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Isolation and identification of phosphate solubilizing bacteria (PSB) from various plant rhizospheres and its ability to dissolve tricalcium phosphate under *in vitro* condition

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Phosphorus (P) is the second most indispensable limiting nutrient in crop production due to a strong retention in soil. Recently, the utilization of microbes is considered as an alternative to improve the bioavailability of soil phosphate for plants. The present study designated to isolate and to identify the phosphate solubilizing bacteria (PSB) from different plant rhizospheres. Isolation of PSB was performed under *in vitro* condition, followed by dilution plate technique using Pikovskaya's solid medium. A series of morphological characterization, physiological and biochemical test, were done in order to identify the bacteria and molecular identification using 16S rRNA was performed for the best PSB in term of quantitative P solubilization. With using clear zone indicator, a total of fourteen PSB were identified from rhizosphere soil sample of gadung (three isolates), bamboo (five isolates), and corn (six isolates). Solubilization index (SI) ranged from 2.56 to 4.5. All isolates were capable of solubilizing insoluble-P. The higher P solubilizer was recorded by BPFG3 isolate (112.24 ppm), followed by BPFJ2 isolate (100.20 ppm). Both BPFG3 and BPFJ2 isolates were the most potential to be a bioinoculant in order to improve the efficiency of chemical P fertilizers in the field condition.

Keywords: Bacteria, organic acid, phosphate, rhizosphere, solubilizers

INTRODUCTION

Phosphorus (P) is one of the major nutrients after nitrogen that plays a number of important roles in plants. It makes up about 0.2% of a plant's dry weight (Alori et al., 2017). Since phosphorus is a component of deoxyribonucleic acid (DNA) and ribonucleic acids (RNA), it plays a vital role in plant reproduction, of which grain production is an important result. It is also critical in biological energy transfer processes that are indispensable for life and growth. The absence of this nutrient may affect the root development and perturbe the flowering proccesses and seed formation (Donahue et al., 1990). Plants absorve this nutrient in the ionic form $H_2PO_4^-$ or HPO_4^{-2} from soil solution. Although soil P is abundant in both organic and inorganic forms, its accessibility to plant root is limited due to poor solubilization and its retention in soil (Illmer and Schinner, 1992). In agricultural practices, the challenge of

soil phosphorus deficiency was addressed by the application of phosphorus fertilizers (Alori et al., 2017). However, it was commonly inefficient because phosphate was complexed with Aluminum (Al) orIron (Fe) in acidic condition and inversely, phosphate was fixed by Calcium (Ca)in alkaline conditions. Most authors have shown that the efficiency of applied P fertilizers in chemical form rarely exceeded 30% due to P precipitation by metal-cation complexes (Adnan et al., 2003).The inefficient P fertilizers were also observed in many croplands in Indonesia, mainly in Southeast Sulawesi, in which the P availability in soil is one of the most constraints for crop production.

Based on the empirical evidences, soil microorganism plays an important role in P-insoluble transformation to soluble P in soil (Prernaet et al.,1997; Rajuand Reddy, 1999). A larger number of soil microbes, including bacteria, fungi, actinomycetes and algae, exhibit P solubilization and mineralization ability. Soil bacteria that have been reported to be poorly mobile in available P through solubilization include *Pseudomonas spp., Agrobacterium spp.,* and *Bacillus circulans (*Babalola and Glick, 2012). Other bacteria documented as P solubilizers were *Azotobacter, Burkholderia, Rhodococcus, Serratia*, and *Thiobacillus (*Kumar et al., 2014; Zhao et al., 2014; Istina et al., 2015; Postmaet et al., 2010; David et al., 2014). Several studies conducted under *in vitro* condition showen that soil bacteria produced some mineral dissolving compounds such as organic acid, siderophores, protons, hydroxil ions and CO_2 (Sharma et al., 2013; Rodríguez and Fraga, 1999). The releasing of these organic acid was accompanied with the pH reduction resulting in the acidification of microbial cell as well as the surroundings, and than P ions were released by substitution of H^+ for Ca^{2+} (Goldstein, 1994). It was also documented that NH_4^+ assimilation within microbial cell was simultaneous with the liberation of protons and this resulted in the P solubilization without the production of any organic acids (Sharma et al., 2013). Many studies performed in the field condition showed that PSB was capable of improving plant yield and increasing phosphorus uptake (Wang et al., 2015), increasing the bioavailability of insoluble P for plant use (Zhu et al., 2011), increasing soil available P and P uptake in plants and plant growth (Gupta et al., 2012) and stimulating the efficiency of biological nitrogen fixation, synthesizing phytohormones and enhancing the availability of some trace elements (Wani et al., 2007). Regarding to its positive benefits, the use of PSB is a promising approach in improving food production through enhancing agricultural yield as it is better to use an environmental-friendly approach to solve the problems of infertile soil (Babalola and Glick, 2012). However, the ability of bacteria to solubilize insoluble P strictly varies depending on the soil bacteria themselves, soil and climatic conditions and cropping history. Nowadays, it is rarely reported the locally adaptive PSB in given environment soil conditions. With the emphasis on the potential PSB from the soil for the agriculture purposes, this study was designed to isolate and to indentify the indigenous PSB from different rhizospheres and its ability to dissolve tricalcium phosphate, which are potential as bioinoculants, mainly for croplands in Southeast Sulawesi in which they have problems with the availability of phosphate.

MATERIALS AND METHODS

Rhizosphere soil sampling.

The present study was carried out from March to July 2018.Rhizosphere soil samples of gadung (*Dioscorea hispida* Dennst) and bamboo (*Dendrocalamus asper*) were randomly collected from agricultural area near to Kendari town. The sites are located between 04^o 01'10, 2" S and 122^o 31' 58, 7" E with an altitude of 48 masl and between 04^o 00' 33, 3" S and 122^o 31' 27, 9" E with an altitude of 44 masl, respectively. Rhizosphere soil samples of maize (*Zea mays* L.) were collected from farmer field in Konda, Konawe Selatan Regency, approximately 25 Km from Kendari, located between 04^o 06'46,3" S and 122^o 27' 06,7" E with an altitude of 57 masl. The rhizosphere soil samples were taken from the upper 25 cm of the soil profile. The soil samples were served for isolation and identification of PSB. The samples were air-dried, grounded and sieved through a 2-mm sieve for physical and chemical analysis. Parameters assessed were; soil texture, pH, organic carbon, total-N, P and K. Soil pH was measured using pH meter equipped with a glass electrode. Measurement of organic carbon was performed by wet digestion (oxidation) method of Walkely-Black (Nelson and Sommers, 1996). Total-N was analysed using the procedure as described by Kjeldal, then P and K were assessed using the procedure described by Bray and Kurtz (Bray and Kurtz, 1945).

Isolation and measurement of solubilization index:

Isolation of phosphate solubilizing bacteria was performed under *in vitro* condition, followed by dilution plate technique using Pikovskaya's solid medium, containing; 0.5 g $(NH_4)_2SO_4$, 0.2 g KCl, 0.1 g $MgSO_4$, 5 g $Ca_3(PO_4)_2$, 0.002 g $MnSO_4$, 0.002 g $FeSO_4$, 10 g glukosa 0.5 g yeast exstract, 20 g agar, 0.2 g NaCl and 1000 mL distilled water (Pikovskaya, 1948). For the isolation of PSB, the rhizoshpere soil samples were serially diluted up to 10^{-5} dilution, and pour plated on Pikovskaya's agar medium and then incubated at 30ºC for 3 days. At the end of incubation, bacterial colonies showing clear zone around the colonies were further purified and maintained in nutrient agar slantsfor the next studies (Cappucino and Sherman, 1987). The qualitative measurement of P solubilized was carried out by observing the clear zone around the colonies, and the results were expressed as the P solubilitation index (SI):

$$\text{Solubilization Index (SI)} : \frac{\text{halo zona diameter} + \text{colony diameter}}{\text{colony diameter}}$$

Identification of PSB:

A series of morphological characterization, physiological, biochemical and molecular tests, were performed in order to identify the bacteria. The morphological characterization, biochemical and physiological test were done by following the standar procedure as described in *Burgey's Manual of Determination Bacteriology* (Holt et al., 1994) and then all these characters were analysed using Multi Variate Statistical Package (MVSP)3.1 version for their similarity by comparing them to reference bacteria.

The molecular identification was carried out for the best PSB in term of quantitative P solubilization in the Pikovskaya's liquid medium. Bacterial DNAs were extracted from pure culture by heating at 90ºC for 15 minutes followed by extraction using silica columns prior to amplification (Simfukwe and Tindwa, 2018). The amplification of 16S rRNA gene fragment was performed using primers27F and 1492R (forward primer 5' AGAGTTTGATCMTGGCTCAG 3' and reverse primer 5 (5' TACGGYTACCTTGTTACGACTT 3'). The reaction conditions included an initial denaturation of 5 minutes at 95ºC followed by 40 cycles of 1 minutes 30 s at 94ºC, 1 min 30 s at 37ºC, and 2 min 30 s at 72ºC with final extension of 10 min 30s at 72ºC. The PCR products were resolved on 1% agarose gel with 110V running volatge for 45 minutes (Chen et al., 2006).

The 16S rRNA purified PCR product (100ng concentration) was subjected to thesequencing using Automated DNA Sequencer ABI PRISM 377 (Perkin Elmer Biosystem, USA). Sequencing of the 16S rRNA gene of the bacterial isolate was performed from both directions. The sequences obtained were compared to other sequences at the GenBank using BLASTn tool to determine the bacterial species.

Determination of P solubilization, pH and organic acid:

The ability of the bacterial isolates to dissolve insoluble P was estimated by growing the selected isolates in Pikovskaya medium without agar and incubated for 3 days. At the end of incubation, the pH medium was measured with a pH meter equipped with a glass electrode and then the culture medium was centrifuged at 3500 rpm for 15 minutes. The supernatant was used for P analysis using UV spectrofotometer with an absorbance of 880 nm.

The total of organic acid produced by phosphate solubilizers was analysed in term of total titrable acidity of bacterial cultures (Dharmwal et al., 1989). Some selected isolates were inoculated in the Pikovskaya's liquid medium and incubated for three days. After incubation, the bacterial culture was centrifuged and removed its cell biomass, pipetted 1 mL of culture filtrate and added 1% phenolphthalein and then titrated with 0.1 N sodium hydroxide. The titration was stopped after a change in color and the total acid was calculated.

Data analysis:

Phenetic characters of bacterial isolates were analyzed using Multi Variate Statistical Package (MVSP) 3. 1 version and the isolates were identified using *Burgey's Manual of Determination Bacteriology*. Quantitative P solubilizations were subjected to Analysis of Variance (Anova) and the difference between their means was analyzed using HSD's tests.

RESULTS

Isolation of PSB and physico-chemical characteristics of rhizosphere soil sample:

Some selected physico-chemical characteristics of three rhizosphere soil samples used in the current study were: sandy loam in texture as described by USDA textural class

triangle (Brady and Weil, 2002) and total-N was 0.09 %. The different characters was soil pH 5.7, total-C 2.13 %, P_2O_5 56 ppm and K_2O 84 ppm for gadung, and soil pH 6.29, total-C 0.96 %, P_2O_5 14 ppm and K_2O 65 ppm for bamboo, and soil pH 5.80, total-C 2.51 %, P_2O_5 64 ppm and K_2O 75 ppm for maize.

Isolation of PSB was carried out under *in vitro* condition using Pikovskaya solid medium and tri calcium phosphate as a sole source of phosphate used. In the present study, fourteen bacterial isolates in total were found from the different plant rhizospheres collected; three isolates from gadung (*Dioscorea hispida* Dennst), five isolates from bamboo (*Dendrocalamus asper)* and six isolates from maize (*Zea mays*. L) rhizospheres. All isolates were capable of dissolving tri-calcium phosphate in agar medium as shown by the appearance of halo zones or clear zones on isolates grown on Pikovskaya's agar medium after three-days incubation.

Solubilization index (SI) varied from 2.56 to 4.5. The clear zone was maximum by BPFJ1 and BPFB1 compared to other isolates and the minimum was noted by BPFJ6 isolate, as shown in Table 1.

Identification of PSB.

Based on phenetic numerical analysis, the PSB strains were identified up to species level. The similarity index of bacterial isolates versus reference species varied from 88.6% to 100%. It was observed fourteen species of P solubilizer that belonging of the genera *Bacillus, Lactobacillus*, and *Burkholderia.*Two strains were identified as *Bacillus megaterium* (BPFB1 and BPFG2), three strains as *B. pumilus* (BPFB4, BPFB3, BPFB2), two strains as *B. larvae* (BPFB5 and BPFG1), BPFJ3, BPFJ4 and BPFJ6 isolates as *B. polymyxa,* and BPFG3, BPFJ1, BPFJ2 and BPFJ5 isolates were identified as *Lactobacillus plantarum, Burkholderia cepacea, B. firmus and B. pantothenticus,* respectively, see Table 2.

Table 1.Solubilization index (SI) of bacterial isolates from Bamboo (BPFB), Gadung (BPFG) and Maize (BPFJ)

Source of Isolates	Isolate code	Solubilization Index (SI)
	BPFB1	4.2
Bamboo	BPFB2	3.2
(*Dendrocalamus asper*)	BPFB3	3.4
	BPFB4	3.5
	BPFB5	3.3
Gadung	BPFG1	3.7
(*Dioscorea hispida* Dennst)	BPFG2	4.0
	BPFG3	4.2
	BPFJ1	4,5
	BPFJ2	2,7
Maize	BPFJ3	2,6
(*Zea mays* L.)	BPFJ4	2,9
	BPFJ5	2,6
	BPFJ6	2,56

Table 2. Bacterial isolates and the corresponding species obtained from gadung, bamboo, and maize rhizosphere.

PSB Isolate	Corresponding species	Simiarity index (%)
BPFB1	*Bacillus megaterium*	88.6
BPFB2	*Bacillus pumilus*	88.6
BPFB3	*B. pumilus*	88.6
BPFB4	*B. pumilus*	88.6
BPFB5	*Bacillus larvae*	88.6
BPFG1	*B. larvae*	88.6
BPFG2	*B. megaterium*	88.6
BPFG3	*Lactobacillus plantarum*	100.0
BPFJ1	*Burkholderia cepacea*	100.0
BPFJ2	*Bacillus firmus*	80.8
BPFJ3	*Bacillus polymyxa*	79.5
BPFJ4	*B. polymyxa*	79.5
BPFJ5	*Bacillus pantothenticus*	78.2
BPFJ6	*B. polymyxa*	79.5

B. megaterium; the cells were rod-shape, Gram positive, growth with spore and motile. BPFB1 isolate was positive in cellulolitic test, but it was negative for BPFG2 isolate. BPFB1 isolate survived till 50^{o}C, but not his counterpart. *Bacillus larvae*; celles were Gram positive, rod-shaped, motile, presence of spore, growth from acidic to neutral conditions (pH 5 to 7), positive growth till 40^{o}C but not BPFG1 isolate. Only BPFG1 isolate was able to ferment sucrose, but BPFB5 was positive to cellulolitic test.*B. pumilus;* the cells were rod-shaped, Gram positive, positive motile and growth with spore. Two strains (BPFB2 and BPFB3) were negative cellulolitic test and starch hydolysis, but it was positive for BPFB4 *isolate*. Only BPFB3 and BPFB4 were positive to mannitol test. BPFB2 and BPFB3 isolates were able to survive till 40^{o}C, except BPFB4 (37^{o}C).*B. polymyxa*; the cells were rod-shaped, Gram positive, generally positive responses in biochemical test, but negative in indole and VP test. These bacteria growth till 40^{o}C, survived in neutral pH, but less resistant to salty condition, see Table 3.

Lactobacillus plantarum; its cells were rod-shaped, Gram positive, absence of spore and non motile, positive to catalase, cellulolitic, methyl red, and Voges-Proskauer test, fermentation of glucose, sucrose and mannitol. However, the negative responses were detected to citrate test, urea hydrolysis and starch hydrolysis and indole test, survived at 5% of NaCl, adaptive till 40 ^{o}C and it was facultative anaerob.

Table 3.Phenetic characteristics of PSB isolates. (+) indicates positive reponses, (-) indicates negative responses.

Character	Isolate code													
	BPFB1	BPFB2	BPFB3	BPFB4	BPFB5	BPFG1	BPFG2	BPFG3	BPFJ1	BPFJ2	BPFJ3	BPFJ4	BPFJ5	BPFJ6
Cell morphology														
Cell shape	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gram stain	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Motility	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Endosporic	+	+	+	+	+	+	+	-	-	+	+	+	+	+
Biochemical test														
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Voges-Proskauer (VP) test	+	-	-	-	-	-	+	+	-	-	-	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Manitol	+	-	+	+	-	-	+	+	+	+	+	+	-	+
Gelatin	+	+	+	+	+	+	+	+	+	+	-	+	-	+
Urea hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrate test	-	-	-	-	-	-	-	-	+	+	+	+	+	-
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Physiological test														
pH 5	+	+	+	+	+	+	+	+	+	+	+	+	+	+
pH 7	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NaCl 2 %	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NaCl 5 %	+	+	+	+	-	-	+	+	-	-	-	-	-	-
NaCl 7 %	-	+	+	+	-	-	-	-	-	-	-	-	-	-
Temperature 5^{o}C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Temperature 37^{o}C	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Temparature 40^{o}C	+	+	+	-	+	-	+	+	+	+	+	+	+	+
Temperature 50^{o}C	+	-	-	-	-	-	-	-	-	-	-	-	-	-

Burkholderia cepacia; the cells were rod-shaped, Gram negative and non endosporic. Biochemically, this strain has a positive responses, except indole, MR, and VP test. This strain growth till 40^{o}C, survive from acidic to alkaline conditions, but negative responses above of 5 % of NaCl. Based on the molecular identification, BPFG3 and BPFJ1 isolates were also identified as *Lactobacillus plantarum* and *Burkholderia cepacia*, with 100 % in term of similarity index.

Quantitative P solubilization, pH and organic acid:

The measurement of quantitative solubilization of tricalcium phosphate, pH culture medium and organic acid produced, was recorded after three days of incubation. The results were presented in Table 4. It was observed that the solubilization of P in Pikovskaya liquid medium varied among isolates. Statistically, it was observed the solubilization of tricalcium phosphate was significantly different after three days incubations. Liberation of insoluble P by BPFG3 isolate from gadung and all isolates from maize rhizoshpere was higher than other isolates. The highest P solubilization was recorded by BPFG3 isolates (112.24 ppm) from gadung rhizosphere, followed by BPFJ2 (100.20 ppm) from maize, and the least was noted by BPFG1 (31.35 ppm). The solubilization of tricalcium phosphate (TCP) in the culture medium was accompanied with the reduction of pH (Table 4). The maximum dropping in pH was noted in BPFG3 and BPFB1 isolates (calculated by a different method), however, the decreasing of pH in both was not significantly different. Interestingly, the measurement of total organic acid produced increased in term of volume of NaOH titration in culture medium after three days of incubation, but the mean values were not significantly different. It was found that both BPFG3 and BPFJ1 isolates (calculated by a different method) were good in organic production in culture medium (Table 4).

Table 4. Available-P, pH medium, and total organic acid in term of NaOH titrated after 3-days incubation

Isolate Code	pH medium	P-available (ppm)	Total organic acid (0.1N NaOH titrated)
	day-3		
BPFG 3	5.10	112.24a	3.90a
BPFJ2	5.39	100.20a	3.5a
BPFJ4	5.50	92.87ab	2.90a
BPFJ5	5.52	88.58abc	4.40a
BPFJ3	5.92	87.61abc	4.20a
BPFJ6	5.44	86.59abc	3.80a
BPFJ1	5.65	86.09abc	5.00a
BPFB 4	5.02	43.03bcdef	3.40a
BPFB 5	5.15	35.97cdef	2.30a
BPFB 1	5.07	34.66def	4.10a
BPFB 2	5.10	33.84def	2.70a
BPFG 2	4.98	33.06ef	3.60a
BPFB 3	4.93	31.45f	3.80a
BPFG 1	5.06	31.35f	3.40a

Note : Values of P-available and organic acid were the mean of 3 replicates.Mean values followed by the same letter within column are not significantly different ($p<0.05$) by HSD test.

DISCUSSION

In natural circumstance, soil microbes including bacteria have been well recognized and they played an important role in P cycling through mineralization and immobilization processes. A number of theories explain the mechanism of inorganic-P solubilization, such as the production and secretion of organic acids by microbes, which complexed the cations bound to release P in soluble form. It has been reported a large number of microbes including bacteria, fungi, actinomycetes, and algae that possess P solubilizing and mineralizing ability (Babalola and Glick, 2012; Kumar et al., 2014; Postma et al., 2010). The potency ofPSB from different rhizospheres was investigated under *in vitro* condition using tricalcium phosphate as asole source of insoluble P. Based on the clear zone forming, fourteen bacterial isolates were positive as PSB. The clear or halo zone was reported as an indicator of solubilization of insoluble phosphates by acidification of association of either proton extrusion or organic acid release (Dharmwalet al., 1989). The formed clear zone varied between phosphate solubilizers (2.56 to 4.50), and it was higher than those observed by some authors (Baliah et al., 2016; Atekan et al., 2014; Karpagam and Nagalakshmi, 2014). Among fourteen isolates, BPFJ1, BPFG3 and BPFB1 isolates were the most efficient in term of solubilization index. As reported by other authors, the bacteria were the microbes solubilizing in soluble phosphate with high efficiency (Narsian et al, 1995; Kapoor et al., 1989). It was documented that *Pseudomonas* and *Bacillus* were the most efficient (Daveand Patel, 1999). The other study recorded that abrupt changes of halo zone sometimes occurred after seven days of incubation (Rashid et al., 2004).

Based on the results of phenetic numerical analysis and molecular identification, it was observed fourteen species of bacteria belonging the genera *Bacillus*, *Burkholderia* and *Lactobacillus*. The wide spacial distribution of PSB in the top soil indicated that the climatic condition, physico-chemical properties of plant rhizospheres, cropping system and plant species played an important role in the distribution of bacterial species in the soil. The different of physico-chemical properties of plant rhizospheres noted in the current study may be attributed to the variation of microbes associated with the plant roots. In another study, Guyasa et al., (2018) reported a large species of bacteria was documented from upland rice rhizosphere.The findings reported here were contradictory to that noted by Keneni et al.(2010) that reported *Pseudomonas* sp as a sole P-solubilizer from four agricultural areas in Ethiopia. In the large experiment, the wide variation in the distribution of PSB might be due to the differences in physical, chemical and biological properties of soil (Yadav and Singh, 1991). Another work noted that plant rhizospheres released some organic compounds such as water soluble sugars, organic acids and amino acids, and they also contained hormones, vitamins, amino compounds, phenolics and sugar phosphate esters. Different microbes living surrounding the rhizospheres indicated the different ecological conditions affecting the excudation and thereby rhizoshpere colonization by phosphate solubilizers (Uren, 2001).

In the present study, the pH reduction of the culture medium was observed for all isolates and this was accompanied with the increasing of organic acid. The maximum pH reduction occurred in BPFG1 and BPFB3 isolates from 7.41 to 5.06 and 7.28 to 4.93, respectively. Statistically, it was obtained a negative correlation ($r= -0.54$; $p\leq 0.05$) between organic acid production and reduction of pH, but it was not as a constant relationship among them. The findings were corresponding to the results obtained by the previous authors (Cunningham et al., 1992; Baliah et al., 2016; Illmer et al., 1995). Quantitative measurement of P solubilization revealed that all isolates were capable of releasing P in culture medium. Solubilization of insoluble P was accompanied with an increasing production of organic acid in term of volume NaOH titrated and a decreasing of pH, see Table 4. A positive correlation ($r=0.66$) between P solubilization and organic acid produced was observed but it can not be established as a constant relationship. A weak negative correlation ($r=-0.02$) between P solubilization and the pH dropping was recorded. This phenomenon was reported by some authors (Rashid et al., 2004; Bar-Yosef et al., 1999; Asea et al., 1988). The soluble-P concentration in the culture medium varied between 31.35 to 112.24 ppm with variations among different isolates. These values were higher than those found by other authors. For example, Karpagam and Nagalakshmi (2014) documented that *Pseuodomas, Bacillus*, and *Rhizobium* produced soluble phosphate by 0.37 mgL-1, 0.30 mgL-1 and 0.28 mgL-1, respectively. The study by Baliah et al. (2016) showed that the releasing of insoluble-P by *Bacillus* and *Pseudomonas* varied from 22.3 to 46.0 mgL-1. The best P solubilizer

(112.24 ppm) reported here was recorded by BPFG3 isolate, identified as *Lactobacillus plantarum*, and followed by BPFJ2 isolate (100.20 ppm), confirmed as *B .firmus*. The findings indicated that organic acid together with their carboxyl and hydroxyl ions chelated cations or reduced pH to release P (Rashid et al., 2004; Seshachala and Tallapragada, 2012). The secretion of organic acid was also accompanied with the pH reduction that resulted in the acidification of the microbial cells and the surroundings. Hence, P ions were released by substitution of H^+ for Ca^{2+}. Interestingly, *Lactobacillus plantarum* reported here was found as the best P solubilizer which was rarely reported in the previous literature.

CONCLUSION

It is concluded from the current study that different plant rhizospheres boosted a diverse group of naturally occuring phosphate-solubilization bacteria. Fourteen bacterial species found were associated with bamboo, gadung and maize rhizosphere. All bacteria were capable to dissolve insoluble-P under in vitro conditions, despite the amount varied between the isolates. The higher P-solubilizer was noted by BPFG3 isolate (*Lactobacillus plantarum*) and followed by BPFJ2 isolate (*Bacillus firmus*), and these strains were the most potential to be proposed as a bioinoculant in order to improve the chemical P fertilizers efficiency.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Darwis Suleman designed and performed the experiments and also prepared the manuscript. Asrul Sani analyzed data and prepared the manuscript. Sri Ambardini reviewed literature on phenetic characterisation of microbial and conducted the measurements. Dirvamena Boer reviewed literature concerning moleculer identification and performed the sequencing processes. Nur Arfa Yanti reviewed literature concerning biochemical activities of microbe in soil and prepared the manuscript. All authors read and approved the final version.

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