African Journal of Biotechnology Vol. 11(11), pp. 2711-2719, 7 February, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2218
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Isolation and characterization of phosphate-solubilizing bacteria from aerobic rice

Qurban Ali Panhwar[1], Radziah Othman[1]*, Zaharah Abdul Rahman[1], Sariah Meon[2] and Mohd Razi Ismail[3]

[1]Soil Microboilogy, Department of Land Management, Faculty of Agriculture University Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[2]Institute of Tropical Agriculture, University Putra Malaysia, 43400 Serdang, Selangor Malaysia.
[3]Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia 43400 Serdang, Selangor, Malaysia.

Accepted 3 June, 2011

Phosphate-solubilizing bacteria are frequently used as plant growth promoters. A study was conducted to isolate phosphate-solubilizing bacteria (PSB) from aerobic rice grown in Penang Malaysia and to determine some biochemical properties of the isolates such as, organic acids, enzymes, indoleacetic acid (IAA), siderophore production and its antagonistic effect against pathogen *Rhizoctonia solani.* Selective media used for the isolation were; *Pseudomonas aeruginosa* (PA), national botanical research institute's phosphate growth medium (NBRIP), Pikovskaya and *Pseudomonas* spp. (PS). Organic acid production was determined using high performance liquid chromatography (HPLC). The PSB populations were higher in rhizosphere than non-rhizospheric soil and the highest population was found in PS and Pikovskaya, while the lowest was found in PA media plates. The highest P solubilizing activity (69.58%) was found in PSB9 strain grown in NBRIP plate. Isolated PSB were able to produce different organic acids and growth hormone such as IAA. A number of PSB isolates belong to the *Bacillus* sp. and proved for the antagonistic effect against *R. solani* (sheath blight) even though most of the isolated strains can grow in nitrogen, free semi-solid medium and able to produce siderophore. PSB inoculants with their beneficial traits would be considered as potential biofertilizer for the sustainable aerobic rice cultivation system.

Key words: Aerobic rice, antagonistic effect, indoleacetic acid, organic acids, phosphorus solubilizing bacteria.

INTRODUCTION

Phosphorus is a primary essential nutrient element for rice production and aerobic rice is a rice production system for water-short environments where adapted rice varieties are grown under aerobic soil conditions (Bouman et al., 2006). Water use is 30 to 70% less than in flooded rice, depending on irrigation water management and anticipated yields (Bouman et al., 2005). Aerobic rice requires the same amount of nutrients like anaerobic rice. However, there could be problem for the availability of phosphorus, due to its fixation with Fe and Al especially in acidic and aerobic soil conditions.

There are some species of bacteria which have potential to mineralize and solubilize organic and inorganic phosphorus in soil (Hilda and Fraga, 2000; Khiari and Parent, 2005). Strains from the genera *Pseudomonas, Bacillus* and *Rhizobium* are among the most powerful phosphate solubilizers (Rodriguez and Fraga, 1999). Phosphorus solubilizing activity is proved by the ability of microbes to release metabolites such as organic acids. These organic acids chelate the cation bound to phosphate and being converted to soluble forms

*Corresponding author. E-mail: radziah@agri.upm.edu.my. Tel: +60163058964. Fax: +603-8943 4419.

Abbreviations: **PSB**, Phosphate solubilizing bacteria; **IAA**, indoleacetic acid; **PA**, *Pseudomonas aeruginosa*; **NBRIP**, National Botanical Research Institute's phosphate; **PS**, *Pseudomonas* spp.

through their hydroxyl and carboxyl groups (Sagoe et al., 1998).

The use of PSB as inoculants simultaneously increases P uptake by the plant and crop yield. The *Pseudomonas* spp. has been used for plant growth promotion and disease control in rice crop (Saikia et al., 2005). *Pseudomonas aeruginosa* is known to enhance the plant growth and suppress many fungal diseases by induced systemic resistance (Audenaert et al., 2002). Strains of *Pseudomonas putida* and *Pseudomonas fluorescens* increased P uptake resulting root and shoot elongation in canola, lettuce, tomato and increased crop yields of potato, radishes, rice, sugar beet, tomato, lettuce, apple, citrus, beans, ornamental plants and wheat (Glick, 1997; Kloepper, 1994). The application of these inoculants by different methods can be positive to enhance the efficiency of naturally and synthetically produced P resources and thus, optimize the chemical fertilizer application for the crop production (Salimpour et al., 2010).

The aerobic rice cultivation is still under research. More effort is needed especially nutrient and disease management in high-yielding aerobic rice varieties with sufficient aerobic adaptation. PSB have a high potential to be used for the management of phosphorus in P deficient soils as well as disease suppression. Therefore, usage of environmental friendly microorganisms is needed for plant-growth promotion and disease control for sustainable agriculture. The research on PSB can be a new approach to improve P uptake in aerobic rice. The aim of this study was to isolate and characterize such environmental friendly and effective PSB isolates which have the ability to solubilize insoluble P and other beneficial traits for sustainable aerobic rice production system.

MATERIALS AND METHODS

Sample collection for isolation of indigenous PSB strains

Soil and plant samples were collected from aerobic rice (M-4 and M-9 varieties) grown in Kepala Batas MARDI Penang, Malaysia. Soils of the sampling area belong to Holyroad soil series with sandy loam texture. The soil was low in organic carbon (0.97%) and nitrogen (0.15%). The composite soil and plant samples were carried to the laboratory in an icebox and kept at 4°C temperature before analyses.

Enumeration of indigenous PSB strains from soil and aerobic rice

A series of 10-fold dilutions were prepared up to 10^{-8} for rhizosphere, non-rhizosphere and endophytic population determination. The PSB populations were determined using total plate count (TPC) method using selective media (1) *P. aeruginosa* (PA); (2) Pikovskaya (Pikovskaya, 1948); (3) National Botanical Research Institute's phosphate growth medium "NBRIP" (Nautiyal, 1999) and(4) *Pseudomonas* spp. (PS) media plates. For rhizosphere population, approximately 3 g of rice plant roots with its adhering soil were transferred to conical flask containing 99 ml sterile distilled water and the content were vigorously shaken. A 10-fold series of dilution was prepared up to 10^{-8} and 0.1 ml aliquots were spread on the selective media and incubated at 28±2°C in incubator.

For endophytic population determination, fresh roots were taken and surface sterilized with 70% ethanol for 5 min and treated with 3% Clorox for 30 s (Naher et al., 2009). Roots were cut in small (5 cm) pieces and surface sterilize by dipping into 95% ethanol and flame. Surface sterilized roots were homogenized using sterilized mortar and pestle. Endophytic PSB populations were determined using total plate count method.

Determination of P solubilization activity

Phosphorus solubilizing activities of each isolate was assayed by spotting 10 µl of cultures on the top of NBRIP, CIRP and PDYA-AlP media plates. The plates were incubated at 30°C for one week and measured according to Nguyen et al. (1992).

$$Solubilization\ efficiency = \frac{solubilization\ diameter}{growth\ diameter} \times 100 \quad (1)$$

Determination of organic acid production

For the organic acid production, PSB strains were cultured in three different P sources of broths; (1) tricalcium phosphate (NBRIP); (2) rock phosphate (CIRP) broth was modified from NBRIP and supplemented with 5 g $^{-1}$ of rock phosphate; (3) aluminum phosphate (PDYA-AlP) broth modified from Katzenelson and Bose, (1959). The inoculated broth cultures were incubated at 30°C on a Kottermann 4020 shaker at a medium speed of 80 cycle's min^{-1}.
After 48 h, cultures were centrifuged at 4000 rpm for 20 min. Supernatant of each culture was filtered through 0.45 µm micro filters. Each culture sample (20 µl) solution was taken for the determination of organic acids and injected in high performance liquid chromatography (HPLC, Jasco Borwin software) with a UV detector set at 210 nm. Rezex ROA-organic acid H^+ (8%) column (250x4.6 mm) made by Phenomenex company was used and H_2SO_4 (0.005 N) was the mobile phase with flow rate of 0.17 ml min^{-1}).

Determination of indoleacetic acid (IAA)

The PSB isolates were inoculated in NBRIP broth with addition of tryptophan (2 mg l^{-1}) and incubated at 28 ± 2°C for 48 h. The culture was centrifuged at 7000 rpm for 7 min and 1 ml of the supernatant was mixed with 2 ml of Salkowsky's reagent (Gordon and Weber, 1950). The IAA concentration was determined using spectrophotometer at 535 nm.

Determination of acid phosphatase activity

Acid phosphatase was assayed as Tabatabai and Bremner (1969) method with a slight modification. Exact 3 ml of aliquot (48 h PSB culture), 1 ml modified universal buffer (MUB) and 1 ml 0.115 M p-NPP were pipetted into a 20 ml reagent vial. The mixture was incubated at 37°C for 1 h. Phosphatase reaction was stopped by the addition of 20 ml 0.5 N NaOH. The mixture was transferred to a 50 ml volumetric flask and the volume was made up to with distilled water. The absorbance (yellow colour intensity) was read with aspectrophotometer at 410 nm along with standards which were

prepared by using 20 µg/ml p-NP.

Determination of phytase enzymes activity

Phytase activity was measured by using a modification of the method of Fiske and Subbarow (1925). After 48 h of incubation, exact 150 µl of PSB culture was added with 600 µl of substrate (3 mM Na-phytate in 0.2 M Naacetate, pH 4.0) and incubated at 45°C temperature (Shimizu, 1992). The reaction was stopped by adding 750 µl of 5% trichloroacetic acid. The released inorganic phosphate was measured by adding 750 µl of colour reagent prepared by mixing four volumes of 1.5% (w/v) ammonium molybdate (Sigma, A7302) in a 5.5% (v/v) sulphuric acid solution (Carlo Erba Reagenti, 410301) and one volume of a 2.7% (w/v) ferrous sulphate (Sigma, F-7002) solution. The absorbance was measured at 700 nm on spectrophotometer. One unit of phytase activity was defined as the amount of enzyme required to liberate 1 nmol of phosphate min-1 under the assay conditions.

Antagonistic effect of PSB strains

Antagonistic effect of PSB against the rice pathogen *Rhizoctonia solani* was determined by employing dual culture technique (Sariah, 1994). An exact 5 mm diameter agar plug with mycelium was placed on the centre of the circle of PDA (BD Difco™) media plates. The PSB isolates were spotted (20 µl) on the same plate, approximately 3 cm distance from the pathogen. The plates were incubated at room temperature and checked zones of inhibition of mycelium growth after seven (7) days when the fungal mycelium had reached the edge of the plate. The measurement of growth inhibition zone was done by using following formula:

$$\% \text{ Inhibition in radial growth} = 100 \times \frac{r1 - r2}{r1}$$

Where, r1 is the radial mycelia growth in control and r2 is the radial mycelia growth in treatment.

Determination of siderophore production and N_2 fixation activity

Siderophore production was determined as described by Schwyn and Neilands (1987) using blue indicator dye, crome azurol S. Bacterial isolates exhibiting an orange halo after 5 days of incubation at 28 ± 2°C were considered positive for the production of siderophores. N_2 fixing activity was tested by using the Nfb semisolid liquid medium (Prasad et al., 2001).

Statistical analysis

All data were analyzed using the SAS statistical software and mean differences were tested using Tukey's studentized range test at the 5% level of probability.

RESULTS

Isolation and enumeration of PSB population

Forty three (43) strains were isolated and screened from aerobic rice soil and plant samples in four different PSB media plates. There were significant differences found in PSB strains in various media plates (Table 1). The higher bacterial populations were found in rhizosphere compared with non rhizosphere soil. Among the four media plates, the highest population was found in the PS and Pikovskaya, while lowest population was in (PA) plates. Aerobic rice genotype, M-9 produced higher non-rhizosphere population (6.98 x 10^6) in PS plates, while the highest rhizosphere populations was found in both PS and pikovskaya media plates. However, the highest endosphere population (2.71 x 10^8) was found in M-4 aerobic rice variety in PS followed by pikovskaya (2.56 x 10^8) media plates, respectively.

Biochemical properties of PSB isolates

Phosphorus solubilizing activities

A number of sixteen (16) isolated PSB strains were tested for P solubilizing activity in NBRIP media plates, a clear halo zone indicating P-solubilizing activity (Figure 1). The highest P solubilizing activity was found in PSB9 (69.58%) followed by PSB1 (62.5%), while the lowest activity (12%) was found in PSB2 (Table 2). There were no colonies exhibiting a clear halo zone in agar plates supplemented with aluminum phosphate (PDYA-AlP) and phosphate rock (CIRP).

Organic acid production

The PSB strains isolated from aerobic rice were able to produce organic acids in the broth culture containing different P sources during 48 h of incubation period. A number of seven PSB strains were selected for the organic acids determination. After analysis of broth culture filtrates by using HPLC, the presence of succinic, oxalic, malic and propionic acids were found. The highest amount of organic acids production was found in NBRIP broth containing tricalcium phosphate (Figure 2a) and very less amount of organic acids produced in PDYA-AlP broth containing aluminum phosphate. The succinic acid production was found highest, followed by malic acid among all PSB isolates in NBRIP broth. In case of CIRP broth (rock phosphate), the production of organic acids were found lesser than NBRIP, while succinic acid was the highest followed by propionic acid (Figure 2b). The amount of propionic acid production was found less in all sources of P broths during the incubation period (Figure 2c).

Enzymes production

The PSB strains were able to produce phosphatase and phytase enzymes in the broth culture during 48 h of

Table 1. PSB population (cfu g^{-1}) in rhizosphere, non rhizosphere and endosphere.

Media plate	Non-rhizosphere			Rhizosphere		Endosphere	
	M-9	M-4	F/land	M-9	M-4	M-9	M-4
P. aerugonisa (PA)	$1.66x10^{2}$h	$3.65x10^{2}$g	$1.36x10^{3}$f	$5.63x10^{3}$e	$3.96x10^{2}$f	$2.82x10^{3}$e	$3.19x10^{3}$e
Pikovskaya	$4.28x10^{6}$bc	$5.57x10^{6}$b	$1.67x10^{6}$d	$6.51x10^{8}$a	$3.28x10^{8}$b	$2.25x10^{8}$b	$2.56x10^{8}$a
NBRIP	$2.82x10^{6}$dc	$2.59x10^{6}$dc	$4.24x10^{5}$f	$1.96x10^{8}$c	$3.51x10^{7}$d	$3.85x10^{7}$d	$6.14x10^{7}$c
*Pseudomonas.*spp.(PS)	$6.98x10^{6}$a	$5.68x10^{6}$b	$2.25x10^{6}$d	$8x10^{8}$a	$4.22x10^{8}$b	$2.33x10^{8}$b	$2.71x10^{8}$a

M9, M4, are aerobic rice genotypes and F represents fallow land. Values are the mean of 5 replications with in column sharing the same letters are not significantly different according to Tuckey's HSD at P ≤ 0.05.

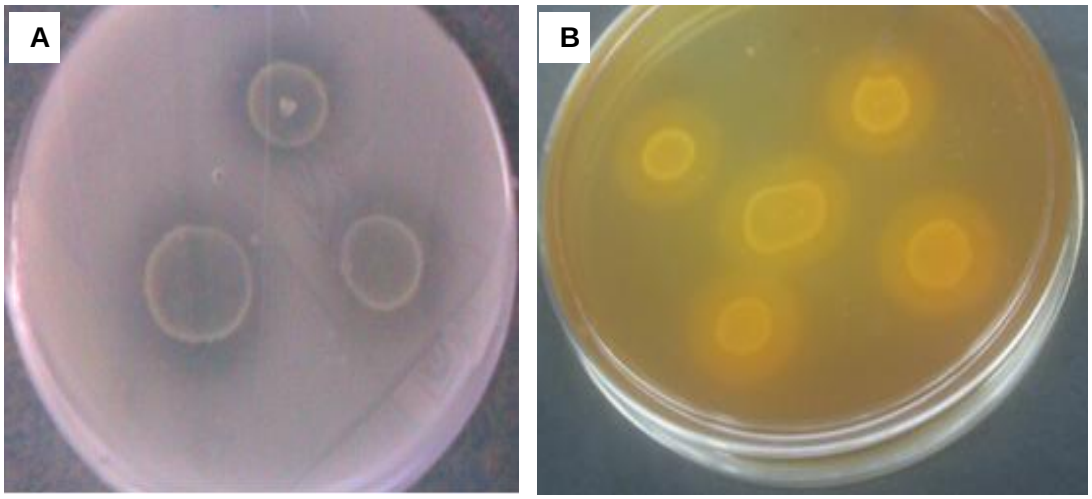

Figure 1. (a) P solubilization on NBRIP agar media plate; (b) siderophore production on CAS agar media plate.

Table 2. Biochemical properties of indigenous isolated PSB strains.

Isolate	Gram staining	Bacterial genera	IAA production (mgl^{-1})	P solubilization (%)	Antagonistic effect (% mycelial inhibition)	Siderophore production	Growth on Nfb media
PSB 1	+ve	*Bacillus* spp.	2.97^{bc}	62.50	50.63	-	+
PSB 2	+ve	ND	4.13^{a}	12.00	20.18	-	+
PSB 3	+ve	ND	4.34^{a}	23.30	12.00	-	+
PSB 4	-ve	ND	3.53^{ab}	34.47	8.33	-	+
PSB 5	-ve	ND	3.07^{bc}	60.83	35.44	-	+
PSB 6	+ve	*Bacillus* spp.	1.118^{f}	33.33	40.50	-	+
PSB 7	-ve	ND	1.67^{def}	18.18	32.34	-	+
PSB 8	-ve	ND	1.55^{ef}	27.27	17.78	-	+
PSB 9	+ve	*Bacillus* spp.	2.69^{cbd}	69.58	58.53	+	+
PSB 10	+ve	*Bacillus* spp.	0.746	53.59	59.16	+	+
PSB 11	-ve	ND	2.17^{cde}	27.53	11.09	-	+
PSB 12	-ve	ND	1.55^{ef}	30.00	12.84	-	+
PSB 13	-ve	ND	1.41^{ef}	25.00	14.02	-	+
PSB 14	+ve	*Bacillus* spp.	1.37^{ef}	12.50	31.44	-	+
PSB 15	+ve	*Bacillus* spp.	0.996^{f}	43.93	55.54	+	-
PSB 16	+ve	*Bacillus* spp.	2.22^{cde}	44.82	72.08	+	+

Data values are means of five replicates. Means in each column followed by the same letters are not significantly different according to Tukey's HSD at P ≤ 0.05. (+) For presence and (-) for absence and Gram stain (+ve) positive, (-ve) for negative and ND = not done.

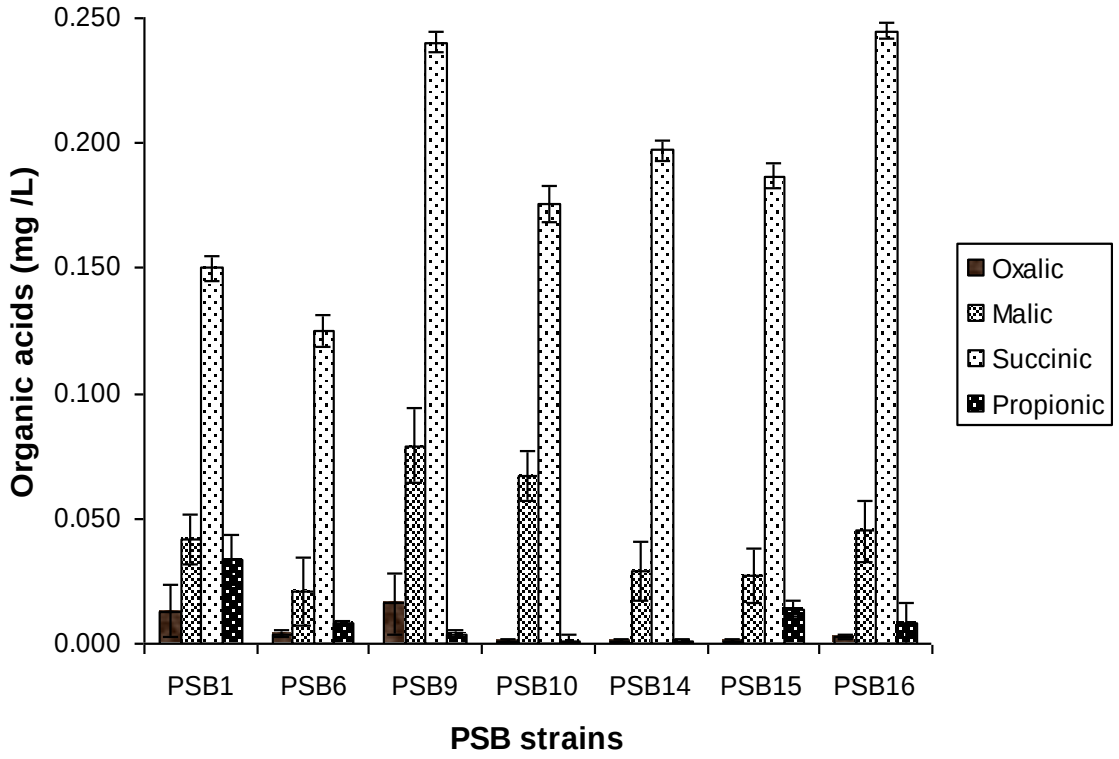

Figure 2a. Production of organic acids by PSB isolates grown in NBRIP broth. Bars in the line indicate standard error (n=6).

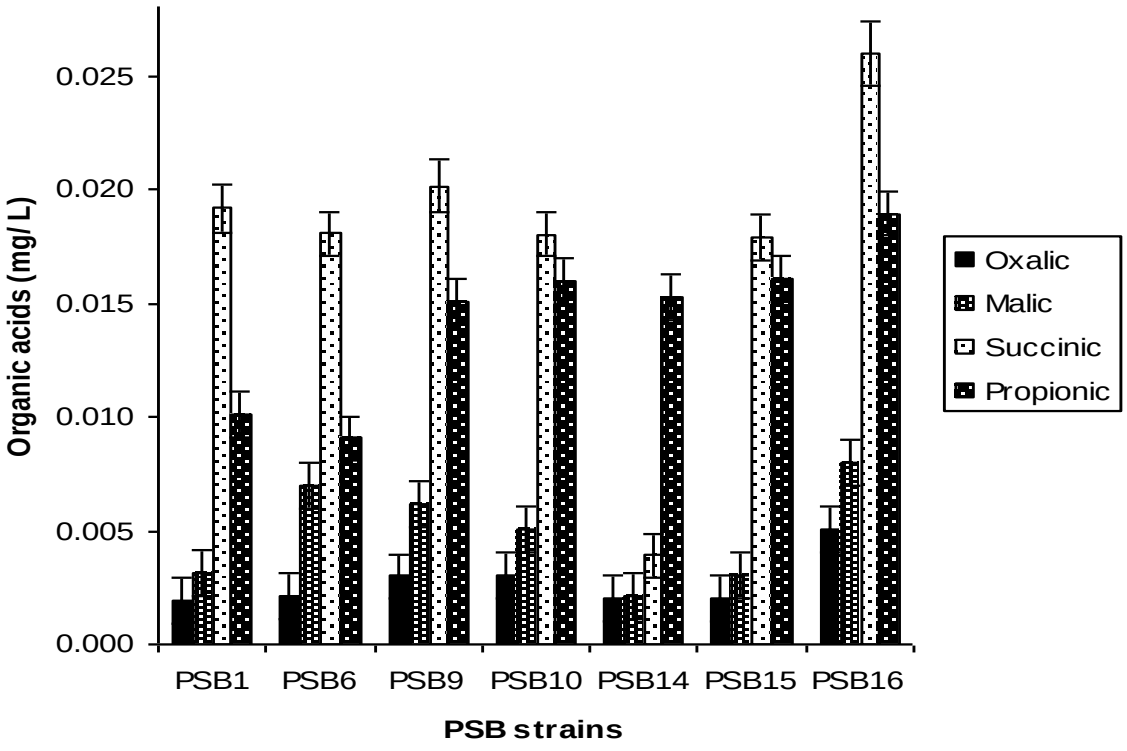

Figure 2b. Production of organic acids by PSB isolates grown in CIRP broth. Bars in the line indicate standard error (n=6).

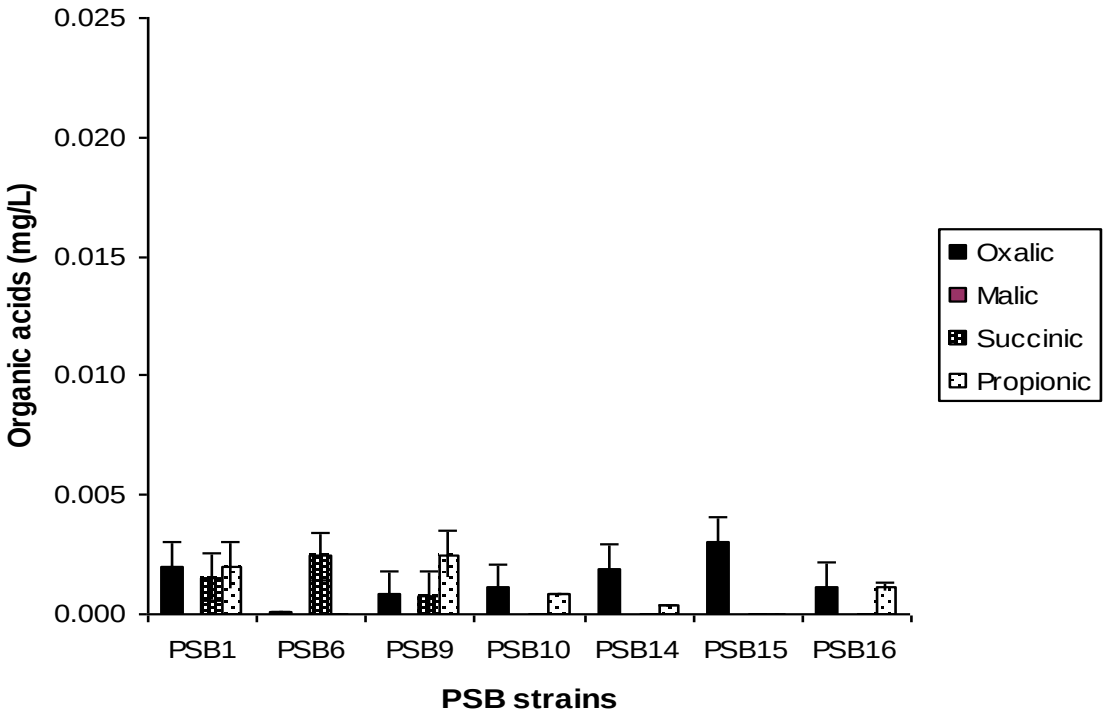

Figure 2c. Production of organic acids by PSB isolates grown in PDYA-AlP broth. Bars in the line indicate standard error (n=6).

incubation period (Figure 3). Between two enzymes, the production of phosphatase was higher than phytase. The highest phosphatase (417.89 EU ml^{-1}) and phytase (142.25 EU ml^{-1}) was found by PSB16, whereas PSB14 showed lowest phosphatase and phytase activity.

Gram staining, indoleacetic acid (IAA), siderophore production and N_2 fixing activity of PSB strains

There were sixteen (16) PSB isolates selected for the Gram staining. Among the isolates, seven were shown to be as Gram-negative and nine isolates were Gram-positive, chain and rod shaped, while seven isolated strains were identified as *Bacillus* spp. (Table 2).

The indigenous PSB isolates produced indoleacetic acid (IAA). The highest IAA (4.34 mgl^{-1}) was produced by PSB3, followed by PSB2 (4.13 mgl^{-1}) and the lowest IAA (0.74 mgl^{-1}) was produced by PSB10. Among all PSB strains, only four strains PSB9, PSB10, PSB15 and PSB16 were found positive for siderophore production activity. After the inoculation of PSB strains in Nfb media, all PSB strains were able to grow in nitrogen free semi-solid medium except PSB-15 (Table 2).

Antagonistic effect against *Rhizoctonia solani*

The PSB isolates were tested for their ability to inhibit the mycelium growth of *R. solani in vitro* by dual culture technique on PDA plates (Table 2). The dual culture plate showed that all PSB strains were able to inhibit the fungal growth. The highest antagonistic effect was found in PSB16 (71.14%) followed by PSB10 (59.31%) and PSB9 (58.33%), respectively. After seven days incubation of the dual culture, fungal hyphae of *R. solani* were unable to reach the bacterial culture and inhibition zone was established with the dimension of inhibition circle, which obtained ranges from 4 to 10 mm. While control plates not treated with the PSB isolates were completely covered by the pathogen showing no antagonistic effect.

DISCUSSION

The soils of sampling area were deficient in OC% and N, while the rests of the other nutrients were in adequate quantities. The populations of PSB differ according to the sampling part of the plant not to the soil. The higher PSB populations were found in rhizosphere than non-rhizosphere soil. Reyes et al. (2006) also found a significantly higher content of PSB population in the rhizosphere in comparison with non rhizosphere or bulk soil. It has been also observed that a high percentage of PSB population concentrated in the rhizosphere of plants (Whipps and Lynch, 1986). The PSB population (rhizosphere and endosphere) given better results in pikovskaya media plates might be due to its composition.

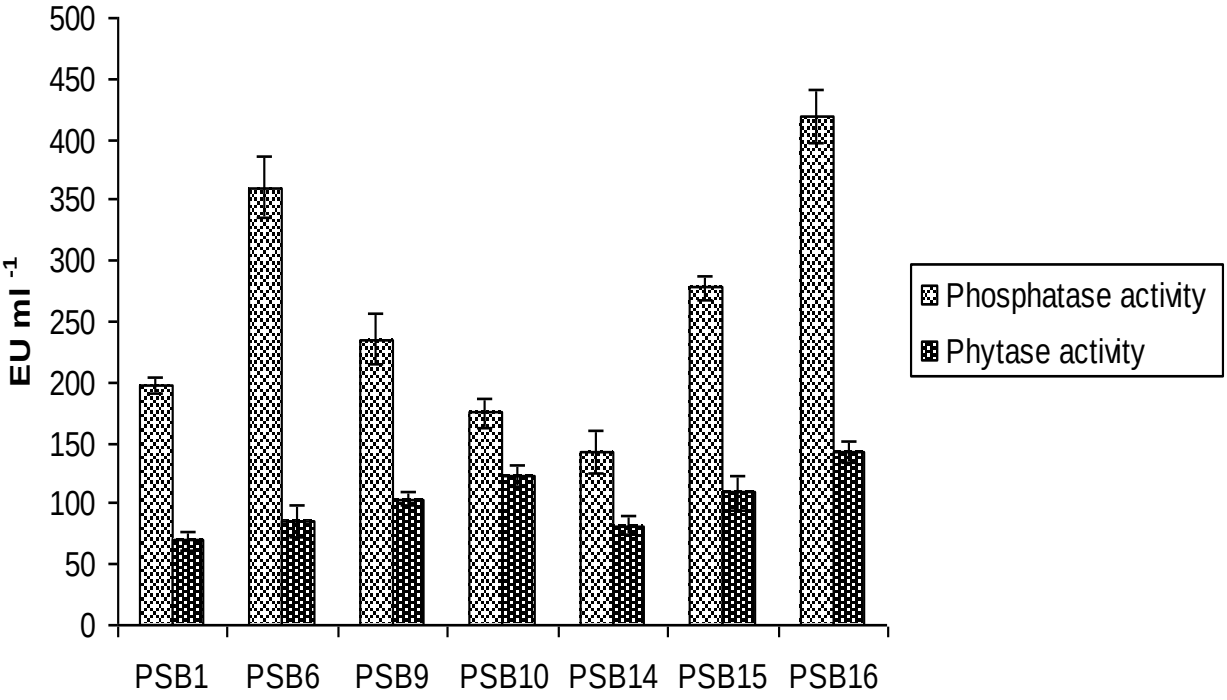

Figure 3. Enzymes activity by phosphate solubilizing bacteria (PSB).

PSB are important components of soil and directly or indirectly influence the soil's health through their useful activities. It is known that rhizosphere microorganisms mediate many soil processes such as decomposition, nutrient mineralization and nitrogen fixation (Pradhan and Sukla, 2005).

In this study, sixteen (16) PSB strains were selected for P solubilization activity. All strains were able to solubilize P on NBRIP media plates, while there was no P solubilization found on PDYA-AlP and CIRP media plates. Perez et al. (2007) found similar findings that NBRIP media plates showed positive for P solubilizing activity, however, PDYA-AlP plates showed negative results. The PSB isolates solubilized tricalcium phosphate to a greater extent than rock phosphate, aluminum phosphate and iron phosphate with $AlPO_4$ exhibiting poor solubilization (Chakraborty et al., 2010; Chung et al., 2005). These results are in agreement with the findings of Kumar et al. (2010) and Parasanna et al. (2011). This solubility of P might be the activity of certain microbes in preferable phosphate sources or due to the activity of phosphatase enzyme. Mostly, all PSB strains were able to produce organic acids (succinic, oxalic, malic and propionic acids) in different sources of broth cultures and it is known that one of the mechanisms for P solubilization is the production of organic acid. The microorganisms capable to form a halo zone due to organic acids production in the media plates (Singal et al., 1991) and are selected as potential phosphate solubilizers (Das, 1989). The microbes produced halo zones in phosphate media plates are able to produce organic acids in broth culture. This is also in agreement with Vikram et al. (2007) and Leyval and Berthelin (1989) who found the production of organic acids including oxalic, citric, butyric, malonic, lactic, succinic, malic, gluconic, acetic, glyconic, fumaric, adipic and 2-ketogluconic acid by PSB in the broth culture. The indigenous strains isolated from aerobic rice were able to produce phosphatase and phytase enzymes, which are known to have prominent effects on P solubilization as well as plant growth (Glick, 2005). Production of phosphatase enzyme by PSB and microbial phytases activity was reported by Ponmurugan and Gopi (2006).

PSB isolated strains had potential for other beneficial characteristics and positive effects for the aerobic rice. The capacity to synthesize IAA is widespread among soil and plant-associated bacteria. The indigenous PSB strains isolated from aerobic rice were able to produce IAA, which are known to have prominent effects on plant growth and development (Glick, 2005). Other researchers found similar results that isolated strains from rice field have potential for IAA production and N_2 fixing ability (Naher et al., 2009). The PSB strains were also able to produce siderophores and capable for the antagonistic effect against *R. solani* by dual culture technique on PDA plates.

These results were supported by the findings of Woo et al. (2010) that isolated PSB strains from the rhizosphere of Chinese cabbage were found to solubilize P in the media and besides this, they were able to produce IAA and siderophores. Nur Azura et al. (2008) have previously reported same observation that PSB strains

(*Pseudomonas* spp.) exhibited the best antagonistic properties against *R. solani* among the all isolates collected from paddy location in Seberang Perai, Penang, Malaysia. Some bacteria (PGPR) can produce inhibition zones to reduce the fungal growth on culture media due to release of materials like antifungal substances and cell wall degrading enzymes (Fatima et al., 2009). PSB can have a direct consequence on plant growth other than the mechanism of phosphate solubilization like, production of phytohormones (IAA), biological N_2 fixation, enhance the availability of other trace elements, increased iron nutrition through iron-chelating siderophores and volatile compounds that affects the plant signaling pathways (Hariprasad and Niranjana, 2009; Gyaneshwar et al., 2002). Most of the isolated strains grow in nitrogen free media showed the potentiality of nitrogen fixation.

Conclusions

The study area of aerobic rice was rich in potential PSB strains. The PSB population was found higher in rhizosphere and endosphere when compared with non-rhizosphere soil. Pikovskaya media was more effective for the isolation of PSB compared with other media plates. Isolated PSB strains were able to solubilize P, produce organic acids, enzymes, IAA, siderophore and competent for antagonistic effect against pathogen *R. solani*. These beneficial characteristics would be considered as potential biofertilizer of isolated PSB for aerobic rice production.

ACKNOWLEDGEMENTS

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

REFERENCES

Audenaert T, Cornelis P, Hofte M (2002). Induction of systemic resistance to Botry cinerea in tomato by *Psuedomonas aeruginosa* 7NSK2. Role of salicylic acid, pyochelin and pycoyanin. Molecular Plant-Microbe Interaction, 12: 720-727.

Bouman BAM, Yang XG, Wang HQ, Wang ZM, Zhao JF, Chen B (2006). Performance of aerobic rice varieties under irrigated conditions in North China. Field Crops Res. 97: 53-65.

Bouman BAM, Peng S, Castaneda AR, Visperas RM (2005). Yield and water use of irrigated tropical aerobic rice systems. AgriC. Water Manage. 74: 87-105.

Chakraborty BN, Chakraborty U, Saha A, Sunar K, Dey PL (2010). Evaluation of Phosphate Solubilizers from Soils of North Bengal and Their Diversity Analysis. World J. Agric. Sci. 6(2): 195-200.

Chung MP, Munusamy M, Sundaram S, Jaekyeong S, Hyunsuk C, Tongmin S (2005). Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil Biol. Biochem. 37: 1970-1974.

Das AC (1989). Utilization of insoluble phosphates by soil fungi. Indian Soc. Soil Sci. 58: 1208-1211.

Fatima Z, Saleemi M, Zia M, Sultan T, Aslam M, Chaudhary MF (2009). Antifungal activity of plant growth-promoting rhizobacteria isolates against *Rhizoctonia solani* in wheat. Afr. J. Biotechnol. 8(2): 219-225.

Fiske CH, Subbarow YP (1925). The colorimetric determination of phosphorus. J. Biol. Chem. 66: 375-410.

Glick BR (2005). Modulation of plant ethylene levels by the enzyme ACC deaminase. FEMS Microbiol. Lett. 251: 1-7.

Glick BR, Changping L, Sibdas G, Dumbroff EB (1997). Early development of canola seedlings in the presence of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2. Soil Biol. Biochem. 29: 1233-1239.

Gordon AS, Weber RP (1950). Colorometric estimation of indoleacetic acid. Plant Physiol. 26: 192-195.

Gyaneshwar P, Kumar GN, Parekh LJ, Poole PS (2002). Role of soil microorganisms in improving P nutrition of plants. Plant Soil, 245: 83-93.

Hariprasad P, Niranjana SR (2009). Isolation and characterization of phosphate solubilizing rhizobacteria to improve plant health of tomato. Plant Soil, 316: 13-24.

Hilda R, Fraga R (2000). Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv. 17: 319-359.

Katzenelson H, Bose B (1959). Metabolic activity and phosphate dissolving ability of bacterial isolates from wheat roots rhizosphere and non-rhizosphere soil. Can. J. Microbiol. 5: 79-85.

Khiari L, Parent LE (2005). Phosphorus transformations in acid light-textured soils treated with dry swine manure. Can. J. Soil Sci. Soc. 85: 75-87.

Kloepper JW (1994). Plant growth promoting bacteria (other systems). In: Okon J Editor, *Azospirillum/Plant Association,* CRC Press. Boca Raton. pp. 137-154.

Kumar A, Bhargava P, Rai LC (2010). Isolation and molecular characterization of phosphate solubilizing Enterobacter and Exiguobacterium species from paddy fields of Eastern Uttar Pradesh, India. Afr. J. Microbiol. Res. 4(9): 820-829.

Leyval C, Berthelin J (1989). Interaction between *Laccaria laccata*, *Agrobacterium radiobacter* and beech roots: influence on P, K, Mg and Fe movilization from minerals and plant growth. Plant Soil, 117: 103-110.

Naher UA, Radziah O, Shamsuddin ZH, Halimi MS, Mohd Razi I (2009). Isolation of diazotrophs from different soils of Tanjong Karang Rice growing area in Malaysia. Int. J. Agric. Biol. 11(5): 547-552.

Nautiyal CS (1999). An efficient microbiological growth medium for screening phosphate-solubilizing microorganisms. FEMS Microbiol. Lett. 170: 265-270.

Nguyen C, Yan W, Le Tacon F, Lapeyrie F (1992). Genetic variability of phosphate solubilizing activity by monocaryotic and dicaryotic mycelia of the ectomyccorhizal fungus *Laccaria bicolor* (Maire) P.D. Orton. Plant Soil. 143: 193-199.

Nur Azura MS, Gayah MS, Marzukhi H, Siti N, Noor A, Noriha, MA (2008). Antagonistic property of *Pseudomonas spp.* Isolated from Seberang Perai's paddy field soil against sheath blight disease (*Rhizoctonia solani*). Sustaining soil ecosystems with emphasis on coastal soils, Proceedings Soils 2008, Ipoh, Perak, Malaysia.

Perez E, Miguel S, Maria MB, Yarzabal LA (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.

Pikovskaya RI (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Microbiologiya, 17: 362-370.

Ponmurugan P, Gopi C (2006). In vitro production of growth regulators and phosphatase activity by phosphate solubilizing bacteria. Afr. J. Biotechnol. 5(4): 348-350.

Pradhan N, Sukla LB (2005). Solubilization of inorganic phosphate by fungi isolated from agriculture soil. Afr. J. Biotechnol. 5: 850-854.

Prasad G, James EK, Mathan N, Reddy PM, Barbara Reinhold-Hurek B, Ladha JK (2001). Endophytic Colonization of Rice by a Diazotrophic Strain of *Serratia marcescens.* J. Bacteriol. 183(8): 2634-2645.

Parasanna A, Deepa V, Balakirashna Murthy P, Deecaraman M, Sridhar R, Dhandapani P (2011). Insoluble phosphate solubilization by bacterial strains isolated form rice rhizosphere soils from southern India. Int. J. Soil Sci. 6(2): 134-141.

Rodriguez H, Fraga R (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv. 17(4-5): 319-339.
Reyes I, Valery A, Valduz Z (2006). Phosphate-solubilizing microorganisms isolated from rhizospheric and bulk soils of colonizer plants at an abandoned rock phosphate mine. Plant Soil, 287: 69-75.
Sagoe CI, Ando T, Kouno K, Nagaoka T (1998). Effects of organic acid treatment of phosphate rocks on the phosphorus availability to Italian ryegrass. Soil Sci. Plant Nut. 43: 1067-1072.
Saikia Singh BP, Kumar R, Arora DK (2005). Detection of pathonesis related proteins, chitinase and B-1, 3-glucanse in induced chickpea. Curr. Sci. 89: 659-663.
Salimpour S, Khavazi K, Nadian H, Besharati H, Miransari M (2010). Enhancing phosphorous availability to canola (*Brassica napus* L.) using P solubilizing and sulfur oxidizing bacteria. Aust. J. Crop Sci. 4(5): 330-334.
Sariah M (1994). Potential of *Bacillus spp*. as biocontrol agent for anthracnose fruit rot of chilli. Malaysian Appl. Biol. 23: 53-60.
Schwyn B, Neilands JB (1987). Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160: 47-56.
Shimizu M (1992). Purification and characterization of phytase from Bacillus subtilis (Natto) n-77. Biosci. Biotechnol.Biochem. 6: 1266-1269.
Singal R, Gupta R, Kuhad RC, Saxena RK (1991). Solubilization of in organic phophates by a Basidiomyceteous fungus *Cauthus.* Indian J. Microbiol. 31: 397-401.
Tabatabai MA, Bremner JM (1969). Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1: 301-307.
Vikram A, Hamzeehzarghani H, Alagawadi AR, Krishnaraj PU, Chandrasshekar BS (2007). Production of plant growth promoting substances by phosphate solubilizing bacteria isolated from vertisols. J. Plant Sci. 2(3): 326-333.
Whipps JM, Lynch JM (1986). The influence of the rhizosphere on crop productivity. Adv. Microbiol. Ecol. 9: 187-244.
Woo SM, Lee MK, Hong IS, Poonguzhali S, Sa TM (2010). Isolation and characterization of phosphate solubilizing bacteria from Chinese cabbage. 19th World Congress of Soil Science, Soil Solutions for a Changing World 1-6 August 2010, Brisbane, Australia.