ISOLATION AND CHARACTERIZATION OF PEAT SOILS PHOSPHATE SOLVENT BACTERIA IN GIAM SIAK KECIL BIOSPHERE CONSERVATION-BUKIT BATU, BENGKALIS, RIAU

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SUMMARY

Phosphate (P) is the essential nutrient which has an important role in the process of photosynthesis and root development. The availability of this element is limited in the tropical land. One of the alternative processes to improve the efficiency of phosphate availability in the soil is using phosphate solvent microorganisms. Phosphate solvent bacteria is one of the microorganisms that has a role in providing the P element for plants. This study aims to obtain the phosphate solvent bacteria isolate and determine the potential of the bacteria in dissolving phosphate. The result of the isolation and bacteria selection, purified by streak plate method on Pikovskaya media, obtained 16 phosphate solvent bacteria isolates. The result of clear zone observation on Pikovskaya media, there are 7 isolates that have PSI index ≥ 2.5. Based on the result of morphology, physiology and biochemistry observation, the selected phosphate solvent bacteria, categorized seems like to the genus of Bacillus and Pseudomonas.

Keywords: Phosphate solvent bacteria, Peat soil, Giam Siak Kecil Biosphere conservatory

INTRODUCTION

As a nutrients source, nutrient is an essential requirement to produce agricultural products. For agricultural activities, the use of nutrient is highly recommended from organic fertilizers. Organic fertilizer largely consists of organic materials derived from plants and/or animals that used to provide useful organic materials to improve the physical, chemical and biological elements of soil. Phosphate (P) is a macro nutrients required by plants in large quantities. Phosphate is the second essential nutrient after N which has an important role in the process of photosynthesis and root development. The availability of this element is limited in the tropical land, only about 0.1% of the total P available for plants because phosphate is chemically bonded and has a low solubility. One of the alternative processes to improve the efficiency of phosphate to overcome its low availability in the soil is using phosphate solvent microorganisms so that it can be absorbed by plants. Phosphate solvent microorganisms utilization is expected to overcome the P problem in acid soils (Tilak *et al.*, 2005).

Phosphate Solvent Bacteria (PSB/BPF) is a group of soil microorganisms which capable to dissolve the fixed P in the soil and turn it into an available form so that can be absorbed by plants. Phosphate Solvent Bacteria (PSB/BPF) as Bacillus sp. and Pseudomonas sp. is soil microorganisms that have the greatest ability to dissolve unavailable P to be available (Subba-Rao, 1982; Widawati, 1999; Whitelaw, 2000). It can happen because the bacteria are able to secrete organic acids that can form a stable complex with P cations binding in soil and organic acids can lower the pH and break the bonds of some phosphate compounds form so it will increase the phosphate availability in land solvent (Subba-Rao, 1982).

The BFP work mechanism can dissolve the P soil and P original fertilizer applied allegedly based on bacterial secretion systems such as organic acids. The increasing of organic acid usually followed by the chelate formation of Ca with the organic acids so that P is soluble and P soil availability increased. According to Illmer *et al.* (1995) stated that the phosphate dissolution mechanism of hard soluble materials are linked to the ability of microbial activity to produce the phosphatase, phytase enzyme, and organic acid from metabolism such as acetic acid, propionic acid, glycolic, fumarate, oxalate, succinate, tartrate, citrate, lactate, and ketoglutarate.

Several researched of the use of phosphate solvent bacteria as an effort to increase the availability of phosphate (P) for the plant have widely done. Some bacteria such as *Pseudomonas* sp. and *Bacillus* sp. included in a group of organic acids secrete bacteria such as formic acid, acetic, propionic, lactic, glycolic, glyoxylic, fumaric, tartat, ketobutirat, succinate and citrate which is able to dissolve a hard soluble phosphate form to be available to plants . The existence of these bacteria can reach 30% of the total population which can be cultured from the rhizosphere soil (Whitelaw, 2000).

This study aims to obtain the phosphate solvent bacteria isolate on peat soil and identify also determine its potential were taken from Giam Siak Kecil Biosphere Conservatory-Bukit Batu.

MATERIALS AND METHODS

The materials used for isolated is soil samples which originated from Giam Siak Kecil Biosphere Conservation-Bukit Batu. The materials used for media making and physiological test are Nutrient Agar, Nutrient broth powder, NaCl 0.85%, $Ca_3 (PO_4)_2$, $(NH_4)_2SO_4$, NaCl, KCl, $MgSO_4.7H_2O$, $MnSO_4.7H_2O$, $FeSO_4.7H_2O$, yeast extract, glucose, sucrose, maltose, mannitol, jelly stem, alcohol 70%, alcohol 96%, crystal violet, iodine, safranin, KH_2PO_4, phenol red, peptone, gelatin, reagent Kovacks, H_2O_2 3%.

Soil samples used is a soil sample collection from Soil Laboratory of the Faculty of Agriculture, University of Riau. The soil samples originated from Giam Siak Kecil Biosphere Conservatory-Bukit Batu.

The obtained soil samples were cleaned by hand to separate from the dirt. The cleaned soil samples weighed as much as 10 g and added to Erlenmeyer containing 90 ml physiological saline solvent (NaCl 0,85%), each soil sample is taken of 1 ml from a 10^{-1} dilution and put in 9 ml of saline physiological as 10^{-2} dilution. The dilution is done up to 10^{-7} series.

A total of 1 ml dilution suspension with 10^{-4} - 10^{-7} serial, inserted into a sterile Petri dish, then put the pikovskaya media in a Petri dish, homogenized and incubated at room temperature for 4 days. Isolate bacterial purification by taking a bacterial colony and grown on sloping media Nutrient Agar (NA) by scratch method then incubated for 3 days. Then proceed with morphology and physiology test on each isolate. Morphology test is done by taking 1 ose of pure breed bacterial isolates then scratched to a glass object, isolates were fixed over a Bunsen flame and then gram staining done. Physiology test is done to determine the physiological character of the isolate. Physiology test included: Glucose, Mannitol, Sucrose, Maltose, Indole, Urease, MR, VP, Catalyses. Isolate bacteria that has been known its physiological character then matched with Bergey's Manual of Determinative Bacteriology book.

The ability test of phosphate solvent bacteria done by growing the isolate on the pikovskaya media with dotted method, then observed the isolate growth and media color changed around the isolate. Clear zone formed was measured using a caliper, and then divided by the diameter of bacterial colonies growing. Furthermore, calculate the PSI value (Phosphate Solubilization Index) based on Premono method (1994) to get the criteria of clear zone bacteria.

RESULTS AND DISCUSSION

The isolation and selection of bacteria that has been purified by a scratch method on pikovskaya media obtained 11 phosphate solvent isolates. It can be identified up to the genus level and did a potential test by looking at the clear zone ratio of the obtained isolates. The bacteria phosphate dissolving ability characterized by the formation of a clear zone around the bacterial colonies, the formed clear zone will fade the media feculent white color to be clear around bacterial colonies (Figure 1).

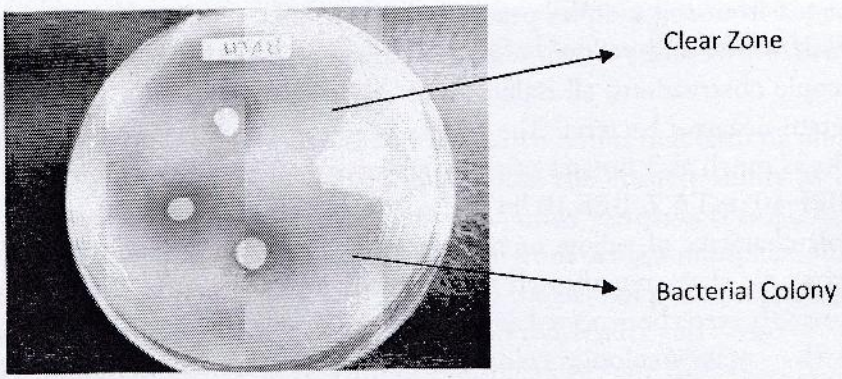

Figure 1. Clear zone formed by the solvent phosphate isolates

Figure 1 presents the representation of a clear zone formed in bacteria. Clear zone formed ratio is calculated based on the comparison between the clear zone formed diameter with bacterial colonies diameter (Premono, 1994). Formed clear zone indicated that the bacteria can utilize the phosphate in the media to the needs of the nutrients needed by bacteria to growth. The highest clear zone ratio produced by isolates BPF 10^{-4} HTA 3 at 4.3 while the lowest clear zone ratio produced by isolates BPF 10^{-7} HTA 4 at 1.5 (Table 1).

Table 1. The Observation of Phosphate Solvent Bacteria's Clear Zone

Isolate Code	Colony Diameter	Clear Zone Diameter	Clear Zone Ratio
BPF 10^{-4}HTA 3	4.6 mm	19.8 mm	4.3
BPF 10^{-4}HTA 5	3.7 mm	11.8 mm	3.18
BPF 10^{-4}HTA 1	3.8 mm	11.2 mm	2.94
BPF 10^{-6}HTA 3	3.0 mm	8.6 mm	2.8
BPF 10^{-5}HTA 1	4.4 mm	12.1 mm	2.75
BPF 10^{-6}HTA 1	3.2 mm	8.2 mm	2.56
BPF 10^{-6}HTA 2	3.3 mm	8.3 mm	2.51
BPF 10^{-5}HTA 3	1.6 mm	2.9 mm	1.81
BPF 10^{-7}HTA 1	3.4 mm	6.1 mm	1.79
BPF 10^{-7}HTA 5	2.0 mm	3.2 mm	1.6
BPF 10^{-7}HTA 4	2.4 mm	3.6 mm	1.5

The amount of clear zone is formed is influenced by the amount of phosphate described by the phosphatase enzyme produced by bacteria. Rachmiati (1995) qualitatively wide clear zone indicates the size of the bacteria's ability to dissolve P from insoluble phosphate. BPF activity in dissolving insoluble phosphates in the soil is influenced by several factors: temperature, temperature, humidity, pH of the soil, environmental conditions and food supply for growth.

Characterization of Morphology and Physiology Biochemistry Bacteria Phosphate Solvent on Peat

Based on the observation of macroscopic morphology of bacteria, all isolates solvent phosphate isolated from soil samples peat colony morphology were grouped into three colors (white, yellowish white, and yellow), elevation convex, the edges smooth and round shape. While microscopic observations, all isolates were rod-shaped and included in 2 groups: gram-positive and gram-negative bacteria. The results of gram stain for bacteria phosphate solvent obtained results as much as 3 isolates of gram-negative and gram-positive isolates contained 9. Isolates with BPF 10^{-7}HTA 2, BPF 10^{-4}HTA 3, BPF 10^{-6}HTA 4 and BPF 10^{-4}HTA 5 code has the macroscopic character of yellow or slightly yellowish colonies color and rounded-shaped colonies. Microscopic characters showed stem cells shape and showed Gram-negative bacteria. The Gram-negative bacteria be expected as genus Pseudomonas that have morphological features: slightly yellowish or yellow colonies colour, Gram-negative, and in the group of straight and stem-shaped cells, measuring of 0.5–1.0 x 1.5 to 5.0 μm. Many species can decipher poly-2-hydroxybutyrate while absorbing carbon in the material, facultative, positive catalyses test, positive methyl red, and the optimum growth temperature at 30–37°C. Bacteria is including to the genus Pseudomonas (Feliatra and Suryadi, 2004). While the Gram-positive bacteria be expected to genus of Bacillus that have gram-positive stem-shaped bacteria, it is usually an aerobic or facultative anaerobic bacteria (Wolf and Barker, 1968). Morphology test is still not enough to identify the bacteria. Therefore, these isolates physiological testing. Physiological test is needed to support the necessary data to identify the bacteria obtained (Table 2).

Table 2. The observation of biochemical test bacteria

Isolates code	Catalase Test	Sucrose Test	Glucose Test	Manitol Test	Maltosa Test	Urease Test	Indole Test	MR Test	VP Test
BPF 10^{-4}HTA 1	+	+	+	+	+	+	+	-	+
BPF 10^{-5}HTA 1	+	+	+	+	+	+	-	-	+
BPF 10^{-6}HTA 1	+	+	+	+	+	+	-	-	+
BPF 10^{-7}HTA 1	+	+	+	+	+	-	-	-	+
BPF 10^{-6}HTA 2	+	+	+	+	+	+	-	-	+
BPF 10^{-4}HTA 3	+	+	+	+	+	+	+	-	+
BPF 10^{-5}HTA 3	+	+	+	+	-	+	+	+	+
BPF 10^{-6}HTA 3	+	+	+	+	+	-	+	-	-
BPF 10^{-7}HTA 4	+	+	+	+	+	+	+	-	-
BPF 10^{-4}HTA 5	+	+	+	+	+	+	+	-	-
BPF 10^{-7}HTA 5	+	+	+	+	+	-	-	-	-

Description:
+: Positive reaction for biochemical tests
-: Negative reaction to biochemical tests

Catalase test results obtained throughout the bacteria capable of breaking down hydrogen peroxide (H_2O_2) into H_2O and O_2, as evidenced by the emergence of air bubbles on the bacteria after a few drops of 3% H_2O_2 solution. Hydrogen peroxide is toxic to cells because these materials menginaktivasikan enzymes inside cells (Seeley *et al.* 2001). Catalase test conducted by Sharma *et al.* (2007) in phosphate solvent bacterial isolates also showed positive results in *Pseudomonas fluorescens* and *Bacillus megatherium*. Other physiological test conducted on the isolates showed a lot of closeness to the genus Pseudomonas and Bacillus.

According to Widawati and Suliasih (2006) research results that bacteria phosphate solvent bacteria are genus of Pseudomonas and Bacillus. It has the greatest ability as biofertilizer by dissolving phosphate elements which is bound to the other elements (Fe, Al, Ca, and Mg), so that the P element becomes available for plants. Aside from being a phosphate solvent, bacterial genus *Pseudomonas* sp. and *Bacillus* sp. widely used in the field of plant breeding, for example as a biological agent in a plant growth promoter (PGPR) (Saylendra and Firnia, 2013). Therefore, this study as a basic step for the utilization of phosphate solvent bacteria as embellishment at once will be developed for the use of this bacterium as a biocontrol agent for plant breeding.

CONCLUSION AND SUGGESTION

The result of isolation researched and identification of phosphate solvent bacteria from the peat soils of Giam Siak Kecil Biosphere-Bukit Batu, phosphate solvent bacteria isolates obtained and identified as a genus Bacillus and Pseudomonas. There is isolate that potentially dissolving the phosphate which is characterized by the formation of clear zone. There are 7 isolates that have the PSI index ≥ 2.5. To determine the species of bacteria are needed molecular tests are needed to determine the species of bacteria obtained so that can know the species of bacteria.

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REFERENCES

Feliatra E, Suryadi E. (2004). Isolasi dan Identifikasi Bakteri Probiotik dari Kerapu Macan (*Ephinephelus fuscogatus*) dalam Upaya Efisiensi Pakan Ikan. *Jurnal Natur Indonesia.* 6(2): 75–80.

Illmer P, Barbato A, Schinner F. (1995). Solubilizing of Hardly Soluble $AlPO^4$ with P-Solubilizing Microorganisms. *Soil Biol. Biochem.* Vol 27: 265–270.

Premono EM. (1994). Jasad Renik Pelarut Fosfat, Pengaruhnya terhadap Tanah dan Efisiensi Pemupukan P Tanaman Tebu. Phd Thesis. Graduate School Bogor Agricultural University, Bogor. (Unpublished).

Rachmiati Y. (1995). Bakteri Pelarut Fosfat dari Rhizosfer Tanaman dan Kemampuannya dalam Melarutkan Fosfat. In: prosiding Kongres Nasional VI HITI. 12–15 Desember 1995. Jakarta.

Saylendra A, Firnia D. (2013). *Bacillus* sp. dan *Pseudomonas* sp. Asal Endofit Akar Jagung (*Zea mays* L.) yang Berpotensi sebagai Pemacu Pertumbuhan Tanaman. *Jurnal Pertanian dan Perikanan.* 2(1): 19–27.

Seeley HW, Paul JV, John JL. (2001). Microbes in action fourth edition. W.H.Freeman and Company, New York.

Sharma K, Dak G, Agrawal A, Bhatnagar M, Sharma R. (2007). Effect of phosphate solubilizing bacteria on the germination of *Cicerarietinum* seeds and seedling growth. *Journal of Herbal Medicine and Toxicology.* 1(1): 61–63.

Subba-Rao NS. (1982). Mikrobiolgi Tanah dan Pertumbuhan Tanaman. UI Press, Jakarta.

Tilak KVBR, Ranganayaki N, Pal KK, Saxena De R, Natiyal AK, Mittal CS, Tripathi SAK, John BN. (2005). Diversity of Plant Growth and Soil Health Supporting Bacteria. *Current Science.* 89: 136–150.

Whitelaw MA. (2000). Growth Promotion of Plants Inoculated with Phosphate Solubilizing Fungi. *Advances in Agronomy.* 68 :99–151.

Widawati S. (1999). Penggunaan Introduksi Mikrobia Tanah terhadap Pertumbuhan dan Produksi Kedelai (*Glycine max* L.) di Tanah Masam. Jurnal Mikrobiologi Tropika, Volume 2 (2).

Widawati S, Suliasih. (2006). Populasi Bakteri Pelarut Fosfat (BPF) di Cikaniki, Gunung Botol, dan Ciptarasa, serta Kemampuannya Melarutkan P Terikat di Media Pikovskaya Padat, Biodiversitas, 7 (2): 109–113.

Wolf J, A N Barker. (1968). The genus *Bacillus*: Aids to identifiction of its species. Pages 93–109 in: B.M. Gibbs and D.A. Shapton, eds., Identification Methods for Microbiologists, Part B. Academic Press, New York.