Environmental Science and Pollution Research (2021) 28:21633–21649
https://doi.org/10.1007/s11356-020-11816-7

RESEARCH ARTICLE

Abatement of arsenic-induced phytotoxic effects in rice seedlings by an arsenic-resistant *Pantoea dispersa* strain

Antara Ghosh[1] · Krishnendu Pramanik[2] · Shatabda Bhattacharya[3] · Sayanta Mondal[1] · Sudip Kumar Ghosh[1] · Pallab Kumar Ghosh[4] · Tushar Kanti Maiti[1]

Received: 5 June 2020 / Accepted: 23 November 2020 / Published online: 7 January 2021

Abstract

Population detonation and rapid industrialization are the major factors behind the reduction in cultivable land that affects crop production seriously. This situation is further being deteriorated due to the negative effects of abiotic stresses. Under such conditions, plant growth-promoting rhizobacteria (PGPR) are found to improve crop production which is essential for sustainable agriculture. This study is focused on the isolation of potent arsenic (As)-resistant PGPR from the agricultural land of West Bengal, India, and its application to reduce As translocation in rice seedlings. After screening, an As-resistant PGPR strain AS18 was identified by phenotypic characters and 16S rDNA sequence-based homology as *Pantoea dispersa*. This strain displayed nitrogen fixation, phosphate solubilization, 1-aminocyclopropane-1-carboxylic acid deaminase (ACCD) activity, indole-3-acetic acid (IAA) production, in addition to As (III) resistance up to 3750 μg/mL. The As removal efficiency of this strain was up to 93.12% from the culture medium as evidenced by AAS. The bioaccumulation property of AS18 strain was further validated by TEM-EDAX-XRD-XRF-FTIR studies. This strain showed significant morpho-biochemical improvements including antioxidant enzymatic activities and As-minimization in plant (rice) cells. Thus, being an As-resistant potent PGPR, AS18 strain is expected to be applied in As-spiked agricultural fields for bioremediation and phytostimulation.

Keywords PGPR · Arsenic-resistant bacteria · *Pantoea dispersa* · Antioxidant · Bioaccumulation

Introduction

Arsenic (As)-contaminated ecosystem possesses serious impendence towards food security and human health largely due to their perseverance, toxicity, and widespread distribution in the environment. It was observed that As contamination affects plant growth causing a reduction in crop productivity. But food production should be significantly increased worldwide to fulfill the necessities of a rapidly growing population like India.

The metalloid, As, is a soil contaminant and among the potential toxic elements affecting both plants and animals. Arsenic affects plant growth and productivity by interefering with the physiological, biochemical, and molecular mechanisms of plants. The harmful effects of As include surge of reactive oxygen species (ROS) at sub-cellular levels (Abbas et al. 2018). This increased production of ROS (such as superoxide radicals, hydroxyl radicals, hydrogen peroxide) causes irreparable damages to DNA, proteins, membrane lipids, and various cell organelles including chloroplast (Finnegan and Chen 2012). Phytotoxic effect of As includes chlorosis, necrosis, delayed flowering, defoliation, leaf senescence, stunted growth, reduction in fertility, inhibition of photosynthesis, nutrient depletion, reduced stomatal conductance, chloroplast membrane disintegration to name a few. All these together ultimately cause reduced crop production in As-spiked soil (Abbas et al. 2018). Furthermore, this metalloid can be easily transferred into humans via food chain

Responsible Editor: Gangrong Shi

✉ Tushar Kanti Maiti
tkmbu@yahoo.co.in

[1] Microbiology Laboratory, Department of Botany, The University of Burdwan, Purba Bardhaman, West Bengal 713104, India

[2] Mycology and Plant Pathology Laboratory, Department of Botany, Siksha Bhavana, Visva-Bharati, Santiniketan, Birbhum, West Bengal 731235, India

[3] Nanospinics Laboratory, Department of Materials Science & Engineering, Seoul National University, Seoul 151-744, South Korea

[4] DODL, University of Kalyani, Nadia, Kalyani, West Bengal 741235, India

and got accumulated within the human body showing its long-term effects such as discoloration and thickening of the skin, numbness in the hands and feet, pain in the stomach, vomiting, diarrhea, queasiness, partial paralysis, blindness, and various types of cancers (bladder, kidney, liver, prostate, lungs, and skin) (Das and Sarkar 2018; Rahman et al. 2020).

In nature, As mainly exist in two forms. The trivalent form of arsenic [As (III)] is found mostly in the aqueous state in the environment, soluble, and thus easily taken up by the plants. However, the pentavalent form As (V) is usually found in bound form with minerals in the solid-state and is less available to plants (Abbas et al. 2018). The conventional remediation methods come with a burden of toxic secondary products and huge monetary investments. The emergence of cost-effective bioremediation techniques provides a much-needed impetus for the rise in crop production in metalloid-contaminated soil. In this context, plant growth-promoting rhizobacteria (PGPR), which are root-colonizing soil bacteria exerting multiple useful effects on plant growth and development, became the need of the hour. PGPR promotes sustainable agriculture by virtue of having numerous plant growth-promoting traits. They tend to increase plant biomass directly through their growth-promoting traits like nitrogen (N_2) fixation, solubilization of minerals such as phosphate (P), iron sequestration via siderophore production, phytohormone production like that of indole-3-acetic acid (IAA), stress amelioration by 1-aminocyclopropane-1-carboxylic acid deaminase (ACCD) activity and indirectly by hydrocyanic acid (HCN) production, chitinase (cell-wall degrading enzyme) production, and induction of induced systemic resistance (ISR) through detoxification of virulence factors of various pathogenic agents (Glick et al. 2012). These indigenous growth-promoting rhizobacteria are well adapted to adverse environmental conditions and thereby reduces deleterious effects of multiple abiotic stress factors increasing plants' survival as well as crop productivity.

Solubilization of As in soil is regulated by As-resistant microbial strains which occur mainly through oxidation-reduction and methylation reactions (Cavalca et al. 2015). Instances of As-resistant bacteria include *Bacillus nealsonii*, *B. tequilensis* (Pandey et al. 2020), *Bacillus* sp. GIS1, *Acinetobacter* sp. GIS3, *Acinetobacter* sp. GIS5 (Rahman et al. 2020), *Azospirillum brasilense* Cd (Vezza et al. 2020), *Pseudomonas mosselii*, *Bacillus thuringiensis*, *Bacillus* sp. JBS-28 (Xiao et al. 2020), *Acinetobacter lwoffii* (Das and Sarkar 2018), *Bacillus aryabhattai* (Ghosh et al. 2018), *Kocuria flava, Bacillus vietnamensis* (Mallick et al. 2018), and *Bacillus flexus* ASO-6 (Das et al. 2016). The application of such As-resistant PGPR is of great ecological importance that abates As toxicity to support plant growth indispensable for sustainable agriculture (Pandey et al. 2020; Ghosh et al. 2018). However, due to the niche specificity of certain isolates, environmental influence, and degree of metal tolerance, isolation of native PGPR is still a challenging issue that needs to be addressed. Therefore, the present study is concentrated on isolation, identification, and characterization of a potent As-resistant PGPR strain from As-contaminated rice field. Further efforts were also made to evaluate its effect on As uptake and growth of rice seedling under As stress.

Material and methods

Site characterization and soil analysis

The soil sample was collected from the rice rhizosphere (23° 12′ 22″ N, 87° 50′ 44″ E) of Purba Bardhaman district, West Bengal, India. Different physicochemical properties of the soil samples were measured and selected heavy metal(s)/metalloid (As, Cd, Pb) contents were estimated using an atomic absorption spectrophotometer (GBC Avanta, Australia) after the aqua-regia digestion (Hseu et al. 2002).

Isolation and screening of arsenic-resistant PGPR

The isolates were first screened on Pikovskaya's agar (PVK) plates containing 150 μg/mL As (III) for P-solubilization. All the isolates were inoculated on the plates kept aside for 3 days at 30 ± 2 °C. Distinct colonies (AS17, AS18, and AS30) that produced transparent zones in PVK medium were further tested for ACCD activity, IAA production, N_2-fixation, siderophore production, and HCN production according to the standard protocols as described by Pramanik et al. (2017).

Test for tolerance to heavy metals/metalloids and sodium chloride (NaCl)

All these selected bacterial strains (AS17, AS18, and AS30) were grown individually in both liquid and solid Davis Mingioli (DM) (Davis and Mingioli 1950) medium supplemented with either arsenite ($NaAsO_2$) or cadmium ($CdCl_2$) or Lead [$Pb(NO_3)_2$] of different concentrations. Minimum inhibitory concentration (MIC) of each metal for the said strains was determined after 7 days of incubation (Andrews 2001). The NaCl tolerance ability of these strains was also determined in DM medium supplemented with different NaCl concentrations (Sarkar et al. 2018).

PGP characteristics of the isolates

As part of the screening process and to select the most efficient strain among the three isolates (AS17, AS18, and AS30), selected PGP traits were evaluated. Estimation of IAA production was carried out following Bric et al. (1991). The solubilization of phosphate was measured using the ammonium molybdate method of Fiske and Subbarow (1925). Acetylene

reduction assay (ARA) in gas chromatography (VARIAN CP3800) equipped with a flame ionization detector (FID) was used for the detection of nitrogenase activity (Kaushal and Kaushal 2015). ACCD activity was measured by the quantity of α-ketobutyrate liberated from ACC degradation (Penrose and Glic 2003). Considering the PGP traits together with As resistance capacity, AS18 strain was selected for subsequent experiments.

Molecular identification of strain AS18

The phenotypic characterization of AS18 was done as per standard methods of Benson (1990). Phenol/chloroform extraction method (Sambrook et al. 1989) was followed for genomic DNA isolation of AS18. The amplification of the 16S rDNA gene was performed using universal primers 16F27 and 16R1492. The amplified PCR product was further purified by PEG-NaCl precipitation followed by sequencing (Applied Biosystems, Inc., Foster City, CA). Assembling of the nucleotide sequence for identification of AS18 was done using the Laser gene package to a final length of 1510 nucleotides base and was compared by EzTaxon-e server (Kim et al. 2012) and the phylogenetic tree was prepared by MEGA6 (Kumar et al. 2016). The strain AS18 and 16S rDNA sequence of the said strain were deposited to National Centre for Microbial Resource (NCMR), National Centre for Cell Science (NCCS), Pune, India, and to NCBI database for accession number respectively.

Effect of As on growth and IAA production of strain AS18

The strain was grown at different concentrations (0, 1000, 2000, 3000 μg/mL) of As at 30 ± 2 °C and optical density (OD) of bacterial growth was measured at 540 nm in a spectrophotometer (Shimadzu 190, Japan) up to 72 h (12-h interval). The IAA production in presence of different As levels was also quantified up to 72 h (12-h interval) (Bric et al. 1991).

As oxidation-reduction test

The potentiality of AS18 strain to oxidize As (III) and to reduce As (V) was performed by using a silver nitrate solution (Simeonova et al. 2004). For this, the strain AS18 was grown in Brunner's mineral medium (Atlas 2005) containing 375 μg/mL As (III) and 375 μg/mL As (V) for oxidation and reduction respectively. After 3 days, culture plates were drenched with 0.1 M $AgNO_3$ solution. In general, the appearance of brown-colored colonies indicates the formation of silver arsenate (colonies express arsenite oxidase), whereas yellow color formed due to silver arsenite (colonies express arsenate reductase) (Banerjee et al. 2011). For the estimation of As transformation by the strain AS18, the method described in Qamar et al. (2017) was followed with some minor modification. Inoculum was prepared in Brunner's mineral medium containing 375 μg/mL As (III) and 375 μg/mL As (V) for oxidation and reduction respectively and incubated at 30 ± 2 °C at 100 rpm. Bacterial growth and As transformation were recorded in 12-h interval. As (V) determination was done by acidifying the samples whereas KIO_3 treament was followed for As (III) determination spectrophotometrically (Cummings et al. 1999). The difference between the oxidized and unoxidized As (III) in case of oxidation and the difference between the reduced and non-reduced As (V) in case of reduction estimates the As oxidation or reduction potential of the strain respectively (Qamar et al. 2017).

As bioaccumulation studies

As-accumulation by the strain AS18 was confirmed by TEM (transmission electron microscopy) study with two conditions (without As (III) as control and 1500 μg/mL As (III) as treatment). The method of Chen et al. (2016) was followed for sample preparation. For TEM analysis, JEOL-2011 field emission instrument was used at an operating voltage of 120 kV. A carbon-coated Cu grid was used as a sample holder. EDAX (energy-dispersive absorption x-ray) analysis was done using Bruker X Flash 6130. Powder X-ray diffraction (XRD) study was carried out using lyophilized bacterial samples (Arivalagan et al. 2014). For XRD analysis, the isolates were grown (without As (III) as control and 1500 μg/mL As (III) as treated) for 24 h at 30 ± 2 °C in DM medium. The bacterial cells were collected after centrifugation at 6000 rpm for 6 min followed by washing with phosphate buffer (PBS1X, pH 7.2) several times and then bacterial pellets were lyophilized and dried. For measurement, X-ray diffractometer (RICH SEIFERT-XRD 3000P) with X-Ray Generator-Cu, 10 kV, 10 mA, and wavelength 1.5418 Å was used. XRD profile was observed with an angular province of 10° to 80° and 2° min^{-1}. The phase identification number was found using X'PertRigaku PDXL software version 2.4.2.0. For FTIR study, we have taken the powder samples that were used during XRD in addition to potassium bromide (KBr) pellets at 27 °C (Deokar et al. 2013) using an FTIR spectrometer (NICOLET MAGNA IR 750 system). The mass ratio for the sample to KBr was in between 4000 and 200 cm^{-1} range with an increment of 4 cm^{-1}. For X-ray fluorescence spectra (XRF) analysis, bacterial lyophilized samples [AS18, AS18 + As (III)] and plant samples (control, EC50,

EC50 + AS18) were used with XRF spectrometer Bruker ARTAX-ELEMENT ANALYSER (Current-698 μA, Time-300 S, Voltage-50 kV).

As removal study

To determine As removal efficacy, the strain was grown in DM broth supplemented with 1000, 2000, 3000, and 3500 μg/mL As (III). The cultures were kept in a rotary incubator shaker at 32 ± 1 °C up to 72 h. Residual As concentration in each culture medium was recorded by AAS at every 12-h interval. For this, each bacterial culture suspension was centrifuged at 10,000 rpm for 15 min and the culture supernatant was taken for AAS (PerkinElmer, USA) analysis. Simultaneously bacterial growth of each set was measured by spectrophotometer (Shimadzu, Japan) at 540 nm. As removal efficiency was calculated according to Pandey and Bhatt (2015):

$$\text{As removal}\% = \frac{\text{IC}-\text{FC}}{\text{IC}} \times 100$$

Here, IC is the initial As concentration (μg/mL) supplemented in the medium at time zero, and FC is the final concentration (μg/mL) of As in medium after growth in 12-h interval up to 72 h.

Viability test

For the viability confirmation of AS18 strain at higher doses of As(III), it was grown in DM agar plate supplemented with 1500 μg/mL As(III) and compared with another plate with the same medium without any As. After the incubation period of 24 h at 30 °C, the triphenyl tetrazolium chloride (TTC) test was performed after the appearance of bacterial growth. Viable cells were detected by observing red-colored colonies that appeared on the agar plate (Pandey and Bhatt 2015).

Plant growth-promotion study

EC50 determination of As on seed germination

Pratikshya rice cultivar (IET-15191) was obtained from Krishi Vigyan Kendra (KVK), ICAR, Chinsurah, Hooghly, West Bengal, India, for determination of different plant growth-promoting effects of strain AS18. EC50 (50% germination was inhibited on effective concentration) value of the rice seeds was evaluated using $NaASO_2$ as As (III) source in different concentration gradients (0–25 μg/mL). Surface disinfection of the seeds was performed using sodium hypochlorite solution (2%, 15 min). The disinfected seeds were washed repeatedly with sterile distilled water and placed in blotting paper containing Petri dishes for incubation in dark condition at 30 ± 2 °C. After 3 days, the germination percentage was recorded.

$$\text{Germination percentage} = \frac{\text{No.of seeds germinated}}{\text{Total no.of seeds}} \times 100$$

Inoculation of strain AS18 on rice seedling under As stress

For in vitro plant growth-promoting experiment, disinfected seeds were imbibed in sterile Millipore water for 6 h. One third of the imbibed seeds were taken into a sterile 100-mL beaker containing overnight cultured bacterial suspension ($OD_{540} = 0.01$)(1×10^6 CFU/mL) for seed bacterization up to 1 h. The remaining seeds were kept in a separate 100-mL beaker containing sterile Millipore water for 1 h. The whole seed lot was then divided into three different seedbeds in triplicates—one seedbed containing bacterized seeds while the other two devoid of it. The formation of seedbeds was somewhat similar for all the three sets, i.e., 200-mL glass beakers containing sterile absorbent cotton (approximately 2 cm height), filled with 25 mL of sterile Hoagland's solution (Ahmad et al. 2016) and Whatman filter paper on top of it bearing 80 seeds. The whole setup (in triplicates) was designated as *control*—[without As(III) and AS18 strain], *EC50*—[with As(III) but without AS18 strain], and *EC50 + AS18*—[with As(III) at EC50 concentration and AS18 strain] which was maintained in a plant growth chamber at 30 ± 2 °C in dark condition for 3 days and after that, all these sets were kept inside the same chamber with intermittent light (light/dark = 10 h/14 h) for 7 days more.

Monitoring morphological parameters in seedlings

After a 10-day growth period, growth parameters viz. shoot length, root length, fresh and dry weights of shoots, fresh and dry weights of roots, and seedling vigor index (Bal et al. 2012) were recorded accordingly.

$$\text{SVI} = (\text{mean shoot length} + \text{mean root length}) \times \text{germination percentage}$$

Monitoring biochemical parameters in seedlings

The phenol-sulfuric acid method proposed by Dubois et al. (1956) was implied to determine the total sugar content of seedlings with the help of a calibration curve of glucose at 490 nm. The total protein content was

estimated accordingly (Bradford 1976) at 595 nm. In this case, bovine serum albumin (BSA) was used for making the standard curve. The enzyme extraction for the determination of α-amylase and protease activity was done following Snell and Snell 1971. The analysis of enzymes and proteins was done following Khan and Faust (1967) and Bradford (1976), respectively. The calculation was done according to Fick and Qualset (1975).

$$\text{Enzyme activity} = \frac{\Delta A \times Tv}{t \times v} \text{ units/min/g fw}$$

where ΔA = O.D. difference of reaction set − control set, Tv = total volume of extracted enzyme, t = incubation time, and v = volume of extracted enzyme taken for reaction.

The total chlorophyll content of shoot and leaves was measured by spectrophotometric method following acetone extraction method (Arnon 1949). The enzymes were extracted from the seedlings following the method of Pandey et al. (2013). From the extracted aliquots, proline content and catalase activity were measured following the methods of Bates et al. (1973) and Aebi (1984) respectively. The malondialdehyde (MDA) content was estimated according to Heath and Packer (1968). Superoxide dismutase (SOD) activity was measured following the protocol of Giannopolitis and Ries (1977). The calculation of SOD activity was done following the formula of Fick and Qualset (1975).

In addition to three seedbed setup stated earlier, a fourth set (treated with $NaAsO_2$ along with $CoCl_2$ but without AS18 strain) was marked as EC50 + $CoCl_2$ ($CoCl_2$ was used as an inhibitor of ethylene biosynthesis) for performing the said experiment (Chmielowska-bąk et al. 2014; Pramanik et al. 2017). Rice seedlings were grown up to 8 days without bacterial inoculation and As stress. On the ninth day, As treatment and bacterial inoculation (AS18 strain) were given for respective sets. After 24 h, seedlings were taken out from the beaker from the respective sets carefully and restrained in 60-mL culture tubes with rubber stopper for estimation of ethylene production via GC after 3 h.

As accumulation study in rice seedling

The amount of accumulated As in rice seedlings was also determined by AAS (PerkinElmer, USA). For this, seedlings were dried in a hot air oven at 70 °C for 4 h. Then, the dried seedlings were subsequently ground and digested in a flask containing HNO_3 and $HClO_4$ at the ratio of 3:1 (*v*/*v*). After digestion, the samples were heated on a hot plate at 350 °C until white fumes appeared. The flasks containing the samples were cooled off and diluted for further filtration for AAS analysis to detect As concentration following Yang et al. (2009). Bioaccumulation of As was measured using the following formula proposed by Ahmad et al. (2014).

$$\text{Bioaccumulation factor} = \text{As concentration in rice tissues} \div \text{As concentration given in seedbed.}$$

Statistical analysis

The statistical analysis was done by using the MS-EXCEL 2010 statistical analytical tool and Origin 8.5 software. The mean values (of triplicate data in every case) are presented with the standard error bars in figures and standard error (SE) as ± in the tables. The significance of differences between the control and treatment was calculated by Student's *t* test and differences between groups were estimated by one-way analysis of variance (ANOVA) test (Pramanik et al. 2017). Differences at $p \leq 0.05$ were treated as statistically significant.

Results and discussion

Site characterization and soil analysis

Crop production is highly impeded due to NaCl and As contamination of agricultural lands (Mallick et al. 2018). The presence of metal-resistant microbial community in contaminated rhizospheric soils is the main reason for particular site selection. The soil analysis of the study site found contaminated with three heavy metals/metalloids (As, Cd, Pb) established from the AAS study (Supplementary Table 1). Rhizospheric microflora are known to be greatly affected by soil characteristics (Goswami et al. 2014). The presence of ~ 33-μg/g arsenic in soil could be the reason for the enrichment of As-resistant PGPR in the rice rhizosphere (Supplementary Table 1).

Screening of As-resistant PGPR, metal/metalloid, and NaCl tolerance

Primarily, three stains (AS17, AS18, AS30) were selected which showed various PGP traits and As, Cd, and Pb resistance of varying degrees. Further screening of the isolates was done by determining MIC in different heavy metals/metalloids (As, Cd, and Pb). The maximum tolerance levels of AS18 strain for As, Cd, and Pb are 3750 (50 mM), 4140 (40 mM), and 4496 (20 mM) μg/mL respectively (Fig. 1a). The strain AS18 also showed high salt-tolerant property among the three preliminary selected strains and the maximum tolerance level of this strain was found as 6% (Fig.

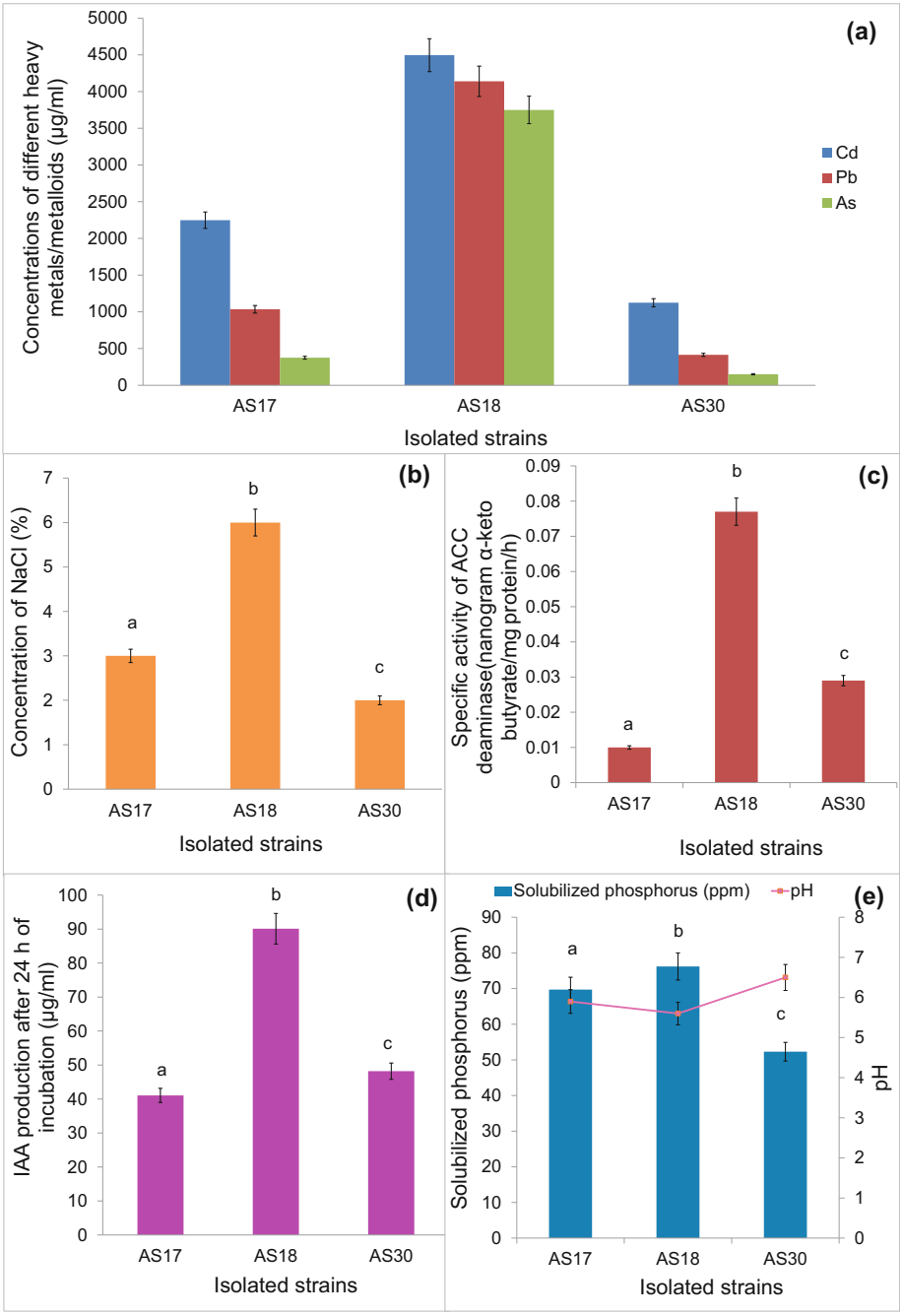
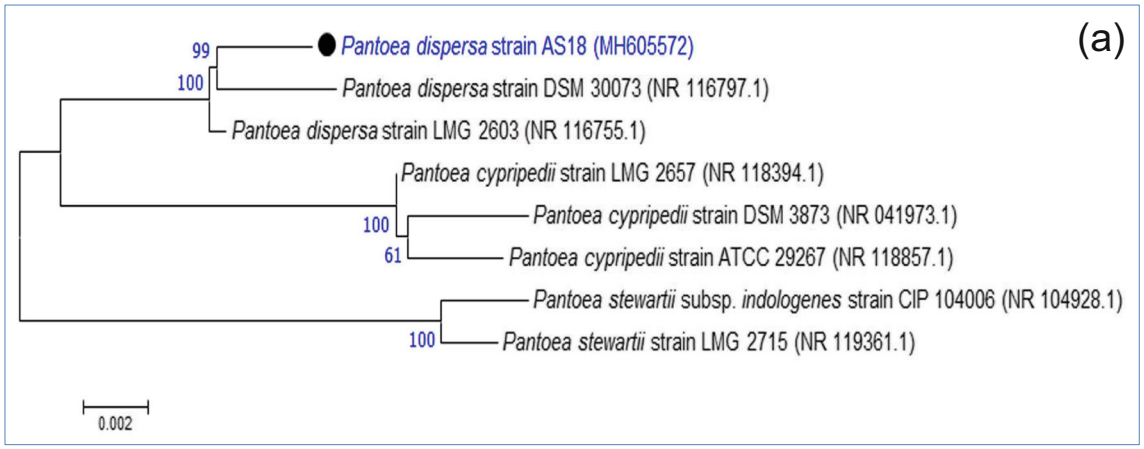


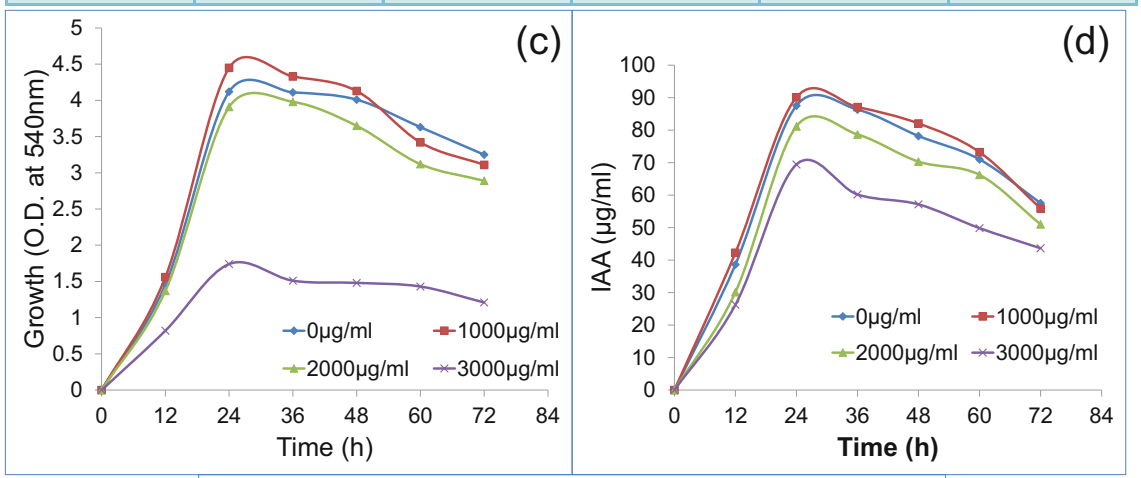
Fig. 1 Determination of MIC and PGP traits of selected multi heavy metal-resistant isolates. (a) Heavy metal/metalloid resistance, (b) salt tolerance (c) ACC deaminase activity, (d) IAA production, (e) phosphate solubilization. The results show that AS18 has the highest capacity in the tested traits among the isolates

1b). From this result, it is evident that AS18 (*Pantoea dispersa*) is a multi-metal/metalloid-resistant strain. Besides, it also tolerates high doses of NaCl. The strain AS18 was found to be metabolically active withstanding high concentrations of the aforementioned contaminants during our work. Several other strains were also reported with such characteristics in previous studies conducted by separate group of researchers (Paredes-Páliz et al. 2016; Mallick et al. 2018). Heavy metal/metalloid- and salt-contaminated environment generates selection pressure on microbes that enables them to develop intrinsic resistance mechanisms to tolerate high doses of toxicants. These resistance mechanisms not only favor their survival but also help to retain their PGP traits of utmost agronomic importance in metal/metalloid and salt stressed conditions. Therefore, PGPR can cause betterment of crop yield in heavy metal- and salt-stressed conditions (Sengupta et al. 2015; Mallick et al. 2018). The strain AS18 was unanimously selected for further study that aims to evaluate the effect of PGPR on rice seedling under As (III)-stressed condition. The strain exhibits multiple plant growth-promoting traits viz. ACCD activity (Fig. 1c), IAA production (Fig. 1d), P-solubilization (Fig. 1e), N_2-fixation



(b)

Molecular Identification of AS18 strain					
Strain No.	**16S rDNA sequence length (bp)**	**Closest relative with accession number (EZ-taxon)**	**Pair wise similarity (%)**	**NCBI accession number**	**MCC strain accession number**
AS18	**1510**	***Pantoea dispersa* (NR116797.1)**	**99%**	**MH605572**	**MCC4044**



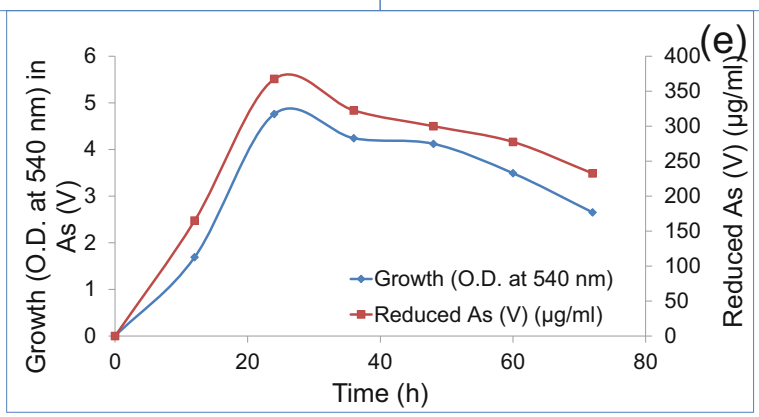
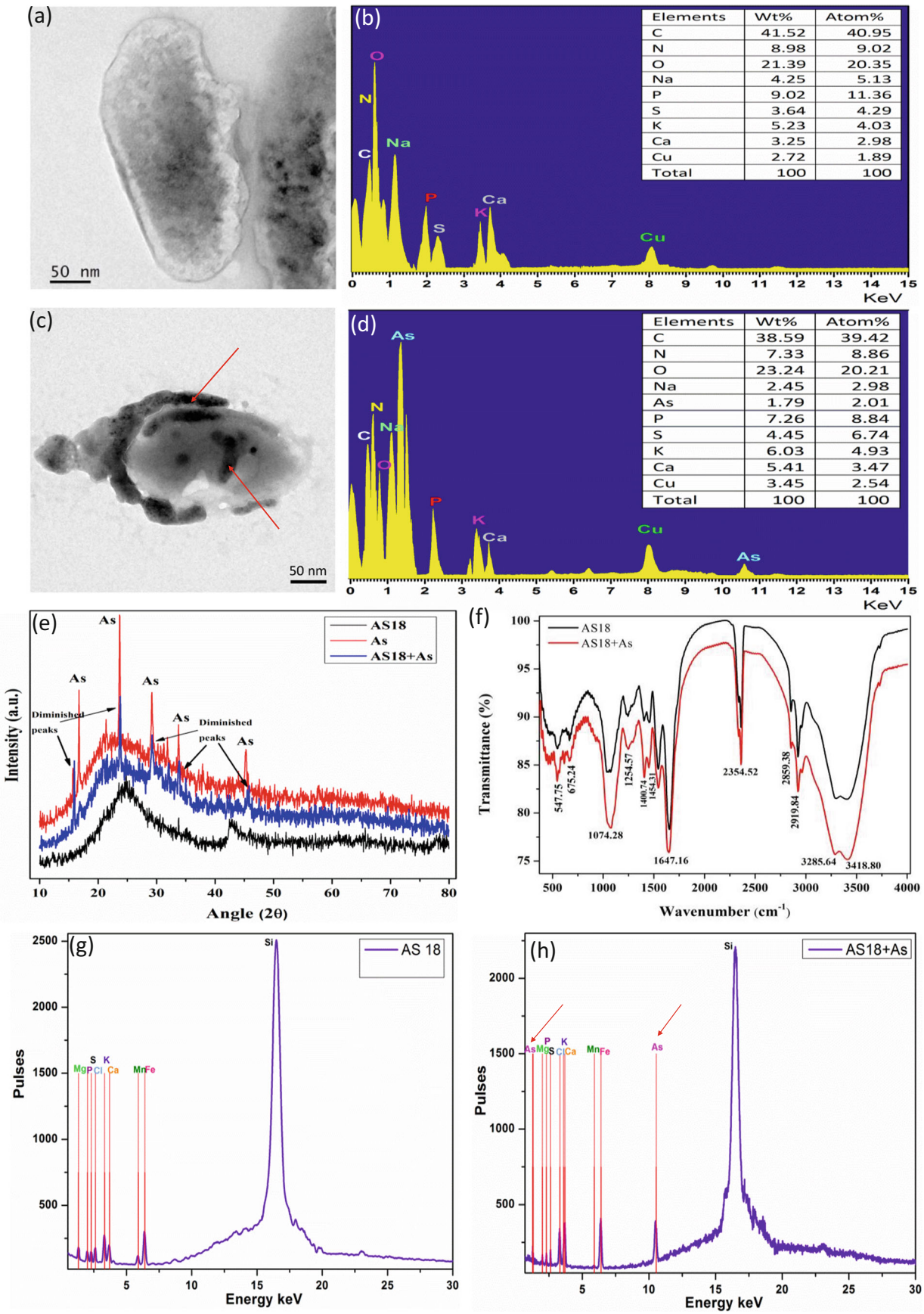


Fig. 2 Identification and biochemical characterization of AS18 strain. (a) Phylogenetic tree, (b) molecular identification, (c) effect of various As concentrations on bacterial growth, (d) IAA production, (e) reduction of As(V). Identification reveals the AS18 strain to be a species of *Pantoea* which has sustained IAA production under As-stress and As(V) reducing potential


(a)
50 nm
(b)
Elements Wt% Atom%
C 41.52 40.95
N 8.98 9.02
O 21.39 20.35
Na 4.25 5.13
P 9.02 11.36
S 3.64 4.29
K 5.23 4.03
Ca 3.25 2.98
Cu 2.72 1.89
Total 100 100
KeV
(c)
50 nm
(d)
Elements Wt% Atom%
C 38.59 39.42
N 7.33 8.86
O 23.24 20.21
Na 2.45 2.98
As 1.79 2.01
P 7.26 8.84
S 4.45 6.74
K 6.03 4.93
Ca 5.41 3.47
Cu 3.45 2.54
Total 100 100
KeV
(e)
AS18
As
AS18+As
Diminished peaks
Intensity (a.u.)
Angle (2θ)
(f)
AS18
AS18+As
Transmittance (%)
Wavenumber (cm-1)
547.75
675.24
1074.28
1254.57
1400.74
1454.31
1647.16
2354.52
2859.38
2919.84
3285.64
3418.80
(g)
AS 18
Pulses
Energy keV
(h)
AS18+As
Pulses
Energy keV

◀ **Fig. 3** Arsenic bioaccumulation confirmatory study of strain AS18. **a**, **b** As-untreated and **c**, **d** As-treated cells of AS18 strain by TEM and EDAX studies. **e** XRD analysis of As-resistant *Pantoea dispersa* (AS18) strain. **f** FTIR spectra analysis. **g** XRF spectra analysis of As-untreated bacterial cells. **h** XRF of As-treated bacterial cells. These observation suggest As bioaccumulation inside the bacterial cells

(Supplementary Table 2), siderophore production, ammonia production, and HCN production (Supplementary Table 3; Fig. 1c–e). As per previous reports, many rhizospheric soil bacteria that possess heavy metal-resistant property were also found to promote plant growth (Pandey and Bhatt 2016; Pramanik et al. 2017; Das and Sarkar 2018; Ghosh et al. 2018; Pramanik et al. 2018; Rahman et al. 2020; Xiao et al. 2020; Pandey et al. 2020; Vezza et al. 2020) as found in this study. Therefore, the strain *Pantoea dispersa* isolated during this work should be further exploited for bioremediation and plant growth promotion in salt- and metalloid-contaminated agricultural fields.

PGP characteristics of the selected strain AS18

In this present study, the strain AS18 is found to produce IAA which is known to be essential for regular cell division, enlargement, and normal root development processes (Glick 2012). This trait was also proved to be beneficial for rice seedling growth under Cd stress (Bhattacharyya and Jha 2012; Pramanik et al. 2017). The AS18 strain was also found to produce ACCD that splits ACC (the precursor of ethylene) into α-ketobutyrate and ammonia (Glick et al. 1998). The lowering of stress ethylene by ACCD facilitates longer root growth formation and better nutrient acquisition (Pandey et al. 2013; Das et al. 2014; Ghosh et al. 2018). Furthermore, the ability of the strain AS18 to solubilize phosphate might be helpful in augmentation of plant growth promotion. The predominant insoluble form (such as phytate, phosphate monoesters) of phosphorus present in soil is not usually taken up by the plants directly (Glick 2012). The P-solubilizing capability of some soil-borne bacteria makes phosphorus more available to plants. Phosphate-solubilizing bacteria (PSB) convert phosphate from insoluble to soluble form (monobasic, dibasic ionic forms) in As-amended soil through phosphatase enzyme and makes it more available to plants (Ghosh et al. 2015). As-resistant strain AS18 also possesses N_2-fixing capability and it performs as persuasive bio-fertilizer. PGPR have the ability to fix atmospheric nitrogen which is an added advantage that can be used as an alternative for nitrogen-based chemical fertilizers (Bhattacharyya and Jha 2012). As-resistant PGPR *Bacillus flexus* ASO-6 has also shown IAA, siderophore, and ACC deaminase-producing ability along with P-solubiliing activity (Das et al. 2016). The siderophores produced by microbes often act as biogenic chelator and form complexes with Fe (III), increasing iron availability for the host plant. The novelty of AS18 lies in its high degree of metal/metalloid tolerance and simultaneous plant growth promotion through its multiferous PGP traits.

Molecular identification of strain AS18

Morphological and physio-biochemical studies of strain AS18 show that the strain is a gram-negative, rod-shaped, round-formed colonies which is identified by 16S rDNA sequence-based homology. The phylogenetic analysis confirmed 99% clustering with strain *Pantoea dispersa* DSM 30073 (NR 116797.1) (Fig. 2a). Thus, it is trustworthy to say that the strain AS18 can be *Pantoea dispersa*. The 16S rDNA sequence of this strain was deposited to NCBI (16S rDNA sequence accession number is MH605572) and the strain was deposited to NCMR, NCCS, Pune, India (strain accession number is MCC 4044) (Fig. 2b).

Effect of As on growth and IAA production of strain AS18

Effect of As stress on growth and IAA production was essential for future perspectives. The log phase of bacterial growth was found to be reduced and is correlated with the occurrence of the stationary phase (Fig. 2c). It was found that IAA production under As stress (1000 μg/mL) was increased at 24 h and is decreased thereafter (Fig. 2d). The decrease in IAA production was also found in *Enterobacter* sp. (Chen et al. 2016) and *Klebsiella* sp. (Pramanik et al. 2017) under Cd stress.

Arsenate-reducing property of strain AS18

It was found that the bacterial strain AS18 reduces from As(V) to As(III) due to the interaction of $AgNO_3$ with AsO_3 in the medium resulted in the bright yellow precipitate of Ag_3AsO_3 in the medium. The strain generates arsenate reductase enzyme and the amount of maximum conversion from As(V) to As(III) was 367.5 μg/mL after 24 h growth of bacteria (Fig. 2e). The qualitative assay was confirmed by this quantitative test where the maximum reduction was obtained after 24 h. This result corresponds with the redox transformation of arsenate by other *Bacillus* spp. (Banerjee et al. 2011; Qamar et al. 2017; Ghosh et al. 2018). However, the reduction of As(V) may or may not be required if it does not harm the bacterial cell. Similarly, if reduction occurs, the extrusion of As(III) is also not obvious unless it reaches a threshold level in the cells. After reduction, As(III) may conjugate with glutathione, non-protein thiol, and/or metallothionenis or accumulate intracellularly (Pratush et al. 2018; Sher and Rehman 2019).

As bioaccumulation by strain AS18

The high-resolution image of As-untreated and As-treated bacterial samples of AS18 strain was showed in TEM study (Fig. 3) and clearly reveals the accumulation of As successfully within the bacterial cell in the form of the electron-dense element. The bioaccumulation of As in the strain AS18 was further established by EDAX analysis through the presence of acute As peaks in As-treated bacterial cell (Fig. 3a, c); however, no peak was found in the untreated cell (Fig. 3b, d). Thus, As-contaminated agricultural fields could be reduced or eliminated from As content with the application of this AS18 strain. This result agrees with others using halophilic plant growth-promoting *Bacillus* sp. (Mallick et al. 2018). Comparative study of XRD profiles of treated, untreated bacterial samples and As precursor salt was carried out to detect the accumulation of As into the bacterial sample (Fig. 3e). Profile in red color shows the pristine As phase of the salt along with sharp diffraction peaks that reveal the crystalline phase of As. The black-colored profile is for As-untreated bacterial strain AS18 where no sharp diffraction peaks were observed. The profile of As-treated bacterial strain AS18 + As is shown in blue color. Here, it is clearly observed that after As attachment, the characteristic As peaks were diminished in the treated sample which confirms the accumulation of As in the bacterial strain from the medium in the form of intensity reduction. Figure 3f shows the FTIR spectra of As-treated and As-untreated bacterial samples to differentiate the associated reaction between As and cell surfaces present in the bacteria. Based on principal IR transmission, spectra at around 3418.80 cm^{-1} are due to O–H groups and 3285.64 cm^{-1} is for N–H bonding. The position of peak at 2919.84 cm^{-1} is due to C–H stretching frequently arises because of alkyl functional group (Murthy et al. 2014). C=O arises at 1647.16 cm^{-1} and 1400.74 cm^{-1} is due to C–N mode. Bands around 1254.57–1074.28 cm^{-1} are due to phosphate groups. It was clearly observed that due to As attachment, all the peaks are intensified which proofs the interaction of As with the bacterial cell. This kind of interaction was also observed in *Rhodococcus* sp. (Prasad et al. 2011) and *B. aryabhattai* (Singh et al. 2016) previously. For elemental detection of untreated and treated bacterial samples (AS18 and AS18 + As), XRF analysis was carried out (Fig. 3g and h). In the untreated sample (AS18), the As peak was absent while in the treated sample (AS18 + As), an As peak was detected. The As accumulation in bacterial cell pellets was confirmed by this XRF analysis; however, all other elements remained the same in both the samples and no peak was noticed in the untreated control set. This confirms the accumulation of As in the AS18 bacterial cell. Ghosh et al. (2018) also showed As bioaccumulation through XRF analysis while working with *B. aryabhattai.*

As removal efficiency of AS18

The MIC of AS18 was 3750 μg/mL of As (III) and thus the bacteria were able to grow efficiently in 1000, 2000, 3000, and 3500 μg/mL As (III)-supplemented liquid medium. This study also reveals that AS18 not only resisted high As concentration but also removed the As from the culture medium. The maximum As (III) removal efficiencies as measured by AAS in 1000, 2000, 3000, and 3500 μg/mL As (III)-supplemented medium were 93.12%, 78.11%, 73.26%, and 62.19%, respectively (Fig. 4) each after 72-h incubation. However, it was found that the As removal efficiency of AS18 strain was high in 1000 μg/mL among the said concentration so far studied. This study also reveals that in every case, the removal efficiency gradually increased with a simultaneous increase of incubation time. This is also an indicative of increase in bacterial growth. The ability of As bioaccumulation of this strain and As removal efficiency could be further explored in contaminated crop fields. These results are at par with previous works on As removal and bioaccumulation by the following microorganisms—*Bacillus* (Majumder et al. 2013; Pandey and Bhatt 2015), *Lysinibacillus* (Rahman et al. 2014).

Hence, TEM-EDAX, XRD, FTIR, XRF, and AAS studies confirmed that *Pantoea dispersa* AS18 accumulated As(III) when grown in liquid media (Figs. 3, 4). On the other hand, arsenate reductase activity of this strain (Fig. 2e) is associated with the conversion of As (V) to As (III) (Qamar et al. 2017; Ghosh et al. 2018) that is known to conjugate with the thiols or to be utilized as energy source essential for its growth (Tsai et al. 2009). Thus, the strain in rhizospheric association would help its host plant by reducing the As level in rhizosphere. This important property bundled with PGP traits would accelerate the growth of host plant under As-spiked soil (Qamar et al. 2017; Ghosh et al. 2018).

Viable cells of strain AS18 under As stress

Strain AS18 has the ability to tolerate upto 3750 μg/mL of As (III) (Fig. 1a). Thus, it was indispensable to determine the viability of the strain at such exalted concentration of As. The results obtained from the tetrazolium test in which colorless and soluble TTC converted to red color in both As-treated and As-untreated Petri plate due to the formation of red formazan used as a metabolic indicator. Moreover, this finding was also reflected in the viability test of AS18 (Supplementary Fig. 1). The current investigation is sustained by earlier authors (Chen et al. 2016) and also justified the ability to alleviate As (III) from the medium. Thus, AS18 strain can be further explored in As-contaminated fields to remove As toxicity and promote plant growth.

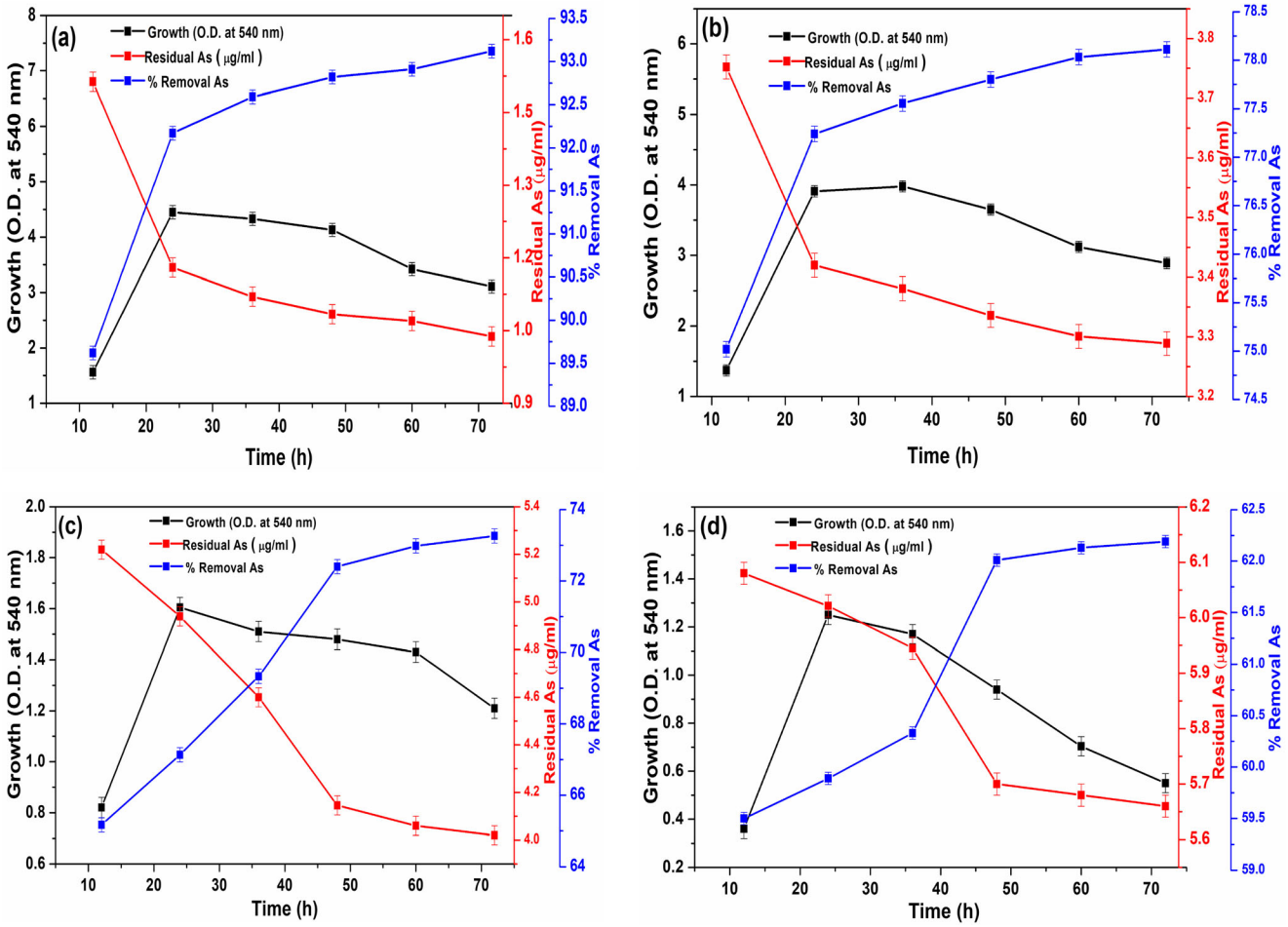


Fig. 4 Determination of As removal efficiency of AS18 bacterial strain by AAS at various concentrations of As-supplemented culture medium. **a** 1000 µg/mL As, **b** 2000 µg/mL As, **c** 3000 µg/mL As, and **d** 3500 µg/mL As. This observation indicates the removal of As from the culture medium

Rice seedling growth promotion by strain AS18 under arsenite stress

The combined effect of PGP traits and As bioaccumulation by AS18 displays a positive influence on rice seedling growth under As stress. The plant growth-promoting trait of AS18 was clearly perceived in various morphological and biochemical growth parameters. Starting from seed germination (100%) (Fig. 5a), significantly ($p < 0.05$) enhanced shoot length (> 1.3 fold) (Fig. 5b), root length (> 1.4 fold) (Fig. 5b), shoot fresh weight (> 1.44 fold) (Fig. 5c), root fresh weight (> 1.44 fold) (Fig. 5c), shoot dry weight (> 1.33 fold) (Fig. 5d), root dry weight (1.2 fold) (Fig. 5d), and seedling vigor index (SVI) (Fig. 5e) when compared against As-stressed condition at EC50 (of the rice cultivar) of 15 µg/mL. The visible morphological improvements of rice seedlings under As stress might be due to combined effect of PGP traits viz. N_2-fixation, P-solubilization, IAA production, and ACCD activity is possessed by the strain AS18. Here, PGPR act as a phytostimulator and assist plants to draw nutrition from contaminated soils thereby promoting their overall growth and development. This result is positively correlated with other works also (Das et al. 2016; Pandey and Bhatt 2016; Ghosh et al. 2018; Mallick et al. 2018; and Xiao et al. 2020).

Biochemical improvements in rice seedlings

The total sugar content in AS18-inoculated seedlings was found to be increased > 1.82 fold when compared to As-treated (EC50) seedlings (Fig. 6a). This significant ($p < 0.05$) rise in total sugar content in AS18 inoculated seedlings might have a role to play in relieving As toxicity by scavenging free radicals (Singh et al. 2016). On the contrary, total protein content was dropped in AS18-inoculated seedlings compared to EC50 but it was significantly ($p < 0.05$) higher than control (Fig. 6b). Controlled proteolysis is obligatory to sustain the intracellular protein homeostasis which promotes plant growth under abiotic stresses (Kidric et al. 2014). Besides, plant cells synthesize antioxidant enzyme superoxide dismutase (SOD) which reduces stress-induced damages by

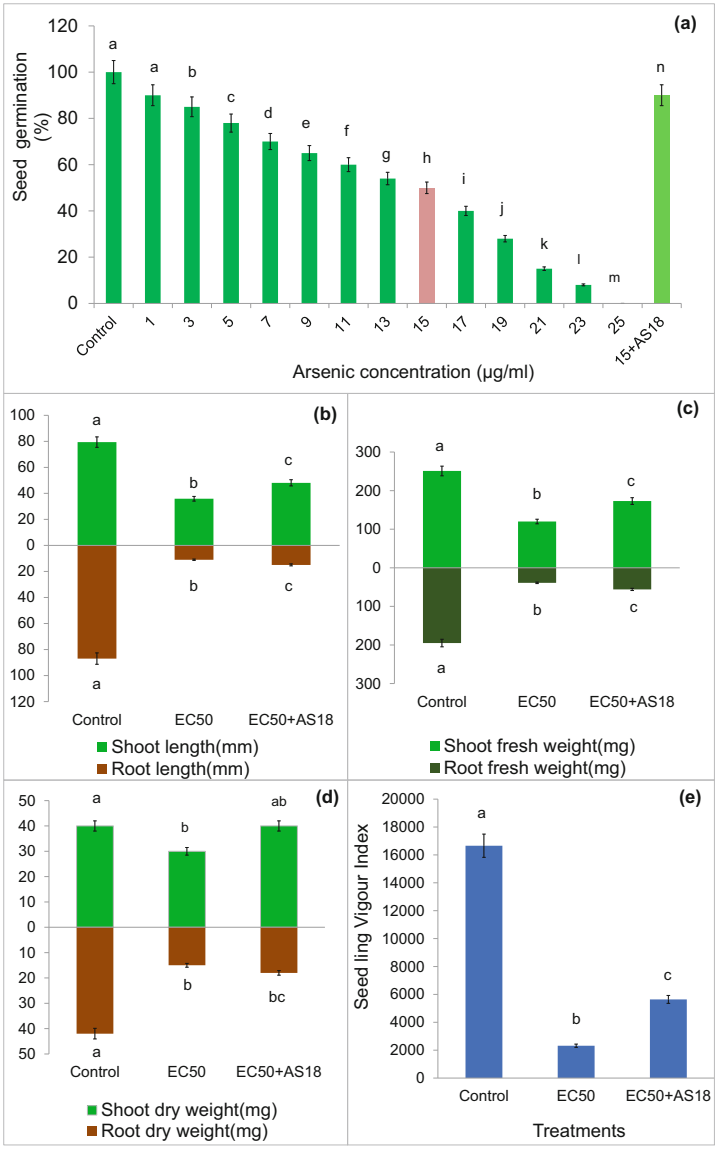


Fig. 5 Effect of application of strain AS18 on various plant growth-promoting parameters. **a** Seed germination, **b** shoot-root length, **c** shoot-root fresh weight, **d** shoot-root dry weight, and **e** seedling vigor index on rice seedlings under As stress. The results specify that AS18 has an influence in improving rice seedlings growth under As stress

scavenging ROS (reactive oxygen species). The study showed AS18-inoculated seedlings had significantly ($p < 0.05$) higher activity of SOD compared to EC50 (Fig. 6c). Correspondingly, the protease activity was decreased in AS18-inoculated seedlings compared to EC50 (Fig. 6d). This observation is similar to protease activity on rice seedling under heavy metal stress (Pandey et al. 2013; Pramanik et al. 2017). The osmoprotectant proline is generally overproduced due to various environmental stresses (Hashem et al. 2016). Strain AS18-inoculated seedlings showed a significant ($p < 0.05$) increase in proline content compared to both EC50 and control set (Fig. 6e). Proline is a stress-induced non-enzymatic osmolyte essential for adaption in plants (Abbas et al. 2018). Raised proline levels in rice seedlings act as an antioxidant defense molecule. The MDA (the end product of lipid peroxidation) content was significantly ($p < 0.05$) declined in inoculated plants (Fig. 6f) that hints about minimization of cell membrane damage. The reduction in lipid peroxidation levels further helps to maintain cellular integrity. As-induced membrane damage due to chain-like peroxidation of unsaturated fatty acids in the membrane and its reclamination using bacterial inoculant has also been reported by various authors (Pandey and Bhatt 2016; Singh et al. 2016; Das and Sarkar 2018; Ghosh et al. 2018). Besides, catalase activity was found significantly ($p < 0.05$) increased with the comparison to both EC50 and control (Fig. 6g). The As-resistant PGPR helps to maintain redox homeostasis in plants in such adverse conditions (Pandey et al. 2013; Pandey and Bhatt 2016). The strain also significantly ($p < 0.05$) improved α-amylase activity compared to both control and EC50 set (Fig. 6h). This activity might be associated with the increased germination percentage in AS18 inoculated seeds and decreased germination percentage in As-treated (EC50) seeds (Pandey et al. 2013; Pramanik et al. 2017). The reduction in

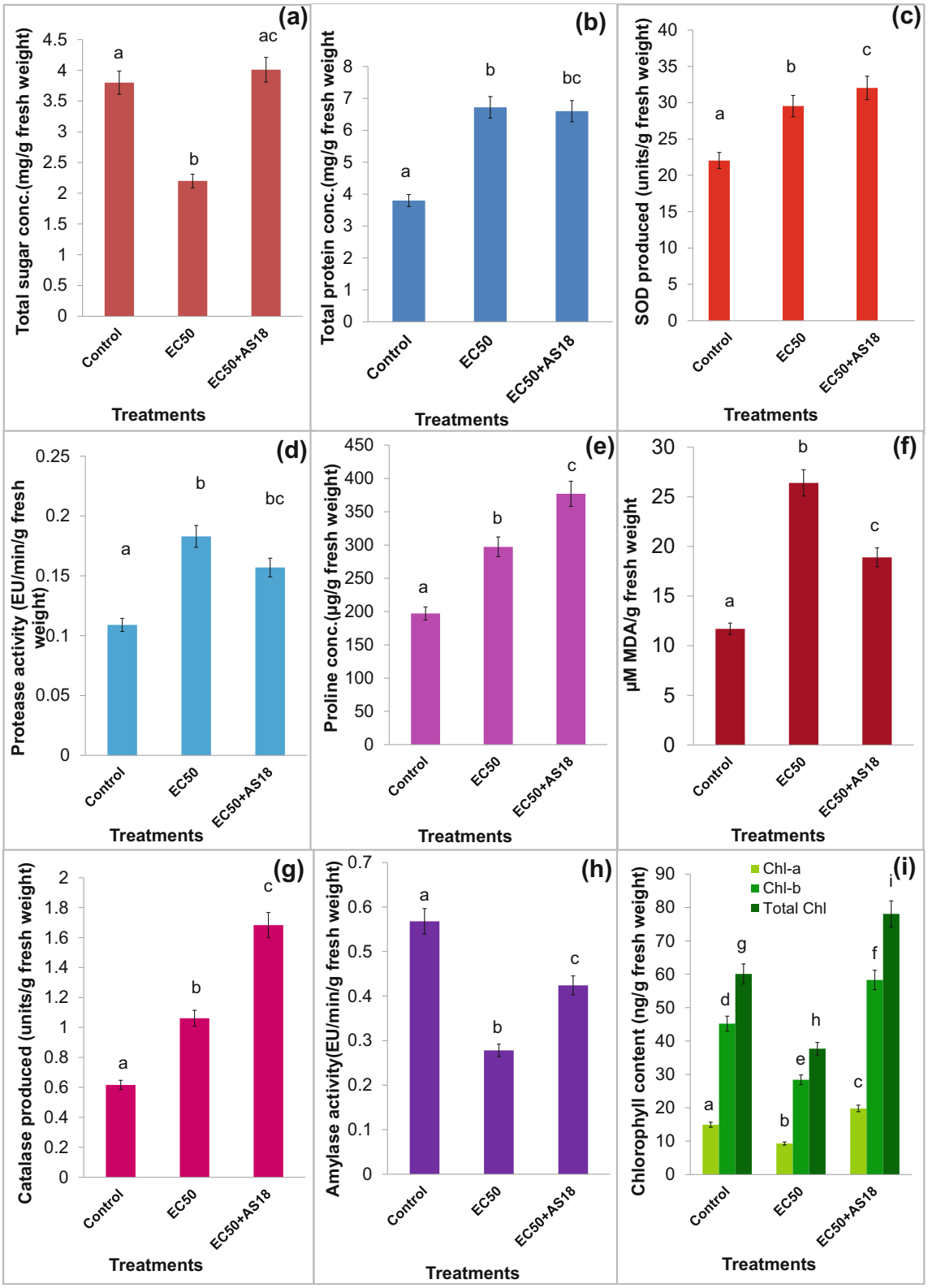


Fig. 6 Effect of application of strain AS18 on various plant growth-promoting parameters including antioxidants. **a** Total sugar content, **b** total protein content, **c** SOD activity, **d** protease activity, and **e** proline content, **f** MDA content, **g** catalase activity, **h** amylase activity, **i** chlorophyll (Chl-a, Chl-b, and total Chl) contents of rice seedlings under As stress. These biochemical improvements suggest stress alleviation in rice seedlings by AS18 strain

chlorophyll content is an indication of As-induced impairment of photosynthesis in leaves of rice seedings (EC50). However, inoculated seedlings showed significantly ($p < 0.05$) increased Chl-a, Chl-b, and total chlorophyll content under As-stressed condition.

From primary screening, it was evident that the strain AS18 possesses ACCD activity (Fig. 1c). When the strain was applied to As-treated rice seedlings, stress ethylene production found to be reduced due to the mobilization of ethylene precursor ACC by the strain (Das et al. 2014). AS18-induced reduction in stress ethylene levels was found to be comparable with inhibitor-treated control sets (Fig. 7a). As-induced stress ethylene production causes root growth inhibition and stunted plant growth (Ghosh et al. 2018). The IAA production ability and ACCD activity of AS18 might be the reason behind enhanced root growth in rice seedlings under As-stressed environment (Das et al. 2016). Previous studies have revealed that inoculation with ACCD-producing bacteria

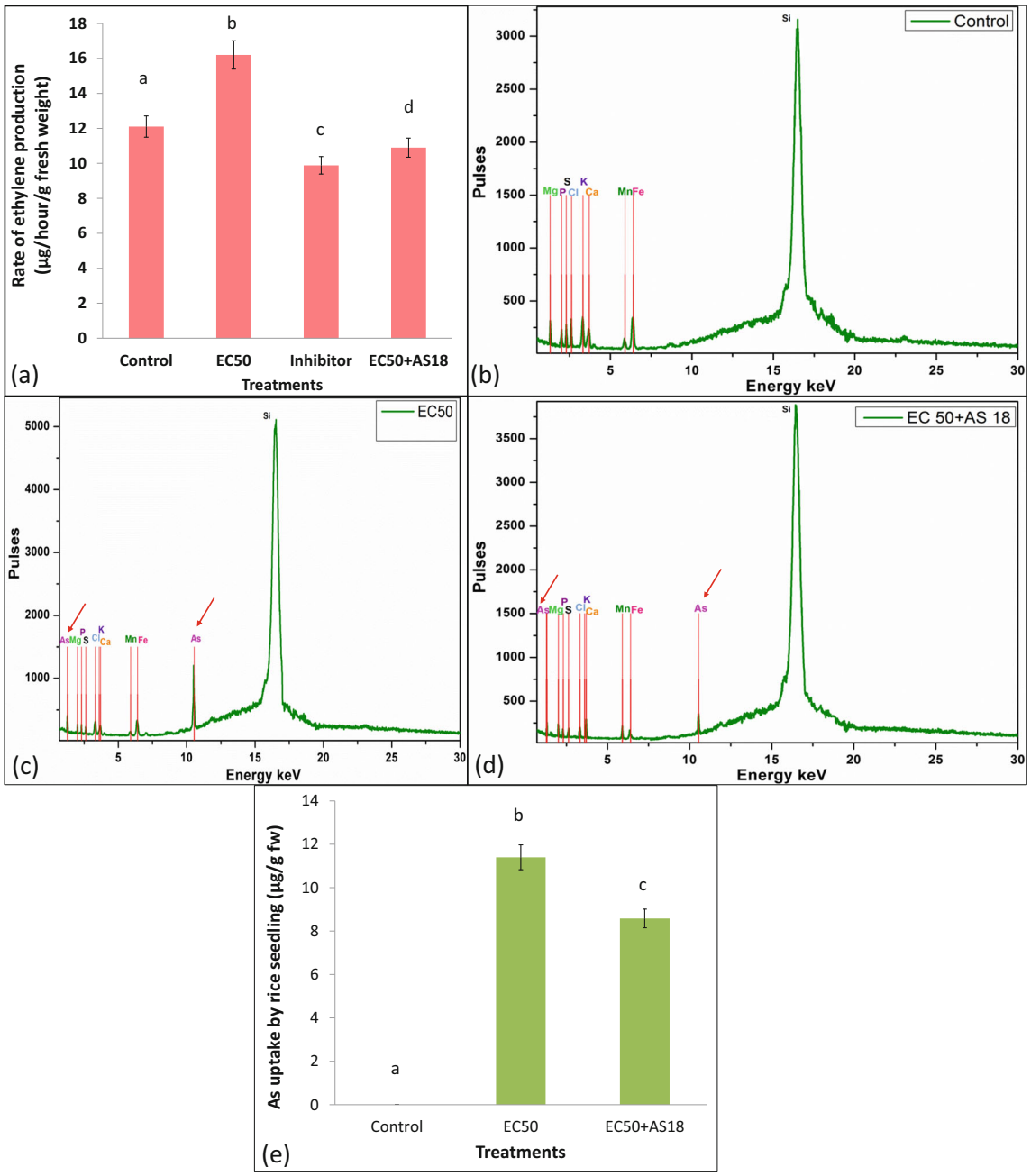


Fig. 7 Effect of AS18 strain on ethylene production and As uptake in rice seedlings. **a** Rate of stress ethylene production under As-stress, **b** XRF of As-untreated rice seedlings (control), **c** XRF of As-treated rice seedlings (EC50), **d** XRF of As-treated rice seedlings with bacterial strain (EC50 + AS18), **e** influence of PGPR strains AS18 on As uptake by rice seedlings. Minimization of both stress ethylene and As-content is evident from these studies

(such as *Bacillus flexus*, *B. aryabhattai*, *Pseudomonas* sp., *Enterobacter* sp., etc.) assist plants to thrive in As-spiked conditions (Das et al. 2014; Das et al. 2016; Ghosh et al. 2018).

Reduced As content in rice seedlings by strain AS18

From the XRF analysis of plant samples with different treatments (control, EC50, EC50 + AS18), it was found that As peak was prominent in EC50- and AS18-treated set while in control it was not detected (Fig. 7b–d). However, the peak height is less in AS18-treated set compared to EC50. This finding can be correlated with As bioaccumulation efficacy of the said strain as revealed from TEM-XRD-FTIR-XRF studies (Fig. 3). Furthermore, it appears that AS18 influences As mobilization into rice seedlings resulted in a reduction of As concentration in bacteria-treated seedlings. Reduction of As accumulation in rice by *Pantoea* sp. has also been reported by Lakshmanan et al. (2015). Furthermore, PGPR-mediated reduction in heavy metal content was perceived in rice seedlings along with phytostimulation under Cd stress (Pramanik et al. 2017, 2018). Das et al. (2016) also showed the As-resistant *Bacillus flexus* ASO-6 to reduce As accumulation in shoots and grains of rice. Application of AS18 strain on rice seedling showed decreased As uptake in As-treated seedbed compared to the As-treated non-inoculated seedbed set (Fig. 7e). The results clearly suggest the bacteria-induced reduction in As toxicity in plants. The selected strain could be explored for As elimination from the environment and reduced As uptake by plants for better growth. The results are correlated to the findings of the *Exiguobacterium* strain applied in the *Vigna radiata* plant under As stress (Pandey and Bhatt 2016).

Conclusion

In this study, the *Pantoea dispersa* strain AS18 was screened as a multi-heavy metal resistant, As bioaccmulating, and halotolerant bacterium that possesses numerous PGP attributes of great agroeconomic importance including phosphate solubilization, IAA production, ACC deaminase activity, and N_2 fixation which are essential for both stress tolerance and plant growth promotion. Improved chlorophyll contents, antioxidant enzyme activities, enhanced seedling growth with a simultaneous reduction in As uptake, and stress ethylene levels in plants upon AS18 inoculation also reflected the same. The combined effect of these growth-promoting parameters attenuates As-induced phytotoxicity of rice seedlings. Therefore, the strain might be helpful in bioremediation of As and could be seen as a part of As removal strategy in the As-contaminated crop fields for sustainable agriculture.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-020-11816-7.

Authors' contributions AG performed most of the experiments starting from soil sample collection, isolation, screening, and characterization of rhizobacterial strain; perfomed plant growth promotion experiments; and analyzed and interpreted the data including writing the first draft of the manuscript. KP has monitored antioxidant enzymes of rice seedlings, performed the statististical analysis, prepared the phylogenetic tree of AS18 strain, and analyzed and interpreted the data including the revision and editing of the MS. SB has contributed to all the experiments related to As bioaccumulation of AS18 strain (TEM, EDAX, XRD, FTIR and AAS) except XRF analysis, AAS analysis of rice seedlings, analyzed and interpreted the data including the revision of the MS. SM performed XRF analysis of AS18 strain as well as revised the MS. SKG has performed the viability test of AS18 strain. PKG has performed the qualititative test for As oxidation-reduction experiment of AS18 strain. TKM has contributed to the experimental conception, design, supervision, validation of the data editing, and revision of the MS. All authors read and approved the final manuscript.

Funding The first author is thankful to the University of Burdwan, West Bengal, India, for awarding the institutional fellowship (No.: 2018/75). The authors are also gratefully acknowledged to Council of Scientific and Industrial Research (CSIR), India, for financial support for this work [vide letter No. 38 (1469)/18/EMR-II, dt.04.04.18]. KP gratefully acknowledges the financial assistance from the University Grants Commission (UGC), New Delhi, for UGC—Dr. D. S. Kothari Post-Doctoral Fellowship [No.F.4-2/2006 (BSR)/BL/19-20/0072 dated October 21, 2019].

Data availability All data generated or analyzed during this study are included in this published article (and its supplementary information files). The 16S rDNA sequence of *Pantoea dispersa* strain AS18 is available in NCBI database vide accession number—MH605572 and the strain is available at NCMR, NCCS, Pune, India, with strain accession—MCC 4044.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

References

Abbas G, Murtaza B, Bibi I, Shahid M, Niazi NK, Khan MI, Amzad M, Hossain M, Natasha (2018) Arsenic uptake, toxicity, detoxification, and speciation in plants: physiological, biochemical, and molecular aspects. Int J Environ Res Public Health 15:59

Aebi H (1984) Oxygen radicals in biological systems. Methods Enzymol 105:121–126

Ahmad I, Akhtar MJ, Zahir ZA, Naveed M, Mitter B, Sessitsch A (2014) Cadmium-tolerant bacteria induce metalstress tolerance in cereals. Environ Sci Pollut Res 21:11054–11065

Ahmad I, Akhtar MJ, Asghar HN, Ghafor U, Shahid M (2016) Differential effects of plant growth-promoting rhizobacteria on maize growth and cadmium uptake. J Plant Growth Regul 35:303–315

Andrews JM (2001) Determination of minimum inhibitory concentrations. J Antimicrob Chemother 48:5–16

Arivalagan P, Singaraj D, Haridass V, Kaliannan T (2014) Removal of cadmium from aqueous solution by batch studies using *Bacillus cereus*. Ecol Eng 71:728–735

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24:1–15

Atlas, R.M. (2005) Handbook of Media for Environmental Microbiology, Second Edition. USA: CRC Press

Bal HB, Nayak L, Das S, Adhya TK (2012) Isolation of ACC deaminase producing PGPR from rice rhizosphere and evaluating their plant growth promoting activity under salt stress. Plant Soil 366:93–105

Banerjee S, Datta S, Chattyopadhyay D, Sarkar P (2011) Arsenic accumulating and transforming bacteria isolated from contaminated soil for potential use in bioremediation. J Environ Sci Health A Tox Hazard Subst Environ Eng 46:1736–1747

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207

Benson HJ (1990) Microbiological application—a lab manual in general microbiology. Brown Publishers, USA, W. C

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350

Bradford MM (1976) A rapid and sensitive method for the quantitation microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Bric JM, Bostock RM, Silverstone SE (1991) Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. Appl Environ Microbiol 57:535–538

Cavalca L, Corsini A, Enrica C, Zanchi R (2015) Rhizobacterial communities associated with spontaneous plant species in long-term arsenic contaminated soils. World J Microbiol Biotechnol 31:735–746

Chen Y, Chao Y, Li Y, Lin Q, Bai J, Tang L, Wang S, Ying R, Qiu R (2016) Survival strategies of the plant-associated bacterium *Enterobacter* sp. strain EG16 under cadmium stress. Appl Environ Microbiol 82:1734–1744

Chmielowska-bąk J, Lefèvre I, Lutts S, Kulik A, Deckert J (2014) Effect of cobalt chloride on soybean seedlings subjected to cadmium stress. Acta Soc Bot Pol 83:201–207

Cummings DE, Caccavo F, Fendorf S, Rosenzweig RF (1999) Arsenic mobilization by the dissimilatory Fe (III)-reducing bacterium *Shewanella alga* BrY. Environ Sci Technol 33:723–729

Das J, Sarkar P (2018) Remediation of arsenic in mung bean (*Vigna radiata*) with growth enhancement by unique arsenic-resistant bacterium *Acinetobacter lwoffii*. Sci Total Environ 624:1106–1118

Das S, Jean JS, Kar S, Chou ML, Chen CY (2014) Screening of plant growth-promoting traits in arsenic-resistant bacteria isolated from agricultural soil and their potential implication for arsenic bioremediation. J Hazard Mater 272:112–120

Das S, Jean JS, Chou ML, Rathod J, Liu CC (2016) Arsenite-oxidizing bacteria exhibiting plant growth promoting traits isolated from the rhizosphere of *Oryza sativa* L.: implications for mitigation of arsenic contamination in paddies. J Hazard Mater 302:10–18

Davis BD, Mingioli ES (1950) Mutants of Escherichia coli requiring methionine or vitamin B12. J Bacteriol 60:17–28

Deokar AR, Lin LY, Chang CC, Ling YC (2013) Single-walled carbon nanotube coated antibacterial paper: preparation and mechanistic study. J Mater Chem B 1:2639–2646

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Fick GN, Qualset CO (1975) Genetic control of endosperm amylase activity and gibberellic acid reoponses in standard-height and short-statured wheats. Proc Natl Acad Sci U S A 72:892–895

Finnegan PM, Chen W (2012) Arsenic toxicity: the effects on plant metabolism. Front Physiol 3:1–18

Fiske CH, Subbarow Y (1925) The colorimetric determination of phosphorus. J Biol Chem 66:375–400

Ghosh P, Rathinasabapathi B, Ma LQ (2015) Phosphorus solubilization and plant growth enhancement by arsenic-resistant bacteria. Chemosphere 134:1–6

Ghosh PK, Maiti TK, Pramanik K, Ghosh SK, Mitra S, De TK (2018) The role of arsenic resistant *Bacillus aryabhattai* MCC3374 in promotion of rice seedlings growth and alleviation of arsenic phytotoxicity. Chemosphere 211:407–419

Giannopolitis CN, Ries SK (1977) Superoxide dismutases: I. Occurrence in higher plants. Plant Physiol 59:309–314

Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012:1–15

Glick BR, Penrose DM, Li J (1998) A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J Theor Biol 190:63–68

Goswami D, Dhandhukia P, Patel P, Thakker JN (2014) Screening of PGPR from the saline desert of Kutch: growth promotion in *Arachis hypogea* by *Bacillus lichenoformis* A2. Microbiol Res 169:66–75

Hashem A, Abd Allah EF, Alqarawi AA, Eqamberdieva D (2016) Bioremediation of adverse impact of cadmium toxicity on *Cassia italica* Mill by arbuscular mycorrhizal fungi. Saudi J Biol Sci 23:39–47

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189–198

Hseu Z, Chen Z, Tsai C, Tsui C, Cheng S, Liu C, Lin H (2002) Digestion methods for total heavy metals in sediments and soils. Water Air Soil Pollut 141:189–205

Kaushal M, Kaushal R (2015) Acetylene reductase activity and molecular characterization of plant growth promoting rhizobacteria to know efficacy in integrated nutrient management system. Indian J Biotechnol 14:221–227

Khan AA, Faust MA (1967) Effect of growth retardants on alpha-amylase production in germinating barley seed. Physiol Plant 20:673–681

Kidric M, Kos J, Sabotic J (2014) Proteases and their endogenous inhibitors in the plant response to abiotic stress. Bot Serb 38:139–158

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Lakshmanan V, Shantharaj D, Li G, Seyfferth AL, Sherrier DJ, Bais HP (2015) A natural rice rhizospheric bacterium abates arsenic accumulation in rice (*Oryza sativa* L) Planta 242:1037–1050

Majumder A, Ghosh S, Saha N, Kole SC, Sarkar S (2013) Arsenic accumulating bacteria isolated from soil for possible application in bioremediation. J Environ Biol 34:841–846

Mallick I, Bhattacharyya C, Mukherji S, Dey D, Sarkar SC, Mukhopadhyay UK, Ghosh A (2018) Effective rhizoinoculation and biofilm formation by arsenic immobilizing halophilic plant growth promoting bacteria (PGPB) isolated from mangrove rhizosphere: a step towards arsenic rhizoremediation. Sci Total Environ 610–611:1239–1250

Murthy S, Bali G, Sarangi SK (2014) Effect of lead on growth, protein and biosorption capacity of *Bacillus cereus* isolated from industrial effluent. J Environ Biol 35:407–411

Pandey N, Bhatt R (2015) Arsenic resistance and accumulation by two bacteria isolated from a natural arsenic contaminated site. J Basic Microbiol 55:1275–1286

Pandey N, Bhatt R (2016) Role of soil associated *Exiguobacterium* in reducing arsenic toxicity and promoting plant growth in *Vigna radiata*. Eur J Soil Biol 75:142–150

Pandey S, Ghosh PK, Ghosh S, De TK, Maiti TK (2013) Role of heavy metal resistant *Ochrobactrum* sp. and *Bacillus* spp. strains in bioremediation of a rice cultivar and their PGPR like activities. J Microbiol 51:11–17

Pandey N, Manjunath K, Sahu K (2020) Screening of plant growth promoting attributes and arsenic remediation efficacy of bacteria isolated from agricultural soils of Chhattisgarh. Arch Microbiol 202:567–578

Paredes-Páliz KI, Caviedes MA, Doukkali B, Mateos-Naranjo E, Rodríguez-Llorente ID, Pajuelo E (2016) Screening beneficial rhizobacteria from *Spartina maritima* for phytoremediation of metal polluted salt marshes: comparison of gram-positive and gram-negative strains. Environ Sci Pollut Res 23:19825–19837

Penrose DM, Glic BR (2003) Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Physiol Plant 118:10–15

Pramanik K, Mitra S, Sarkar A, Soren T, Maiti TK (2017) Characterization of cadmium-resistant *Klebsiella pneumoniae* MCC 3091 promoted rice seedling growth by alleviating phytotoxicity of cadmium. Environ Sci Pollut Res 24:24419–24437

Pramanik K, Mitra S, Sarkar A, Maiti TK (2018) Alleviation of phytotoxic effects of cadmium on rice seedlings by cadmium resistant PGPR strain *Enterobacter aerogenes* MCC 3092. J Hazard Mater 351:317–329

Prasad KS, Srivastava P, Subramanian V, Paul J (2011) Biosorption of As (III) ion on *Rhodococcus* sp. WB-12: biomass characterization and kinetic studies. Sep Sci Technol 46:2517–2525

Pratush A, Kumar A, Hu Z (2018) Adverse effect of heavy metals (aAs, Pb, Hg, and Cr) on health and their bioremediation strategies: a review. Int Microbiol 21:97–106

Qamar N, Rehman Y, Hasnain S (2017) Arsenic-resistant and plant growth-promoting firmicutes and γ-proteobacteria species from industrially polluted irrigation water and corresponding crop land. J Appl Microbiol 123:748–758

Rahman A, Nahar N, Nawani NN, Jass J, Desale P, Kapadnis BP, Saha AK, Ghosh S, Olsson B, Mandal A (2014) Isolation and characterization of a *Lysinibacillus* strain B1- CDA showing potential for bioremediation of arsenics from contaminated water. J Environ Sci Health A Tox Hazard Subst Environ Eng 49:1349–1360

Rahman MS, Jamal MAHM, Biswas PK, Rahman SM, Sharma SP, Saha SK, Hong ST, Islam MR (2020) Arsenic remediation in Bangladeshi rice varieties with enhance plant growth by unique arsenic-resistant bacterial isolates. Geomicrobiol J 37:130–142

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York

Sarkar A, Ghosh PK, Pramanik K, Mitra S, Soren T, Pandey S, Mondal MH, Maiti TK (2018) A halotolerant *Enterobacter* sp. displaying ACC deaminase activity promotes rice seedling growth under salt stress. Res Microbiol 169:20–32

Sengupta K, Maiti TK, Saha P (2015) Degradation of 4-nitrophenol in presence of heavy metals by a halotolerant *Bacillus* sp. strain BUPNP2, having plant growth promoting traits. Symbiosis 65: 157–163

Sher S, Rehman A (2019) Use of heavy metals resistant bacteria—a strategy for arsenic bioremediation. Appl Microbiol Biotechnol 103:6007–6021

Simeonova DD, Lièvremont D, Lagarde F, Muller DAE, Groudeva VI, Lett MC (2004) Microplate screening assay for the detection of arsenite-oxidizing and arsenate-reducing bacteria. FEMS Microbiol Lett 237:249–253

Singh N, Gupta S, Marwa N, Pandey V, Verma PC, Rathaur S, Singh N (2016) Arsenic mediated modifications in *Bacillus aryabhattai* and their biotechnological applications for arsenic bioremediation. Chemosphere 164:524–534

Snell FD, Snell CT (1971) Colorimetric methods of analysis. Van Nostard Reinford Co., New York

Tsai SL, Singh S, Chen W (2009) Arsenic metabolism by microbes in nature and the impact on arsenic remediation. Curr Opin Biotech 20: 659–667

Vezza ME, Olmos Nicotra MF, Agostini E, Talano MA (2020) Biochemical and molecular characterization of arsenic response from *Azospirillum brasilense* Cd, a bacterial strain used as plant inoculant. Environ Sci Pollut Res 27:2287–2300

Xiao AW, Li Z, Li WC, Ye ZH (2020) The effect of plant growth-promoting rhizobacteria (PGPR) on arsenic accumulation and the growth of rice plants (*Oryza sativa* L.). Chemosphere 242:125136

Yang Y, Zhang FS, Li HF, Jiang RF (2009) Accumulation of cadmium in the edible parts of six vegetable species grown in Cd-contaminated soils. J Environ Manag 90:1117–1122