b** BcAl-1 vs arsenate concentration (mM); **c** JN 73 vs arsenate concentration (mM); **d** control (SAR-05) vs arsenite concentration (mM); **e** BcAl-1 vs arsenite concentration (mM) and **f** JN 73 vs arsenite concentration (mM)

genetic system. The As oxidizing capacity of *Alcaligenes* (Amann et al. 1995) and *Burkholderia* also have been identified (Quéméneur et al. 2008).

Plant growth-promoting attributes in As resistant, As (III) oxidizing bacterial strains

Recent studies have shed some light on the PGP traits shown by the As oxidizing bacteria. Strains of *Acinetobacter, Klebsiella, Pseudomonas, Enterobacter,* and *Comamonas*

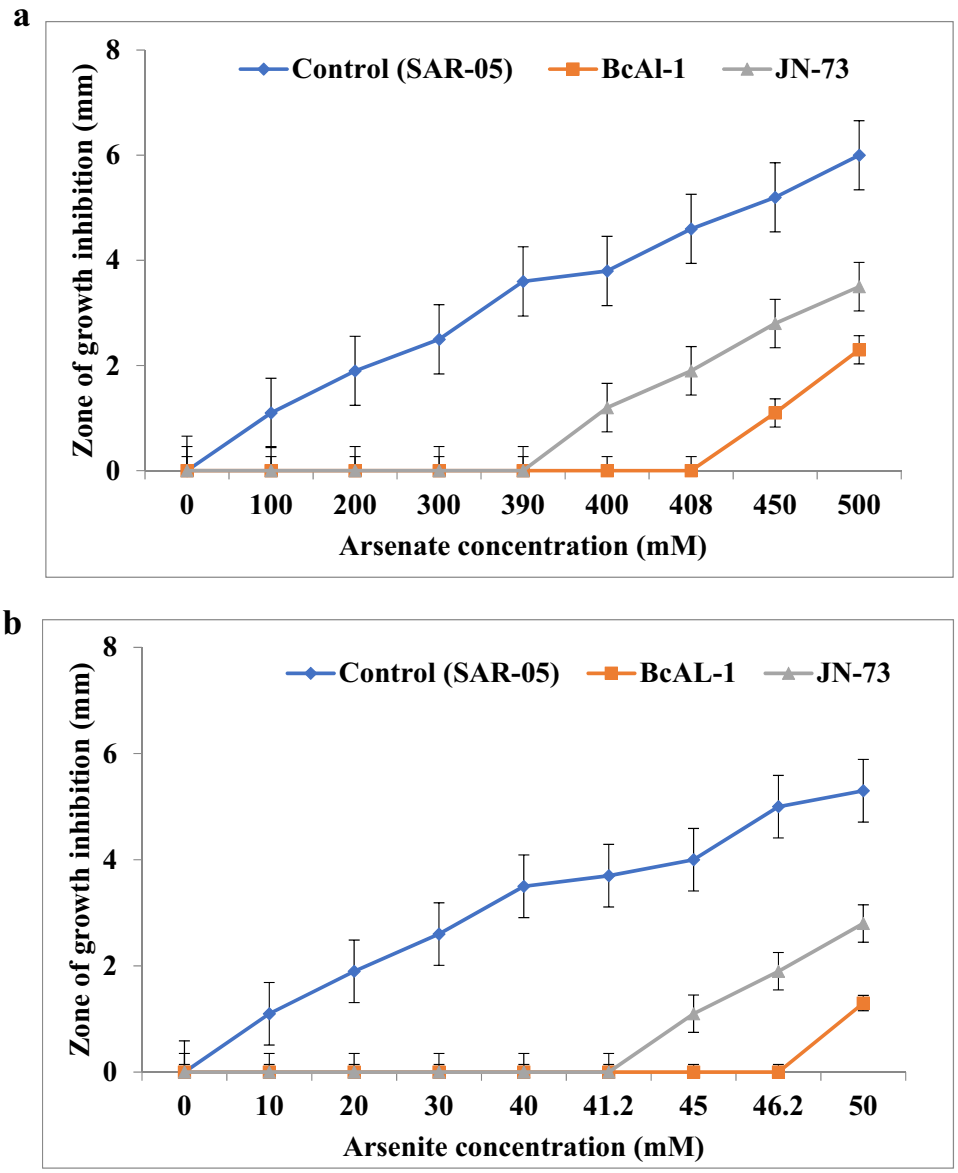

Fig. 3 **a** BcAL-1, JN 73 and control (SAR-05) in agar diffusion assay: plots of arsenate concentration (mM) vs zone of bacterial growth inhibition (mm). Results represented as the mean of three observations ± standard deviations. **b** BcAl-1, JN 73 and control (SAR-05) in agar diffusion assay: plots of arsenite concentration (mM) vs zone of bacterial growth inhibition (mm). Results represented as the mean of three observations ± standard deviations

Table 4 Phenotypic and biochemical characterization of the isolates

Isolates	Gram stain	Cell shape	Cell length (μ)	Colony color	Oxidase	Catalase	Urease
BcAl-1	–	Rod	2	Milky white	+	+	+
JN 73	–	Rod	2	Milky white	+	+	+
AR-30	–	Rod	2	White	+	+	+
LAR-2	–	Rod	2	White	+	+	+
SAR-05	–	Rod	1.5	Light pink	–	+	–

isolated from As contaminated agricultural soil in Thailand possess both As tolerance and the ability to produce siderophores (Ghosh et al. 2011; Das et al. 2014). *Burkholderia* sp. has been previously reported to survive in lead and cadmium contaminated soils and can also decrease cadmium translocation and enhance photosynthetic efficiency in rice (Jiang et al. 2008). The two candidate isolates BcAl-1 and JN 73

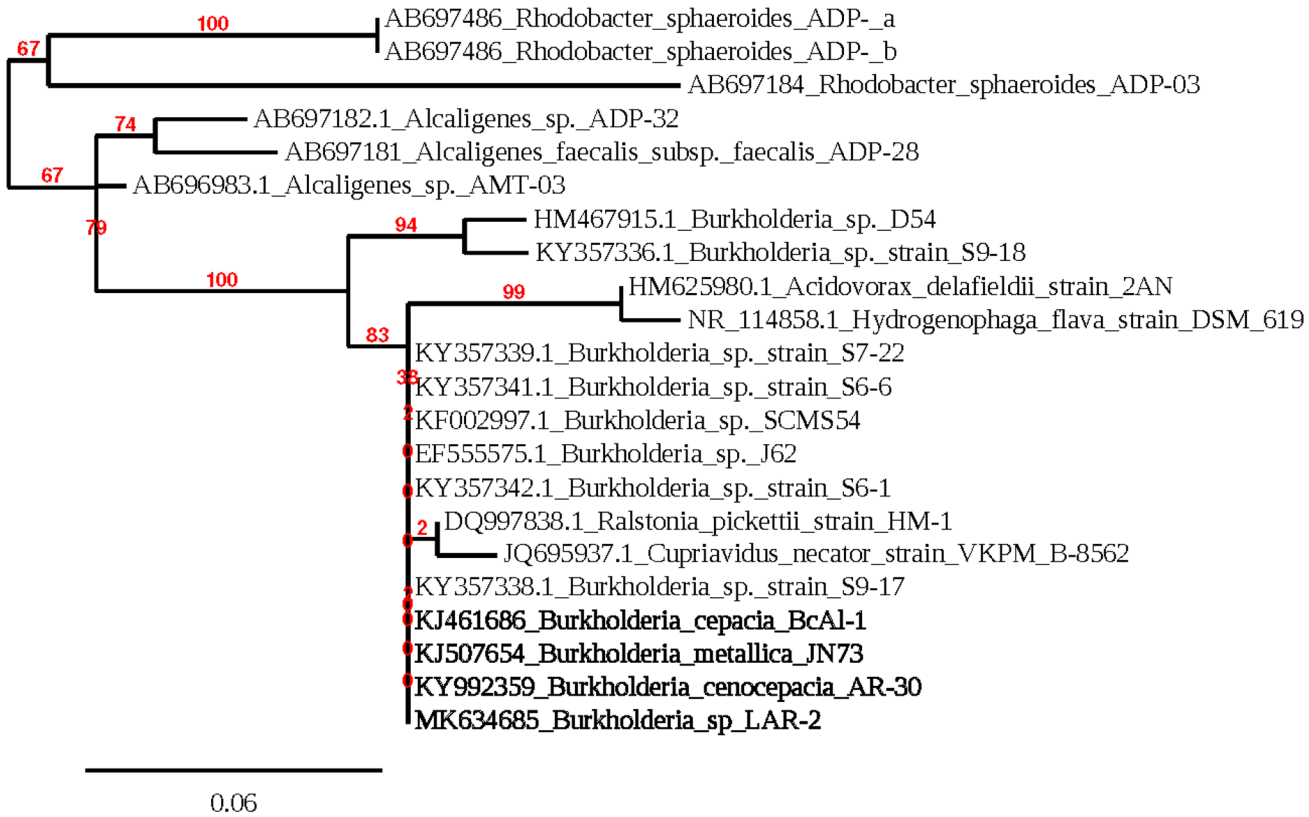

Fig. 4 Phylogenetic tree based on partial 16S rRNA gene sequences of arsenic and heavy metal tolerant bacterial isolates from arsenic-contaminated soil and other arsenic oxidizing bacterial isolates from the database. The database accession numbers are indicated before the name of the bacteria

Table 5 Identification of the arsenic resistant plant growth promoting bacteria

Isolates	Sequence accession number	Identifying bacteria	Source of isolation	Laboratory-based identity of the strain	Arsenite oxidase gene sequence number
BcAl-1	KJ461686	*Burkholderia cepacia*	Groundnut rhizosphere	AOX-1	MT995198
JN 73	KJ507654	*Burkholderia metallica*	Groundnut rhizosphere	AOX-2	MT991554
AR-30	KY992359	*Burkholderia cenocepacia*	Groundnut rhizosphere	AOX-3	MT991558
LAR-2	MK634685	*Burkholderia* sp	Lentil rhizosphere	AOX-4	MT991559
Control (SAR-05)	MK713764	*Escherichia coli*	Soil	–	–

have the ability to solubilize a significant amount of phosphate and produce IAA. Most of the bacteria under *Pseudomonas* sp., *Acinetobacter* sp., and *Paenibacillus* sp. were reported to be potential plant growth promoters (Das et al. 2014). *Bacillus aryabhattai* is another important As resistant plant growth promoter (Ghosh et al. 2018).

Similar observations were also obtained with As-resistant bacteria pertaining to Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria manifesting potential PGP attributes (Cavalca et al. 2010; Ghosh et al. 2011). *Staphylococus arlettae* is another well-known plant growth-promoter

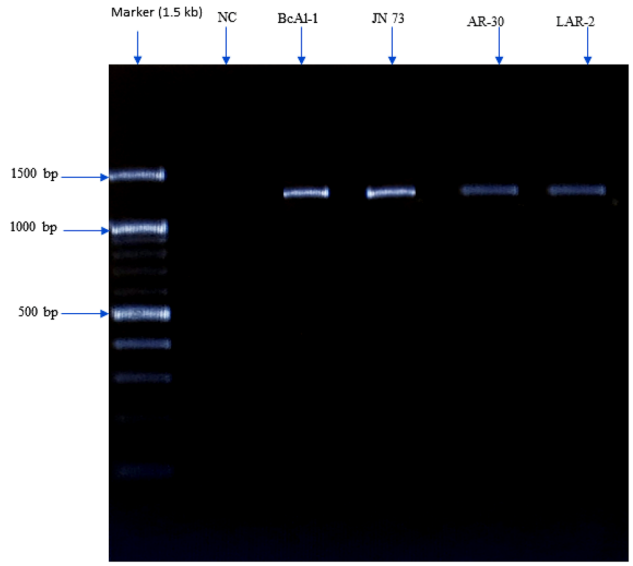

Fig. 5 Agarose gel electrophoresis of PCR product obtained by amplifying aoxB from genomic DNA of four selected arsenic resistant bacterial isolates (lanes 3–6); negative control (lane 2); marker 1001.5 kb DNA ladder (lane 1)

found in As laden soils, which increase plant protein, chlorophyll, and carotenoids in *Brassica* (Srivastava et al. 2013). *Pseudomonas sp*. has been reported to possess high As(III) oxidizing capacity while at the same time found to solubilize a significant amount of phosphate, indulge in siderophores, IAA-like molecules and ACC deaminase production (Das et al. 2014).

The present investigation has indisputably established the manifestation of PGP traits of As tolerant, As oxidizing bacterial isolates *Burkholderia metallica*, *Burkholderia cepacia, Burkholderia cenocepacia, Burkholderia* sp. in solubilizing phosphate, producing siderophores, root nodule, IAA-like molecules, and ACC deaminase under As stress. *Burkholderia cepacia* (BcAl-1) and *Burkholderia metallica* (JN 73) had emerged as best performing candidate isolates concerning As resistance and PGP traits.

Conclusion

To provide an environmental safeguard, restore food safety and sustain food security to the burgeoning population and combat abiotic pollution, a low-cost alternative to exorbitant pollution control strategies remained an absolute priority. The outcome of the present investigation envisioned that the two candidate bacterial isolates *Burkholderia cepacia* (BcAl-1) and *Burkholderia metallica* (JN 73) might be helpful in As decontamination and plant growth promotion through the fulfillment of mass production and field validation protocols. This is quite a novel finding as the strains of *Burkholderia* have never been reported as arsenic-resistant potential PGPR. By virtue of being the

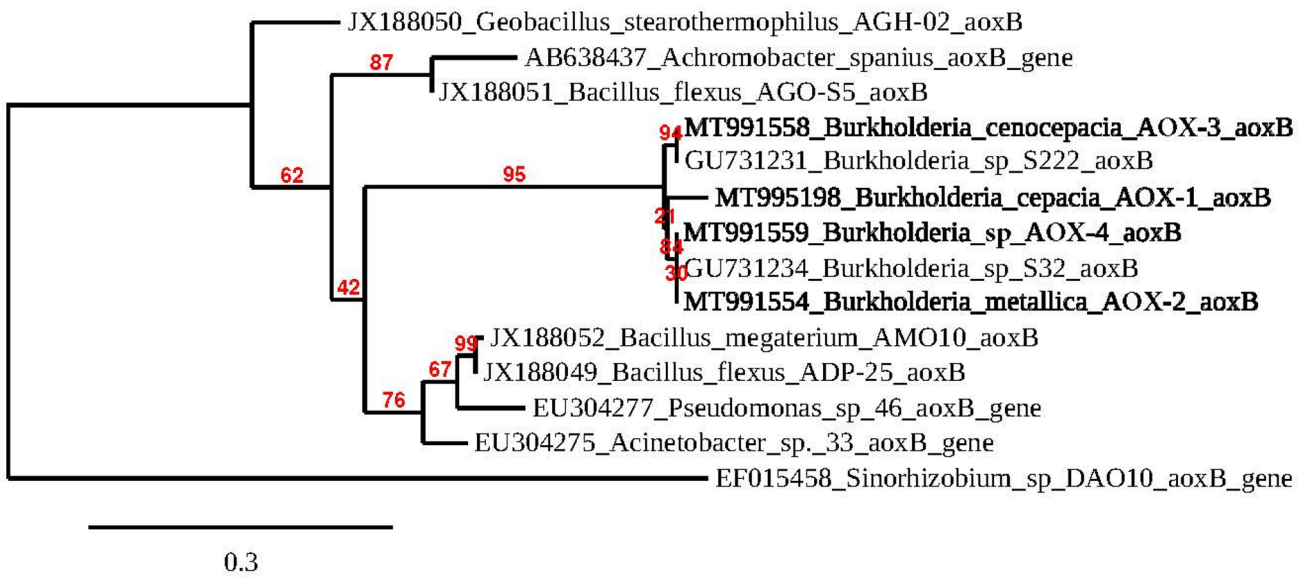

Fig. 6 Phylogenetic tree based on partial *aoxB* gene sequences, including aoxB gene sequences of screened arsenic oxidizing bacterial isolates from arsenic contaminated soil and aoxB of other arsenic oxidizing bacterial isolates from the database. The database accession numbers are indicated before the name of the bacteria. Here the terms AOX-1, AOX-2, AOX-3, and AOX-4 refer to the laboratory based identification of the arsenic oxidizing bacterial isolates and has no connection whatsoever to aoxB gene sequences

Table 6 PGP characteristics of the selected bacteria under varying levels of arsenate spiking

Isolates	As spike (mg/kg)	Phosphate solubilization (µg/L)	IAA Production (µM IAA/mL)	Acc deaminase activity (nM α-ketobutyrate mg/protein/h)	Number of nodules per root	Sidero-phore production
BcAl-1	0	570 ± 1.00^{a}	18.5 ± 0.10^{a}	21.7 ± 0.11^{b}	112 ± 1.73^{bc}	+
	15	568 ± 1.00^{b}	18.6 ± 0.20^{a}	22.0 ± 0.10^{a}	115 ± 1.00^{a}	+
	30	568 ± 1.00^{b}	18.4 ± 0.15^{a}	22.0 ± 0.10^{a}	114 ± 1.52^{ab}	+
JN 73	0	563 ± 1.00^{c}	17.9 ± 0.10^{bc}	19.0 ± 0.10^{c}	111 ± 1.00^{c}	+
	15	563 ± 1.00^{c}	18.0 ± 0.20^{b}	19.0 ± 0.10^{c}	112 ± 1.00^{bc}	+
	30	561 ± 1.00^{d}	17.7 ± 0.17^{cde}	19.0 ± 0.10^{c}	110 ± 1.00^{c}	+
AR-30	0	553 ± 1.00^{g}	17.4 ± 0.11^{f}	19.1 ± 0.10^{c}	112 ± 1.00^{bc}	+
	15	553 ± 1.00^{g}	18.0 ± 0.10^{b}	19.1 ± 0.05^{c}	110 ± 1.73^{c}	+
	30	553 ± 1.00^{g}	17.6 ± 0.15^{def}	19.1 ± 0.10^{c}	111 ± 1.52^{c}	–
LAR-2	0	560 ± 1.00^{de}	17.4 ± 0.17^{f}	17.9 ± 0.10^{d}	111 ± 2.00^{c}	+
	15	558 ± 0.577^{f}	17.8 ± 0.10^{bcd}	17.8 ± 0.10^{d}	114 ± 1.00^{ab}	+
	30	559 ± 0.577^{ef}	17.5 ± 0.10^{ef}	17.8 ± 0.10^{d}	114 ± 1.00^{ab}	–
SAR-05	0	0.00 ± 0.00^{h}	0.00 ± 0.00^{g}	0.00 ± 0.00^{e}	1.00 ± 0.00^{d}	–
	15	0.00 ± 0.00^{h}	0.00 ± 0.00^{g}	0.00 ± 0.00^{e}	1.00 ± 0.00^{d}	–
	30	0.00 ± 0.00^{h}	0.00 ± 0.00^{g}	0.00 ± 0.00^{e}	1.00 ± 0.00^{d}	–
SEm (±)		0.497	0.076	0.051	0.708	–
CD ($p = 0.05$)		1.440	0.220	0.148	2.051	–

Values with different alphabets are significantly different from each other according to the DMRT test ($p < 0.05$). Each value is a mean of three replicates

Table 7 PGP characteristics of selected bacteria under varying levels of arsenite spiking

Isolates	As spike (Mm)	Phosphate solubilization (µg/L)	IAA Production (µM IAA/mL)	Acc deaminase activity (nM α-ketobutyrate mg/protein/h)	Number of nodules per root	Sidero-phore production
BcAl-1	0	570 ± 106.78^{a}	18.5 ± 4.09^{a}	21.7 ± 6.40^{a}	112 ± 3.26^{a}	+
	15	268 ± 107.30^{b}	8.60 ± 4.55^{b}	12.00 ± 2.00^{b}	104 ± 3.08^{b}	+
	30	168 ± 112.65^{bc}	8.4 ± 4.10^{b}	12.0 ± 4.25^{b}	100 ± 3.06^{b}	+
JN 73	0	563 ± 110.12^{a}	17.9 ± 3.80^{a}	19.08 ± 4.55^{a}	111 ± 2.82^{a}	+
	15	163 ± 114.05^{bc}	8.00 ± 3.89^{b}	12.06 ± 1.60^{b}	102 ± 3.53^{b}	+
	30	161 ± 112.53^{bc}	7.70 ± 4.67^{b}	12.06 ± 1.45^{b}	100 ± 2.91^{b}	+
AR-30	0	553 ± 112.06^{a}	17.5 ± 4.06^{a}	19.1 ± 4.37^{a}	112 ± 3.34^{a}	+
	15	248 ± 113.56^{b}	8.60 ± 4.34^{b}	12.00 ± 1.35^{b}	104 ± 3.27^{b}	–
	30	168 ± 111.33^{bc}	8.4 ± 4.75^{b}	12.0 ± 4.25^{b}	100 ± 3.55^{b}	–
LAR-2	0	560 ± 117.01^{a}	17.4 ± 3.75^{a}	17.9 ± 4.56^{a}	111 ± 2.76^{a}	+
	15	163 ± 114.66^{bc}	8.00 ± 4.40^{b}	12.06 ± 1.45^{b}	102 ± 2.60^{b}	+
	30	161 ± 118.50^{bc}	7.70 ± 3.45^{b}	12.06 ± 1.50^{b}	100 ± 3.15^{b}	–
SAR-05	0	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	1.00 ± 0.36^{c}	–
	15	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	–
	30	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	–
SEm (±)		60.180	2.216	1.906	1.652	–
CD ($p = 0.05$)		174.335	6.419	5.521	4.786	–

Values with different alphabets are significantly different from each other according to the DMRT test ($p < 0.05$). Each value is a mean of three replicates

most promising PGPR, the strains provide a great deal of novelty in the research area by merging high As resistant properties and exhibition of several important PGP traits. In terms of sustainable agricultural and novel crop production, the strain can even solve the productivity problem in the contaminated study areas. This result can usher much confidence for use in the As-contaminated field by employing further thorough field-level investigations to support our laboratory results.

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Author contributions AL: methodology, investigation, data curation, software, and writing- original draft preparation; SB: conceptualization, methodology, supervision; SS: data curation, visualization, software, writing- reviewing and editing; KB: conceptualization, methodology, validation, writing- reviewing and editing; SG: methodology, supervision.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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