Phosphate Solubilization and Detoxification Mechanism of Arsenic-resistant Bacteria

การละลายฟอสเฟตและกลไกลดความเป็นพิษของแบคทีเรียต้านทานอาร์ซินิก

Yupa Chromkaew and Arawan Shutsrirung*

ยุพา จอมแก้ว และ อรวรรณ ฉัตรสีรุ้ง*

Department of Plant and Soil Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand
ภาควิชาพืชศาสตร์และปฐพีศาสตร์ คณะเกษตรศาสตร์ มหาวิทยาลัยเชียงใหม่ จ. เชียงใหม่ 50200
**Corresponding author: Email: arawan.s@cmu.ac.th*

(Received: 5 June 2018; Accepted: 29 August 2018)

บทคัดย่อ: อาร์ซินิกซึ่งรู้จักกันดีว่าเป็น “ราชาแห่งสารพิษ” พบว่า มีการปนเปื้อนในดินที่ใช้ทำการเกษตรในเขตภาคเหนือของประเทศไทย อย่างแพร่หลายมากกว่าโลหะหนักชนิดอื่น ดังนั้นจึงมีความเสี่ยงสูงที่จะปนเปื้อนในห่วงโซ่อาหารและเป็นอันตรายต่อสุขภาพของประชาชน จุลินทรีย์หลายชนิดเปลี่ยนรูปอาร์ซินิกให้อยู่ในรูปที่เป็นพิษน้อยลงซึ่งเป็นกลไกหนึ่งในการต้านทาน ในการทดลองครั้งนี้ ได้ทำการประเมินความสามารถในการเปลี่ยนแปลงพีเอช (pH) และ การละลายฟอสเฟตภายใต้ความเข้มข้นของอาร์ซิไนท์หลายระดับ ของเชื้อคัดเลือก 5 ไอโซเลท คือ BAs7, BAs8, BAs11, BAs19 และ BAs29 ผลการทดลองชี้ให้เห็นว่า แต่ละไอโซเลทสามารถเจริญได้ในอาหารที่ผสมอาร์ซิไนท์ก็ต่อเมื่อเชื้อนั้นสามารถเพิ่มค่า pH ของอาหารให้เป็นด่างมากขึ้น (ประมาณ ≥ 5.0-6.0) แสดงให้เห็นถึงการปลดปล่อยสารประกอบที่เป็นด่างเพื่อเป็นกลไกลดความเป็นพิษ ความสามารถในการละลายฟอสเฟตของแต่ละไอโซเลทเพิ่มขึ้นเมื่อสัมผัสกับอาร์ซิไนท์ที่มีความเข้มข้นเพิ่มมากขึ้นซึ่งแสดงถึงการดูดใช้อาร์ซินิกที่ลดลงจึงทำให้ความต้านทานสูงขึ้น อย่างไรก็ตาม ความสามารถในการละลายฟอสเฟตลดลงอย่างชัดเจนที่ความเข้มข้นวิกฤตของอาร์ซิไนท์ซึ่งแตกต่างกันไปในแต่ละไอโซเลท สำหรับการทดลองนี้ ความเข้มข้นวิกฤตที่ทำให้ความสามารถในการละลายฟอสเฟตลดลงอย่างเด่นชัดของไอโซเลท BAs7, BAs8, BAs11, BAs19 และ BAs29 คือ 50, 100, 50, 100 และ 100 mg Na-As(III) ตามลำดับ ผลการทดลองที่สำคัญในครั้งนี้คือ กลไกการลดความเป็นพิษสองกลไก ได้แก่ การเปลี่ยนแปลงค่าของ pH ให้มีสภาพเป็นด่าง และ การเพิ่มความสามารถในการละลายฟอสเฟตของแบคทีเรียต้านทานอาร์ซินิก

คำสำคัญ: แบคทีเรียต้านทานอาร์ซินิก กลไกลดความเป็นพิษ การละลายฟอสเฟต การเปลี่ยนแปลงค่า pH

Abstract: Arsenic (As), known as the 'king of poisons', is the most prevalent heavy metals in most contaminated agricultural-soils of northern Thailand, thus a high risk of food-chain contamination and people health. Many microbes transform arsenic to a less toxic form as a means of resistance. In this study, evaluation of pH alteration and phosphate solubilizing ability under various concentrations of arsenite by five selected isolates i.e. BAs7, BAs8, BAs11, BAs19 and BAs29, was performed. The results indicated that each isolate was able to grow in the medium containing arsenite only when they were able to increase the pH to be more alkali (around ≥ 5.0-6.0) indicating an alkali substance(s) might be released as a detoxification mechanism. Phosphate solubilizing ability of each isolate was increased when exposed to higher arsenite concentration indicating less arsenic uptake thus higher resistance ability. However, the P solubilizing ability was obviously decreased at a critical arsenite levels which were varied from isolate to isolate. In the present study, the critical arsenic levels that markedly decreased the ability to solubilize P of BAs7, BAs 8, BAs11, BAs19 and BAs29 were 50, 100, 50, 100 and 100 mg Na-As(III), respectively. The results highlighted the two detoxification mechanisms; pH alteration towards alkali condition and capability increment of P solubilization, of the arsenic resistant isolates.

Keywords: Arsenic-resistant bacteria, detoxification mechanism, phosphate solubilization, pH alteration

Introduction

The widespread existence of elevated heavy metals concentration 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 (Akhtar *et al*., 2013; Zhao *et al*., 2010). Arsenic is known to cause cancer, as well as many other serious health problems, and is primarily found in various environments including cultivated soils thus a high risk for crop uptake. In many cases, arsenic is found as two inorganic forms; arsenite (As(III): $H_2AsO_3^-$ and $H_3AsO_3^0$) and arsenate (As(V): $HAsO_4^{2-}$ and $H_2AsO_4^-$). Arsenic compounds with a +3 oxidation state (As(III)) are more toxic than analogous compounds with a +5 oxidation state (As(V)) (Oremland *et al*. 2005; Wang *et al*. 2002a). Arsenic has been reported as the most prevalent heavy metal contaminant in agricultural soils of northern Thailand. Various anthropogenic activities including excessive use of chemical fertilizers, arsenical herbicide, pesticides, fresh manures and immature compost have ultimately increased the arsenic concentrations of current productive Thailand agricultural-soils (Shutsrirung, 2012). 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 (Abou-Shanab *et al*., 2003;

Akhtar *et al*., 2013; Smith and Read, 1997). Since As(III) is known to be much more toxic than As(V) therefore bacterial oxidation of As(III) to As(V) has long been recognized as a means of detoxification (Aposhian *et al*., 2003; Silver and Phung, 2005). Adequate P levels are required to enhance shoot and root growth and promote early maturity (Ratanaprommanee *et al*., 2017). Phosphate ion is similar to arsenate ion thus is uptake via phosphate transport system (Farwell *et al*., 2007) therefore more phosphate ions could inhibit arsenate uptake. Arsenic transforming bacteria not only have a key role on the movement of arsenic in soil but they also regulate phosphate solubilization (Bano and Masarrat, 2003; Passardi *et al*., 2004) as a means of avoiding arsenic uptake. Microbial detoxification of arsenic by increasing phosphate uptake has recently received more attention. Various microorganisms including bacteria have evolved many mechanisms to cope with arsenic exposure thus arsenic-resistant bacteria seemed to have a vital role in the transformation of As, movement of As in soils and the availability of arsenic to plants.

The aim of this study was to investigate responses of bacterial isolates to various concentrations of arsenic and pH values using nine selected isolates; BAs7, BAs8, BAs11, BAs19, BAs20, BAs22, BAs29, BAs30 and BAs36 which was isolated and screened in our previous study (Shutsrirung, 2012). Phosphate solubilizing ability of all the isolates was also evaluated. The promising isolates may be employed as potential inoculum for detoxifying arsenic and improving the growth of plants in As contaminated soils.

Materials and Methods

From the results of our previous investigation (Shutsrirung, 2012), eight arsenic resistant isolates i.e. BAs8, BAs11, BAs19, BAs20, BAs22, BAs29, BAs30 and BAs36 were selected for this study. An arsenic sensitive isolate, BAs7 was also selected for comparison because resistant and sensitive isolates might response differently to arsenic. Therefore, the total number of tested bacteria were 9 isolates (eight resistant isolates plus one sensitive isolate)

Qualitative evaluation of pH alteration in agar medium by arsenic-resistant bacteria

Culture solution (0.01 ml) of each isolate was dropped on nutrient agar (NA) plate containing indicator (0.05 g/L bromocresol green) (Shutsrirung, 2012). Responses to different pH levels (4.5, 4.7, 5.5 and 6.0) under various Na-As(III) concentrations (0, 10, 15, 25, 50, 100, 250 and 650 mg/L) of the nine selected isolates were recorded after 7 days of incubation. Bromocresol green is a pH indicator, the medium is yellow at pH 3.5 - 3.8, yellowish at pH 4.0 - 4.2, green at pH 4.5 - 4.6, ocean blue at pH 5.0 and bright blue at pH 5.5 - 6.0. Observation of pH change was made by comparing with the controlled plate without culture. A standardized color chart was used as an aid in determining the color changes.

Determination for phosphate-solubilization under different levels of arsenite

The selected isolates from the above experiment were tested for their phosphate (P) solubilizing ability in Pikovskaya's broth medium (PKVb) (Gaur, 1990). Various concentrations of sodium arsenite ($NaAsO_2$: Na-As(III)) were applied in the PKVb medium 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 Na-As(III) 0, 15, 50 and 100 mg/L (pH 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 in the dark for 30 minutes. The optical density was measured at 820 nm using spectrophotometer (Thermo Scientific, mod. GENESYS 20, USA).

Solubilization of various insoluble phosphate sources

The selected isolates were also examined for their ability to solubilized various sources of insoluble phosphate (P) (0.5% $Ca_3(PO_4)_2$, $AlPO_4$ or rock phosphate in PVKb medium (modified from Gaur, 1990). The medium contained Al (50 µM) and Na-As(III) (15 mg/L) (pH 7.0). Determination of solubilized P was done according to the method described above.

Results

1. Growth of arsenic-resistant bacteria and changes in color of the nutrient agar medium

All the isolates were evaluated for their growth (colony appearance) at different pH levels under various Na-As (III) concentrations. The change in pH of NA medium (color of the medium) containing bromocresol green was also evaluated. The medium is green at pH 4.5 - 4.6, ocean blue at pH 5.0 and bright blue at pH 5.5 - 6.0. The results showed that at pH 4.5, four isolates; BAs8, BAs11, BAs19, and BAs29 could change the pH of the medium from green (pH 4.5) to ocean blue to bright blue (pH ≥ 5.5 - 6.0) at the arsenite concentration of 0 to 100 mg/L (Table 1). In general, the good to very good growth of each isolate was observed only when the color of the medium was changed to ocean blue or bright blue (data not shown). When the pH of the medium was set as 4.7, BAs11 exhibited good growth with pH change from 4.7 to ≥ 5.5 - 6.0 at higher arsenite concentration (250 mg/L) as compared at pH 4.5 (100 mg/L). Isolate BAs22 showed much more increased in arsenite resistance when the pH increased from 4.5 (turned the pH of the medium to blue at only 10 mg/L arsenite) to 4.7 (turned the pH of the medium to blue at up to 100 mg/L arsenite) (Table 1). A slightly increase in the pH of the medium from 4.5 to 4.7 resulted in higher arsenic tolerance (increased the pH of the medium with growth appearance) of several isolates (BAs7, BAs11, BAs20, BAs22). Isolate BAs7 appeared to be sensitive to high arsenic concentration although the pH of the medium was raised to 5.5 or 6.0, no growth of BAs7 was observed on the medium with Na-As(III) higher than 15 mg/L. It was observed that isolates BAs8, BAs11, BAs19, BAs22 and BAs29 exhibited high level of resistance to Na-As(III) (increased the pH of the medium with growth appearance) at the highest tested concentration of 650 mg/L both at pH 5.5 and 6.0. In contrast, BAs7, BAs20 and BAs36 could not increase the pH of the medium at high level of arsenite. Isolate BAs8, BAs11, BAs19 and BAs29 exhibited high level of resistance to Na-As(III) at all level of pH tested particularly at very low pH level (4.5) thus were selected for further experiments. BAs7 was also selected to test a long with the high resistant isolates for comparison.

Table 1. The color change (pH change) in the nutrient agar (NA) medium containing bromocresol green and growth of arsenic-resistant bacteria under different levels of $NaAsO_2$

Bacterial isolates	Changes in color of the NA medium at different pH levels under various Na-As(III) concentrations: 0, 10, 15, 25, 50, 100, 250 and 650 mg/L			
	pH 4.5 (Green: G)[1]	pH 4.7 (Green: G)	pH 5.5 (Ocean blue: OB)	pH 6.0 (Bright blue: BB)
Control	G	G	OB	BB
BAs 7	OB with growth at 0-10[2] Green ≥ 50[3]	OB with growth at 0-15 Green ≥ 250	OB with growth at ≥ 15[2]	BB with growth at ≥ 15
BAs 8	BB with growth at 0-100 Green ≥ 250	BB with growth at 0-100 Green ≥ 250	BB with growth at ≥ 650	BB with growth at ≥ 650
BAs 11	BB with growth at 0-100 Green ≥ 250	BB with growth at 0-250 Green ≥ 650	BB with growth at ≥ 650	BB with growth at ≥ 650
BAs 19	BB with growth at 0-100 Green ≥ 250	BB with growth at 0-100 Green ≥ 650	BB with growth at ≥ 650	BB with growth at ≥ 650
BAs 20	BB with growth at 0-10 Green ≥ 15	BB with growth at 0-15 Green ≥ 25	BB with growth at ≥ 100	BB with growth at ≥ 250
BAs 22	BB with growth at 0-10 Green ≥ 250	BB with growth at 0-100 Green ≥ 650	BB with growth at ≥ 650	BB with growth at ≥ 650
BAs 29	BB with growth at 0-100 Green ≥ 250	BB with growth at 0-100 Green ≥ 250	BB with growth at ≥ 650	BB with growth at ≥ 650
BAs 36	BB with growth at 0-25 Green ≥ 50	BB with growth at 0-25 Green ≥ 25	BB with growth at ≥ 250	BB with growth at ≥ 250

[1] The initial color of NA medium (The medium is yellow at pH 3.5 - 3.8, yellowish at pH 4.0 - 4.2, green at pH 4.5 - 4.6, ocean blue at pH 5.0 and bright blue at pH 5.5 - 6.0. Observation of pH change was made by comparing with the controlled plate without culture.

[2] The figures in each column was Na-As(III) concentrations (mg/L). Abbreviations (OB, BB) are the color which was changed from initial color at each pH level. Growth was observed only in this Na-As(III) concentrations range. [3]No growth at this concentration without change in the medium-color (no pH change) by the bacterial isolates.

2. Phosphate solubilization under various concentrations of arsenite

In this study five selected isolates i.e. BAs7, BAs8, BAs11, BAs19 and BAs29 were evaluated for their phosphate (P) solubilizing ability in Pikovskaya's broth medium (PKVb) and PKVb plus Na-As(III) (0, 15, 50 and 100 mg/L) (PKVbp). Isolate BAs7 (sensitive isolate) gave the highest solubilized P at 0 and 15 mg/L of Na-As(III) (55.6 and 100.0 mg P/L, respectively) (Figure 1) however when the concentration of Na-As(III) exceed 15 mg/L the ability to solubilized P of this isolate was reduced (Figure 2). The P solubilizing ability of high arsenic tolerant isolate; BAs8, BAs19 and BAs29 was increased with increasing Na-As(III) concentrations however the amount of solubilized P of these isolates was decreased at 100 mg/L Na-As(III). Among high tolerant isolates, BAs29 gave the highest values of

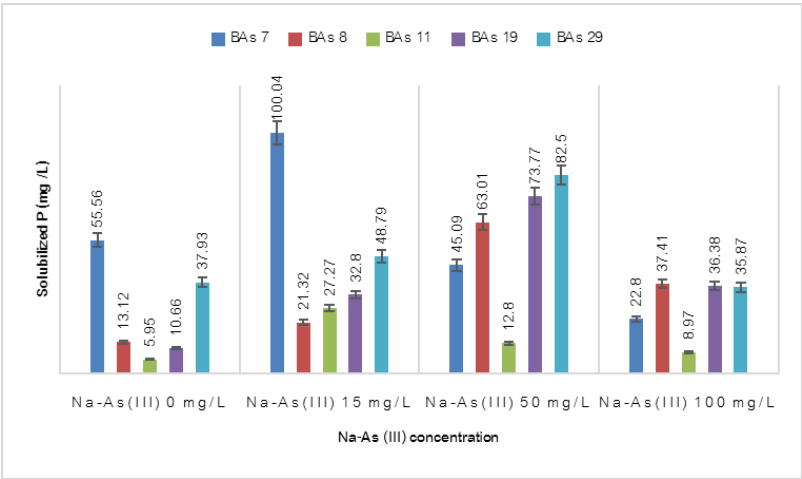


Figure 1. Phosphate solubilizing ability of arsenic-resistant bacteria i.e. BAs7, BAs8, BAs11, BAs19 and BAs29, under various levels of Na-As(III)

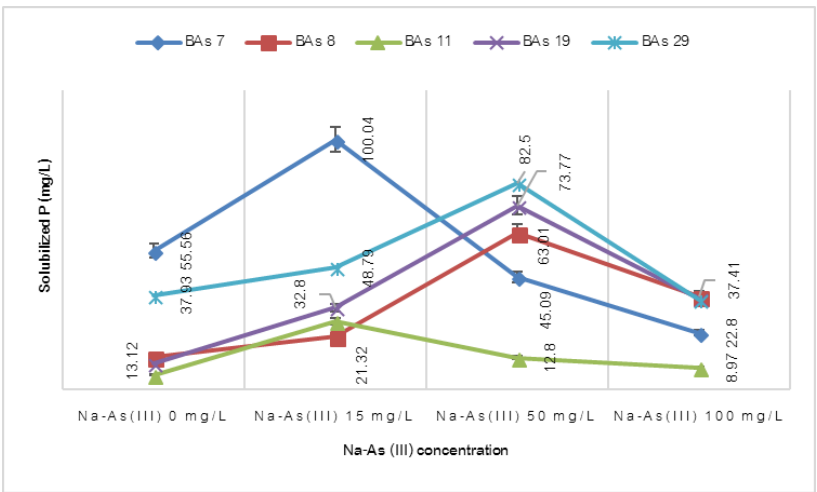


Figure 2. Variation in phosphate solubilizing ability of arsenic-resistant bacteria i.e. BAs7, BAs8, BAs11, BAs19 and BAs29, under various levels of Na-As(III)

solubilized P with values of 37.9, 48.7 and 82.5 mg P/L at 0, 15 and 50 mg/L Na-As(III), respectively. At the highest concentration of Na-As(III) (100 mg/L), BAs8, BAs19 and BAs29 gave similar values of solubilized P (35.9 to 37.4 mg P/L) while BAs7 gave the value of 22.8 mg P/L. BAs11 gave the lowest value of solubilized P at 0, 50 and 100 mg/L Na-As(III), with values of 5.9, 12.8 and 8.9 mg P/L, respectively. The solubilized P of BAs11 was increased when Na-As(III) increased from 0 to 15 mg/L however when the concentration of Na-As(III) higher than 15 mg/L, the ability to solubilize P of BAs11 was markedly reduced.

3. Phosphate solubilization under various sources of insoluble phosphate

In this experiment, different sources of insoluble phosphate i.e. $Ca_3(PO_4)_2$, $AlPO_4$ or rock phosphate were used to evaluate P solubilizing ability of selected isolated.

The results showed that in the medium containing $Ca_3(PO_4)_2$, arsenic-sensitive isolate, BAs7 gave the highest phosphate (P) solubilizing ability both in Pikovskaya's broth medium (PKVb) and PKVb plus Na-As(III) (15 mg/L) (PKVbp) (Table 2, Figure 3). This sensitive isolate exhibited double values of solubilized P in PKVbp (100 mgP/L) as compared to the value obtained in PKVb (55.5 mg/L). The P values obtained by BAs7 were significantly higher than those obtained by the rest of the isolates (Table 2). Among high arsenic-resistant isolates, BAs29 gave the highest ability in P solubilization with values of 37.9 and 48.8 mgP/L in PKVb and PKVbp, respectively.

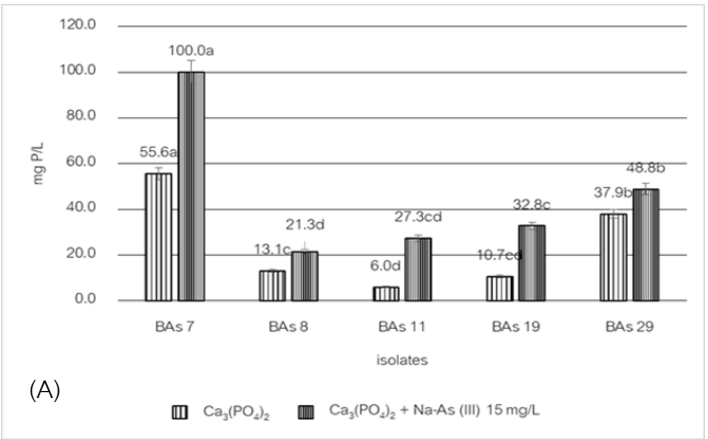
It was observed that in the medium containing $AlPO_4$, BAs19 gave the highest P solubilizing ability with values of 3.8 and 5.1 mgP/L in PKVb and PKVbp, respectively. Isolate BAs7 gave similar value of solubilized as BAs19 in the PKVb medium (3.5 mgP/L). All the isolates gave much lower solubilized P when $AlPO_4$ was used as the P source compared to the values obtained using $Ca_3(PO_4)_2$ as the P source.

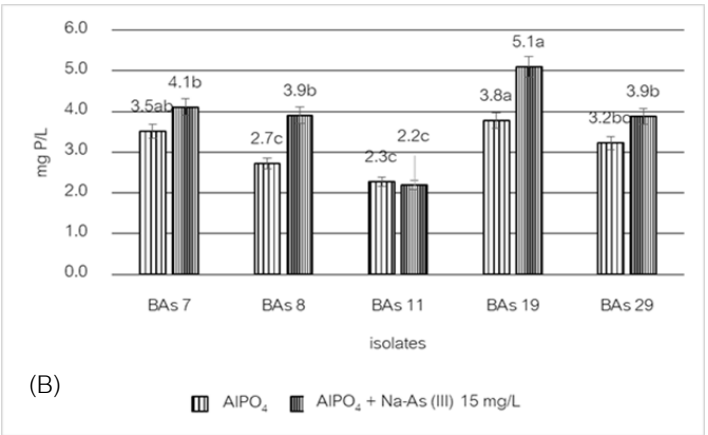
In the medium containing rock phosphate without $NaAsO_2$ (PKVb), arsenic-resistant isolate, BAs8, BAs11, BAs19 and BAs29, showed non-significant solubilized P among them with values of 4.5, 5.3, 5.7 and 6.8 mg P/L, respectively. However, these values are significantly higher than that obtained by sensitive isolate, BAs7 (1.6 mg P/L). The amount of solubilized P was decreased when Na-As(III) was added to the medium (PKVbp) and BAs7 gave the lowest values of solubilized P.

Table 2. Effect of insoluble phosphate substrates on solubilization by arsenic-resistant bacteria

Isolate	Solubilized P (mg/L)					
	Na-As(III) 0 mg/L			Na-As(III) 15 mg/L		
	$Ca_3(PO_4)_2$	$AlPO_4$	Rock phosphate	$Ca_3(PO_4)_2$	$AlPO_4$	Rock phosphate
BAs 7	55.5a	3.5ab	1.6b	100.0a	4.1b	0.6b
BAs 8	13.1c	2.7c	4.5ab	21.3d	3.9b	1.4ab
BAs 11	5.9d	2.3c	5.3a	27.3cd	2.2c	3.7a
BAs 19	10.7cd	3.8a	5.7a	32.8c	5.1a	1.2ab
BAs 29	37.9b	3.3bc	6.8a	48.8b	3.9b	1.0ab
F-test	**	**	**	**	**	**
C.V.	15.41	9.77	37.36	11.31	10.28	82.23

F-test at $P<0.05$; the symbol "**" is significantly different at the $P<0.01$





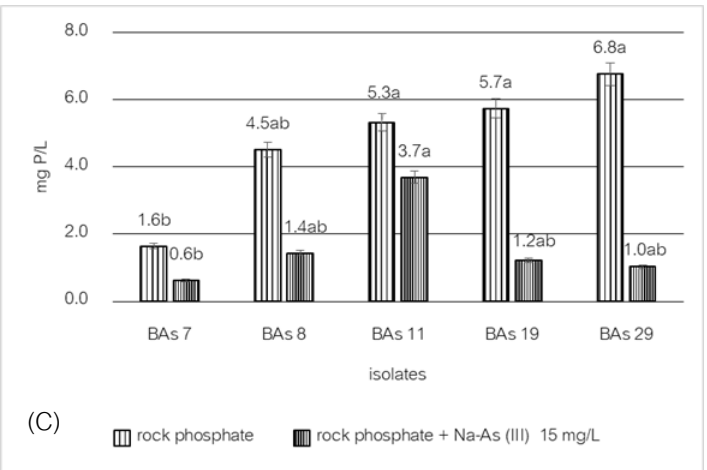


Figure 3. Phosphate solubilizing ability of arsenic-resistant bacteria under various sources of insoluble phosphate; (A) $Ca_3(PO_4)_2$; (B) $AlPO_4$ and (C) rock phosphate

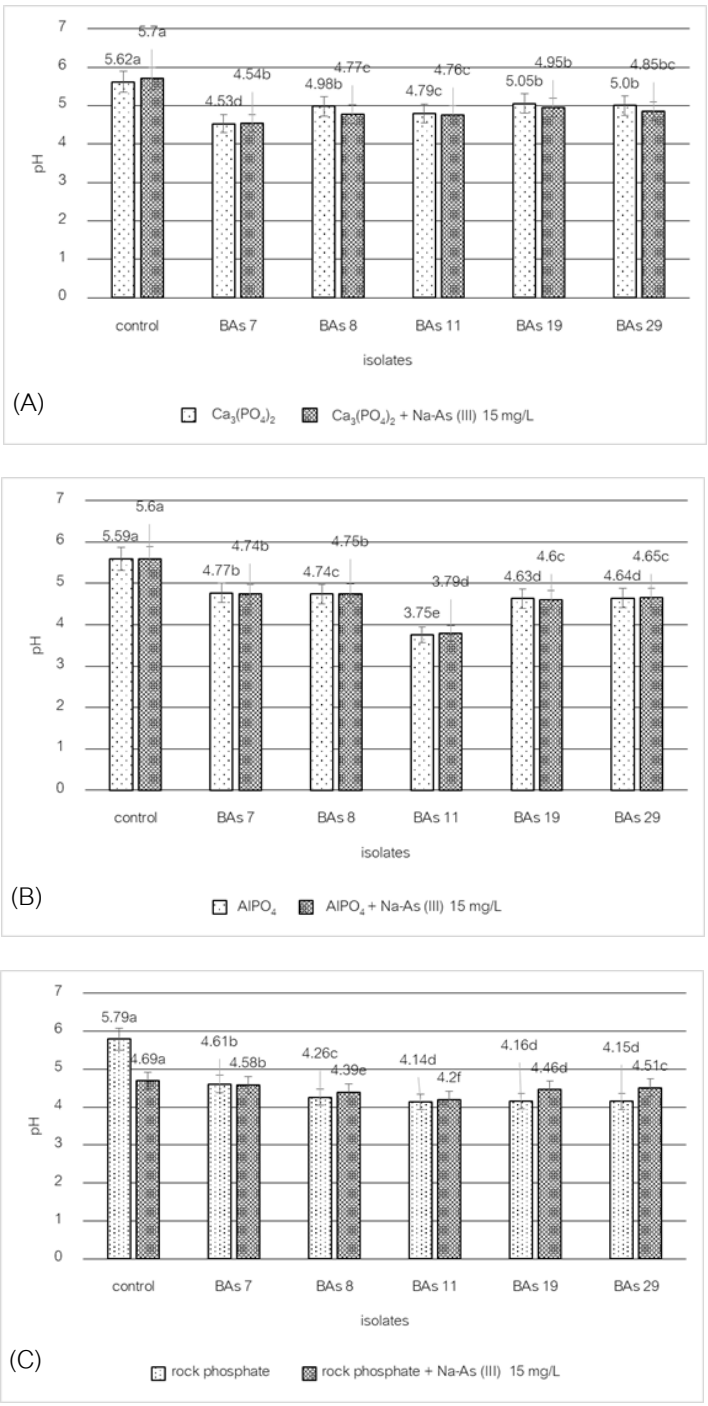


Figure 4. The medium pH changes by arsenic-resistant bacteria under various sources of insoluble phosphate; (A) $Ca_3(PO_4)_2$; (B) $AlPO_4$ and (C) rock phosphate

Discussion

The high arsenic resistant isolates (BAs8, BAs11, BAs19 and BAs29) tested in this study were able to increase the pH of the agar medium containing various concentrations of Na-As(III) plus bromocresol green, from 4.5 (green) to around ≥ 5.0 (ocean blue - bright blue). A slightly increase in the pH of the medium from 4.5 to 4.7 resulted in higher arsenic tolerance of several isolates. The growth appearance of the tested isolates was observed only when they were able to alter the pH of the agar medium to be around ≥ 5.0 - 6.0 (ocean blue - bright blue) suggesting an alkali substance(s) might be released by the isolates as a detoxification and survival mechanism. Arsenic concentration and species are influenced by pH (Mandal and Suzuki, 2002) and the solubility of arsenic is decreased when the pH increase thus less toxic to bacteria. Shutsrirung (2003) concluded that rhizobium group Bj-A released alkali substance as a mechanism to alter the medium-pH for toxicities detoxification and this phenomenon occurred with the growth of the native bacterial isolates. The level of this growth depended on the capability of the native isolates to alter the pH of the medium.

In the present study, arsenic-resistant isolates reduced the pH values to be acidic to enable P solubilization of Ca-phosphate, Al-phosphate and rock phosphate. The results implied that organic acid(s) might be produced to facilitate the solubilization (Khan *et al*., 2009). Based on previous studies, the primary mode of P solubilization by microbes is by production of organic acids (Chen *et al*., 2016; Vassilev *et al*., 2004), which is supported by pH reduction in the solution. Chen *et al*. (2016) 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 was due to the exudation of organic acids like citric, gluconic, succinic and lactic acid. Phosphate ion is similar to arsenate ion thus is uptake via phosphate transport system (Farwell *et al*., 2007). The increase in phosphate in the environment markedly decreased arsenic uptake by the plant (Wang *et al*., 2002b). In the present study, the tested isolates solubilized more Ca-phosphate and Al-phosphate in the medium containing higher concentrations of Na-As(III) thus less toxicity of arsenic in their cells.

Conclusion

The growth appearance of the tested isolates was observed only when they were able to alter the pH of the agar medium to be much more alkali. High arsenic resistant isolates i.e. BAs8, BAs11, BAs19 and BAs29 increased the pH of the agar medium from 4.5 and 4.7 to around ≥ 5.0 - 6.0 in the agar medium with high concentration of Na-As(III) indicating ability to detoxify arsenic. High P solubilizing (Ca-phosphate, Al-phosphate) 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.

Acknowledgements

The authors wish to express special thanks to Highland Research and Development Institute (Public Organization), Chiang Mai, Thailand, for financial support.

References

Abou-Shanab, R.A., J.S. Angle, T.A. Delorme, R.L. Chaney, P. Van Berkum, H. Moawad, K. Ghanem and H.A. Ghozlan. 2003. Rhizobacterial effects on nickel extraction from soil and uptake by *Alyssum murale*. New Phytologist 158(1): 219-224.

Akhtar, M.S., B. Chali and T. Azam. 2013. Bioremediation of arsenic and lead by plants and microbes from contaminated soil. Research in Plant Sciences 1(3): 68-73.

Aposhian, H.V., R.A. Zakharyan, M.D. Avram, M.J. Kopplin and M.L. Wollenberg. 2003. Oxidation and detoxification of trivalent arsenic species. Toxicology and Applied Pharmacology 193(1): 1-8.

Bano, N. and J. Masarrat. 2003. Characterization of a new *Pseudomonas aerunginosa* strain NJ-15 as a potential biocontrol agent. Current Microbiology 46(5): 324-328.

Chen, W., F. Yang, L. Zhang and J. Wang. 2016. Organic acid secretion and phosphate solubilizing efficiency of *Pseudomonas* sp. PSB12: Effects of phosphorus forms and carbon sources. Geomicrobiology Journal 33(10): 870-877.

Das, S., J.S. Jean, S. Kar and C.C. Liu. 2013. Changes in bacterial community structure and abundance in agricultural soils under varying levels of arsenic contamination. Geomicrobiology Journal 30(7): 635-644.

Farwell, A.J., S. Vesely, V. Nero, H. Rodriguez, K. McCormack, S. Shah, D.G. Dixon and B.R. Glick. 2007. Tolerance of transgenic canola plants (*Brassica napus*) amended with plant growth-promoting bacteria to flooding stress at a metal-contaminated field site. Environmental Pollution 147(3): 540-545.

Gaur, A.C. 1990. Phosphate solubilizing microorganisms as biofertilizer. Omega Scientific Publishers, New Delhi.

Khan, A.A., G. Jilani, M.S. Akhtar, S.M.S Naqvi and M. Rasheed. 2009. Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. Journal of Agriculture and Biological Sciences 1(1): 48-58.

Mandal, B.K. and K.T. Suzuki. 2002. Arsenic around the world: a review. Talanta 58(1): 201-235.

Oremland, R.S. and J.F. Stolz. 2005. Arsenic, microbes and contaminated aquifers. Trends in Microbiology 13(2): 45-49.

Passardi, F., C. Penel and C. Dunand. 2004. Performing the paradoxical: how plant peroxidases modify the cell wall. Trends in Plant Science 9(11): 534-540.

Ratanaprommanee, C., K. Chinachanta and A. Shutsrirung. 2017. Effects of leonadite on soil quality and rice yield improvement. Journal of Agriculture 33(2): 215-224.

Shutsrirung, A. 2003. Characterization of native bradyrhizobia in soybean-growing areas of northern Thailand. Ph.D. Thesis. Mie University, Mie, Japan. 150 p.

Shutsrirung, A. 2012. Selection of microorganism in highland for soil quality improvement in acid and high arsenic soils. Final Report. Highland Research and Development Institute (Public Organization), Chiang Mai. 52 p.

Silver, S. and L.T. Phung, 2005. Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. Applied and Environmental Microbiology 71(2): 599-608.

Smith, S.E. and D.J. Read. 1997. Mycorrhizal Symbiosis. Academic Press, San Diego. 605 p.

Vassilev, A., J.P. Schwitzguébel, T. Thewys, D. van der Lelie and J. Vangronsveld. 2004. The use of plants for remediation of metal contaminated soils. Scientific World Journal 4: 9-34.

Wang, J.P., L. Qi, M.R. Moore and J.C. Ng. 2002a. A review of animal models for the study of arsenic carcinogenesis. Toxicology Letters 133(1): 17-31.

Wang, J., F.J. Zhao, A.A. Meharg, A. Raab, J. Feldmann and S.P. McGrath. 2002b. Mechanisms of arsenic hyperaccumulation in *Pteris vittata*. Uptake kinetics, interactions with phosphate, and arsenic speciation. Plant Physiology 130(3): 1552-1561.

Zhao, F.J., S.P. McGrath and A.A. Meharg. 2010. Arsenic as a food chain contaminant: mechanisms of plant uptake and metabolism and mitigation strategies. Annual Review of Plant Biology 61: 535–559.