CHAPTER 4

EVALUATION OF PLANT-GROWTH PROMOTING TRAITS OF ARSENIC-RESISTANT BACTERIA

4.1 Introduction

The widespread existence of elevated of heavy metals in cultivated soils as the result of human activities has raised more concern about their potential effects on human health and the environment. Amongst the various heavy metal contaminants arsenic are recognized as the leading toxicants worldwide thus it is an important current public health issue due to its toxic effects and accumulation through the food chain (Zhao *et al.*, 2010; Akhtar *et al*., 2013). Arsenic have been reported as the most prevalent heavy metal contaminant in agricultural soils of northern Thailand (Shutsrirung, 2013). Besides agrochemical usage, arsenic is also accumulated in the topsoil of cultivated land through irrigation. Due to its low solubility and low volatility the contaminated topsoil may have influence on the entry of arsenic into the food chain (Das *et al*., 2013).

Bioremediation of arsenic in contaminated soil by microorganisms is a natural process to alter contaminants by rendering the contaminants harmless or less toxic products. The activities of microorganisms to alter the toxicity of arsenic through various mechanisms, e.g. release of chelating agents, acidification and phosphate solubilization, enhance arsenic mobilization in soils thus play a critical role in arsenic mobility and availability to the plant (Smith and Read, 1997; Abou-Shanab, 2003; Akhtar *et al*., 2013). Several investigations have shown that microorganisms not only affect the mobility and availability of heavy metals but also exhibit plant growth promoting abilities such as producing phytohormone and solubilizing phosphorus and other nutrients (Bano and Masarrat, 2003; Passardi *et al*., 2004). For these reasons, mechanisms involved in microbial detoxification of arsenic and heavy metals have recently received more attention. In the context of increasing international concern for food and environmental

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quality, the use of arsenic-resistant bacteria which are also cable of plant growth promotion is of interest.

The aim of this study was to investigate plant growth promoting traits performed using five selected isolates; BAs7, BAs8, BAs11, BAs19 and BAs29 which was identified in the previous chapters. The selected strains were evaluated for their ability in plant growth promotion, i.e. phosphate solubilization, indole acetic acid (IAA) production, and seed germination enhancement. The promising strains may be employed as potential bio-inoculants for detoxifying arsenic and improving the growth of plants in As contaminated soils. Accordingly, 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.

4.2 Materials and methods

The five bacterial strains i.e. one arsenic sensitive isolates (BAs7) and four arsenic resistant isolates; BAs8, BAs11, BAs19 and BAs29 were used in the following experiments.

4.2.1 Evaluation for phosphate-solubilization under different level of arsenite

The five selected isolates were tested of quantitative estimation of phosphate (P) solubilization in Pikovskaya's broth medium, was modified by adding $Ca_3(PO_4)_2$ (PKVb) (Gaur, 1990). Various concentration of sodium arsenite ($NaAsO_2$) was applied in the PKVb medium in order to observe the effect of arsenic on P solubilization. The 0.50 mL of 7-day-old culture broth of each isolate was inoculated into 50 mL of PVKb medium containing Al (50 µM) and $NaAsO_2$ 0, 15, 50 and 100 mg/L. The medium pH was adjusted to 7.0. After 5 days on incubator shaker (120 rpm) at room temperature (≈ 30°C) the cultures were centrifuged at 5,000 rpm for 15 minutes (ORTO ALRESA, Spain). One ml of supernatant was mixed with 4 ml of color reagent (1:1:1:2 ratio of 6N H_2SO_4, 2.5% $(NH_4)_2MoO_4$, 10% ascorbic acid and distilled water), incubated at room temperature in the dark for 30 minutes and observed by measuring optical density at 820 nm using spectrophotometer (Thermo Scientific, mod. GENESYS 20, USA). The amount of soluble phosphorus was determined from the standard curve of KH_2PO_4.

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4.2.2 Solubilization of various insoluble phosphate sources

The five selected isolates were examined for their ability to solubilize various sources of insoluble phosphate (P). Quantitative estimation of phosphate solubilization was performed using PVKb medium (Gaur, 1990). The medium was modified by adding $Ca_3(PO_4)_2$, $AlPO_4$ or rock phosphate as a sole source of insoluble phosphate in the culture broth. The 0.50 mL of 7 day-old culture broth of each isolate was inoculated into 50 mL of PVKb containing Al (50 µM), $NaAsO_2$ 0, 15 mg/L and the media was suspended with a source of insoluble phosphate [0.5% $Ca_3PO_4)_2$ (AP, Sigma, USA), 0.5% $AlPO_4$ (AP, Sigma, USA) or 0.5% rock phosphate (Lamphun, Thailand), the pH was adjusted to 7.0. After 5 days on incubator shaker (120 rpm) at room temperature (≈ 30°C) the cultures were centrifuged at 5,000 rpm for 15 minutes (ORTO ALRESA, Spain). One ml of supernatant was mixed with 4 ml of color reagent (1:1:1:2 ratio of 6N H_2SO_4, 2.5% $(NH_4)_2MoO_4$, 10% ascorbic acid and distilled water), incubated at room temperature in the dark for 30 minutes and observed by measuring optical density at 820 nm using spectrophotometer (Thermo Scientific, mod. GENESYS 20, USA). The amount of soluble phosphorus was determined from the standard curve of KH_2PO_4

4.2.3 Indole-3-acetic acid production

The ability of five selected isolates to produce indole-3-acetic acid (IAA) was determined by the method as described by Gordon and Weber (1951). All the isolates were multiplied in nutrient broth until reach maximum growth. After that, 0.50 ml of the culture solution was inoculated in 50 ml of NB containing tryptophan (0.1 g/L), Al (50 µM) and various concentration of $NaAsO_2$ was applied (0, 15, 50 and 100 mg/L). The medium pH was adjusted to 7.0. All the culture broth was incubated with shaking (120 rpm) at room temperature (≈ 30°C) for 5 days. The cultures were centrifuged at 5,000 rpm for 15 minutes (ORTO ALRESA, Spain). One ml of supernatant was mixed with 2 ml of Salkowski's reagent (2% 0.5 M $FeCl_3$ in 35% perchloric acid) incubated at room temperature in the dark for 30 minutes for development of pink color. The IAA-like molecule concentration in the culture was determined using a calibration curve of pure IAA as a standard following the linear regression. The standard IAA series; 0, 10, 20,

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50, 100, 150 µM were used. The optical density (OD) of the isolates and the standard was recorded at 530 nm after 30 min using spectrophotometer (Thermo Scientific, mod. GENESYS 20, USA).

4.2.4 Evaluation of siderophore production using the Chrome Azurole S Assay

Siderophore production by the selected isolates was evaluated using the Chrome Azural S (CAS) approach (Schwyn and Neiland, 1987). Briefly, siderophore production was qualitatively tested on Petri dishes containing CAS-agar. Modified CAS agar plate was prepared as follow: 60.5 mg of CAS was dissolved in 50 ml deionized water then mixed with 10 ml iron (III) solution (1 mM $FeCl_3.6H_2O$, 10 mM HCl). Under stirring, this solution was slowly mixed with 72.9 mg HDTMA (Sigma, USA) dissolved in 40 ml distilled water. The resultant dark blue solution was autoclaved and mixed with an autoclaved mixture of 900 ml distilled water, 18 g of Agar, 5 g of glucose, 1 g of KNO_3, 0.1 g of NaCl, 0.5 g of K_2HPO_4, and 0.1 g of $MgSO_4$ (pH 6.8). The 7-day-old culture broth of each isolate (20 µl) was dropped on CAS-agar plates and incubated at 30°C for one week in the dark. The colonies with orange zones were considered as siderophore-producing ability of the isolate.

4.2.5 Seed germination test

This experiment was performed to determine the inoculation effects of the selected isolates on seed germination and root growth. The Chinese Kale seeds were surface-sterilized by 3% sodium hypochlorite ($NaClO_4$) and rinsed by gently shaking several times in sterile distilled water. The sterile-seeds were placed on sterile filter paper in Petri dished. The bacterial suspension was diluted 50 times with sterile water and then 2 ml of each isolate, the medium broth and sterile distilled water (control), were added separately to the filter paper. All the plates were placed in the incubator for 3 days and the temperature was maintained at 30°C. Seed germination and root length of Chinese Kale were measured after incubation.

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4.3 Results

4.3.1 Phosphate solubilization under various concentration of arsenite

In this study five selected isolates were evaluated for their phosphate (P) solubilizing ability in Pikovskaya's broth medium (PKVb) and PKVb plus $NaAsO_2$ (0, 15, 50 and 100 mg/L) and Al (50 µM) (PKVbp). All the selected arsenic-resistant strains showed a certain ability to solubilize P in both PKVb and PKVbp. It was interesting to note that all the strains solubilized more P under increasing concentration of $NaAsO_2$ in PKVbp as compared to PKVb. Each individual isolate exhibit its highest ability to solubilized P at different increasing $NaAsO_2$ concentrations; BAs7, 100.04 mg P/L at 15 mg/L $NaAsO_2$; BAs8 showed the highest ability to solubilize P (63.01 mg/L) in PKVbp 50 mg/L $NaAsO_2$, BAs11 showed the highest ability to solubilize P (27.27 mg/L) in PKVbp 15 mg/L $NaAsO_2$, BAs19 showed the highest ability to solubilize P (73.77 mg/L) in PKVbp 50 mg/L $NaAsO_2$ and BA 29 showed the highest ability to solubilize P (82.5 mg/L) in PKVbp 50 mg/L $NaAsO_2$. This value was higher than those of all the isolates in PKVb and PKVbp at all the $NaAsO_2$ concentrations (Table 4.1).

The sensitive isolate, BAs7 gave significant higher solubilized P than the rest of the isolates at 0 and 15 mg/L $NaAsO_2$. However, on the average, BAs29 appeared to gave significant higher P values than the rest of the isolates at higher $NaAsO_2$ concentration (50 mg/L).

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Table 4.1 Phosphate solubilizing ability of arsenic-resistant bacteria under different levels of $NaAsO_2$

Isolate	Solubilize P (mg/L)			
	$NaAsO_2$ 0 mg/L	$NaAsO_2$ 15 mg/L	$NaAsO_2$ 50 mg/L	$NaAsO_2$ 100 mg/L
BAs7	55.56a	100.04a	45.09b	22.8b
BAs8	13.12c	21.32d	63.01a	37.41a
BAs11	5.95d	27.27cd	12.8c	8.97c
BAs19	10.66cd	32.80c	73.77a	36.38a
BAs29	37.93b	48.79b	82.5a	35.87a
F-test	**	**	**	**
C.V.	15.41	11.31	10.51	7.28

F-test at P<0.05 the symbol "**" is significantly different at the P<0.05

The ability of the selected isolates in phosphate solubilization of various sources i.e. $Ca_3(PO_4)_2$, $AlPO_4$ and rock phosphate by arsenic-resistant bacteria was examined.

Five isolates were evaluated for their phosphate (P) solubilizing ability in Pikovskaya's broth medium (PKVb) and PKVb plus $NaAsO_2$ (15 mg/L) and Al (50 µM) (PKVbp). All the selected arsenic-resistant isolates showed a certain ability to solubilize P in both PKVb and PKVbp, isolate BAs7 showed the highest ability to solubilize P and released about twice the amount of solubilized P (100.04 mg/L) in PKVbp greater than that in PKVb (55.56 mg/L) (Table 4.2). The same phenomenon was observed in the rest of the isolates. Isolates BAs11 and BAs19 solubilized around 3 to 4.5 times of P in PKVbp (27.27 and 32.80 mg/L, respectively) higher than those in PKVb (5.95 and 10.66 mg/L, respectively.

Phosphate (P) solubilizing ability of the selected isolates was also evaluated in Pikovskaya's broth medium (PKVb ($AlPO_4$)) and PKVb ($AlPO_4$)) plus $NaAsO_2$ (15 mg/L) (PKVb ($AlPO_4$)p). On the average the ability to solubilize P of each isolate in PKVb ($AlPO_4$)p was higher than in PKVb ($AlPO_4$). In PKVb ($AlPO_4$)p, isolate BAs19 showed the highest ability to solubilize P (5.10 mg/L) followed by BAs7, BAs8 BAs29 and BAs11 with values of 4.11, 3.91, 3.88 and 2.19, respectively. In PKVb ($AlPO_4$) the

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highest of solubilized P was obtained with BAs19 (3.78 mg/L) followed by BAs7, BAs29, BAs8 and BAs11 with values of 3.52, 2.72, 3.23, and 2.28 mg/L, respectively (Table 4.2).

Phosphate (P) solubilizing ability of the selected isolates was also evaluated in Pikovskaya's broth medium (PKVb (rock phosphate) and PKVb (rock phosphate) plus $NaAsO_2$ (15 mg/L) and Al (50 µM) (PKVb (rock phosphate)p. All the isolates in PKVb (rock phosphate) BAs29 exhibited highest ability to solubilize P (6.77 mg/L) followed by BAs19 (5.74 mg/L), BAs11(5.33 mg/L), BAs8 (4.51 mg/L) and BAs7 (1.64 mg/L). In PKVb (rock phosphate)p, BAs11 gave the highest solubilized P (3.69 mg/L) while BAs7 gave the lowest value (0.62 mg/L) (Table 4.2).

The pH of PKVb and PKVbp, PKVb ($AlPO_4$)) and PKVb ($AlPO_4$)p and PKVb (rock phosphate) and PKVb (rock phosphate)p were decreased by all the selected isolates when compared to the control treatment. In PKVb and PKVbp, the pH was lowest when inoculated with BAs7 (4.53 and 4.54), while in PKVb($AlPO_4$)) and PKVb ($AlPO_4$)p the pH was lowest when inoculated with BAs11 (3.75 and 3.79). Inoculation of BAs11 in PKVb (rock phosphate) gave the lowest pH value (4.14) while BAs11 gave the lowest pH value (4.20) in PKVb (rock phosphate)p (Table 4.3).

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Table 4.2 Effect of different phosphate substrates on solubilization by arsenic-resistant bacteria

Isolate	Solubilized P (mg/L)					
	$Ca_3(PO_4)_2$	$AlPO_4$	rock phosphate	$Ca_3(PO_4)_2$[1]	$AlPO_4$[1]	rock phosphate[1]
BAs7	55.56a	3.52ab	1.64b	100.04a	4.11b	0.62b
BAs8	13.12c	2.72c	4.51ab	21.32d	3.91b	1.44ab
BAs11	5.95d	2.28c	5.33a	27.27cd	2.19c	3.69a
BAs19	10.66cd	3.78a	5.74a	32.80c	5.10a	1.23ab
BAs29	37.93b	3.23bc	6.77a	48.79b	3.88b	1.03ab
F-test	**	**	**	**	**	**
C.V.	15.41	9.77	37.36	11.31	10.28	82.23

[1] P source supplemented with $NaAsO_2$ 15 mg/L

F-test at P<0.05 the symbol "**" is significantly different at the P<0.05

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Table 4.3 Effect of different phosphate substrates on pH changed by arsenic-resistant bacteria.

Isolate	pH					
	$Ca_{3(PO_4)_2}$	$AlPO_4$	rock phosphate	$Ca_{3(PO_4)_2}$[1]	$AlPO_4$[1]	rock phosphate[1]
control	5.62a	5.59a	5.79a	5.7a	5.60a	4.69a
BAs7	4.53d	4.77b	4.61b	4.54d	4.74b	4.58b
BAs8	4.98b	4.74c	4.26c	4.77c	4.75b	4.39e
BAs11	4.79c	3.75e	4.14d	4.76c	3.79d	4.20f
BAs19	5.05b	4.63d	4.16d	4.95b	4.6c	4.46d
BAs29	5.0b	4.64d	4.15d	4.85bc	4.65c	4.51c
F-test	**	**	**	**	**	**
C.V.	0.88	0.39	0.88	1.71	0.37	0.57

[1] P source supplemented with $NaAsO_2$ 15 mg/L

F-test at P<0.05 the symbol "**" is significantly different at the P<0.05

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4.3.2 IAA production of arsenic-resistant bacteria

IAA production of five arsenic-resistant isolates were determined and compared between the NB medium and NB medium plus $NaAsO_2$ 15 mg/L and Al 50 µM. The results obtained were mentioned in Table 5.4. All the isolates showed ability for IAA production. The highest IAA producing isolate (20.32 mg/L) was obtained with isolate BAs19 in NB medium plus $NaAsO_2$ and Al, followed by isolate BAs29 (12.65 mg/L), isolate BAs7 (12.49 mg/L), isolate BAs8 (12.39 mg/L) and isolate BAs11 (9.86 mg/L). In the NB medium isolate BAs11 performed highest IAA production (18.41 mg/L), followed by BAs7 (10.22 mg/L), BAs8 (9.53 mg/L), BAs19 (9.12 mg/L) and BAs29 (9.06 mg/L).

Table 4.4 Indole-3-acetic acid (IAA) produced by arsenic-resistant bacteria

Isolate	pH	IAA (mg/L)	pH	IAA (mg/L)
control	7.0	-	7.0	-
BAs7	8.50	10.22b	8.09	12.49b
BAs8	8.43	9.53b	8.08	12.39b
BAs11	8.34	18.41a	7.98	9.86c
BAs19	8.46	9.12b	8.14	20.32a
BAs29	8.46	9.06b	8.01	12.65b
F-test		**		**
C.V.		7.70		6.06

F-test at P<0.05 the symbol "**" is significantly different at the P<0.05

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4.3.3 Siderophore production by arsenic-resistant bacteria

Siderophores also indirectly stimulates the biosynthesis of the other antimicrobial compounds by making these minerals (Fe^{+3}) easily available to bacteria (Dikin *et al*., 2007). We found that only strain BAs11 could grow on CAS blue agar and the color was changed from blue to orange. Some of our strains selected in this study did not grow on CAS agar, it is suggested that this problem was caused by the toxicity of HDTMA (72.9 mg/L) present in CAS agar which affects mainly fungi and Gram-positive bacteria (Milagres *et al*., 1999).

Table 4.5 Siderophore production by arsenic-resistant bacteria.

Strains	Growth (day)[a]	CAS blue agar (color change)	CAS reduction[b]
BAs 7	No growth	-	-
BAs 8	No growth	-	-
BAs 11	7	orange	+
BAs 19	No growth	-	-
BAs 29	No growth	-	-

[a]Time (day) required for the bacteria colonies to grow

[b]Color changed of the CAS blue agar; + positive, - negative

control strain BAs 11

Fig 4.1 Siderophore production by strain BAs11

4.3.5 Seed germination test

All the isolates positively affected the germination of Chinese *Kale* seed but the values are not significantly different (P< 0.05). They increased the seed germination by 6.67 -16.67% over the control (Table 6). The highest percentage of seed germination was obtained with strain BAs29 (96.67%) followed by BAs7 (93.33%), BAs11 (90%) and BAs8 and BAs19 (86.67%). The control (sterile distilled water) gave only 80% seed germination therefore BAs29 increased 16.67% germination over the control.

Table 4.6 Effects of arsenic-resistant isolates on seed germination and root length of *Chinese Kale*.

Treatment	OD (600 nm)	pH	Diluted culture solution (1:50)	
			Seed germination (%)	Root length (cm)
Control	0	6.91	80.00	1.55 ± 0.35
BAs7	0.963	8.66	93.33	1.98 ± 0.37
BAs8	0.932	8.72	86.67	1.45 ± 0.15
BAs11	0.613	7.56	90.00	1.74 ± 0.26
BAs19	1.214	8.72	86.67	1.85 ± 0.29
BAs29	0.910	8.74	96.67	1.72 ± 0.39
F-test				ns
C.V.				18.17

Values are means of three replications ± SE

Mean with the same letter are not significantly different (P< 0.05)

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4.4 Discussion

Our results indicated that arsenic tolerant isolates could solubilize Ca-phosphate and rock phosphate into available P form by lowering the pH of the medium suggesting organic acid(s) might be produced to increase phosphate availability under P deficiency. The principal mechanism in the soil for mineral phosphate solubilization is lowering of soil pH by the microbial production of organic acids and mineralization of organic P by acid phosphatases (Khan *et al*., 2009). Based on previous studies, the primary mode of P solubilization by microbes is by production of organic acids (Vassilev *et al*., 2006; Chen *et al*., 2006), which is supported by pH reduction in the solution. Chen *et al*. (2006) also found an inverse relationship between the pH and P solubilization by bacterial strains *Arthrobacter* sp., *Bacillus megaterium*, and *Serratia marcescens*. The decrease in pH (6.8–7 to 4.9–6) was due to the exudation of organic acids like citric, gluconic, succinic and lactic acid. Arsenate is taken up via phosphate transport system in both prokaryotes and eukaryotes since phosphate ion is similar to arsenate ion (Dixon, 1997). Substitutions for phosphate and subsequent inhibition of oxidative phosphorylation is the major toxicity of arsenate. In the plant, it was observed that increasing phosphate supply decreased arsenic uptake markedly in arsenic hyperaccumulation *Pteris vittata* (Wang *et al*., 2002). It was seen in this study that arsenic tolerant bacterial isolates solubilized much higher P (2 to 6 times) in medium with $NaAsO_2$ than in medium without $NaAsO_2$. There seemed to be no report on the relation between soluble phosphate and arsenic uptake by bacteria. However, the results of this study implied that the isolates needed more available phosphate to compete with arsenate uptake. The isolates solubilized more phosphate for lowering arsenate absorption and thus less toxicity in their cells. Our results seemed to be the first report on arsenic detoxify phenomenon outside the bacterial cell by enhancing phosphate-solubilizing ability when exposed to arsenic.

Indole-3-acetic acid (IAA) is a naturally occurring auxin with broad physiological effects and is known to enhance plant growth. Many arsenic-resistant bacteria exhibited the ability to produce IAA with the value range from 3.28 to 36.5 mg/L (Zhu *et al*., 2014). In our study, all the selected isolates could produce IAA (9.53 to 20.32 mg/L) indicating that the IAA levels found were slightly lower than the literature. It was interesting to note that IAA production by all the isolates (except for BAs 11) was increased when $NaAsO_2$ was added to the medium. This might be another mechanism of bacteria to cope with

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arsenic toxicity. It is possible that the increase of plant growth promoting the ability of highly tolerant and sensitive isolates is related to its heavy metal resistance. Bohui *et al*. (2012) determined plant growth promotion ability of arsenic-tolerant bacteria isolated from arsenic-contaminated soil of Jaanghang, South Korea. They reported that the strain JS126 performed IAA production of 3.82 mg/L (1 mg/ml of tryptophan). The ability of IAA production appeared to depend on type and strain of microorganisms, and tryptophan concentration. In addition to bacteria, Shutsrirung et al. (2013) concluded that different genera of *actinomycetes* showed different ability in IAA production. The highest IAA production was obtained with genus Nocardiopsis (62.2 to 222.8 mg/L). Zaidi *et al*. (2006) reported that a *Bacillus subtilis* strain was able to promote the growth of *Brassica juncea* and thereby increased Ni accumulation. The strain was shown to produce IAA, which might have been responsible for plant growth promotion.

We found that only BAs 11 could grow on CAS blue agar and the color was changed from blue to orange. Siderophore-producing bacteria have been shown to enhance chlorophyll content and growth of various crop plants in contaminated soil by selectively supporting iron uptake from the pool of trace element cations competing for import (Burd *et al*.,1998, 2000; Dimkpa *et al*., 2009). Moreover, complexation of trace elements by bacterial siderophores in the rhizosphere likely prevents generation of free radicals and oxidative stress. This strategy bears certain advantages over inoculation with living cells, as it does not require establishment of a population in the rhizosphere, does not raise biosafety issues and guarantees siderophore synthesis under controlled conditions. Inoculation of plants with siderophore producing bacteria has been observed to both promote and reduce heavy metal uptake, depending on the combination of plant, bacterium and metal (Ma *et al.*, 2011).

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Various physicochemical techniques, e.g., oxidation and reduction, chemical precipitation and filtration have been applied for the removal of toxic heavy metals. However high input cost with some disadvantages associated with such techniques resulting in ineffective output and secondary environmental pollution. Using high tolerant bacterial isolates could be a realistic and desirable strategy for maintaining crop production in arsenic-contaminated soils as well as reduction of arsenic uptake by crops. The tolerant isolates obtained in this study are excellent candidates for the bioremediation process of the arsenic polluted areas. Nevertheless, additional research would be

necessary to identify effects of these arsenic-tolerant and plant growth-promoting bacterial isolates on detoxification of soil arsenic and enhancement of plant growth and yield under controlled and field conditions.

4.5 Conclusions

Five strains, i.e. BAs 7, BAs 8, BAs 11, BAs 19 and BAs 29 performed promising tolerant ability under various pH values. High P solubilizing ability was observed in all the selected isolates, and the ability was increased markedly when they were exposed to arsenite, particularly sensitive isolate (BAs7), suggesting less arsenic uptake thus higher tolerant ability. It was interesting to note that IAA production by all the isolates (except for BAs 11) was also increased when $NaAsO_2$ was added to the medium. The clear phenomenon of increment in P solubilization and IAA production by the arsenic-resistant isolates in this study seemed to be the first report on simple mechanisms to cope with high arsenic environments. It is possible that plant growth promoting the ability of high arsenic tolerant and sensitive isolates is related to its heavy metal resistance. The results of this study reveal the plant growth-promoting potentials of arsenic-tolerant bacteria along with their positive effects on seed germination and root length of *Chinese Kale* seedlings. These bacterial strains may have a potential to be used in the field to improve plant P and Fe nutrition and there by enhance plant growth and use of these strains in crop production would lead to a less uptake of arsenic by plant signify their potential application for sustainable bioremediation of arsenic in the environment.

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