ISOLATION AND CHARACTERIZATION OF PHOSPHATE SOLUBILIZING BACTERIA FROM PINEAPPLE RIZOSPHERE IN LAMPUNG

Kusuma Handayani1, Dwi Andreas Santosa2, NisaRachmania Mubarik3, Atang Sutandi2, Untung Sudadi2

1Study program Soil Science, Graduate School, University of IPB,

Kampus IPB Dramaga, Bogor 16680, Indonesia

2Department of Soil Science & Land Resources, University of IPB, Kampus IPB Dramaga, Bogor 16680, Indonesia

3Department of Biology, Faculty of Mathematics and Natural Sciences, University of IPB, Kampus IPB Dramaga, Bogor 16680, Indonesia

Email: kusumahandayani@yahoo.co.id

**Abstract**

Pineapple is one of the most cultivated plants that use fertilizers in its management system. Excessive use of chemical fertilizers can change soil structure and reduce soil fertility. Therefore, a method is used to address the problem, i.e. using phosphate solubilizing bacteria (PSB) found in soil around the roots (rhizosphere) of pineapple plants. PSB can dissolve bound phosphates, into exchangeable or water-soluble phosphate available for plants. The purposes of this study were to determine the number of PSB in pineapple rhizosphere, to isolate PSBwith high solubility index (SI), and to determine the characteristics of PSB isolated from pineapple rhizosphere. This study used descriptive method by taking rhizosphere soil samples from 4 different block locations at pineapple plantation in Lampung. The results showed that the PSB population was around 103 CFU/g. Out of total 12 PSB isolates tested for their P solubilizing ability, isolate RV1 had the lowest SI(0.7) while isolate RG2 had the highest (1.2). Morphological characteristics of PSB isolateswere mostly white, yellowish, gram-positive, and rod-shaped. The optimum pH for average growth was at pH 5.0, while the optimum temperature was at 30°C. Based on 16S rRNA identification, isolate RG2 was similar to *Bacillus altitudinis.*

Keywords: gram positive, solubility index (SI),soluble phosphate

**INTRODUCTION**

Pineapple cultivation in a location reduces soil fertility over the years due to continuous fertilizer and pesticide application for each pineapple planting cycle. However, such condition should be addressed by improving hard, lumpy soil as the negative impact of long term fertilizer and pesticide application. One of the efforts to address declining soil quality is utilizing interaction between plant and microbes in the rhizosphere (Dell’mour *et al*. 2010). One of the most frequent efforts is adding potential bacteria as biofertilizer, e.g. PSB. *Pseudomonas* sp., *Bacillus* sp., *B. megaterium*, and *Chromobacterium* sp. are PSB with high ability as biofertilizer, making P available for plants (Widawati *et al.* 2005). Betty *et al.*(2008)utilized PSB as biofertilizer, resulting in increasing phosphatase enzyme activity in soil because the biofertilizer is capable of increasing plant growth. PSB are soil microbe groups often utilized to improve soil that was previously in bad condition due to fertilizer application (Setiadi, 2001). More soil biological, physical, and chemical reactions are in rhizosphere because activities in rhizosphere are supported by nutrients in their soluble exudate forms secreted by plants, such as carbohydrate, amino acid, low molecular weight organic acid,and other photosynthates(Dilfuza *et al*. 2011). Rhizosphere’s ecology is driving factors, combining soil matrix’s physical factors, rhizodeposit (root exudate), proton (H+), gas, and root’s role in absorbing water and nutrients, that are spatially and temporally well-distributed, allowing root areas become sites where soil microbes, plant roots, and soil components interact (Richter *et al.* 2010). The purpose of the study was to determine the number of PSB in pineapple rhizosphere, to isolate PSB with high SI, and to understand the characteristics of PSB isolated from pineapple rhizosphere.

**METHOD**

The present study was carried out in January to July 2018 using descriptive method by obtaining soil samples from soil rhizosphere in 4 blocks of PT Great Giant Pineapple (GGP), Lampung Tengah, Lampung Province, and analyzing the samples at Laboratory of Soil Biology, Biotechnology, Faculty of Agriculture, Bogor Agricultural University.

**Method**

**Soil Sampling**

Random samplingswere carried out in 4 locations with 5 replications each, totaling 20 samples. Soil samples of 100 gr each were obtained from around pineapple root system areas at 0-15 cm deep (Figure 1) and put in labelled plastic bags for biological analysis. The 4 sampling locations were Block RV (4°47' S, 105°18' E), RG (4°48' S, 105°15' E), TV (4°49' S, 105°11' E), and TG (4°47' S, 105°12' E).



 25 cm

 12.5 cm

 15 cm





Figure 1 Soil sampling from pineapple rhizosphere

**PSB Isolation and P Solubilizing Test**

A total of 5 gr soil was put in 45 ml sterile physiological solution and shaken for 30 minutes in shaker. 1 ml extract was then put in test tube with 9 ml sterile physiological solution and shaken to homogeneous. 1 ml extract was then put in the next test tube and so on until 10-1-10-6 serial dilutions were obtained. 1 ml extract from each 10-3-10-6 serial dilution was put in sterile petri dish and Pikovskaya selective media was poured in prior to incubation for 3-5 days at ±25℃. Colonies with clear zone were observed and counted. Desired colonies were then purified using quadrant streak method on Pikovskaya medium to obtain single colony. Isolates obtained were stored as stocks for further test (Suliasih 2012, Ulfiyati *et al.* 2015). Pure PSB isolates’ P solubilizing ability was tested to obtain phosphate SI using equation below.

$$SI =\frac{Clear Zone diameter – Colony diameter}{Colony diameter}$$

**PSB Characterization**

 PSB isolates with rapid growth and high SI were then characterized against their colony and cell morphology. Gram staining was used to stain bacteria cells and group them into Gram-positive and Gram-negative (Pelezar 2008).

**Biochemical Test**

 1 loop anti-microbe producing sample bacteria was inoculated on biochemical test media (glucose, fructose, sucrose, lactose, and galactose) prior to incubation for 24 hours at room temperature 25oC. Observation was carried out on media’s color change and Durham tube’s gas bubble.

**Influence of pH on Bacterial Growth (Qualitative)**

 Sample PSB candidates were taken from ultisols with pH 4.5-5.5 (Munir 1996). It is necessary to understand the optimum pH for the growth. Samples obtained were streaked onto Nutrient Agar (NA) media at pH 5 and 7 prior to incubation for 24 hours at room temperature 25oC. After incubation, size of colonies growing on the media was observed.

**Influence of Temperature on Bacterial Growth (Qualitative)**

 Bacterial samples obtained were streaked onto NA media prior to incubation at 30 and 40oC. After incubation, size of colonies growing on the media was observed.

**RESULT AND DISCUSSION**

**Result**

**PSB from Pineapple Rhizosphere**

According to isolation result, PSB populations from the 4 locations were around 103CFU/g where the highest was from location RG and the lowest was from location TV (Figure 2).

 Figure 2. Total PSB from different locations

Isolate RV was found with the lowest SI (0.7) while isolate RG2 was the highest (1.2) (Table 2). Isolate RG2 is Gram-positive bacteria and its colony growth on Pikovskaya medium was surrounded by clear zone (Figure 3), indicating the isolate’s ability to solubilize phosphate in Pikovskaya medium. Isolate RG2 was unable to ferment lactose but can grow well at pH 5 and 7 (Table 3).

 **SI**

Figure 3. PSB colony growth, surrounded by phosphate clear zone on Pikovskaya media

Table 2. Phosphate SI

|  |  |  |  |
| --- | --- | --- | --- |
| **Isolate** | **Phosphate SI** | **Isolate** | **Phosphate SI** |
| TV1 | 1.1 | RV1 | 0.7 |
| TV2 | 0.75 | RV2 | 0.85 |
| TV3 | 0.95 | RV3 | 1.0 |
| TV4 | 1.1 | RV4 | 0.75 |
| TV5 | 0.65 | RV5 | 1.05 |
| Average | 0.9 | Average | 0.76 |
| TG1 | 0.75 | RG1 | 0.85 |
| TG2 | 0.75 | RG2 | 1.2 |
| TG3 | 0.8 | RG3 | 1.0 |
| TG4 | 0.75 | RG4 | 0.80 |
| TG5 | 0.75 | RG5 | 0.80 |
| Average | 0.74 | Average | 0.93 |

Table 3. Bacterial characterization based on biochemical properties, pH test, temperature, and Gram staining

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Isolate Name** | **Biochemical Test** | **pH** | **Temperature (℃)** | **Gram Staining** |
| **Glu** | **Gal** | **Fru** | **Suc** | **Lac** | **5** | **7** | **30** | **40** |  |
| TV1 | +++ | + | +++ | ++ | - | +++ | + | +++ | + |  (-) |
| TV2 | +++ | ++ | ++ | ++ | + | +++ | ++ | +++ | + | (-) |
| TV3 | +++ | ++ | +++ | ++ | ++ | +++ | + | +++ | + | (+) |
| TV4 | +++ | ++ | +++ | ++ | - | +++ | ++ | +++ | + | (-) |
| TV5 | +++ | ++ | +++ | ++ | - | +++ | ++ | +++ | + | (-) |
| TG1 | +++ | ++ | ++ | ++ | - | +++ | ++ | +++ | + | (+) |
| TG2 | +++ | ++ | +++ | ++ | - | +++ | + | +++ | + | (-) |
| TG3 | +++ | + | +++ | ++ | - | +++ | + | +++ | ++ |  (+) |
| TG4 | +++ | ++ | +++ | ++ | - | +++ | ++ | +++ | + | (-) |
| TG5 | +++ | + | +++ | ++ | - | +++ | ++ | +++ | ++ | (-) |
| RV1 | +++ | ++ | +++ | ++ | + | +++ | ++ | +++ | ++ | (-) |
| RV2 | +++ | ++ | ++ | ++ | - | +++ | ++ | +++ | + | (+) |
| RV3 | +++ | ++ | ++ | ++ | + | +++ | ++ | +++ | + | (-) |
| RV4 | +++ | ++ | +++ | ++ | - | +++ | ++ | +++ | ++ | (+) |
| RV5 | +++ | ++ | +++ | ++ | - | +++ | ++ | +++ | + | (+) |
| RG1 | +++ | ++ | +++ | ++ | - | +++ | ++ | +++ | ++ |  (-) |
| RG2 | +++ | ++ | +++ | ++ | - | +++ | ++ | +++ | ++ |  (+) |
| RG3 | +++ | + | +++ | ++ | - | +++ | + | +++ | + |  (-) |
| RG4 | +++ | ++ | ++ | ++ | - | +++ | ++ | +++ | + | (+) |
| RG5 | +++ | ++ | ++ | ++ | - | +++ | ++ | +++ | + | (-) |

Note:

+ = Slightly grow, ++ = Moderately grow, partial fermentation

+++ = Abundantly grow, full fermentation

Glu = glucose, Fru = fructose, Gal = galactose, Suc = sucrose, Lac = lactose

**Discussion**

Total PSB populations from the isolation were different due to different soil type and soil ability to uptake fertilizer in each treatment, relating to soil physical and chemical properties around the rhizosphere. Taha *et al.* (1969) stated that soil chemical and physical factors along with vegetation, plant rotation, and environmental condition highly influence soil microbe population, including PSB. According to Jha *et al.* (1992), PSB population compositions and activities will be diverse due to different soil physical-chemical properties, climate, and vegetation.

According to Farhad *et al*. (2015), the number of microbe population around soil depends on the microbe’s metabolic sensitivity against the surrounding conditions, e.g. humidity, light intensity, and soil temperature. It means PSB population in soil is also influenced by temperature, considering temperature’s role in cell physiological reaction, and environment’s physical-chemical characteristics including soil volume, pressure, oxidation-reduction potential, diffusion, brown movement, viscosity, surface tension, and water structure(Shanware*, et al.* 2014). Other study indicated that there is a seasonal change in rhizosphere microbe population in line with root growth (Semenov, 1999), making newly growing root, mature root, old root, and decaying root have different community compositions. Bacterial community obtains nutrients released by young roots in root hair area.

Bacteria’s different SI is due to bacteria’s different ability. P solubilizing process is presumably due to organic acids production. According to Khan *et al.* (2009), the main mechanism releasing P is due to organic acids productionby PSB. Organic acid production decreases pH, and P is released through proton substitution. Organic acids produced then react with Ca2+, Fe3+, and Al 3+ions that bind phosphate into stable form and phosphates are released in forms available for plants (Yani, 2011). Study by Setiawati and Mihardja (2008) reported that there are several microbe mechanisms in solubilizing phosphate, e.g. orthophosphate anion competition, pH change due to organic acids productions, metal binding to form organic metal, and chelation by organic ligand.

Morphological characteristics of the 12 PSB isolates (Table 2) revealed that most of the PSB colonies wereround-shaped, white, yellow, Gram-positive (5 isolates), and Gram-negative (7 isolates). Physiological characteristics of the isolated PSB revealed that most PSB were capable of fermenting carbohydrates in glucose, fructose, galactose, and sucrose forms, where only isolate RV1 and TV3 were capable of reducing lactose. Optimum pH for bacterial growth was at pH 5 and 7, although isolate RG5 and TV1 only grew slightly at pH 7. According to Widawati and Suliasih (2006), maximum pH for phosphate availability in soil is 6.5, while PSB is still capable of solubilizing phosphate at pH below 5.5. PSB isolates were obtained from pineapple rhizosphere at 0-15 cm deep where the temperature at the soil layer fluctuated from 25 to 33 ℃at noon, especially during dry season. This is due to the soil temperature at the layer is highly influenced by the temperature of the environment above (Balittanah, 2005).

Based on 16S rRNA identification, isolate RG2 with the highest SI was similar to *Bacillus altitudinis* ( Figure 4.).Tripati A and Banu F (2017) also managed to isolate *Bacillus altitudinis* as PSB from coriander rhizosphere.



 Figure 4. Filogenetic tree

**CONCLUSION**

A total of 20 potential PSB isolates were successfully obtained from pineapple rhizosphere. P dissolving test revealed that isolate RV1 had the lowest SI (0.7) and isolate RG2 had the highest (1.2). Morphological characteristics of PSB isolates were mostly white, yellowish, gram-positive, and rod-shaped. The optimum pH for average growth was at pH 5.0, while the optimum temperature was at 30°C. Based on 16S rRNA identification, isolate RG2 with the highest SI was similar to *Bacillus altitudinis.*

**REFERENCES**

Aditi Tripati and Farhat Banu. 2107. Microbial Identification by Molecular Characterization and Screening of Phosphate Solubilizing Bacteria of *Coriandrum sativum* Rhizosphere.*Int. J. Pure App. Biosci.* 5 (5):1286-1297.

BalaiPenelitian Tanah. 2005. *Petunjuk Teknis: Analisis kimia tanah, tanaman, air dan pupuk.* Bogor (ID) Badan Penelitian dan Pengembangan Pertanian Departemen Pertanian.

Betty N, Fitriatin, Reginawanti H, and Pujawati Suryatmana. 2008. Aktivitas Enzim Fosfatase dan Ketersediaan Fosfat Tanah Pada Sistem Tumpang Sari Tanaman Pangan dan Jati (*Tectonagrandis*, L) setelah Aplikasi Pupuk Hayati.*J.Agrik*. 19 (3):161-166.

Dell’mour M, Leonhard J, Eva O, Markus P, Gunda K, Stephan Hann. 2010. Hydrophilic interaction LC combined with electrospray MS for highly sensitive analysis of underivatized amino acids in rhizosphere research. *J. Sep Scie.* 33:911-922.

Dilfuza E, Giancarlo R, Stephan W, Rafiq I. 2011.Enzyme activities in the rhizosphere of plants.*Soil Enz J.*22(1):149-166.

Farhad, I.S.M., M.N. Islam, S. Hoque, M.S.I. Bhuiyan. 2010. Role of potassium and sulphur on the growth, yield, and oil content of soybean (*Glycine max* L.). Ac=. *J.Plant Sci*. 3 (2):99-103.

Jha, D.K., G.D. Sharma, and R.R. Mishara. 1992. Ecology of soil microflora and mycorrhizal symbionts. *Biol.Fertil. Soils* 12:272-278.

Khan, M.S., A. Zaidi, P.A.Wani . 2009. Role of phosphate solubilizing microorganisms in sustainable agriculture [review]. in: E. Lichtfouse*et al.* (eds.). *Sustain.Agric.* New York (US): Springer Science Business Media. 551-570.

Munir, M. 1996. Tanah Ultisol – Tanah Ultisol Di Indonesia. Pustaka Jaya. Jakarta.

Noor, A, 2003, Pengaruh Fosfat Alam dan Kombinasi Bakteri Pelarut Fosfat dengan Pupuk Kandang terhadap P Tersedia dan Pertumbuhan Kedelai pada Ultisol, *Buletin Agronomi*, 31 (3): 100-106.

Pelezar,chan. 2008. Dasar-Dasar Mikrobiologi. UI Press: Jakarta.

# Richter D, Neung-HO, Ryan F, Jason J. 2007. The Rhizosphere and soil formation. Duke University & Yale University.

Semenov, A.M., A.H.C. van Bruggen and V.V. Zelenev. 1999. Moving Waves Bacterial Population andTotal Organic CarbonAlong Roots of Wheat. *Microbiol. Ecol*. 37: 116-128.

Setiadi, Y, 2001. Peranan Mikoriza Arbuskula dalam Rehabilitasi Lahan Kritis di Indonesia. Prosiding Seminar Nasional Mikoriza. Asosiasi Mikoriza Indonesia Cabang Jawa Barat, Hal 1-12.

Setiawati, T.C., A.Mihardja. 2008. Identifikasi dan Kuantifikasi Metabolit Bakteri Pelarut Fosfat dan Pengaruhnya terhadap Aktivitas *Rhizoctoniasolani* pada Tanaman Kedelai. *J. Tanah Trop*. 13:233-240.

Shanware, A.S., S.A. Kalkar, M.M. Trivedi. 2014. Potassium solublisers: occurrence, mechanism and their role as competent biofertilizers. *Intern J Curren Microbiol and App Sci.* 3 (9): 622-629.

Suliasih, 2012. Pelarutan batuan fosfat oleh bakteri pelarut fosfat dan kemampuannya dalam meningkatkan pertumbuhan tanaman sengon buto (*Enterolobiumcyclocarpum*).*J. Tek. Ling*. 4(1) : 21-29.

Taha, S.M. S.A.Z. Machmoud, A. Halim El Damaty and A.M. Abd. El Hafez.1969. Activity of phosphate dissolving bacteria in Egyption soils. *Plantand Soil* 31 (1): 151-160.

Ulfiyati N, Zulaika E. 2015. Isolat *Bacillus* pelarut fosfat dari Kalimas Surabaya.*J.Sains ITS*. 4 (1) :1-3.

Widawati dan Suliasih, 2006. Populasi Bakteri Pelarut Fosfat (BPF) di Cikaniki, Gunung Botol, dan Ciptarasa, serta Kemampuannya Melarutkan P Terikat di Media Pikovskaya Padat. *Biodiver*.7 (6): 109-113.

Whitelaw, MA. 2000. Growth Promotion of Plants Inoculated with Phospate solubilizing Fungi.*adv.Agron*.69:99-151.

Yani R. 2011. Karakterisasi Kemampuan Melarutkan Fosfat Bakteri Pelarut Fosfat Asal Tithonia Diversifolia pada Media Agar Ekstrak Tanah. Fakultas Pertanian, Universitas Andalas. Padang.