Chemosphere 211 (2018) 407–419

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

The role of arsenic resistant *Bacillus aryabhattai* MCC3374 in promotion of rice seedlings growth and alleviation of arsenic phytotoxicity

Pallab Kumar Ghosh [a, *], Tushar Kanti Maiti [b], Krishnendu Pramanik [b], Sudip Kumar Ghosh [b], Soumik Mitra [b], Tarun Kumar De [a]

[a] Department of Marine Science, Ballygunge Science College Campus, Calcutta University, 35, B.C.Road, Kolkata, 700019, India
[b] Microbiology Laboratory, CAS, Department of Botany, Burdwan University, Burdwan, Pin. 713104, WB, India

HIGHLIGHTS

- As resistant *Bacillus aryabhattai* AS6 strain isolated from contaminated rhizosphere.
- AS6 strain could tolerate As (v) and As (III) upto 100 mM and 20 mM respectively.
- It exhibited IAA and siderophore production, P-solubilization and ACCD activity.
- High As removal and bioaccumulation of AS6 confirmed from various *in vitro* studies.
- It improved rice seedling growth under As(V)-spiked soil by reducing phytotoxicity.

ARTICLE INFO

Article history:
Received 28 March 2018
Received in revised form
23 July 2018
Accepted 24 July 2018
Available online 26 July 2018

Handling Editor: T. Cutright

Keywords:
Bioremediation
Arsenate reduction
Heavy metal
1-Aminocyclopropane-1-carboxylate deaminase
Plant growth promotion
Ethylene production

ABSTRACT

The biological agents have been utilized as an affordable alternative to conventional costly metal remediation technologies for last few years. The present investigation introduces arsenic (As) resistant plant growth promoting rhizobacteria (PGPR) isolated from the As-contaminated agricultural field of West Bengal, India that alleviates arsenic-induced toxicity and exhibited many plant growth promoting traits (PGP). The isolated strain designated as AS6 has identified as *Bacillus aryabhattai* based on phenotypic characteristics, physio-biochemical tests, MALDI-TOFMS bio-typing, FAME analysis and 16S rDNA sequence homology. The strain found to exhibit five times more resistance to arsenate than arsenite with minimum inhibitory concentrations (MIC) being 100 mM and 20 mM respectively. The result showed that accumulation of As was evidenced by SEM- EDAX, TEM-EDAX studies. The intracellular accumulation of arsenic was also confirmed as in bacterial biomass by AAS, FTIR, XRD and XRF analyses. The increased rate of As (V) reduction by this strain found to be exploited for the remediation of arsenic in the contaminated agricultural field. The strain also found to exhibit important PGP traits viz., ACC deaminase activity (2022 nmol α-ketobutyrate/mg protein/h), IAA production (166 μg/ml), N_2 fixation (0.32 μgN fixed/h/mg proteins) and siderophore production (72%) etc. Positive influenced of AS6 strain on rice seedlings growth promotion under As stress was observed considering the several morphological, biochemical parameters including antioxidants activities as compared with an uninoculated set. Thus this strain might be exploited for stress amelioration and plant growth enhancement of rice cultivar under arsenic spiked agricultural soil.

1. Introduction

Arsenic is a toxic metalloid caused serious health problems were described as “the greatest mass poisoning in human history” by World Health Organization (WHO, 2001) and recognized as “Class-1 human carcinogen” by the USEPA (United States Environmental Protection Agency) as a global concern (Ng et al., 2003). In the periodic table, Arsenic (As) belongs to a group 15, period 4, P block

Abbreviations: MALDI-TOFMS, Matrix assisted laser desorption ionization-time of flight mass spectrometry; FAME, Fatty acid methyl ester; SEM, Scanning electron microscopy; TEM, Transmission electron microscopy; EDAX, Energy dispersive X-ray spectroscopy; FTIR, Fourier transform infrared spectroscopy; XRF, X-ray fluorescence.

* Corresponding author.
E-mail address: pal.mic.bu@gmail.com (P.K. Ghosh).

https://doi.org/10.1016/j.chemosphere.2018.07.148
0045-6535/

element with an electronic configuration as (Ar) $3d^{10}$ $4s^2$ $4p^3$. In nature, As can occur in four oxidation states i.e., -3, 0, +3 and + 5. In the environment, As (V) and As (III) are exist, most predominant, biologically relevant/bio-available forms. Among these, As (III) is being more toxic and mobile than As (V) (Rhine et al., 2006). Both acute and chronic As poisoning to human has raised great concerns, especially in heavily contaminated areas in different parts of India especially in West Bengal (Majumder et al., 2013). Continuous use of As contaminated groundwater for crop irrigations significantly increased the As level in the soil in some part of West Bengal (Sanyal and Dhillon, 2005). Therefore, there is a potential risk of As contamination in rice crop leading to food chain contamination resulted in the deleterious human health problem (Williams et al., 2009; Li et al., 2018).

Moreover, As accumulation in the soil exceeds natural sources by 3:1 due to excessive use of arsenical pesticides, application of fertilizers, dust from the burning of fossil fuels, medicines, disposal of industrial, metallurgy and animal wastes (Nordstrom, 2002). In another side, elevated concentration of As in soil is absorbed by the roots and transported to different plant parts leading to impaired metabolism (Zhao et al., 2013), reduced growth and decreased crop production (John et al., 2009).

Heavy metals are nondegradable and only change may occur in the nuclear structure of the element. Till now, several methods have been adopted for their remediation, however, no such methods are suitable for practical applications, for their high cost, low efficiency, destruction of soil structure and fertility, high dependence on the contaminants, site conditions etc. In this respect bioremediation is an emerging approach which offers an eco-friendly and low cost technology to mitigate or stabilize the soil contaminants such as As toxicity in the environment.

In nature, microorganisms cope with As toxicity in different ways (act as precipitation, chelation, compartmentalization, extrusion and biochemical transformation) (Paez-Espino et al., 2009; Tsai et al., 2009; Khan et al., 2015) and plays an important role in the As Geo-cycle (Mukhopadhyay et al., 2002). Thus, the bioremediation strategies can be adopted for effective reclamation of As contaminated agricultural sites that helps to increase crop yield essential for food security.

Heavy metals (HMs) resistant PGPR (Plant Growth Promoting Rhizobacteria) can ameliorate metal toxicity by using different mechanisms and modulate plant growth under HMs stress by neutralizing reactive oxygen species (ROS) and producing enzymes viz. 1-aminocyclopropane-1-carboxylate deaminase (ACCD), phosphatase/phytase, secreting phytostimulants viz. indole acetic acid (IAA) and producing siderophores, hydrocyanic acid (HCN), NH_3 and extracellular polysaccharide (EPS) etc. (Ma et al., 2011; De-Bashan et al., 2012). These HMs resistant PGPR are known to increase the tolerance of plants against HMs or metalloid and enhance plant growth in contaminated soils (Ma et al., 2011; De-Bashan et al., 2012).

Bacillus species is a large heterogeneous group and very few numbers of As resistant *Bacillus* spp. exhibiting plant growth promoting (PGP) traits has been studied in relation to their role in PGP activities of rice cultivars (Pandey et al., 2013; Lakshmanan et al., 2015; Das et al., 2016; Tiwari et al., 2016; Mallick et al., 2018). Although other As resistant bacterial spp. viz. *Pseudomonas* sp., *Comamonas* sp. and *Stenotrophomonas* sp. (Ghosh et al., 2011); *Staphylococcus arlettae* strain NBRIEAG-6 (Srivastava et al., 2013); *Brevibacillus* sp. KUMAs2 (Mallick et al., 2014); *Exiguobacterium* sp. (Pandey and Bhatt, 2016); *P. aeruginosa* KUJM (Biswas et al., 2017); *Kocuria flava* AB402 (Mallick et al., 2018) have also been reported to enhance plant growth. However, the search for newer potent As resistant PGPR that can thrive in a specific ecological niche of contaminated soil is always relevant (Srivastava et al., 2013).

The principal objective of the present study is to isolate potent As resistant PGPR strain from contaminated industrial soils and its identification by the polyphasic approach of bacterial taxonomy. Attempts are also made to determine its bioaccumulation ability of As by AAS, EDAX, XRD, XRF and biotransformation ability by oxidation reduction assay. Besides, this investigation was also aimed to determine increased plant growth promotion and amelioration of As stress of rice seedlings by selected strain.

2. Materials and methods

2.1. Soil sample analysis and isolation of arsenic resistant isolates

The rhizospheric (rice) soil samples were collected from a heavy metal polluted agricultural field near the Industrial belt of Durgapur, India. Different physio-chemical properties (APHA, 2005) and HMs content were measured by using Atomic Absorption Spectrophotometer (AAS) according to Abdel-Lateef et al. (2013). Isolation and enumeration of As resistant strains were done according to Aksornchu et al. (2008) using basal salt medium (BSM) and desirable colonies were selected.

2.2. Determination of minimum inhibitory concentration (MIC) and screening of isolates

For determination of MIC, all the isolates were further grown in BSM medium supplemented with increasing concentrations of As (V) and As (III) (Pandey and Bhatt, 2015). Isolates exhibiting maximum MIC were selected for further study.

2.3. Plant growth promoting (PGP) attributes

2.3.1. PGP traits of the selected isolates

The PGP traits viz. production of IAA, siderophore, HCN, EPS and phosphate solubilization of selected isolates (AS4, AS6, AS7, AS11, AS12 and AS14) were performed according to Wevar Oller et al. (2013). The nitrogenase activities of the selected isolates were determined by Acetylene reduction test using Gas Chromatography (VARIAN CP3800) following Kaushal and Kaushal (2015) and ACCD activities were measured according to Pandey et al. (2013). The isolate AS6 was selected based on the highest MIC value and the presence of different PGP traits among initially selected isolates.

2.3.2. Effect of As on some important PGP traits in culture

The effect of As (III) and As (V) on some important PGP traits *viz.* IAA production, ACCD activity, phosphate solubilization and siderophore production in the culture of isolate AS6 was tested. The isolate AS6 was grown in respective culture media supplemented with 5 mM As at $32 \pm 2\,^{\circ}C$ up to 72 h and PGP traits were measured (Paredes-Paliz et al., 2016).

2.4. Arsenic transformation by the AS6 strain

The oxidation-reduction assay was performed according to Simeonova et al. (2004). The development of brown colour and yellow precipitate in the culture media confirmed the formation of silver arsenate (arsenite oxidase) and silver arsenite (arsenate reductase) respectively (Banerjee et al., 2011). The estimation was performed according to Qamar et al. (2017).

2.5. Determination of growth, removal assay and bioaccumulation efficiency

The growth kinetics of AS6 strain was determined in BSM broth containing As (V) and As (III) separately. The AS6 strain was

incubated at 32 ± 2 °C in 120 rpm for 72 h and growth was measured at every 12 h interval. The bioaccumulation of As and its removal were measured according to Pandey and Bhatt (2015). The cell pellets of each treatment were digested with aquaregia (HNO_3: HCl = 1: 3) in Anton Paar MDS (Rotor Model No.12HF100), filtered and finally, accumulation was estimated by AAS. Percentage of As removal was calculated by following equation (Pandey and Bhatt, 2015).

Arsenic removal = [(IC − FC)/IC] X 100 [IC - Initial concentration of arsenic (mM), FC - Final concentration]

2.6. Identification of the selected isolate

2.6.1. Phenotypic and physio-biochemical characterization of the AS6 strain

Different morphological and physio-biochemical characteristics were determined using standard methods of microbiology (Benson, 1990). The AS6 strain was identified by Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis (Pavlovic et al., 2013) followed by fatty acid methyl ester (FAME) analysis (Sasser, 2001) and 16S rDNA sequence homology.

2.6.2. 16S rDNA sequencing and phylogeny

The genomic DNA of AS6 strain was isolated according to Sambrook et al. (1989) and 16S rRNA gene was amplified using universal primers 16F27 (5′-CCAGAGTTTGATCMTGGCTCAG-3′) and 16R1492 (5′-TAC GGYTACCTTGTTACGACTT-3′) following Panday et al. (2011). The PCR product was purified by PEG-NaCl precipitation and sequenced on an automated DNA sequencer (Applied Biosystems 3730XL, Foster City, CA). The assembled nucleotide sequence was compared by the EzTaxon-e server (Kim et al., 2012) and phylogenetic tree was constructed using MEGA 6 software package with calculating the best-fitted model (The Biodesign Institute, Arizona, USA). The AS6 strain and 16S rDNA sequence were deposited to Microbial Culture Collection (MCC), Pune, India and to (The National Centre for Biotechnology Information) NCBI database for accession numbers respectively.

2.7. Microscopic studies and elemental analysis

2.7.1. SEM, TEM and EDAX studies

To study the effect of arsenic on the cell surface morphology, scanning electron microscopy (SEM) was performed. The AS6 strain was grown on BSM broth containing 5 mM of As (V) and As free set as a control. The harvested cells washed with sodium phosphate buffer (pH 7.4) and fixed with 2% glutaraldehyde followed by subsequent dehydration in ethanol from 30 to 50% (v/v). Fixed cells were put on the tab, coated with gold particle and observed under SEM (Zeiss-EVO18). The elemental content was analyzed by energy-dispersive-X-ray spectroscopy (EDAX) with INCA-X-Sight (Oxford Instruments, United Kingdom) probe.

For Transmission electron microscopy (TEM) study, the sample was prepared similarly to SEM preparation. The sample was placed on a copper coated carbon grid (300 mesh) and observed under TEM (JEOL 1200 EX II). EDAX analysis was also performed by the same sample. The viability of AS6 strain was tested with Triphenyl Tetrazolium Chloride (TTC) (1%) and the positive result was confirmed by colour development (Pandey and Bhatt, 2015).

2.7.2. FTIR, XRD and XRF analyses

For FTIR analysis, the AS6 strain was grown in broth with the presence and absence of As (V). The harvested cell was washed with milli-pore water and lyophilized samples were prepared in KBr (Sample: KBr = 1: 3) pellets for FTIR spectrometer (PerkinElmer 2000) analysis.

The lyophilized sample was used for X-ray diffraction (XRD) analysis using a diffractometer Smart Lab (Rigaku) with a diffracted beam of CuKα, diffraction patterns an angular range 5°–80° and 2° min^{-1}. The peaks were identified by X'Pert High Score Plus software (Rigaku PDXL 2.4.2.0 version). The same sample was used for X-ray fluorescence (Bruker-Artex elemental analysis at 50 kv and 698 μA) analysis for detection of As and total elemental content in cell pellets.

2.8. Plant growth promoting activities by AS6 strain under As stress

2.8.1. Determination of EC_{50} of As with respect to seed germination

Certified seeds of rice variety (Swarnamasuri, No.: MTU7029) were procured from Hooghly Krishi Vigyan Kendra, (ICAR centre), West Bengal, India. Surface sterilized seeds (1.5% NaClO for 15 min) were placed on blotting papers amended with increasing concentrations of As (V) aqueous solution. The Petri dishes were then placed in a growth chamber at 24 ± 2 °C and a relative humidity of 70–80% first in dark condition for three consecutive days. As concentration that inhibited germination by 50% was considered as the EC_{50} used for further plant growth promotion experiments and performed in triplicate sets.

2.8.2. Influence of the AS6 strain on relative root and shoot elongation of the cultivar

For pot experiments, soil sample was collected from rural and non-industrial agricultural fields of West Bengal India. Arsenic free soil samples were (determined by XRF analysis) dried in sunlight and sieved through a 2 mm sieve. Eighty percent of pot height was poured by soil (2 kg each) and sterilized fully by autoclave. All the experimental pots were prepared according to Guo and Chi (2014). The AS6 strain was used as an inoculant [AS6 strain grown in BSM broth and serially diluted with 0.9% (w/v) normal saline] and mixed with soil at a level of 10^6 CFU/g soil (Pandey and Bhatt, 2016). The germinated seeds (with 3–5 mm radicals) were again surfaced sterilized and sown in soil of pot experiment (50 seeds in each set). The experimental setup was designed as, control set I - [without As (V) and AS6 strain], EC_{50} set II - [with As (V) but without AS6 strain] and EC_{50} + AS6 set III - [with As (V) + AS6 strain]. Germination percentage, relative root-shoot elongation (RRE and RSE) and seed vigour index (SVI) were determined and calculated according to Bal et al. (2013).

2.8.3. Determination of amylase and protease activity

To determine the amylase and protease activities, 500 mg of seedlings was prepared according to Snell and Snell (1971) and estimated following Khan and Faust (1967). Protein content was estimated by Lowry et al. (1951). Enzyme activities were calculated according to Fick and Qualset (1975). The effectiveness of AS6 strain was studied comparing by inoculation of an *Escherichia coli strain* as negative control.

2.8.4. Determination of antioxidant enzymes

The enzyme extraction from the seedlings was performed according to Pandey et al. (2013). To determine SOD (superoxide dismutase) (EC 1.15.1.1) activity, after extraction the supernatant was used as enzyme source and SOD activity was measured by inhibition of photoreduction of nitroblue tetrazolium (NBT) according to Dhindsa et al. (1981). To measure the MDA content (Malondialdehyde), 1 ml of supernatant was mixed with 3 ml of 5% Trichloroacetic acid (TCA) containing thiobarbituric acid (TBA 1%), heated on a water bath for 30 min. MDA concentration was

measured in a spectrophotometer (Heath and Packer, 1968). Catalase (CAT) (1.11.1.6) activity was measured according to Aebi (1984).

2.8.5. Determination of proline

For proline measurement, samples were prepared according to enzyme extraction. After that ninhydrin solution was added and the mixture was heated in a boiling water bath for 1 h. After cooling, the mixture was extracted with toluene and O.D. was taken at 520 nm (Bates et al., 1973).

2.8.6. Determination of stress ethylene by Gas Chromatography (GC)

The widely accepted mechanism of PGPR strain under abiotic stress (like heavy metal stress) is the expression of ACCD that found to reduce plant stress ethylene level (Glick, 2005). The stress ethylene level under As (V) stress was determined according to Siddikee et al. (2011). Similar three experimental set up was prepared as mentioned above (section 2.8.2) to compare the effect of AS6 strain on stress ethylene production. In addition, a fourth experimental set up of seedlings treated with aminoethoxyvinylglycine (AVG) (a natural C_2H_4 inhibitor) was designed to detect the effect of inhibitor on ethylene production under arsenic stress. However, unlike the previous set up both the inhibitor and arsenic stress were imposed on the ninth day of growing seedlings. After 24 h, the seedlings from each set were carefully taken out of the respective pots and confined into separate airtight sterile glass tubes and kept in dark for 1 h at 30 °C. From each tube 1 ml gas was drawn and injected into Gas Chromatography (VARIAN CP3800) to estimate ethylene production. The amount of C_2H_4 was estimated as nmol of C_2H_4/g fresh weight/h by comparing with the standard pure C_2H_4 (Siddikee et al., 2011).

2.9. Statistical analysis

Each experiment was performed in triplicate and data are expressed as the mean ± standard error (SE). Difference between group means was tested by one-way analysis of variance (ANOVA) assuming equal variances using Microsoft Excel 2010 software package (Microsoft Corp.; Redlands, WA, USA). The differences between the control and treatments were determined by Student's *t*-test. Significant differences at $p < 0.05$ are represented by different letter alphabets above the bars.

3. Results and discussion

3.1. Isolation and selection of arsenic resistant bacteria

The different physiochemical properties including heavy metal contents of soil samples were determined (Supplementary file Table 1). The microflora of contaminated soil had been exposed to greater amounts of metals often leads to developing some metal resistant properties which are often found to be associated with plant growth promotion (Murthy et al., 2014). The physiochemical characters of the soil sample greatly affected the rhizospheric microflora which is dependent on available soil nutrient and environmental factor (Goswami et al., 2014).

Initially, 22 bacterial isolates (designated AS1 to AS22) were isolated from collecting soil samples. Among these, 10 bacterial isolates were screened out based on the tolerance of As (III) above 10 mM level (Supplementary file Table 2). AS4, AS6, AS7, AS11, AS12 and AS14 strains were further selected based on ACCD activities for further study (Table 1). Exposure of these strains to the gradient of As concentrations during enrichment might have exerted a selective pressure on bacteria to develop metal resistance systems for protecting cellular components (Pandey and Bhatt, 2015). The higher MIC value of any As resistant isolate is expected to confer better adaptability in As contaminated soil and better utilized for bioremediation purpose in agricultural fields. Several arsenic-resistant bacteria have been reported to play a key role in bioremediation of As pollutants (Srivastava et al., 2013; Das et al., 2014; Tiwari et al., 2016; Pandey and Bhatt, 2016; Mallick et al., 2018).

3.2. Plant growth promoting attributes

Augmentation of As resistant inoculants, in single or as consortia, comprising of desired metabolic activity will ameliorate the rhizospheric environment for As uptake. Many root-associated bacteria are reported to possess one or more important PGP traits in addition to metal resistant property (Das et al., 2014, 2016; Pandey and Bhatt, 2016; Tiwari et al., 2016; Biswas et al., 2017; Mallick et al., 2018). These traits have a profound role in assisting plants to grow better even in toxic metal contaminated soil.

In this study, among the selected six isolates, AS6 found to exhibit important PGP traits showed the best performance (Table 1) viz. N2 fixation, IAA production, phosphate solubilization, siderophore production, ACC deaminase activity and EPS production (Table 1) that have the direct or indirect influence on plant growth promotion. Sufficient nitrogen supplementation enhanced stress tolerance of plant by increasing photosynthetic enzyme activity and defense against stress has reported by Jalloh et al. (2009). The IAA production and ACCD activity of the AS6 strain are essential important PGP traits to support plant growth promotion in As-stressed environments. PGPR synthesize IAA utilizing tryptophan excreted by the roots in the rhizosphere which in turn (i) participates in plant cell growth and (ii) promotes ACC synthase activity to increase the ethylene titer (Ma et al., 2011). This characteristic

Table 1
Determination of MIC value and PGP traits of selected As resistant isolates.

MIC (mM)	Bacterial isolates					
	AS4	AS6	AS7	AS11	AS12	AS14
Sodium arsenite (**AS III**)	17	20	15	18	15	15
Sodium arsenate (**AS V**)	50	100	70	50	75	60
PGP traits						
Nitrogen fixation (μg N fixed/h/mg protein)	0.06 ± 0.002	0.32 ± 0.030	0.21 ± 0.030	0.11 ± 0.010	0.19 ± 0.020	0.20 ± 0.020
IAA production (μg/mL)	34.0 ± 0.660	166.0 ± 0.500	30.0 ± 0.330	33.0 ± 0.500	36.0 ± 0.330	33.0 ± 0.660
Phosphate solubilization (mg/L)	2.0 ± 0.050	3.0 ± 0.060	1.0 ± 0.003	1.0 ± 0.003	2.0 ± 0.050	2.5 ± 0.060
Siderophore production (%)	52.0 ± 0.330	72.0 ± 0.500	50.0 ± 0.660	45.0 ± 0.330	56.0 ± 0.500	56.0 ± 0.500
ACC deaminase activity (nmolα-ketobutyrate/mg protein/h)	662 ± 1.330	2022 ± 2.500	1024 ± 2.000	1156 ± 0.500	1470 ± 2.000	1485 ± 1.660
EPS production (μg/mL)	28.0 ± 2.000	44.0 ± 1.330	30.0 ± 0.500	26.0 ± 2.000	34.0 ± 1.660	36.0 ± 2.000
HCN production	Positive	Positive	Positive	Positive	Positive	Positive
Ammonium production	Positive	Positive	Positive	Positive	Positive	Positive

Data are mean of three replicates ± standard error.

conferred by many metalloids resistant bacteria and play a significant role in plant establishment in contaminated habitats, particularly nutrient acquisition under As stress (Cavalca et al., 2015; Pandey and Bhatt, 2016). The AS6 strain showed increased IAA production in presence of As (V) compared to As (III) treatment (Fig. 1A). A similar pattern of effects reported by *Halobacillus* spp. and *B. flexus* ASO-6 in culture in presence of different HMs respectively (Desale et al., 2014; Das et al., 2014).

The ACCD activity of AS6 strain was found to increase in presence of As (V) in culture condition (Fig. 1B), however, it is decreased under As (III) treatment. This increasing effect might be the induction of metal stress and help plant better root formation, better

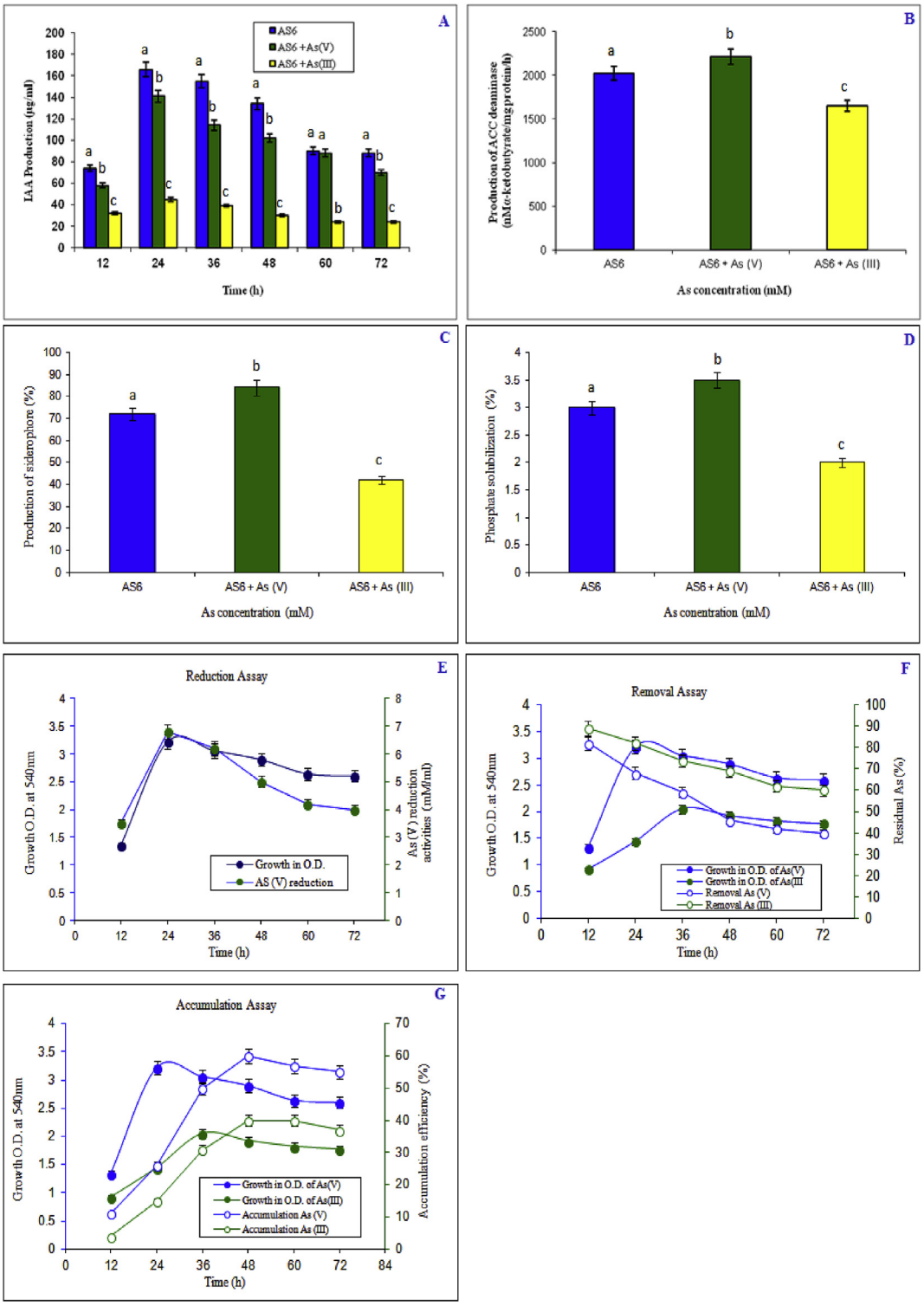

Fig. 1. PGP traits and arsenic removal efficiency of AS6 strain in culture. A) IAA production B) ACCD production C) siderophore production D) phosphate solubilization E) transformation assay F) removal efficiency and G) accumulation of arsenic. Data are mean of three replicates ± standard error.

acquisition of nutrients and able to thrive in As-spiked soils due to less ethylene production under stress condition (Cavalca et al., 2010; Srivastava et al., 2013; Das et al., 2014). Similarly, application of strain *B. flexus* ASO-6 has been reported to produce ACCD and IAA alleviated the inhibition effects of ethylene on plant growth and promoted plant growth under As-stressed environments (Das et al., 2014). The production of the higher amount of IAA and ACCD activity of AS6 strain also corroborated the present findings.

Concerning about increasing effect of siderophore production (Fig. 1C) and phosphate solubilization (Fig. 1D) in As amended condition, the phosphatase enzyme makes the conversion of non-soluble phosphate to soluble form in the soil and make it more available to plants (Nannipieri et al., 2011) whereas under metal stress condition different PGPR produced siderophores to form complexes with different metals, nutrient mobilization and assist in metals availability to plants (Rajkumar et al., 2010; Schalk et al., 2011). On the other hand, As-sensitive plant in As-contaminated soil can be adversely affected because As (V) that reduces the amount of phosphorous availability in plants (Singh and Ma, 2006). This deficiency can be compensated by phosphate solubilization activity of AS6 strain under As stress condition by reducing the pH of the inoculated soil (Singh and Ma, 2006; Das et al., 2014).

Inoculation of phosphate solubilizing bacteria (PSB) enhanced plant growth, higher shoot and root length and biomass production under As stress reported by Srivastava et al. (2013) and Xu et al. (2016). The increasing effect of siderophore production by AS6 strain in As amended condition (Fig. 1C). Several experimental data supported as simultaneous growth promotion and increased Fe uptake by applying siderophore-producing bacteria (SPB) under As stress condition (Srivastava et al., 2013) and increasing the effects by *Pantoea agglomerans* RSO6 in the presence of Cu and As (Paredes-Paliz et al., 2016).

Furthermore, all the strains are positive for HCN and ammonium production (Table 1). The higher EPS production ability of AS6 strain play a key role in binding metals with higher affinity to help plant better adaptation under the As contaminated site (Pandey and Bhatt, 2016). Thus the exhibition of several important PGP traits of this AS6 strain was added advantages to qualify as a potent PGP strain that could be potentially exploited to reduce stress under As contaminated soil for crop production.

3.3. Arsenic tolerance

In contaminated soil, As detoxification by a microorganism, primarily occurs through oxidation, reduction or methylation reaction (Xiong et al., 2010). Under As stress condition, they have evolved a mechanism for As detoxification which is under control of *ars* operon system (Sarangi et al., 2009). The selected AS6 strain was found to reduce As (V) to As (III) and interaction of $AgNO_3$ with AsO_3 ion generates bright yellow precipitate of Ag_3AsO_3 (supplementary file Fig. 1).

It is revealed that strain produced arsenate reductase enzyme and the conversion of As (V) to As (III) is 6.6 mM after 24 h which is comparable to the redox transformation of arsenate by other species of *Bacillus* sp. (Banerjee et al., 2011; Qamar et al., 2017) (Fig. 1E). The higher MIC value of AS6 strain confirmed that this strain possesses an *ars* operon by which it extruded the As from the cell. The gene mediated As resistance potentiality and its redox transformation of As by several species of *Bacillus* has been reported by (Banerjee et al., 2011, 2013; Das et al., 2014; Qamar et al., 2017) and corroborated with this finding.

3.4. Growth kinetic, removal and bioaccumulation efficiency of As

During the growth kinetics study, it has revealed that this strain had different effects on the growth. A high growth rate was observed for AS6 cells in the presence of As (V) and the highest growth occurred at 24 h of incubation (Fig. 1E). On the other hand, the growth rate is reduced in the presence of As (III) with prolonged exponential phase up to 36 h (Fig. 1F) for physiological adaptations to cope up with As stress in the lag phase (Paul et al., 2014). The reduction in the cellular growth rate of the AS6 strain was due to the higher toxicity of As (III) and a similar pattern of growth characteristic was also reported in As resistant *Bacillus* spp. (Banerjee et al., 2011; Pandey and Bhatt, 2015).

Regarding the As removal ability of this AS6 strain it showed higher removal efficiency (41%) of As (V) (Fig. 1F) and comparatively lower removal efficiency of As (III) (26%) was recorded in maximum growth phase at 24 h and 36 h respectively (Fig. 1F). Concerning As accumulation, it was revealed that AS6 strain found to exhibit 50% accumulation of As (V) (Fig. 1G) and 31% accumulation of As (III) (Fig. 1G) with maximum growth phase. This result was agreed with the previous reports that bioaccumulation would be the result of higher uptake of As into the cell (Banerjee et al., 2011; Majumder et al., 2013; Pandey and Bhatt, 2015).

3.5. Identification of the selected As resistant PGPR AS6 strain

Different morphological and biochemical characteristics of the selected AS6 strain are given in Table 2. The taxonomical identification was carried out through MALDI-TOFMS analyzer, FAME analysis and 16S rDNA sequence studies. According to MALDI-TOFMS analysis, the best match organism was suggested by the biotype score value as *B. megaterium* (2.349) and second match organism as *B. aryabhattai* (2.340) (Table 2, Fig. 2A). However, the FAME profile of the AS6 strain identified as *B. aryabhattai* with the highest similarity index 0.684 (GC subgroup A) (Table 2).

The proportion of predominant fatty acids composition of the AS6 strain was compared with *Bacillus* species for its specific epithet (Fig. 2B) (Shivaji et al., 2009) (supplementary file Table 3). The identification of this strain was further confirmed by 16S rDNA sequence-based homology. The phylogenetic analysis confirmed, AS6 strain was 100% clustering with type strain *B. aryabhattai* strain B8W22 (NR115953) and *B. aryabhattai* strain B8W22 (EF114313) (Fig. 2C). Thus the species status of the AS6 strain was assigned as *B. aryabhattai* based on phenotypic characterization, FAME profile analysis and 16S rDNA sequence homology data. The 16S rDNA sequence accession number and strain accession number of AS6 are KY908323 and MCC3374 respectively.

3.6. Microscopic studies and elemental analysis

Regarding about microscopic analysis, SEM study indicated the surface of treated cell wall were irregular, enlarged with rough, wrinkled appearances whereas untreated cell wall were rod-shaped with smooth surface, this can be interpreted as a possible strategy of the cell to accumulate As in order to minimize its toxic effect (Fig. 3A–D). The average length of treated cells was notably smaller as compared to control cells. The energy dispersive X-ray spectroscopy (EDAX) analysis showed a distinct EDAX signal corresponding to As peak was observed in presence of As (V) treated cells. The hypothesis was confirmed through SEM-EDAX and TEM-EDAX (Fig. 3E–F) study, which strongly proved that biosorption of As in cell leads to such distinguishable alterations in the bacterial cell wall (Fig. 3E). This result also agrees with the previous studies of different *Bacillus* spp. (Shakya et al., 2012; Pandey and Bhatt, 2015; Singh et al., 2016; Mallick et al., 2018). Moreover, in TTC test, in spite of high accumulation of As by AS6 strain was remained viable and metabolically active due to its red colour formation as compared with As treated control set. The development of red

Table 2
Phenotypic and molecular identification of selected arsenic resistant AS6 strain.

Characteristics	Bacterial strain		
Morphological characters	Strain AS6		
Gram reaction	Positive		
Motility	Positive		
Cell shape	Large, rod shaped		
Colony diameter (mm)	4–8		
Colony Shape	Entire, round, flat		
Colony colour	Peach		
Physiological-Biochemical characters			
Gelatine hydrolysis	+		
Starch hydrolysis	+		
Urea hydrolysis	+		
Oxidase test	+		
Growth on nitrogen-free medium	+		
Citrate utilization	+		
Indole production	–		
Voges-Proskauer test	+		
Acid production from: Glucose, galactose, mannitol, sorbitol, inositol, lactose, L-ribose, L-xylose, L-arabinose	+		
Lysine decarboxylation	+		
Arginine decarboxylase	–		
Catalase test	+		
Protease test	+		
Amylase test	+		
Carbon-source utilization: D-Glucose, D-galactose, rhamnose, D- and L-arabinose, sucrose, D-mannitol, raffinose, D-mannose, D-xylose,	+		
MALDI-TOFMS analysis of selected AS6 strain	1st Match		2nd Match
Organism matches	*Bacillus megaterium*		*Bacillus aryabhattai*
Biotype Score	2.349		2.340
FAME analysis of selected AS6 strain	*Bacillus aryabhattai* - GC subgroup A		*Bacillus aryabhattai* - GC subgroup B
Similarity index (%)	0.684		0.565
Molecular characterization of selected AS6 strain			
16S rDNA sequence length (bp)	Closest relatives of EzTaxon-e data base	Similarity (%)	NCBI 16S rDNA sequence accession no.	MCC Strain accession no.
1277	*Bacillus aryabhattai* B8W22 (T) (EF114313)	100	KY908323	MCC3374

'+' sign indicates positive result & '-' sign indicates a negative result.

colour TPF (1, 3, 5-triphenyl formazan) by AS6 strain was also corroborated with the findings of earlier study (Pandey and Bhatt, 2015).

To investigate whether the interaction of As with bacterial cells was due to an absorption process of As ions with functional groups of cell surface, an FTIR analysis was carried out and spectra of the As-tolerant AS6 strain grown in the absence or presence of As were recorded. Based on principal IR absorptions for certain functional groups were characterized of each peak with the corresponding functional groups of control (without As) (Fig. 4A, red lines) were with wave numbers at 3440.43 cm^{-1} stretch was assigned to O–H and N–H groups, stretching peak at 2958.24 cm^{-1} assigned to C–H bonds from alkyl groups (Murthy et al., 2014), all these peaks showed a slight shift in the presence of As (Fig. 4B, black line). The FTIR bands assigned to an asymmetric stretch of the C=O at the 1645.60-cm^{-1} range and C–N stretching was at 1409.28-cm^{-1} also displaced in the presence of As (Singh et al., 2016). Finally, a significant displacement of FTIR bands corresponding to phosphate groups located at 1239.45–1084.53 cm^{-1} was observed in presence of As. This result highly in accordance with the results reported in *Rhodococcus* sp. (Prasad et al., 2014) and *B. aryabhattai* NBRI014 (Singh et al., 2016).

Based on the result from both FTIR and SEM-TEM EDAX analyses, it was suggested that As complexes with polarisable functional groups on the cell surface of *B. aryabhattai* AS6. A similar finding was also reported by Singh et al. (2016). The As absorption by AS6 also has been confirmed by X-Ray diffraction analysis (Fig. 4C–D). The presence of peak (32.10) at 2 theta position indicated the accumulation of As by AS6 strain was clearly confirmed in treated condition as compared with control (Fig. 4D). The As absorption in cell pellets was also confirmed by X-Ray fluorescence analysis (Fig. 4E–F), however, no such type of peak found in the untreated control set.

3.7. Amelioration of As toxicity in the rice cultivar by AS6 strain as inoculants

As far as the effect of As (V) on the *Oryza sativa* (L.), the toxicity level was detected by EC_{50} (2.5 mM) of the Swarnamasuri rice cultivar (Fig. 5A). This EC_{50} concentration of As (V) was maintained in all set (except control Set I) for pot experiments prepared with the arsenic free soil (supplementary file Fig. 2), aiming to assess the stress ameliorating efficiencies of AS6 strain. Results showed that AS6 strain improved various morphological and biochemical parameters of the selected rice cultivar under As stress (Fig. 5B–D). Germination related enzyme like amylase (51.35%) and protease (50%) activities were significantly ($p < 0.05$) enhanced in the presence of AS6 strain compared to EC_{50} (Fig. 5E–F). A similar pattern of improvement was observed in *Vigna radiata* by arsenic resistant PGPR inoculation (Pandey and Bhatt, 2016).

This improved morphological and biochemical parameters may be correlated with the high bioaccumulation efficiency of arsenic by AS6 strain (Figs. 3 and 4) and possession of important PGP traits (Fig. 1A–D) as well. The bioaccumulation property of metal tolerant PGPR, in one hand leads to reduced metal mobilization to the plant cells and on the other hand, PGPR-produced phytostimulators like

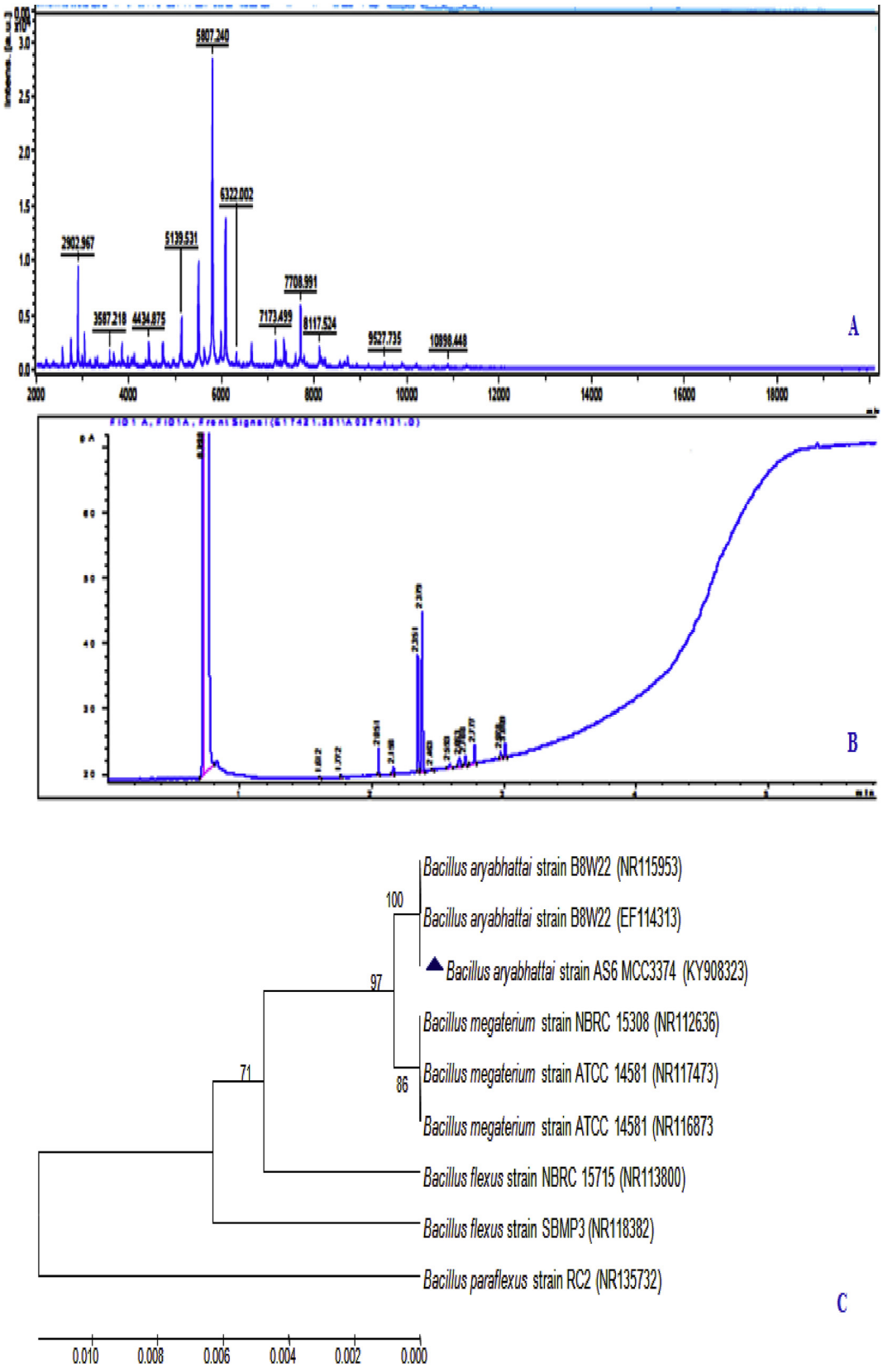

Fig. 2. Identification of AS6 strain. **A)** MALDI-TOFMS analysis. B) GC chromatogram of FAME analysis. C) The phylogenetic tree based on 16S rRNA gene sequence.

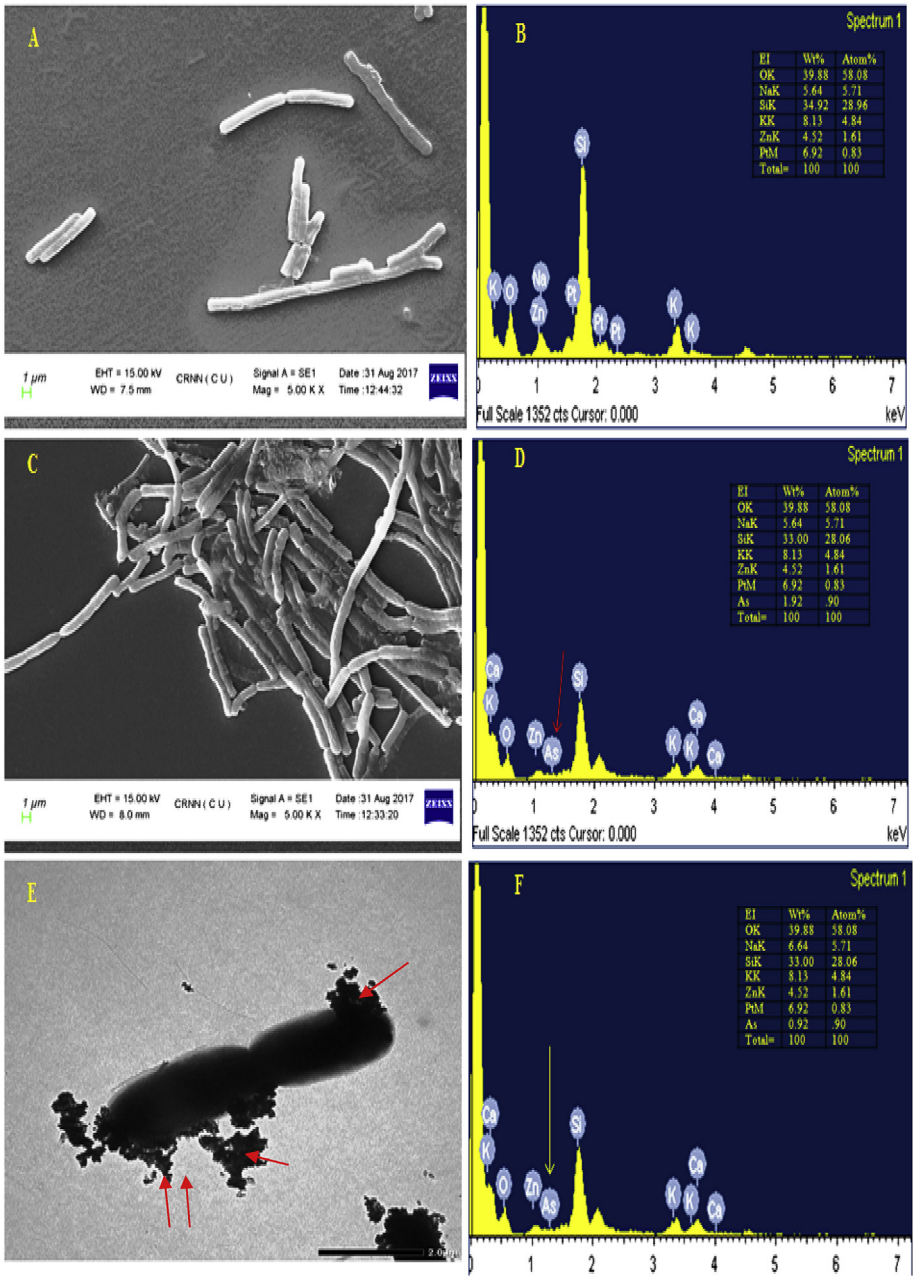

Fig. 3. Scanning electron microscopic study in the presence and absence of As. A) AS6 strain control B) EDAX of control C) AS6 strain treated D) EDAX analysis of AS6 strain in treated E) Transmission electron microscopic study and F) EDAX of TEM.

IAA, efficiently promotes plant growth even under metal stress condition (Pishchik et al., 2002; Pandey and Bhatt, 2016). In this study, As accumulating AS6 strain sufficiently removed As (Fig. 5A–C) from the localized environment at the vicinity of the imbibed seeds, thereby decreased the toxic effect of As which ultimately resulted in enhanced germination parentage and growth of seedlings (Pandey and Bhatt, 2016; Mallick et al., 2018).

Moreover the plant system possesses several anti-oxidative defence systems to scavenge toxic free radicals to protect themselves from oxidative stress that often caused by heavy metals and metalloids (Talukdar, 2013). Overproduction of reactive oxygen species (ROS) production in plants is regulated by antioxidative enzymes, which scavenge excessive free radicals to protect the cells from oxidative damage (Duquesnoy et al., 2010).

In this study, arsenic treatment caused significant alterations in antioxidative enzymes activities in rice cultivar. Although, the As (V) treatment significantly ($p < 0.05$) increased SOD and CAT activities in rice seedlings to combat against stress induced damages, but the activities were improved more (27.27% and 62.26% respectively) by the application of AS6 strain (Fig. 5G and H). The similar pattern of result with increased antioxidative enzymes activities in response to arsenic treatment reported in *Vicia faba*; *Zea mays* (Duquesnoy et al., 2010) and *Vigna radiata* (Pandey and Bhatt, 2016).

However, the MDA content (product of membrane lipid peroxidation) was found to remain in almost the same level as EC_{50} (Fig. 5I). The proline (one of the stress indicating factors) content was found to increase significantly ($p < 0.05$) in each As treated set

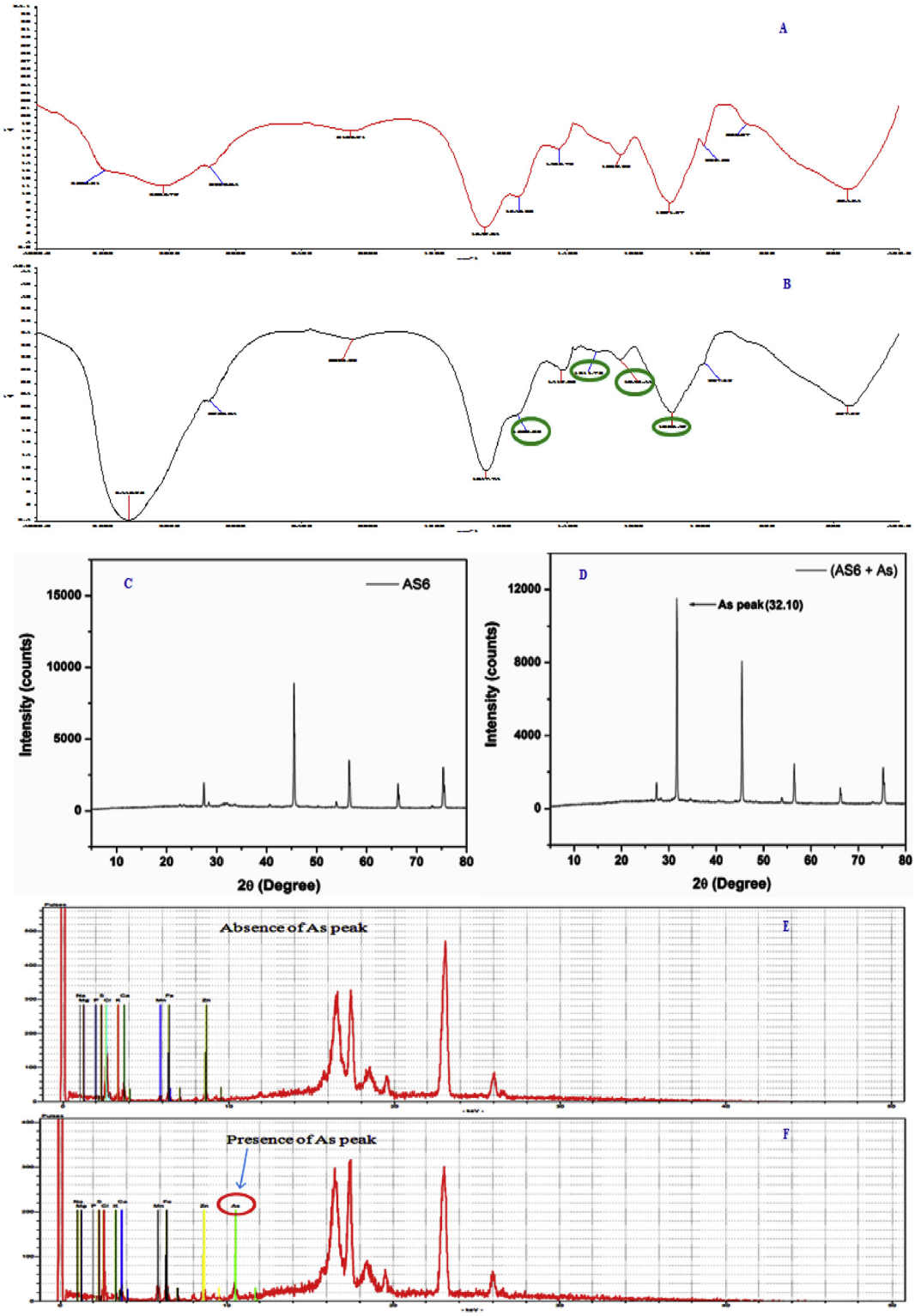

Fig. 4. Arsenic resistant *B. aryabhattai* AS6 strain was growing in absence and presence of As in 3 mM $NaAsO_2$. FT-IR spectra analysis A) untreated cells (red *lines*) B) treated cells (black *line*). X-ray diffraction patterns C) untreated cells D) treated cells. X-ray fluorescence analysis E) untreated cells F) treated cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

compared to control and the highest content of proline was found in AS6 strain treated seedlings (Fig. 5J). Elevated proline level in seedlings is a stress-induced non-enzymatic adaptation in plants acting as metal chelator, signalling and antioxidant defence molecule (Hayat et al., 2012).

This result is consistent with several studies about severe lipid peroxidation in different plant species under As amended condition (Talukdar, 2013; Siddiqui et al., 2015; Pandey and Bhatt, 2016).

Thus, the increased activity of these antioxidative enzymes and MDA content is helpful to the plants under metal stress for the

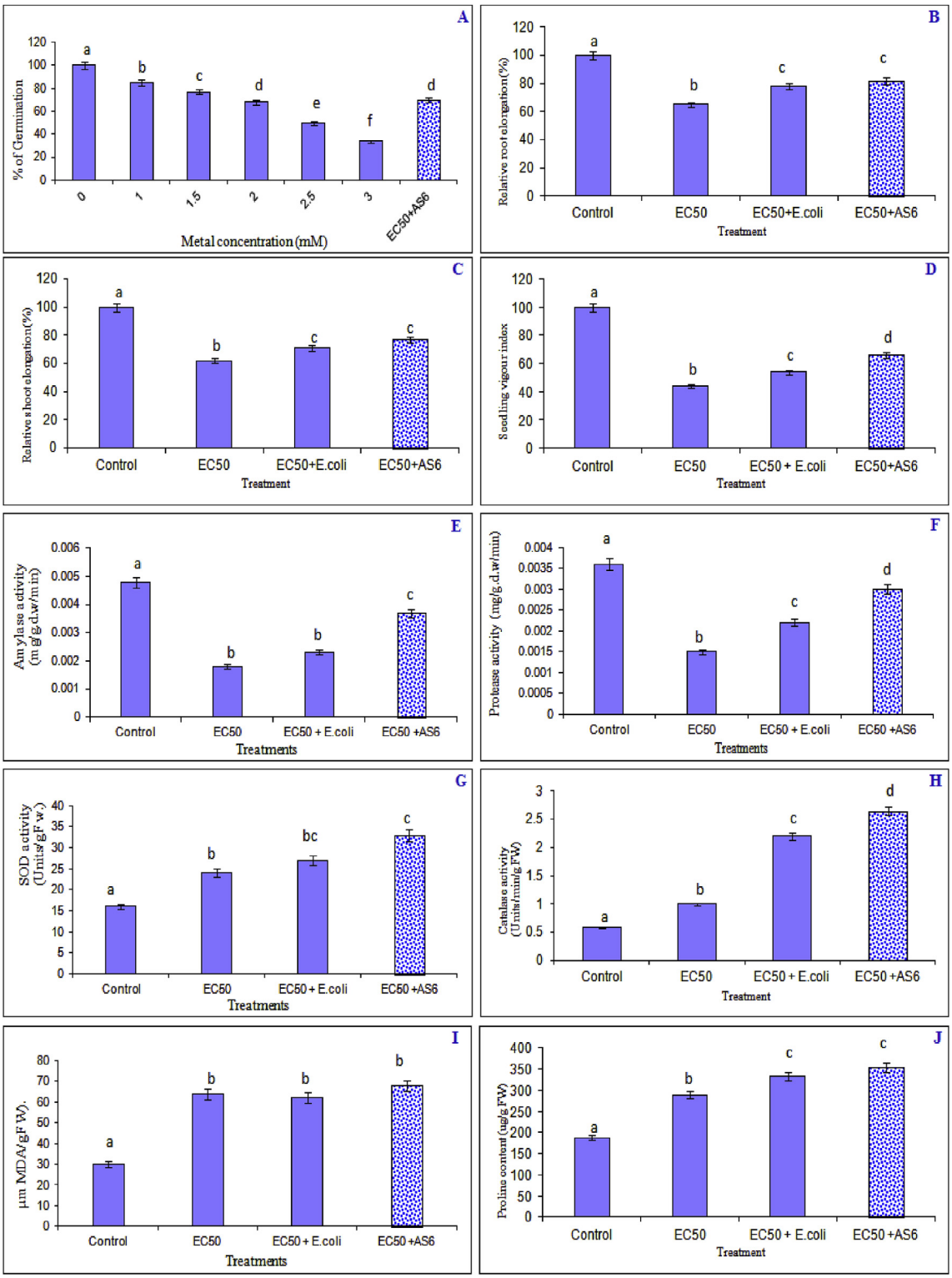

Fig. 5. Plant growth promoting abilities by AS6 strain on rice plant in As amended condition A) percentage of seed germination B) relative root elongation C) relative shoot elongation D) seed vigour index. Phytotoxicity ameliorating activities by AS6 strain with respect to different enzyme activity E) amylase activities F) protease activities G) SOD in roots H) CAT activity I) MDA content and J) proline content. Data are mean of three replicates ± standard error.

efficient recycling of ROS to ensure normal plant growth and protection against stress induced impairments (Talukdar, 2013; Pandey and Bhatt, 2016). This study indicated that the application of AS6 strain prevented the host cells from the metalloid induced oxidative damages with increased antioxidant activities. Therefore, alleviation of As stress-induced toxicity may be considered as one of the possible mechanism by which PGPR enhance plant growth. This result was also supported by several studies such as Zaidi et al. (2006); Pandey et al. (2013) and Pandey and Bhatt (2016). This result is also consistent with several studies about severe lipid

peroxidation in different plant species under As amended condition (Talukdar, 2013; Siddiqui et al., 2015; Pandey and Bhatt, 2016).

3.8. Amelioration of ethylene production under metal stress

The higher amount of stress ethylene production was recorded in arsenic treated uninoculated (control) rice seedlings as compared to the inoculated set under As stress, which indicated that the application of ACCD producing AS6 strain reduced stress ethylene production near the level of inhibitor-treated conditions (data not shown). This accelerated stress ethylene production in response to As might be one of the important causes for inhibition of root growth which resulted in stunted plant growth (Arshad et al., 2007; Bal et al., 2013).

The improved rice seedlings growth even under As (V) stress was observed, possibly due to mitigation of stress induced ethylene production by the ACCD positive PGPR strain AS6 that have the capacity to maintain the ethylene homeostasis within seedlings tissues. In inoculated set could be due to ACCD activity of AS6 strain which regulates the over production of ethylene could modulate the stress ethylene level by increasing root architecture (formation of longer roots and profuse root hair formation) for better adaptation in HMs contaminated soil. These findings also supported by ACCD containing *Bacillus* spp. which were able to reduce As toxicity and potential seedlings growth of rice cultivar as well (Pandey et al., 2013; Lakshmanan et al., 2015; Das et al., 2016; Mallick et al., 2018).

4. Conclusion

The *B. aryabhattai* AS6 strain is the most promising PGPR as it offers great potential to novel crop production strategies due to its high As resistant properties and exhibition of several important PGP traits. The performance in plant growth promotion and reduces As toxicity was tested by application of this bacterium in relation to different physio-biochemical parameters under As amended condition. The arsenate extrudation and higher ACCD production of *B. aryabhattai* AS6 strain might be helpful for bioremediation, better adaptation in As-spiked soils as well as increased growth of the plant. The present investigation established that AS6 strain could be a better choice for application in As contaminated soil for sustainable agronomic production. This result was highly encouraging to its use in As-contaminated agriculture field; however practical field application should be conducted to realize the laboratory results which are ours under scan.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgments

Financial support for the first author provided by DST (SERB), New Delhi, India (NPDF/2016/00323 dt.05.07.2016) through Department of Marine Science, Ballygunge Science College, Calcutta University is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2018.07.148.

References

Abdel-Lateef, A.M., Mohamed, R.A., Mahmoud, H.H., 2013. Determination of arsenic (III) and (V) species in some environmental samples by atomic absorption spectrometry. Adv. Chem. Sci. 2, 110–113.
Aebi, H., 1984. Catalase invitro. In: Methods in Enzymology, vol. 105. Academic Press, Inc. ISBN0.12-182005-X.
Aksornchu, P., Prasertsan, P., Sobhon, V., 2008. Isolation of arsenic-tolerant bacteria from arsenic-contaminated soil. Songklanakarin. J. Sci. Technol. 30, 95–102.
(American Public Health Association, the American Water Works Association and the Water Pollution Control Federation) APHA, AWWA, WPCF, 2005. Standard Methods for the Examination of Water and Wastewater, 21stedn. APHA, AWWA, WPCF, Washington, DC.
Arshad, M., Saleem, M., Hussain, S., 2007. Perspectives of bacterial ACC deaminase in phytoremediation. Trends Biotechnol. 25, 356–362.
Bal, H.B., Nayak, L., Das, S., Adhya, T.K., 2013. 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. 46, 1736–1747.
Banerjee, S., Majumdar, J., Samal, Bhattachariya, A.C., Bhattachariya, P., Santra, S.C., 2013. Biotransformation and bioaccumulation of arsenic by *Brevibacillus brevis* isolated from arsenic contaminated region of West Bengal. J. Environ. Sci. Toxicol. Food Technol. 3, 1–10.
Bates, L.S., Waldron, R.P., Teare, I.D., 1973. Rapid determination of free proline for water stress studies. Plant Soil 39, 205–208.
Benson, H.J., 1990. Microbiological Application-a Lab Manual in General Microbiology, fifth ed. W.C.Brown Publishers, Dubuque, IA, USA.
Biswas, J.K., Mondal, M., Rinklebe, J., Sarkar, S.K., Chaudhuri, P., Rai, M., Shaheen, S.M., Song, H., Rizwan, M., 2017. Multi-metal resistance and plant growth promotion potential of a waste water bacterium *Pseudomonas aeruginosa* and its synergistic benefits. Environ. Geochem. Health 39 (6), 1583–1593.
Cavalca, L., Anna, C., Enrica, C., Raffaella, Z., 2015. Rhizobacterial communities associated with spontaneous plant species in long-term arsenic contaminated soils. World J. Microbiol. Biotechnol. 31, 735–746.
Cavalca, L., Zanchi, R., Corsini, A., Colombo, M., Romagnoli, C., Canzi, E., Andreoni, V., 2010. Arsenic-resistant bacteria associated with roots of the wild *Cirsium arvense* (L.) plant from an arsenic polluted soil, and screening of potential plant growth-promoting characteristics. Syst. Appl. Microbiol. 33, 154–164.
Das, S., Jean, J.S., Chou, M.L., Rathod, J., Liu, C.C., 2016. Arsenite-oxidizing bacteria exhibiting plant growth promoting traits isolated from the rhizosphere of *Oryza sativa* L.: implications form mitigation of arsenic contamination in paddies. J. Hazard Mater. 302, 10–18.
Das, S., Jean, J.S., Kar, S., Chou, M.L., Chen, C.Y., 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.
De-Bashan, L.E., Hernandez, J.P., Bashan, Y., 2012. The potential contribution of plant growth-promoting bacteria to reduce environmental degradation-a comprehensive evaluation. Appl. Soil Ecol. 61, 171–189.
Desale, P., Patel, B., Singh, S., Malhotra, A., Nawani, N., 2014. Plant growth promoting properties of *Halobacillus* sp. and *Halomonas* sp. in presence of salinity and heavy metals. J. Basic Microbiol. 54, 781–791.
Dhindsa, R.H., Plumb-Dhindsa, P., Thorpe, T.A., 1981. Leaf senescence correlated with increased level of membrane permeability, lipid peroxidation and decreased level of SOD and CAT. J. Exp. Bot. 32, 93–101.
Duquesnoy, I., Champeau, G.M., Evray, G., Ledoigt, G., Pissaloux, A.P., 2010. Enzymatic adaptations to arsenic-induced oxidative stress in *Zea mays* and genotoxic effect of arsenic in root tips of *Vicia faba* and *Zea mays*. C. R. Biologies 333, 814–824.
Fick, G.N., Qualset, C.O., 1975. Genetic control of endosperm amylase activity and gibberellic acid Reoponses in standard-height and short-statured wheats. Proc. Nat. Acad. Sci. U. S. A. 72, 892–895.
Ghosh, P., Rathinasabapathi, B., Ma, L.Q., 2011. Arsenic-resistant bacteria solubilised arsenic in the growth media and increased growth of arsenic hyper accumulator *Pteris vittata* L. Bioresour. Technol. 102, 8756–8761.
Glick, B.R., 2005. Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol. Lett. 251, 1–7.
Goswami, D., Vaghela, H., Patel, P., Dhandhukia, P., Thakker, J.N., 2014. Screening of PGPR from the saline desert of Kutch: growth promotion in *Arachis hypogea* by *Bacillus lichenoformis* A2. Microbiol. Res. 169, 66–72.
Guo, J., Chi, J., 2014. Effect of Cd-tolerant plant growth-promoting rhizobium on plant growth and Cd uptake by *Lolium multiflorum* lam. And *Glycine max* (L.) merr. in Cd-contaminated soil. Plant Soil 375, 205–214.
Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J., Ahmad, A., 2012. Role of proline under changing environments. Plant Signal. Behav. 7 (11), 1456–1466.
Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125, 189–198.
Jalloh, M.A., Chen, J., Zhen, F., Zhang, G., 2009. Effect of different N fertilizer forms on antioxidant capacity and grain yield of rice growing under Cd Stress. J. Hazard Mater. 162, 1081–1085.
John, R., Ahmad, P., Gadgil, K., Sarma, S., 2009. Heavy metal toxicity: effect of plant growth, Biochemical parameters and metal accumulation by *Brasica juncea*, L. Int. J. Plant Prod. 3, 65–75.
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, M.A., Faust, M.A., 1967. Effect of growth retardants on - amylase production in germinating barley seeds. Physiol. Plant 20, 673–681.
Khan, S., Waqas, M., Ding, F., Shamshad, I., Arpd Hans, P.H., Li, G., 2015. The influence of various biochars on the bioaccessibility and bioaccumulation of PAHs and potentially toxic elements to turnips (*Brassica rapa* L.). J. Hazard Mater. 300, 243–253.
Kim, O.S., Cho, Y.J., Lee, K., Yoon, S.H., Kim, M., Na, H., Park, S.C., Jeon, Y.S., Lee, J.H., 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.
Lakshmanan, V., Shantharaj, D., Li, G., Seyfferth, A.L., Sherrie, D.J., Bais, H.P., 2015. A natural rice rhizospheric bacterium abates arsenic accumulation in rice (*Oryza sativa* L.). Planta 242, 1037–1050.
Li, G., Khan, S., Ibrahim, M., Sun, T.R., Tang, J.F., Cotner, J.B., Xu, Y.Y., 2018. Biochars induced modification of dissolved organic matter (DOM) in soil and its impact on mobility and bioaccumulation of arsenic and cadmium. J. Hazard Mater. 348, 100–108.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.
Ma, Y., Prasad, M.N.V., Rajkumar, M., Freitas, H., 2011. Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol. Adv. 29, 248–258.
Majumder, A., Ghosh, S., Saha, N., Kole, S.C., 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, S.C., Mukhopadhyay, U.K., 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.
Mallick, I., Hossain, Sk.T., Sinha, S., Mukherjee, S.K., 2014. *Brevibacillus* sp. KUMAs2, a bacterial isolate for possible bioremediation of arsenic in rhizosphere. Ecotoxicol. Environ. Saf. 107, 236–244.
Mukhopadhyay, R., Rosen, B.P., Phung, T., Silver, S., 2002. Microbial arsenic: from geocycle stogene sandenzymes. FEMS Microbiol. Rev. 26, 311–325.
Murthy, S., Bali, G., Sarangi, S.K., 2014. Effect of lead on growth, protein and biosorption capacity of *Bacillus cereus* isolated from industrial effluent. J. Environ. Biol. 35, 407–411.
Nannipieri, P., Giagnoni, L., Landi, L., Renella, G., 2011. Role of phosphatase enzymes in soil. In: Phosphorus in Action. Springer Berlin Heidelberg, pp. 215–243.
Ng, J.C., Wang, J., Sharim, A., 2003. A global health problem caused by arsenic from natural sources. Chemosphere 52 (9), 1353–1359.
Nordstrom, D.K., 2002. Public health-worldwide occurrences of arsenic in ground water. Science 296, 2143–2145.
Paez-Espino, D., Tamames, J., de Lorenzo, V., Canovas, D., 2009. Microbial responses to environmental arsenic. Biometals 22 (1), 117–130.
Panday, D., Schumann, P., Das, S.K., 2011. *Rhizobium pusense* sp. nov., isolated from the rhizosphere of chickpea (*Cicer arietinum* L.). Int. J. Syst. Evol. Microbiol. 61, 2632–2639.
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, P.K., Ghosh, S., De, T.K., Maiti, T.K., 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.
Paredes-Paliz, K.I., Caviedes, M.A., Doukkali, B., Mateos-Naranjo, E., Rodriguez-Llorente, I.D., 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.
Paul, D., Poddar, S., Sar, P., 2014. Characterization of arsenite-oxidizing bacteria isolated from arsenic contaminated groundwater of West Bengal. J. Environ. Sci. Hazard Subst. Environ. Eng. 49, 1481–1492.
Pavlovic, M., Huber, I., Konrad, R., Busch, U., 2013. Application of MALDI-TOF MS for the identification of food borne bacteria. Open Microbiol. J. 7, 135–141.
Pishchik, V.N., Vorobyev, N.I., Chernyaeva, I.I., Timofeeva, S.V., Kozhemyakov, A.P., Alexeev, Y.V., Lukin, S.M., 2002. Experimental and mathematical simulation of plant growth promoting rhizobacteria and plant interaction under cadmium stress. Plant Soil 243, 173–186.
Prasad, K.S., Srivastava, P., Subramanian, V., Paul, J., 2014. Biosorption of as (III) ion on *Rhodococcus* sp. WB- 12: biomass characterization and kinetic studies. Separ. Sci. Technol. 46, 2517–2525.
Qamar, N., Rehman, Y., Hasnain, S., 2017. Arsenic-resistant and plant growth-promoting Firmicutes and c-Proteobacteria species from industrially polluted irrigation water and corresponding crop land. J. Appl. Microbiol. 123, 748–758.
Rajkumar, M., Ae, N., Prasad, M.N.V., Freitas, H., 2010. Potential of siderophore producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol. 28, 142–149.
Rhine, E.D., Phelps, C.D., Young, L.Y., 2006. Anaerobic arsenic oxidation by novel denitrifying isolates. Environ. Microbiol. 8, 899–908.
Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. In: Molecular Cloning a Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.
Sanyal, S.K., Dhillon, K.S., 2005. Arsenic and selenium dynamics in water-soil-plant system: a threat to environmental Quality. In: Indian Society of Soil Science. ICSWEQ-Proceedings, pp. 239–263.
Sarangi, B.K., Kalve, S.K., Pandey, R.A., Chakrabarti, T., 2009. Transgenic plants for phytoremediation of arsenic and chromium to enhance tolerance and hyper accumulation. Transgenic Plant J. 3, 57–86.
Sasser, M., 2001. Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids. MIDI Technical Note. 101.
Schalk, I.J., Hannauer, M., Braud, A., 2011. New roles for bacterial siderophores in metal transport and tolerance. Environ. Microbiol. 13, 2844–2854.
Shakya, S., Pradhan, B., Smith, L., Shrestha, J., Tuladhar, S., 2012. Isolation and characterization of aerobic culturable arsenic resistant bacteria from surface water and ground water of Rautahat District, Nepal. J. Environ. Manag. 95. S250–S250.
Shivaji, S., Chaturvedi, Preeti, Begum, Z., Pindi, P.K., Manorama, R., et al., 2009. *Janibacter hoylei* sp. nov., *Bacillus isronensis* sp. nov. and *Bacillus aryabhattai* sp. nov., isolated from cryo tubes used for collecting air from the upper atmosphere. Int. J. Syst. Evol. Microbiol. 59, 2977–2986.
Siddikee, M.A., Glick, B.R., Chauhan, P.S., Yim, W., Sa, T., 2011. Enhancement of growth and salt tolerance of red pepper seedlings (*Capsicum annuum* L.) by regulating stress ethylene synthesis with halotolerantbacteria containing 1-aminocyclopropane-1-carboxylic acid deaminase activity. Plant Physiol. Biochem. 49, 427–434.
Siddiqui, F., Tandon, P.K., Srivastava, S., 2015. Analysis of arsenic induced physiological and biochemical responses in a medicinal plant, *Withania somnifera*. Physiol. Mol. Biol. Plants 21, 61–69.
Simeonova, D., Lievremon, D., Lagarde, F., Muller, D., Groudeva, V., Lett, M.C., 2004. Microplate screening assay for detection of arsenite oxidizing and arsenate-reducing bacteria. FEMS Microbiol. Lett. 237, 249–253.
Singh, N., Gupta, S., Marwa, N., Pandey, V., Verma, P.C., Rathaur, S., Singh, N., 2016. Arsenic mediated modifications in *Bacillus aryabhattai* and their biotechnological applications for arsenic bioremediation. Chemosphere 164, 524–534.
Singh, N., Ma, L.Q., 2006. Arsenic speciation and arsenic and phosphate distributionin arsenic hyperaccumulator *Pteris vittata* L. and non hyperaccumulator *Pteris ensiformis* L. Environ. Pollut. 141, 238–246.
Snell, F.D., Snell, C.T., 1971. Colorimetric Methods of Analysis, vol. IV. Van Nostrand Reinold Co., New York,USA, pp. 7–145. IAA.
Srivastava, S., Verma, P.C., Chaudhry, V., Singh, N., Abhilash, P.C., Kumar, K.V., Sharma, N., Singh, N., 2013. Singh, Influence of inoculation of arsenic-resistant *Staphylococcus arlettae* on growth and arsenic uptake in *Brassica juncea* (L.) Czern. Var. R-46. J. Hazard Mater. 262, 1039–1047.
Talukdar, D., 2013. Arsenic-induced oxidative stress in the common bean legume, *Phaseolus vulgaris* L. seedlings and its amelioration by exogenous nitric oxide. Physiol. Mol. Biol. Plants 19, 69–79.
Tiwari, S., Sarangi, B.K., Thul, S.T., 2016. Identification of arsenic resistant endophytic bacteria from *Pteris vittata* roots and characterization for arsenic remediation application. J. Environ. Manag. 180, 359–365.
Tsai, S.L., Singh, S., Chen, W., 2009. Arsenic metabolism by microbes in nature and the impact on arsenic remediation. Curr. Opin. Biotechnol. 20, 659–667.
Wevar Oller, A.L., Talano, M.A., Agostini, E., 2013. Screening of plant growth-promoting traits in arsenic-resistant bacteria isolated from the rhizosphere of soybean plants from Argentinean agricultural soil. Plant Soil 369, 93–102.
WHO, 2001. Arsenic in Drinking-water. World Health Organization, Geneva (WHO Fact Sheet No. 210). http://www.who.int/mediacentre/factsheets/fs210/en/print.html.
Williams, P.N., Lei, M., Sun, G., Huang, Q., Lu, Y., Deacon, C., et al., 2009. Occurrence and partitioning of cadmium, arsenic and lead in mine impacted paddy rice: Hunan, China. Environ. Sci.Technol. 43, 637–642.
Xiong, J., Wu, L., Tu, S., Van Nostrand, J.D., He, Z., Zhou, J., Wang, G., 2010. Microbial communities and functional genes associated with soil arsenic contamination and the rhizosphere of the arsenic-hyper accumulating plant *Pteris vittata* L. Appl. Environ. Microbiol. 76, 7277–7284.
Xu, J.Y., Han, Y.H., Chen, Y., Zhu, L.J., Ma, L.Q., 2016. Arsenic transformation and plant growth promotion characteristics of As-resistant endophytic bacteria from As-hyperaccumulator *Pteris vittata*. Chemosphere 144, 1233–1240.
Zaidi, S., Usmani, S., Singh, B.R., Musarrat, J., 2006. Significance of *Bacillus subtilis* strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in *Brassica juncea*. Chemosphere 64, 991–997.
Zhao, F.J., Zhu, Y.G., Meharg, A.A., 2013. Methylated arsenic species in rice: geographical variation, origin and uptake mechanisms. Environ. Sci. Technol. 47 (9), 3957–3966.

Update

Chemosphere

Volume 213, Issue , December 2018, Page 611–612

DOI: https://doi.org/10.1016/j.chemosphere.2018.09.109

Chemosphere 213 (2018) 611–612

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Corrigendum

Corrigendum to "The role of arsenic resistant *Bacillus aryabhattai* MCC3374 in promotion of rice seedlings growth and alleviation of arsenic phytotoxicity" [Chemosphere 211 (2018) 407–419]

Pallab Kumar Ghosh [a, *], Tushar Kanti Maiti [b], Krishnendu Pramanik [b], Sudip Kumar Ghosh [b], Soumik Mitra [b], Tarun Kumar De [a]

[a] Department of Marine Science, Ballygunge Science College Campus, Calcutta University, 35, B.C.Road, Kolkata, 700019, India
[b] Microbiology Laboratory, CAS, Department of Botany, Burdwan University, Burdwan, 713104, WB, India

The authors regret that incorrect version of Fig. 3D and F was published in the original article inadvertently. However, figure legend of Fig. 3 is correct. Fig. 3 along with the corrected version of Fig. 3D and F is given below:

DOI of original article: https://doi.org/10.1016/j.chemosphere.2018.07.148.
* Corresponding author. (P.K. Ghosh).
E-mail address: pal.mic.bu@gmail.com (P.K. Ghosh).

https://doi.org/10.1016/j.chemosphere.2018.09.109

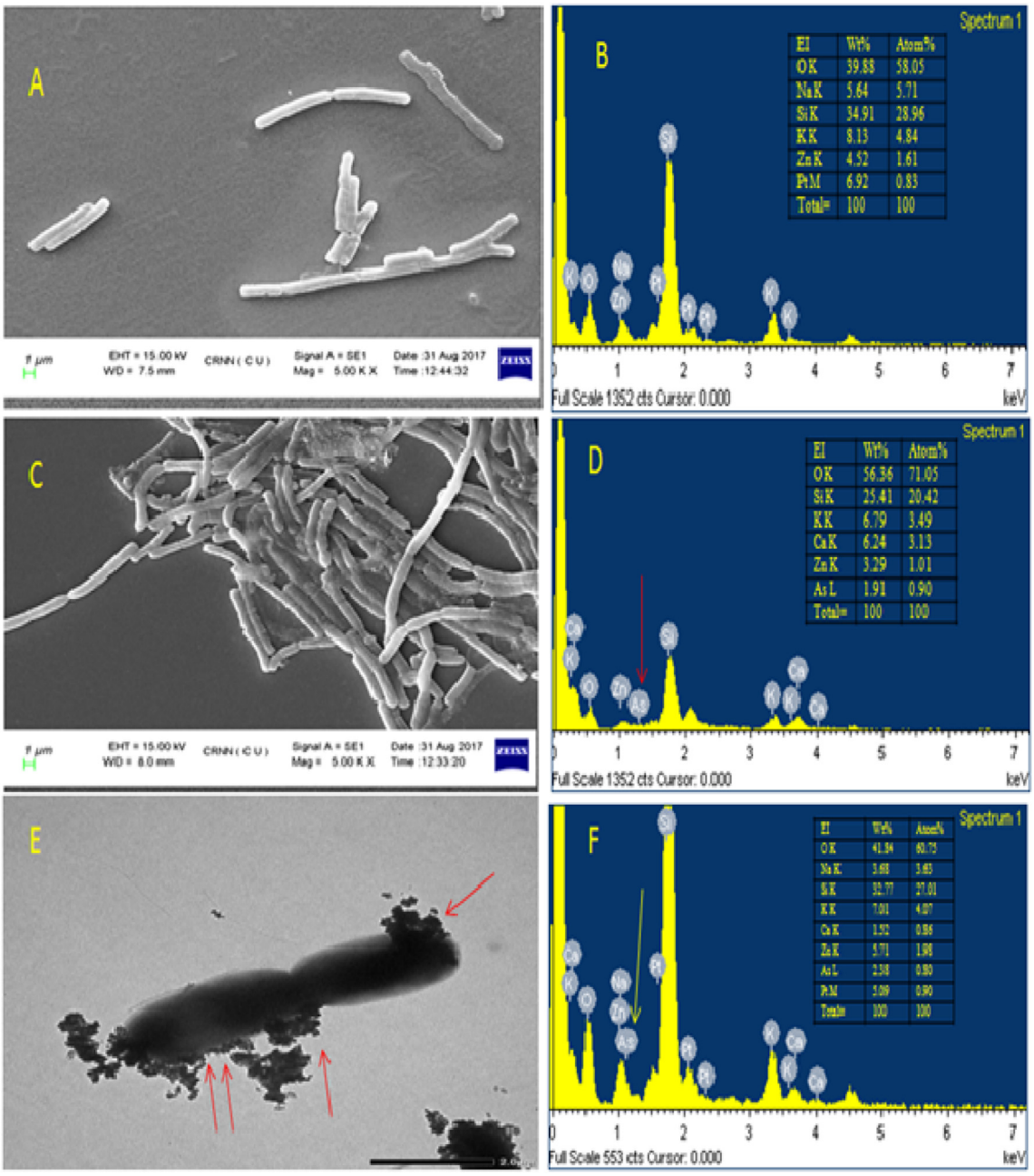

Fig. 3. Scanning electron microscopic study in the presence and absence of As. A) AS6 strain control B) EDAX of control C) AS6 strain treated D) EDAX analysis of AS6 strain in treated E) Transmission electron microscopic study and F) EDAX of TEM.