INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
09–169/MFA/2009/11–6–667–673
http://www.fspublishers.org

Full Length Article

Solubilization of Different Phosphate Forms by Phosphate Solubilizing Bacteria Isolated from Aerobic Rice

Q.A. PANHWAR[1], O. RADZIAH[1], M. SARIAH† AND MOHD RAZI ISMAIL†
Department of Land Management, Universiti Putra Malaysia, 43400 Serdang, Selangor D.E., Malaysia
†Department of Crop Science, Universiti Putra Malaysia, 43400 Serdang, Selangor D.E., Malaysia
[1]Corresponding author's e-mail: radziah@agri.upm.edu.my

ABSTRACT

Phosphorus solubilizing bacteria (PSB) are known to be able to solubilize different forms of inorganic phosphates. An *in vitro* study was conducted to determine the solubilization of phosphorus from inorganic phosphates in three broths containing tricalcium phosphate (NBRIP broth), aluminum phosphate (PDYA-AlP broth) and Christmas island rock phosphate (CRIP broth) by using PSB strains isolated from aerobic rice field. There were differences of bacterial growth found in different forms of phosphate containing media. In general, bacterial populations were higher in NBRIP and CIRP broths and lowest in PDYA-AlP broth. PSB-15 strain showed the highest population in NBRIP, while PSB-10 in CIRP broth. No bacterial growth occurred in PDYA-AlP broth. P solubilizations by the different bacterial strains were significantly influenced by the sources of P used in the broths. Inoculation of NBRIP broth with PSB-10 solubilized the highest P (40.56%), While comparatively lower P was solubilized by the bacteria in CIRP broth. Low amount of soluble P was present in PDYA-AlP even though there was no bacterial growth. Significantly highest pH decrease was found in CIRP broth followed by NBRIP broth, while no pH change occurred in PDYA-AlP broth. The P solubilization rate of different phosphate forms in the broth followed first order kinetics. P solubilization correlated positively with bacterial population and negatively correlated with culture pH. The PSB strains isolated from aerobic rice rhizosphere were able to solubilize P from tricalcium phosphate and rock phosphate, but not from aluminum phosphate.

Key Words: Aerobic rice; Aluminum phosphate; Inorganic phosphate; Christmas island rock phosphate; Phosphate solubilizing bacteria; Tricalcium phosphate

INTRODUCTION

Aerobic rice is water saving rice production technology (Bouman *et al.*, 2005) and phosphorus is a primary essential nutrient element for rice production. A large amount of soluble inorganic phosphate applied to the soil as chemical fertilizers immobilized rapidly and becomes un-available to the plants. The microbial solubilized P can be an integral part of P nutrition in aerobic rice. In seasonally flooded rice soils, phosphorus is released through various processes, however in well-drained aerobic soils P solubilization from insoluble inorganic complexes might be the production and release of protons and chelating agents by microorganisms (He & Zhu, 1998). The plant growth-promoting rhizobacteria have the ability to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite and rock phosphate. Apart from P fertilization, microbial P-mobilization would be the only possible way to increase available phosphate for upland crops (Goldstein, 1986).

Among the bacterial genera *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Micrococcus, Flavobacterium* and *Erwinia* have been reported for plant growth promotion, as wall as inorganic and organic P solubilization from soil (Kucey *et al.*, 1989). *Rhizobium* and phosphate solubilizing bacteria (PSB) have a great importance to plant nutrition and perform a major role as plant growth-promoting rhizobacteria (PGPR) in the biofertilization of crops. (Afzal & Asghari, 2008). It has been reported that inoculation of *Pseudomonas aeruginosa* (PSB) considerably increased the seedling plant growth and root mass (Sapak *et al.*, 2008). The significant genetic diversity in rhizobial strains exists with respect to their efficiency for solubilizing soil phosphate (Sajjad *et al.*, 2008). The use of phosphate solubilizing bacteria as inoculant increased P uptake and crop yield (Rodriguez & Fraga, 1999; Gulati *et al.*, 2007). The bioavailability of soil inorganic phosphorus in the rhizosphere varies considerably with plant species, nutritional status of soil, presence of effective microorganisms and soil conditions. To enhance phosphorus uptake efficiency, PSB play an important role in supplying phosphate to plants, which is environment-friendly and sustainable approach. Although PSB are commonly found in soil, their population may not be high

To cite this paper: Panhwar, Q.A., O. Radziah, M. Sariah and I. Mohd Razi, 2009. Solubilization of phosphate forms by phosphate solubilizing bacteria isolated from aerobic rice. *Int. J. Agric. Biol.*, 11: 667–673

enough to compete with other bacterial community and established in the rhizosphere. Therefore, inoculation with higher concentration of PSB strains would be more beneficial for phosphate solubilizing activity in the root environment.

PSB can be used as biofertilizers, which is friendly for environment and is a possible way to increase the efficiency P fertilizers. Phosphorus biofertilizers may help increase the availability of accumulated phosphate by solubilization process for crop production. This study was therefore undertaken to investigate the efficiency and activity of PSB strains using insoluble P in the form of tricalcium phosphates, aluminum phosphates and Christmas Island Rock Phosphates in *in vitro* conditions. The ability to solubilize different forms of phosphates may be beneficial for the development of biofertilizers for aerobic rice system.

MATERIALS AND METHODS

Preparation of Inoculum. The PSB strains used in the study were isolated from the aerobic rice field of Malaysian Agriculture Research and Development Institute (MARDI) Kepala Batas, Penang, Malaysia. Seven PSB strains (PSB-1, PSB-6, PSB-9, PSB-10, PSB-14, PSB-15 & PSB-16) were grown in nutrient broth (NB) for 48 h. The bacterial cells were harvested by centrifugation at 13500 x *g* for 10 min and washed with 0.85% saline sterilized phosphate buffer (Bacteriological Analytical Manual, 2001). The washed bacterial cells were immediately resuspended in phosphate buffer solution. Optical density (OD_{600}) of the cells were checked and adjusted accordingly. Approximately 5 X 10^9cfu mL^{-1} inoculums live bacterial cells were used for inoculation. The population was confirmed by cell enumeration in drop plate method on nutrient agar (NA) plates (Somasegaran & Hoben, 1985).

Preparation of broths. Tricalcium phosphate was added as a source for phosphorus in NBRIP broth (Nautiyal, 1999). The NBRIP broth contains (g L^{-1}): $MgCl_2.6H_2O$ 5 g, $MgSO_4$. H_2O 0.25 g, KCl 0.2 g, $(NH_4)_2SO_4$ 0.1 g, $Ca_3(PO_4)_2$ 5 g, amended with glucose 10 g. The CIRP broth was modified from NBRIP and supplemented with 5 g L^{-1} of rock phosphate (CIRP) instead of tricalcium phosphate. The PDYA-AlP broth (modified from Katznelson & Bose, 1959) contains (g L^{-1}): PDA agar 39 g, Yeast extract 2 g, sterile 10% K_2HPO_4 50 mL, sterile 10% $AlCl_3$ 100 mL. The different broths were inoculated with the respective x 5 10^9cfu mL^{-1} inoculums. Flasks containing 200 mL of inoculated media were incubated at 30°C on a Kottermann 4020 shaker at a medium speed of 80 (rpm).

Determination of population. One milliliter of broth was taken from the respective flasks at different periods (6, 12, 24 & 48 h) for bacterial population. A series of 10 fold dilution were prepared up to 10^{-10}. Population was determined using drop plate count method according to Somasegaran and Hoben (1985). The pH of the growth culture was also determined at each sampling.

Determination of phosphorus solubilization in broth cultures. At every sampling time, 2 mL of samples were taken for P determination. The samples were first allowed to sediment for 15 min and were centrifuged at 4000 x *g* for 5 min. The supernatant was filtered through 0.2 µm filter paper and kept at -20°C until analysis. Available P was determined following the method of Murphy and Riley (1962).

Kinetics of P solubilization. The P solubilzation rate constant k was determined by using the following equation (Stanford & Smith, 1972).

$$k = \frac{2.303(\log Ct - \log C_0)}{t}$$

Where, k=mineralization rate $\log C_0$=initial P solubilization, log Ct = P solubilization at the time (48), t = time.

Statistical analysis. The experiment was conducted in Completely Randomized Design with (CRD) 5 replications. All data were analyzed using the SAS statistical software and mean differences were separated using Tukey's studentized range test at the 5% level of probability.

RESULTS

Effect of different phosphate broths on population of PSB. Growth of bacteria was significantly affected by the different forms of inorganic phosphorus used in the broths. Bacterial populations were higher in NBRIP broth followed by CIRP broth. In NBRIP broth population of PSB-15 strains was significantly the highest followed by PSB-1 and PSB-6 (Fig. 1a). The populations of PSB-9 and PSB-16 strains were significantly high in CIRP broth (Fig. 1b). In all cases, bacterial growth was concomitant with a significant decreased of pH in growth culture. There was no bacterial growth found in PDYA-AlP broth, where the bacterial population decreased after 6 h of inoculation (Fig. 1c).

P solubilization in different phosphate broths. The PSB isolates were able to solubilize higher P (Table I) from tricalcium phosphate (NBRIP) and Rock Phosphate (CIRP) as compared to aluminum phosphate (PDYA-AlP). The highest P solubilization activity was found in NBRIP broth with PSB-10 (40.56%), followed by PSB-14 (38.91%) isolates. Most strains were able to solubilize P in CIRP broth, but with lower quantities compared to that in NBRIP broth. Among the bacterial strains used higher P solubilization was observed in CIRP broth inoculated with PSB-9 (3.90%) and PSB-16 (3.13%) strains compared to other isolates. There was low amount of soluble P present in PDYA-Al broth even though there was no bacterial growth after 6 h of incubation.

Changes of pH in different phosphate broths. The pH decreased from the initial value 6.77 to 4.27 in NBRIP broth culture after 48 h of incubation period with a decrease of more than 2.5 pH units (Fig. 2a). Where as in CIRP broth there was a higher pH decreased of 4.0 unit from 7.6 to 3.11 (Fig. 2b). There was no significant pH change in PDYA-AlP culture, where the pH value remained about 3.66 - 3.70 through out the incubation period (Fig. 2c).

Fig. 1. Bacterial population in different broths during 48 h of incubation

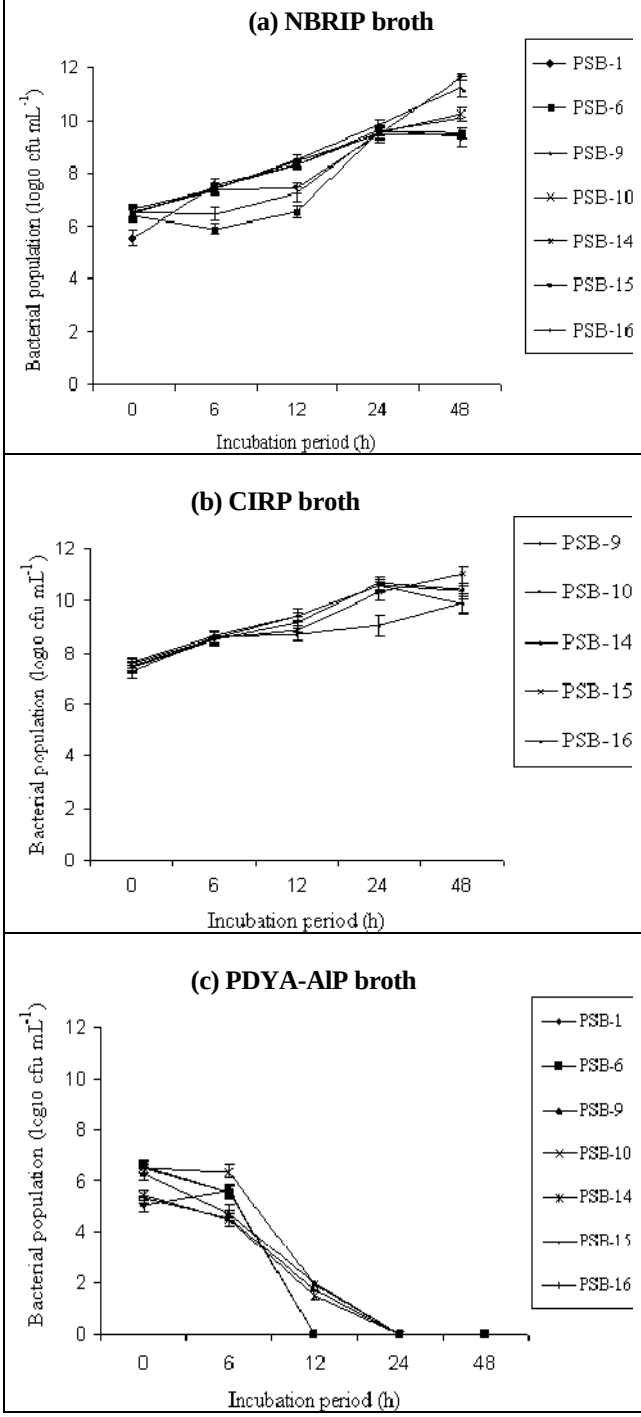
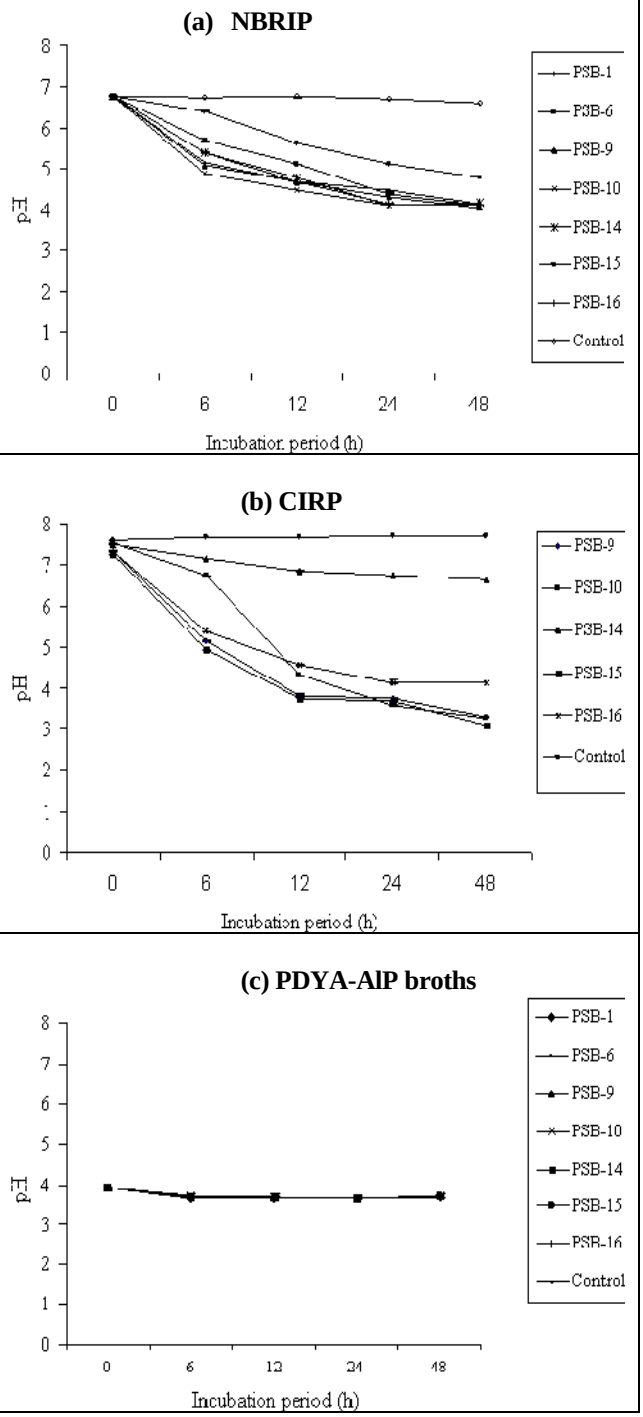


Fig. 2. Changes of pH in different broths during 48 h of incubation



Kinetics of P solubilization in different phosphate broths. The solubilization rate (k) for different times during the incubation period was determined by using first order kinetics. By regression analysis it was found that the kinetics of P solubilization best fitted in power and logarithmic models for both NBRIP and CIRP broths. PSB-mediated $Ca_3(PO_4)_2$ solubilization in NBRIP broth followed two different group of models (Fig. 3). The steep of the curve (k) obtained by PSB-10, PSB-14, PSB-6, PSB-1 and PSB-16 followed power model and most of the P solubilized in culture solution within 20 h of incubation period, while PSB 9 and PSB 15 best fitted in logarithmic model and gradually solubilized P. In CIRP broth PSB-14, PSB-15 and PSB-16 strains solubilized P gradually and

Fig. 3. Kinetics of P solubilization in NBRIP broth during 48 h of incubation

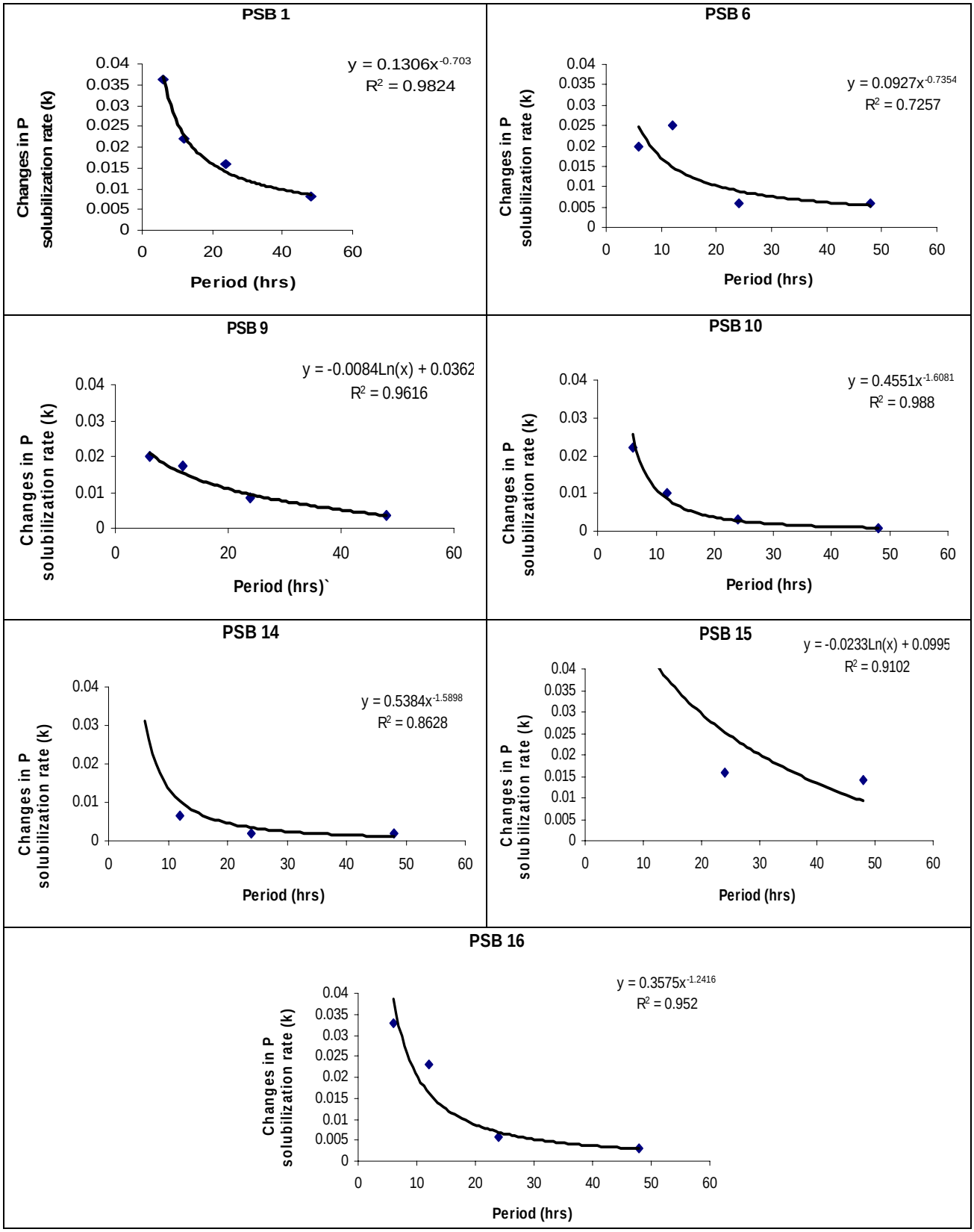
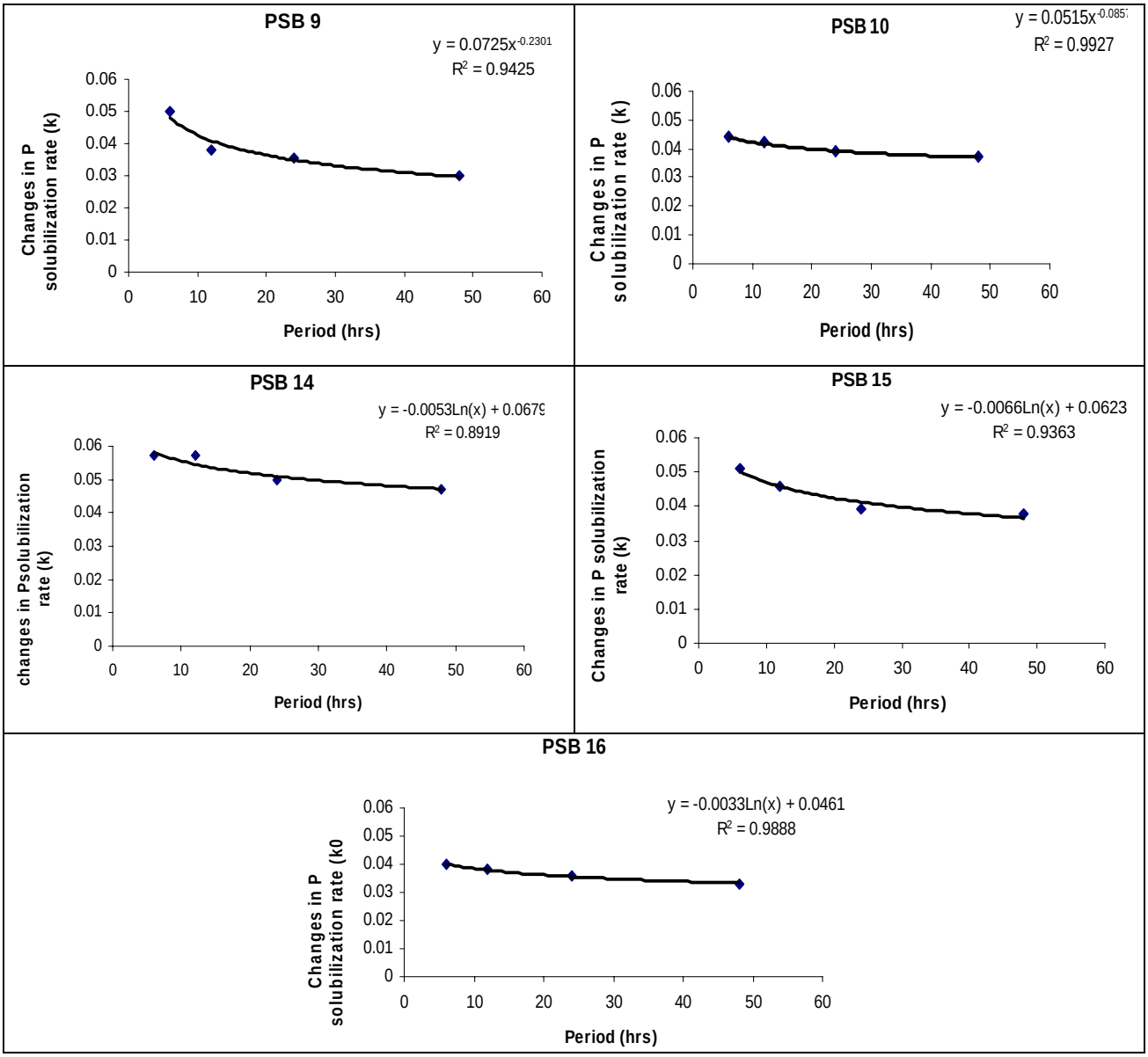


Fig. 4. Kinetics of P solubilization in CIRP broth during 48 h of incubation



followed logarithmic model, where as PSB-9 and PSB-10 were best fitted in power model and they solubilized P faster than other strains in this broth (Fig. 4).

The correlation co-efficient values for P solubilization in NBRIP and CIRP broths (Table II) exhibited significant positive association between bacterial population and P solubilization. Both population and solubilization values showed a significant negative relationship with pH in broth cultures.

DISCUSSION

The PSB strains isolated from aerobic rice were able to grow and solubilize phosphate from different sources of P in broth culture. There were significant differences of population growth and changes of pH found in different broths. In NBRIP and CIRP broth PSB population increased with the decreased of pH. There were more than 2.5 units of pH variation found in tricalcium phosphate containing NBRIP broth, while more than 4.0 units of pH decreased found in CIRP broth containing Christmas Island Rock phosphate. There was a negative correlation between bacterial population and pH of broth culture indicating that P solubilization occurs at low pH. Similarly Perez *et al.* (2007) found 3.2-4.0 pH units decreased with the increased of PSB population during incubation period. The decreased in pH may be due to the production of organic acids during bacterial growth. The increased bacterial growth with decreasing pH and production of organic acids resulted in considerable amount of P solublized. There was a clear

Table I. Percentage (%) of P solubilized from NBRIP, PDYA-AlP and CIRP broths after 48 h of incubation period

Strains	NBRIP	CIRP	PDYA-AlP
PSB1	23.11	ND	2.18
PSB6	20.18	ND	2.84
PSB9	36.26	3.90	1.56
PSB10	40.56	1.96	4.15
PSB14	38.91	1.16	2.27
PSB15	20.21	2.35	1.82
PSB16	36.00	3.13	2.18

ND = Not done

Table II. Correlation co-efficient between bacterial population, pH and P solubilization in NBRIP and CIRP broths

	NBRIP broth		CIRP broth	
	Bacterial population	pH	Bacterial population	pH
Bacterial population	-	-	-	-
pH	-0.801***	-	-0.787***	-
P solubilization	0.807***	-0.803***	0.754***	-0.803***

*** Significant at $p < 0.0001$ ($n = 5$)

relationship established between bacterial growth and P solubilization. These results are consistent with the report of Rodriguez Fraga (1999), Whitelaw (2000), Jeon *et al.* (2003), Maliha *et al.* (2004) and Chen *et al.* (2005), which showed that solubilization of Ca-P complexes were mediated specially by the decreasing pH of the medium. Joseph and Jisha (2008) indicated that phosphate solubilizing organisms are capable of reducing pH of culture medium.

In CIRP broth the amount of soluble P was very low as compared to that in NBRIP, even though the decrease in pH units was higher than that in NBRIP. Similar findings were recorded by Léon *et al.* (1986), where there was higher pH value decreased in CIRP broth (rock phosphate) as compared to NBRIP (calcium phosphate) broth cultures. The low amount of solubilized P in CIRP broth may be due to cessation of PSB activity at lower pH developed as bacterial population increased. In addition, the rock phosphate has low P solubility as compared to calcium phosphate (Nahas, 1996). In CIRP broth the highest P solubilized by PSB-9 and PSB-16 strains, which followed power model of solubilization rate. This indicates rapid P was solubilization at the initial stage of incubation period, which is associated with production of organic acids that causes lower pH in the growth culture medium. This predisposed further P solubilization in the broth culture.

The present study also showed that isolated PSB strains were not able to grow at low pH in PDYA-AlP broth. Bacterial growth decreased in PDYA-AlP broth after 6 h of incubation and no growth found after 48 h of incubation. The pH of medium remained almost the same in all strains, indicating that these bacteria were not able to survive at low pH. Perez *et al.* (2007) also found no bacterial growth and no changes of pH in the aluminum phosphate ($AlPO_4$) and iron phosphate ($FePO_4$) broths. Similar observations were reported by Reyes *et al.* (1999) and Delvasto *et al.* (2006), when iron phosphate and hydroxyapatite were used. Although there was no PSB population found in PDYA-AlP broth after 6 h of incubation period, substantial amount of soluble P was found in the broth culture. This could be due to low pH of the medium that enhance P solubilization from aluminum phosphate.

CONCLUSION

The PSB isolates were able to solubilize P from tricalcium phosphate and Christmas island rock phosphate, but not from aluminum phosphate. The probable mechanism for P solubilization is the production of organic acids during bacterial growth, which decreased the pH of the culture solution and simultaneously solubilized P from the inorganic sources. These PSB isolates could be efficient biofertilizer for improved P-nutrition in aerobic rice cultivation system.

Acknowledgement. The author is grateful to the Universiti Putra Malaysia and the Ministry of Science, Technology and Innovation for providing the financial supports for PhD project.

REFERENCES

Afzal, A. and B. Asghari, 2008. *Rhizobium* and phosphate solubilizing bacteria improve the yield and phosphorus uptake in wheat (*Triticum aestivum*). *Int. J. Agric. Biol.*, 10: 85–88

Bacteriological Analytical Manual, 2001. Washington, D.C.CFSAN/FDA. http://vm.cfsan.fda.gov/~ebam/bam-mm.html.

Bouman, B.A.M., S. Peng, A.R. Castaneda and R.M. Visperas, 2005. Yield and water use of irrigated tropical aerobic rice systems. *Agric. Water Manage.*, 74: 87–105

Chen, Y.P., P.D. Rekha, A.B. Arun, F.T. Shen, W.A. Lai and C.C. Young, 2005. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate abilities. *Appl. Soil Ecol*., 34: 33–41

Delvasto, P., A. Valverde, A. Ballester, J.M. Igual, J.A. Mun Oz, F. Gonza Les, M.L. Bla zquez and C. Garcı, 2006. Characterization of brushite as a re-crystallization product formed during bacterial solubilization of hydroxyapatite in batch cultures. *Soil Biol. Biochem*., 38: 2645–2654

Goldstein, A.H., 1986. Bacterial solubilization of mineral phosphates: historical perspective and future prospects. *Amarican J. Altern. Agric.*, 1: 51–57

Gulati, A., P. Rahi and P. Vyas, 2007. Characterization of phosphate solubilizing *florescent Pseudomonas* from the rhizosphere of sea buckthorn growing in the cold desert of Himalayas. *Curr. Microbiol.*, 56: 73–79

He, Z.L. and J. Zhu, 1998. Microbial utilization and transformation of phosphate adsorbed by variable charge minerals. *Soil Biol. Biochem.*, 30: 917–923

Jeon, J.S., S. Lee, H.Y. Kim, T.S. Ahn and H. Song, 2003. Plant growth promotion in soil by some inoculated microorganisms. *The J. Microbiol. Soc. Korea*, 41: 271–276

Joseph, S. and M.S. Jisha, 2008. Buffering reduces phosphate solubilizing ability of selected strains of bacteria. *American-Eurasian J. Agric. Environ. Sci.*, 4: 110–112

Kucey, R.M.N., H.H. Jenzen and M. Leggett, 1989. Microbially mediated increases in plant available phosphorus. *Adv. Agron*., 42: 199–228

Léon, L.A., Fenster, W.E. & L.L. Hammond, 1986. Agronomic potential of eleven phosphate rocks from Brazil, Colombia, Peru, and Venezuela. *Soil Sci. Soc. American J*., 50: 798–802

Maliha, R., K. Samina, A. Najma, A. Sadia and L. Farooq, 2004. Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms under *in vitro* conditions. *Pakistan J. Biol. Sci*., 7: 187–196

Murphy, J. and J. P. Riley, 1962. A modified single solution method for determination of phosphate in natural water. *Anal. Chim. Acta*, 27: 31–36

Nahas, E., 1996. Factors determining rock phosphate solubilization by microorganisms isolated from soil. *World J. Microbiol. Biotechnol*., 12: 567–572

Nautiyal, C.S., 1999. An efficient microbiological growth medium for screening phosphate-solubilizing microorganisms. *FEMS Microbiol. Letters*, 170: 265–270

Perez, E., S. Miguel, M.B. Marıa and L.A. Yarzabal, 2007. Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. *Soil Biol. Biochem.*, 39: 2905–2914

Reyes, I., L. Bernier, R. Simard and H. Antoun, 1999. Effect of nitrogen source on the solubilization of different inorganic phosphates by an isolate of Penicillium rugulosum and two UV-induced mutants. *FEMS Microbiol. Ecol.*, 28: 281–290

Rodriguez, H. and R. Fraga, 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotech. Adv*., 17: 319–339

Sajjad, M., T.A. Malik, M. Arshad, Z.A. Zahir, F. Yusuf and S.U. Rahman, 2008. PCR studies on genetic diversity of rhizobial strains. *Int. J. Agric. Biol.*, 10: 505–510

Sapak, Z., S. Meon and Z.A.M. Ahmad, 2008. Effect of endophytic bacteria on growth and suppression of *Ganoderma* infection in oil Palm. *Int. J. Agric. Biol.*, 10: 127–132

Somasegaran, P. and H.J. Hoben, 1985. *General Microbiology of Rhizobium*, pp: 39–53. Methods in Legume- *Rhizobium* Technology

Whitelaw, M.A., 2000. Growth promotion of plants inoculated with phosphate solubilizing fungi. *Adv. Agron.*, 69: 99–151

(Received 16 May 2009; Accepted 25 May 2009)