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Isolation and Characterization of Plant Growth Promoting Arsenic-resistant Bacteria and Possible Application in Bioremediation in West Bengal

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ABSTRACT

Soil arsenic contamination is a widespread problem in many developing countries including Bangladesh and India. In recent years development of modern innovative technologies for the removal of arsenic from soil has become an interesting topic for research. In this present study, ten bacteria were isolated besed on their arsenic resistant capacity. Among of them two rod shaped Gram-negative bacteria are being reported, isolated from arsenic affected soil of Chakdaha block of Nadia, West Bengal, India, which can tolerate arsenate concentration up to 409mM and 46mM of arsenite concentration. From biochemical analysis and 16S rRNA sequencing, they were identified as *Burkholderia cepacia* and *Burkholderia metallica* respectively. The isolates AL-1 and AL73 can remove 29% of arsenite and 30% and 29% of arsenate, respectively from arsenic containing culture media. Both of the isolate can oxidize arsenite to less toxic arsenate. These two also possessed plant growth-promoting traits,including phosphate-solubilization, nodule formation and IAA-like molecules. In addition, the ability of As-resistant isolates to grow over wide ranges of pH and temperatures signify their potential application for sustainable bioremediation of As in the environment.

Keywords: Soil, arsenic, Chakdaha, *Burkholderia cepacia*, *Burkholderia metallica*

Arsenic (As) is known to be one of the most hazardous substances in the environment and the attention of which has increased in the past two decades due to the mounting number of affected people exposed to it (Hughes *et al.* 2011). It is a toxic metalloid which is present in the soil as insoluble sulfides and sulfosalts such as Arsenopyrite, Orpiment, Realgar, Lollingite and Tennantite (Elangovan *et al.* 2006). Arsenic is naturally present in the earth and the contamination occurs mainly due to various anthropogenic activities, like excessive use of arsenic in pesticide for agriculture system, herbicide, smelting, ore processing, chromated copper arsenate in wood preservatives and medicinal product (Mandal *et al.* 2002). Arsine (III), elemental arsenic (0), arsenite (III) and arsenate (V) are the main four forms of arsenic which occur in environment. Arsenate (As (V)) and arsenite (As(III)) are reported to be more predominantly present in polluted soils (Pacyna and Pacyna 2001). Arsenate and arsenite both are very toxic in nature, arsenite being more toxic than arsenate.

In India, West Bengal the elevated concentration of As in the soil and in plants is mainly due to the irrigation of agricultural soil with As-enriched groundwater (Mandal *et al.* 2002). The incidence of high As in the groundwater of the Nadia is known for generating unique cases of peripheral vascular disease (i.e., gangrene) (Tseng, 1997). At present, even though As-rich groundwater has not been used for drinking, it is still extensively used for irrigation, aquaculture and industrial purposes (Kar *et al.* 2013). Agricultural soil acts as a principal sink

of As through irrigation of crop land, and most of the arsenical residues have low solubility and low volatility, generally accumulating in the top soil layers (Das *et al.* 2013). Top soil thus contaminated with As may have influence on the entry of As into the food chain (Das *et al.* 2013).

Microbes play an important role in the cycling of As in the environment as they can either oxidize As(III) to As(V) or reduce As(V) to As(III). The more widespread chromosomal and plasmid Ars system takes up As(V) into the cell and reduces it to As(III) and exudes the As(III) through an ATP-dependent efflux pump. Anaerobic periplasmic arsenate reductase also occurs in few bacteria, which enables these bacteria to use arsenate as terminal electron donor (Silver *et al.* 2002). These mechanisms affect speciation and mobility of As which can therefore affect its bioavailability (Cai and Ma 2003; Borch *et al.* 2010).

It becomes essential to mitigate the hazardous As from the contaminated soils. As cannot be easily degraded as any other organic pollutants, and thus, requires apposite methods for its removal (Rajkumar *et al.* 2009). Remediation technologies such as physical and chemical techniques are not only costly but also affect soil physical, biological, and chemical properties (Pulford and Watson, 2003). An alternative technology that has advanced in recent years is bioremediation. The discovery of microbes that can take up heavy metals in large amounts created optimism for the remediation of polluted lands.

Some of the bacterial isolates obtained from such heavy metal-contaminated soils were found to have additional advantage of plant-growth-promoting (PGP) traits such as indole-3-acetic acid production (IAA) production, P solubilization, nitrogen fixation, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase production (Burd *et al.* 1998; Sheng and Xia 2006; Zaidi *et al.* 2006; Jiang *et al.* 2011). Thus, they can solve two purpuses one is bioremediation of arsenic and other is plant growth promotion.

So, the aim of our study was to investigate Plant Growth Promoting (PGP microbes) in As-resistant bacterial strains isolated from the agricultural soil of the West Bengal that had been irrigated with As-enriched groundwater for subsequent studies of plant–microbe interactions and the development of strategies that minimize health risks in food production and lead to better and more sustainable agricultural practices.

MATERIALS AND METHODS

Study area

Chakdaha block of Nadia district, West Bengal, India was chosen for the present study which was previously reported for arsenic contamination (Sarkar *et al.* 2012). The Study area is situated at New Alluvial Zone of West Bengal at 23°5.3'N latitude and 83°5.3'E longitude.

Soil sampling and Physico-chemical analysis

Soil samples (0–10 cm depth) were collected from As-affected areas of the Chakdah, West Bengal. As concentrations in the ground water exceed WHO-defined permissible limits (Sarkar *et al.* 2012). Individual soil cores (2 cm diameter, 10 cm depth) were taken with a sample probe from four different places within each As-enriched site. Each sample was divided into two subsamples (for soil physiochemical and microbial analyses).

The physiochemical properties of the soil such as pH (Jackson, 1967), oxidisable organic carbon (Walkley and Black, 1934), available N (Subbiah and Asija, 1956), K (Brown and Warncke, 1934) and P (Olsen and Sommers, 1982) were determined using standard protocols. Total As (Sparks *et al.* 2006) and $NaHCO_3$-extractable As (Johnson and Barnard, 1979) levels were determined using Atomic Absorption Spectrophotometer (model: Perkin Elmer Analyst 200, USA) coupled with FIAS 400. Microbial biomass carbon (Jenkinson and Ladd, 1981), total and As-tolerant microbial populations of the soil samples were also determined (Bachate *et al.* 2009).

Enrichment and isolation of As-resistant bacteria

Arsenic-contaminated soils (1 g) were suspended in Yeast Extract Mannitol liquid medium supplemented with 1 mMAs(III) and As(V) and incubated at 30 °C for 48 h (Kinegam *et al.* 2008). The cultures were enriched by transferring 2 mL of culture into the same medium.

This process was repeated twice, and the final

enriched culture was used for the isolation of bacteria. Approximately 0.1 mL of enriched culture was plated on Yeast Extract Mannitol Agar medium amended with As and ten distinct colonies were selected for isolation.

Characterization of isolates

The strains were initially checked for colony morphology, Gram reaction, and characterized for oxidase, catalase activities following standard procedures (Holt *et al.* 1994).

Determination of minimum inhibitory concentration of As(III) and As(V)

Minimum inhibitory concentration (MIC) has been defined as the lowest concentration of As(III) or As(V) added that completely inhibits bacterial growth (Daims *et al.* 1999). In this study, As(III) and As(V) resistance in isolated bacterial strains was evaluated using MIC tests. Aliquots of 1.0 mL of overnight cultures were incubated in 99.0 mL of LB medium and Yeast Extract Mannitol liquid medium supplemented with either As(III)as $NaAsO_2$ (1–50 mM) or As(V) as $Na_2HASO_4{\cdot}7H_2O$ (1–500 mM) and incubated at 30◦C with shaking (170 rev/min) for 48 h. The optical density of the cultures, as a measure of micro-bial growth, was detected at a wavelength of 600 nm (OD600) byan UV–vis spectrophotometer (BIO-RAD Smart SpecTM3000, USA); a blank with only the medium culture without bacteria was also analyzed. Experiments were carried out in triplicate.

Bioremediation test

Isolated bacteria were inoculated in the nutrient broth media prepared in 100 ppm arsenate and arsenite solution and incubated at 30°C. After 24 h, 48 h and 72 h of incubation the solution were centrifuged at 10,000 rpm for 10 min to separate the bacterial biomass from the media and the arsenic concentration of the media was measured (Ellis *et al.* 2003).

Oxidation and reduction of arsenic by the isolates

Arsenic-oxidising bacterial isolates were screened using the standard silver nitrate ($AgNO_3$) method (Lett *et al.* 2001). The isolates were cultured on solidified CDM (chemically defined medium) that was supplemented with 1 mMAs(III) for 48 h at 30 °C. The plates were flooded with 0.1 M AgNO3 solutions and the colour changes of the respective colonies were recorded. $AgNO_3$ reacts with As(III), producing a bright yellow silver orthoarsenite (Ag_3AsO_3) precipitate, whereas the brownish silver orthoarsenate (Ag_3AsO_4) precipitate is produced at by the reaction of $AgNO_3$ with As(V). Arsenic-oxidising ability was confirmed using a modified microplate technique (Simeonova *et al.* 2004). Each assay was performed in triplicate, and the isolates that produced brownish coloured precipitates were confirmed as As-oxidising strains.

Arsenite oxidase assay

The bacterial strains exhibiting As-oxidising activity were grownin MMS in the presence of 30 mM of As(III). Cells at late log-phasewere harvested by centrifugation at 10,500 ×g rpm for 2 min. The collected cells were washed with 50 mMTris–HCl buffer (pH 8.0) andsuspended in 2 mL buffer containing 0.5 mM phenyl methyl sulfonylfluoride (PMSF) and 1mg/ml lysozyme. Cell suspensions were incubated for 2 h with occasional stirring. The cells were lysed using sonication and centrifuged at 10,500 × g at 4°C for 30 min. Cell debris was removed by centrifugation at 10, 500× g rpm for 30 min (Bachate *et al.* 2012). Protein concentrations in the supernatants were determined by Bradford assay (Bradford, 1976) using bovine-serum albumin (Sigma) as a standard. The arsenite oxidase assaywas performed using a method previously described by Anderson *et al.* (1992).

Quantitative determination of potential plant-growth promoting traits of As-resistant bacteria

The As-resistant isolates were tested for their ability to solubilize phosphate, to produce siderophores and IAA-like molecules and to grow on ACC as the sole nitrogen source.

Screening for phosphate-solubilization

The ability of As-resistant bacterial isolates to solubilize phosphate was tested by growing the strains in modified Pikovskaya's medium (Gihring and Banfield, 2001) with 0.5% of tri-calcium phosphate (TCP) at 30°C for 5 days at 170 rev/min in order to reach a stationary phase (determined

by measuring absorbance at 600 nm). The cultures supernatants were collected by centrifugation at 6,500 × g for10 min. The soluble phosphate in the culture supernatant was estimated according to the method of Zaidi *et al.* (Glick and Penrose, 1998).

Screening for IAA-like molecules production

As-resistant bacterial strains were cultured in a minimal medium (KH_2PO_4 0.4 g L−1, K_2HPO_{42} g L−1, $MgSO_4{\cdot}7H_2O$ 0.2 g L−1, $FeSO_4{\cdot}7H_2O$ 0.1 g L−1, $CaCl_2$0.1 g L−1, NaCl 0.2 g L−1, $NaMoO_4{\cdot}2H_2O$ 0.005 g L−1, glucose 10 g L−1) with 0.5 mg mL−1l-tryptophan, a precursor of IAA-like molecules. After 5 days of incubation at 30°C in the dark, 2 mL of the cell suspension was transferred into a tube and then mixed vigorously with 100 _L of 10 m Morthophosphoric acid and 4 mL of Salkowski's reagent (2% 0.5 M $FeCl_3$ in 35% perchloric acid) and incubated for 45 min for development of pink color. Then the absorbance was read at 530 nm. The IAA-like molecule concentration in the cultures was determined using a calibration curve of pure IAA as a standard following the linear regression (Grichko *et al.* 2000).

Nodule formation

The bacterial culture were inoculated in sterilized soil in a pot. The seeds of ground nut also inoculated in this pot. An uninoculated bacterial culture soil with ground nut and lentil seeds were also kept as a positive control.

RESULTS AND DISCUSSION

In this study, soil samples were collected from four different locations within the Chakdah block of West Bengal,where As-contaminated groundwater has been used for irrigation for years. The physico-chemical properties and As status of the experimental soils are presented in Table 1. Total and extractable As concentrations of the soils varied from 13.2 to 17.2 mg kg^{-1} and from 1.3 to 2.2 mg kg^{-1}, respectively. The experimental soils were neutral in reaction (pH 6.9 to 7.6), moderate-to-high in organic C (6.8 to 11.4 g kg^{-1}), and low in available N (118–189 kg ha^{-1}). The level of available P (21– 28 kg ha^{-1}) was moderate-to-high, whereas that of the available K (124–134 kg ha^{-1}) was low-to-moderate. The Electrical conductivity (ds/m) of experimental soil varies from 0.61 to 0.63 and Available phosphorus (kg/ha) varies from 21 to 28. The microbial biomass carbon (213–439 μg g^{-1}) varied significantly among the experimental sites. Total microbial count (6.39–6.41 log CFU), As resistant microbial count (3.57–3.61 log CFU) and Arsenic resistant PGPR bacterial count (0.96 to 0.97) of the experimental soils did not exhibit significant differences.

Enrichment and isolation of As-resistant bacteria and Determination of minimum inhibitory concentration of As(III) and As(V)

Arsenic-resistant bacteria from agricultural soil in the Nadia were isolated using enrichment

Table 1: Physiochemical and microbiological properties of experimental soils

Soil parameters	Site-1	Site-2	Site-3	Site-4
Electrical conductivity (ds/m)	.6300[a]	.6167[a]	.6267[a]	.6300[a]
Soil PH	7.5[a]	6.9[a]	7.4[ab]	7.6[a]
Organic carbon (gm/kg)	11.4[a]	6.8[b]	7.2[b]	9.9[ab]
Available nitrogen (kg/ha)	118[b]	178[b]	170[ab]	189[a]
Available phosphorus(kg/ha)	21[b]	26[ab]	23[ab]	28[a]
Available potassium(kg/ha)	124[b]	133[ab]	127[ab]	134[a]
Total arsenic (mg/kg)	17.1[a]	17.2[a]	16.7[ab]	13.2[b]
Available arsenic(mg/kg)	2.2[a]	1.5[ab]	1.3[b]	1.8[ab]
Microbial biomass carbon (MBC) (μg g^{-1} of soil)	439[a]	290[ab]	313[ab]	213[b]
Total bacterial population (log CFU)	6.4[a]	6.3[a]	6.3[a]	6.4[a]
Arsenic resistant bacterial population (Log CFU)	3.6[a]	3.6[a]	3.5[a]	3.5[a]
Arsenic resistant PGPR bacterial population	0.96[b]	0.9633	0.96[ab]	0.97[a]

Means followed by a different letter are significantly different at p≤0.05 by Tukey's HSD (honest significant difference) test.

techniques. Several hundred bacterial colonies were able to grow on the YEMA agar plates containing different arsenate and arsenite concentration. Colonies different in shape, color and margins (10 bacterial colonies) were picked from the most diluted plates (10^{-6}–10^{-5} dilutions) and screened for their MICs. Arsenic-resistant isolates, which had MICs ≥ 19 mM and ≥200 mM for As(III) and As(V), respectively, were selected for further characterization and identification. From the ten As-resistant isolates (MIC ranged from 19 to 46 mM and 168 to 409 mM for As(III) and As(V), respectively), isolate AL^{-1} had the highest MICs of 46 mM and 409.66 mM for As(III) and As(V), respectively, where as isolates AR-33 and AR-46 had the lowest MICs of 19 mM and 213, 21 mM and 168 mM for As(III) and As(V), respectively (Table 2).

Table 2: Minimal Inhibitory Concentration (MIC) on Agar (Yeast Extract Mannitol Agar or YEMA) plates

Bacterial Isolates	MIC of As^{V} (mM)	MIC of As^{III} (mM)
AL-1	409.4a*	46.1a
AL-73	390b	41.3b
AR -17	299.4d	30.3d
AR -30	278.3e	28d
AR -33	213.1g	19.6e
AR -42	382c	41.6b
AR -46	168i	21e
AR -57	380.2c	33.65c
AR -63	239.6f	22.66e
AR -71	199.66h	20.2e
Control (uninoculated)	ND (not detected)	ND (not detected)

Means followed by a different letter are significantly different at p≤0.05 by Tukey's HSD (honest significant difference) test.

Phenotypic and biochemical characterization of the isolates

Both isolates were milky white in color, have a smooth surface, and flat colonies and size. Both the isolates were Gram-negative and rod shaped (Table 3).

Identification of the isolates

Based on phenotypic, biochemical the isolates BcAL-1 and JN73 were identified as *Burkholderia cepacia* and *Burkholderia metallica* respectively.

Bioremediation of arsenate and arsenite by the isolates

The two isolates were resistant against high concentration of both As(III) and As(V). They also exhibit the potentiality to reduce arsenic concentration from nutrient broth media containing 100 ppm of arsenate and arsenite. They both removed 29% arsenite. However, removal of arsenate between AL-1 (30%) and AL-73 (29%) was observed from the media after 72 h of incubation.

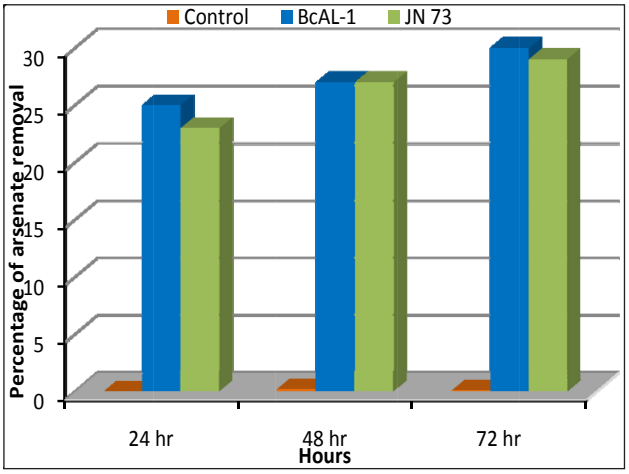

Fig. 1: Arsenate removal by isolates AL-1 and AL-73

Table 3: Morphological and biochemical characterization of isolated As-resistant bacterial strain

Characters	Isolates									
	BcAL-1	JN73	AR-17	AR-30	AR-33	AR-42	AR-46	AR-57	AR-63	AR-71
Gram stain	–	–	–	–	–	–	–	–	–	–
Cell shape	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod
Cell length (μ)	2μm	2μm	2μm	2μm	2μm	2μm	2μm	2μm	2μm	2μm
Colony color	Milky white	Milky white	white	white	white	white	white	white	white	white
Oxidase	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+

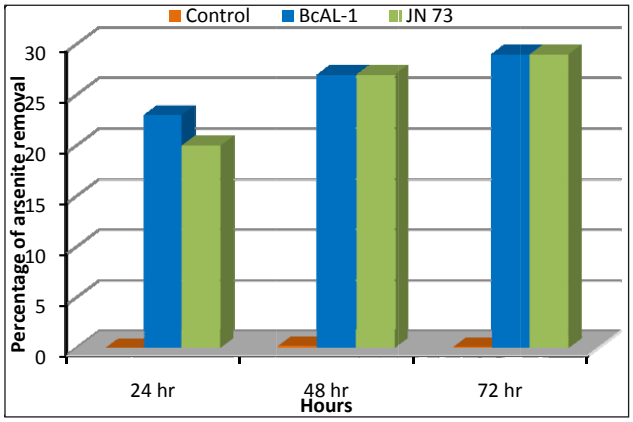

Fig. 2: Arsenite removal by isolates AL-1 andAL-73

Oxidation and reduction of arsenic

After the addition of silver nitrate into 72 h old culture plate containing arsenite, the media slowly turned brown which confirmed the presence of silver arsenate in the media. But when silver nitrate was mixed with the culture containing arsenate, it also turned brown confirming the presence of silver arsenate. Hence, it is observed that none of the bacteria has the ability to reduce arsenate to arsenite, but both of them can oxidize arsenite to arsenate.

As(III)-oxidase assay

The specific As(III)-oxidase activity in the cellular lysates of the As(III)-oxidizing bacterial isolates varied from 0.11 to 5.8 nM $min^{-1}mg^{-1}$ of protein. BcAL-1 exhibited the highest As(III)-oxidase enzyme activity (5.8 nM $min^{-1}mg^{-1}$ protein), followed by JN73 (5.2 nM $min^{-1}mg^{-1}$ protein), AR-17 (4.9 nM $min^{-1}mg^{-1}$ protein), AR-30 (4.1 nM $min^{-1}mg^{-1}$ protein), AR-33 (1.9 nM $min^{-1}mg^{-1}$ protein), It was noted that isolates having higher As(III) oxidation capacity also exhibited higher levels of As(III)-oxidase enzyme activity. As expected, isolates screened negative for As(III)-oxidizing activities did not exhibit specific As(III)-oxidase enzyme activity (Table 4).

Table 4: Arsenic Oxidase Activity

Bacterial Isolates	Enzyme activity (Nm $min^{-1}mg^{-1}$ protein)
AR -1	5.8[a]
AR -8	5.2[b]
AR -17	4.9[c]
AR -30	4.1[d]
AR -33	1.9[e]
AR -42	0.99[f]
AR -46	0.95[f]
AR -57	0.55[g]
AR -63	0.11[h]
AR -71	0.34[h]
Control (uninoculated)	0.11[i]

Means followed by a different letter are significantly different at p≤0.05 by Tukey's HSD (honest significant difference) test.

Screening of potential plant growth promoting As-resistant bacteria

All ten isolates were assayed for one or more characteristics considered to be important for PGP activity. From the ten As-resistant bacterial isolates, AL-1 *Burkholderia capasia*, AL-73 *Burkholderia metalica*, AR-17, AR-33, were able to solubilize phosphate, produce IAA-like molecules. Isolates AL-1, AL-73 possessed one or more than one PGP trait. However isolates AR-33, AR-42, AR-46 possess IAA production, nodulation but did not showed good amount of phosphate solubilization capacity. Notably, the max-imum phosphate-solubilization and, IAA-like molecules were observed in AL-1 *Burkholderia capasia*, AL-73 *Burkholderia metalica* and they are high resistant to arsenic.

Table 5: Quantitative test (IAA Production)

Isolate	IAA production (μml^{-1})	
	Without tryptophan	With tryptophan
AL-1	2.8a	5.5bc
AL-73	2.2a	5.7b
AR-**17**	0.8b	0.8333g
AR-**30**	2.5a	4.6bcd
AR-**33**	0.3b	2.3f
AR-**42**	2.9a	7.3a
AR-**46**	2.3a	4.2cde
AR-57	2.3a	3.8333de
AR-63	0.6b	1.6fg
AR-71	0.8b	1.5fg
Control (uninoculated)	0.6b	2.9ef

Means followed by a different letter are significantly different at p≤0.05 by Tukey's HSD (honest significant difference).

Table 6: Quantitative test [Soluble Phosphate (gL^{-1}), Number of nodule in Ground nut]

Isolate	Soluble Phosphate (gL^{-1})	Number of nodule in Ground nut
BcAL-1	3.2ab	67a
JN73	2.7abc	60ab
AR-17	3.1ab	57abc
AR-30	3.6a	50abc
AR-33	1.2ef	46.2abc
AR-42	2.1cde	35.6bcd
AR-46	0.6f	45abc
AR-57	1.5def	56abc
AR-63	0.5f	31.6cd
AR-71	1.9cde	36.3bcd
Control	2.4bcd	18d

Means followed by a different letter are significantly different at p≤0.05 by Tukey's HSD.

From the experimental data it can be observed that plant growth promoting bacteria also found arsenic contaminated soil. When a microbial community stays under a selective stress condition like high concentration of arsenic for a very long period, they must develop some mechanism to detoxify it and overcome the restriction for growth (Huang *et al.* 2010). It is reported that sometimes microbial community gain protection against toxic agents through the formation of microbial biofilms (Mahand Toole, 2001). Hence, it may be possible that through the formation of biofilms the isolated bacterial strains can resist the toxicity of arsenic. The arsenic resistance mechanism of bacteria can be plasmid associated (Tsai, *et al.* 1997) or by ars operon, containing the genes arsRBC (Carlin *et al.* 1997) when it can resist higher concentrations. A carrier protein, arsB also helps in the extrusion of arsenic from cell. The bacterium which possesses arsA gene also coupled with arsB and significantly increases the arsenic resistant capacity of the bacteria (Rosen, 2002).

In 2004, Anderson and Cook (Anderson and Cook, 2004) have isolated seventeen bacterial strains including *Bacillus licheniformis Bacillus polimyxa*, etc. which were able to resist up to 100 ppm arsenic. Incidence of an arsenic hyper tolerant bacterium from well water which was able to tolerate up to 2000 ppm arsenate was also reported by Zelibor (Zelibor *et al.* 1987). Other bacteria from *Bacillus* groups reported over time also showed the gene mediated arsenic resistance potentiality (Anyanwu, and Ugwu, 2010). These two bacteria were arsenic oxidizing bacteria. The increased growth pattern of the bacteria with increasing arsenite concentration suggested that the bacteria get additional energy from the oxidation process (Mullar *et al.* 2009). Once the bacteria converted the arsenite to arsenate, they easily resist its effect as they are already resistant to higher concentration of arsenate. Both the isolates were Gram-negative in nature. The isolate BcAL-1 and JN73 revealed that the strain *Burkholderia capecia* and *Burkholderia metallica* respectively. The isolate AL-1 and AL-73 can remove 29% arsenite and 30% and 29% arsenate, respectively from the media after 72 h of incubation. Arsenic resistant microbes can reduce the arsenic concentration from the media by developing a number of detoxifying mechanisms including metal reduction, metal efflux, bacterial cell membrane binding, adsorption of heavy metals on to cell surface and complexation of the metal with exopoly sacharides (Anyanwu and Ugwu, 2010). Complexation of heavy metal with carboxyl and phosphate group in the inner portion of metal resistant bacteria has also been reported as a mechanism of bioremediation (Fang *et al.* 2011).

In addition, arsenic resistant bacteria sometimes use both arsenite and arsenate as their natual primary substrate by some specific genes (Brettar *et al.* 2002). From SEM study it was observed that both BcAL-1 and JN73 formed chain like arrangement when treated with arsenic. Both the bacteria were able to oxidize arsenite to less toxic form arsenate but none of them has the ability to reduce arsenate to arsenite. The arsenic resistant bacteria have some specific enzymes, arsenic reductase and arsenic oxidase by which they can oxidize or reduce arsenic. Some bacteria have been reported for containing both these enzymes and can oxidize as well as reduce arsenic (Banerjee *et al.* 2011).

Thus As-resistant microorganisms might have a selective advantage with regard to survival under As-stressed conditions. In addition tosiderophore production, phosphate-solubilization by As-resistant bacteria has also been reported to play an important role in plant growth and survival of bacteria under As-stressed conditions (Srivastava *et al.* 2013). The growth of As-sensitive plants in As-contaminated soil could be adversely affected because As(V) in soils reduces the amount of phosphorous in plants

(Singh *et al.* 2006). The ability of As-resistant bacteria to produce IAA has been reported to induce higher shoot length and higher numbers of leaves and total chlorophyll content in inoculated plants (Srivastava *et al.* 2013). Plant growth-promoting bacteria synthesize IAA utilizing tryptophan excreted by roots in the rhizosphere. The synthesized IAA is then secreted and transported into the plant cells, (i) participates in plant cell growth and (ii) promotes ACC synthase activity to increase the ethylenetiter (Ma *et al.* 2011). The bacteria possessing ACC deaminase metabolize the ethylene precursor, ACC, and lower the stress ethylene production in plants, thus facilitating the formation of longer roots in plants growing in heavy metals/metalloids-contaminated soil (Cavalca *et al.* 2010). Recent studies have revealed that plants inoculated with bacteria containing ACC deaminase are better able to thrive in As-spiked soils.

CONCLUSION

The isolated bacterial strains *Burkholderia capecia*, *Burkholderia metallica* because they offer great potential in regard to novel crop production strategies due to their resistance to As and the presence of several potential PGP traits. The isolate BcAL-1, which is comparatively more resistant to As, possesses greater As(III)-oxidizing activity and exhibits higher phosphate-solubilization, IAA-like molecules and ACC deaminase as compared to other As-resistant isolates. It could therefore be a better choice for potential application in As remediation as well as for sustainable agronomic production programs in As-contaminated soils.

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