



The effect of phosphate solubilizing microbe producing growth regulators on soil phosphate, growth and yield of maize and fertilizer efficiency on Ultisol

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Abstract

Ultisol is soil that have problem of soil acidity, low organic matter and low available macro nutrient in particularly low P availability. This is only small part of phosphorus is available for plant because most of the phosphate was still present in the soil were fixed by soil colloids. Some soil microbes are known phosphate solubilizing microbes have capability to dissolve phosphate that transform of P fixed into the soluble P. The field experiment conducted at Jatinangor, West Java Indonesia to determine the effect of PSM on phosphate solubility, growth and yield of maize and phosphorus fertilizer efficiency. Design experiment of Randomized Block Design (RBD) was used in field experiment, consisted of two factors and three replications. Phosphate solubilizing microbe as the first factor consisted four levels i.e without, phosphate solubilizing bacteria (PSB), phosphate solubilizing fungi (PSF), mixture of PSB and PSF. While the second factor was P fertilizer consists five levels (0%, 25%, 50%, 75% and 100% doses of recommendation). The results of the field experiment showed that application of phosphate solubilizing microbes significantly improve the yield of maize in Ultisol Jatinangor, but did not significantly affect on soil P, available soil P, phosphatase and P uptake of plants. Application of a mixture of PSB and PSF better effect on soil available P and yield of maize. Phosphate fertilizer dosing at a dose of 25 % , 50 % , 75 % and 100 % recommendations increased available soil P. Fertilizer of P could inhibit the activity of phosphatases. Phosphate fertilizer with dose 50 % recommendation gave better effect on soil P and yield of maize.

Keywords: maize, phosphate solubilizing microbe, soil phosphate, phosphatase, Ultisol

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Introduction

Many difficulties found on marginal soils in Indonesia. One of the difficulty that usually appeared in Indonesia marginal soil is the availability of macro nutrients. Ultisol is one of soil ordo in Indonesia which has a distribution area reached 45,794,000 ha or 25 % of Indonesia total land (Subagyo et al, 2004) and most part of that planted with maize. Utilize this type of soil, there are several difficulties such as the quality of its physics, chemical, and biological that gives less in supporting plant growth. The value of acidity of this soil, and also its deficient in P-availability because of P fixation become a constraint for plant growth. Soil that has high value of pH usually consists of low availability-P because of its fixation by soil calcium (Tan, 2008).

Many efforts had given for increasing Ultisol productivity such as fertilizing it with synthetic fertilizer or natural fertilizer. However, there were still many obstacles that found by using this synthetic fertilizer. The

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effect of fertilizer residue is one of obstacles that can pollute the environment, so continuous fertilizing has bad effect on soil and environment.

To Improve the efficiency of fertilization and enhance plant growth, soil biotechnology need to be developed, one of the example is microbial utilization which play a role in the transformation of nutrient elements of P in the soil, known as phosphate solublizing microbia (PSM) that consists of bacteria and fungi phosphate solubilizer. The important role of this microbia is transforming P in mineralizing P organic compounds by releasing inorganic P, changing the solubility of inorganic P compounds, oxidation or reduction of inorganic P compounds and also in immobilizing P. The role of this microbia in mineralizing organic P is because they can produce phosphatase enzym (Cookson, 2002; Sundara et al. 2002; Sarapatka, 2003). While it's role in dissolving a fixed-P because of organic acids produced by this bacteria (Whitelaw, 2000).

The ability of bacteria solublizing phosphate in solublize phosphate depending on the organic acid that is produced as well as phosphatase enzyme activity (Sitepu et al., 2007). Research has been done about soil microbial isolation from food crops rhizosfir and tested its ability to dissolve inorganic soil P i.e. *Pseudomonas* sp., *Bacillus subtilis*, *Aspergillus niger* and *Penicillium* sp. (Fitriatin, 2006). This PSM has been characterized in Biochemistry and test its phosphatase activity in dissolving P medium with various synthetics organic P substrate (Fitriatin et al., 2008). Application of bacteria solublizing phosphate that produced phosphatase and Phytase in improving solubility of soil P was done on Andisol (Fitriatin et al., 2007a). Phosphatase enzymes activity of soil bacteria isolated from rhizosfir of perennial plant has tested the ability of mineralizing P-organic (Fitriatin et al., 2008).

Research by Ponnurugan and Gopi (2006) showed that phosphate solublizing bacteria are capable of producing phosphate and growth regulator substances in the form of IAA and giberelin in vitro. But the study of PSB that can produce phosphatase and growth regulator substances that applied to enhance the dissolving and mineralized P as well as plant growth has not been much done. Preliminary research has been conducted in testing PSB producing growth regulator substances in improving growth of upland rice in agar media (Fitriatin and Simarmata, 2005).

Utilization of PSM-producing growth regulator substance is expected to increase the solubility of P in Ultisol and can spur the growth of the maize so it can obtained maximum yield and reduce the need for inorganic fertilizer. Because of this, it is necessary to do a research about application of Phosphate solublizing bacteria in increases the soil quality and plant growth based on organic acids, enzymes, phosphatase and growth regulator substances produced by this bacterium.

Material and Methods

This research used Randomized factorial Design factorial that consists of two factors. The first factor is inoculants while the second factor is P fertilizer doses. Arrangement of treatments are as follows:

- Factor I : Inoculant PSM (Phosphate Solublizing Microbia)
- i_0 = without inoculant
 - i_1 = PSB (*Pseudomonas cepaceae* and *Pseudomonas mallei*)
 - i_2 = PSF (*Penicillium* sp. and *Aspergillus* sp.)
 - i_3 = PSB and PSF

- Factor II : P fertilizer doses (Super Phosphate/SP)
- p_0 = without
 - p_1 = 25 kg SP ha⁻¹ (25% recommendation dose)
 - p_2 = 50 kg SP ha⁻¹(50% recommendation dose)
 - p_3 = 75 kg SP ha⁻¹(75% recommendation dose)
 - p_4 = 100 kg SP ha⁻¹(100% recommendation dose)

Field experiments carried out up to the end of the generative phase. Observation of the observed parameters (at the end of the vegetative phase) include:

- Phosphatase Enzyme Activity. Analyzases of phosphatase enzymes was determined based on the method of Eivazi, Tabatabai and by giving the substrate p-nitrofenilfosfat p-nitrophenol compounds that are formed as a result of the activity of the enzyme then at all stained by a solution of sodium hydroxide that can measure with spectrophotometer 400 nm (Margesin, 1996).

- Soil Phosphate (method of determination of P with extracts of HCl 25%)
- Availability-P (method of determination of P with extract of Bray I and extract Olsen)
- Plant Growth (plant height, stem diameter, plant roots)
- Maize Yields (observed at the end of generative phase)

Research Implementation

Production of PSM inoculant

Phosphate solubilizing bacteria (*Pseudomonas mallei* and *Pseudomonas cepaciae*) produced in the Nutrient Broth (NB) medium. Production of PSF done with solid media because it aims to get the spores of PSF. Isolates of PSF (*Penicillium* sp. and *Aspergillus* sp.) cultivated on potato dextrose petridish with agar media. After incubated for 48 hours, then the sprouting spore were harvested for disbursed on physiological NaCl.

The results of production of PSM inoculant subsequently inserted into the carrier material. Carrier materials used are a mixture of compost and peat with a 1: 1 comparison. As many as 100 gr of carrier materials is packaged in aluminum foil packaging and sterilized by autoclave. After that, add inoculant of PSM as much as 10% a total of carrier material volume (10 ml). Inoculant of PSM were packed in containers labeled as shown in Figure 1.



Figure 1. Package of mix inoculant (left) and inoculant PSF (right)

Tillage : Land that used for this experiment was at ciparanje testing field, Faculty of Agriculture, University Padjadjaran, which ordo is Ultisols. The area of land that used for this experiment was about 5 m x 2 m per plot with 70 cm x 20 cm spacing.

Treatment and Planting : Inoculant of PSM treatment was done by mixing the manure (2 tons/ha dose) given at the time of 2 days prior to planting (for greenhouse experiment, the manure is in steril state). While the doses of fertilizer P is given at the time of planting. The maize seed that is used is a cultivar of Pertiwi II.

Results and Discussion

Characteristic of Ultisol from Jatiningor, Indonesia

Results of Ultisol showed that available-P is low, only 100 mg g⁻¹, but the potential-P reached 33,37 mg 100 g⁻¹ with moderate criteria. Low available P content reflect that many of phosphate are being disfixate by soil colloids. So that PSM is needed in providing useful P in order to support the growth and development of plants. Ultisol has a pH of 5.8 (slightly acid). Condition of soil pH is an important factor that determines the solubility of element. solubility of hidrous oxides of Fe and Al are directly dependent on the concentration of the hydroxyl (OH⁻) and pH, in addition, pH also controls the solubility of carbonates and silicates, affect redox reactions, micoorganisms activity, and determine the chemical forms of phosphates and carbonates in the soil solution. The content of C-Organic in Ultisol of Jatiningor is very low (0.30%). This indicates that these lands need the addition of organic matter, Clay dominate the texture of Ultisol Jatiningor up to 53%, while the content of dust is 7 % and sand is 40%. It can be inferred that the texture of Ultisol Jatiningor is clay dust because the clay fraction is more dominate.

Soil Phosphate

The results showed that treatment with PSB inoculant gave the better result in soil P up to 7,66 mg 100 g⁻¹ compared to controls 6,87 mg 100 g⁻¹ (Table 1). Mix inoculant of PSB and PSF showed an average result of soil P up to 6.98 mg 100 g⁻¹. This shows the effect of the PSB towards the content of soil P tend to be higher than those by Mix inoculant of PSB and PSF.

Table 1. The effect of PSM inoculant and P fertilizer on soil P

Treatment	Soil P (mg 100 g ⁻¹)
Inoculant of PSM	
Control	6,87
PSB (<i>Pseudomonas maleii</i> + <i>P. cepacea</i>)	7,66
PSF (<i>Penicillium sp</i> + <i>Aspergillus sp</i>)	7,39
Mix inoculant (PSB+PSF)	6,98
Fertilizer P	
0 % recommendation	6,97 ab
25 % recommendation	6,48 a
50 % recommendation	7,95 b
75 % recommendation	7,30 ab
100 % recommendation	7,41 ab

Description: average value followed by the same letter does not differ markedly according to Multiple Range Test Duncan at 5% level.

Doses of P fertilizer 50% recommendation gave better effect on soil P up to 12.37% compared to controls. Increasing doses of fertilizer P more than 50% recommendation were not increasing content of soil P (Table 1). Fertilizer dose 75% and 100% of recommendations were only able to increase content of soil P up to 4,52 % and 5,94% respectively as compared with the control, this is thought to be due to the giving of excessive P fertilizer causes the saturation on the ground so that the phosphate from fertilizer wasted and could not be saved into content of soil P.

Soil Available-P

Application of PSM on Ultisol did not gave significant effects to increased available-P in soil (Table 2). It is expected to be due to the application of the PSM only once at the beginning of the planting which cause activity of PSM was not optimum in the end of vegetative phase. The content of Ultisol available-P in the end of vegetative phase due to treatment in this research ranged from 0.61 mg kg⁻¹ to 1.79 mg kg⁻¹. While the content of available-P in sterile soil in a greenhouse experiment have ranged from 1.42 mg kg⁻¹ to 3, 10 mg kg⁻¹.

Table 2. The effect of PSM inoculant and P fertilizer on soil available-P

Treatment	Available-P (mg kg ⁻¹)
Inoculant of PSM	
Control	1,12
PSB (<i>Pseudomonas maleii</i> + <i>P. cepacea</i>)	1,27
PSF (<i>Penicillium sp</i> + <i>Aspergillus sp</i>)	1,47
Mix inoculant (PSB+PSF)	1,52
Fertilizer P	
0 % recommendation	0,64 a
25 % recommendation	1,34 b
50 % recommendation	1,41 b
75 % recommendation	1,55 b
100 % recommendation	1,79 b

Description: average value followed by the same letter does not differ markedly according to Multiple Range Test Duncan at 5% level.

The results showed that mix inoculant of PSB and PSF gave better influence than just PSB or PSF. Treatment of mix inoculant of PSB and PSF increased soil available-P up to 9.09% compared to controls, while treatment of the PSB and PSF independently only able to improve soil available-P up to 11,81% and 23.81% respectively. This is expected to be the synergism between PSB and PSF in transforming of insoluble P which is immobile into available-P which is relatively mobile. Phosphate solubilizing microorganisms solubilized P to be available-P with it's ability to secrete organic acids that can break complex P compounds in the soil (Whitelaw, 2000). Quantity and Quality of organic acids depends on the type of microorganisms itself. Therefore the synergism of PSB and PSF in dissolving P in the soil is closely related to the respective capabilities in producing organic acids.

Result showed that the decrease of early potential-P from 33,37 mg 100 g⁻¹ up to 6.98 mg 100 g⁻¹ proved mix inoculant of PSB and PSF activity in dissolving P in Ultisol. Giving of various inoculant did not give significant results against available-P, although the results showed an increasing number from control to mix inoculant of PSF and PSB (Table 2). According to Fitriatin et al. (2013), the higher the population of PSB would increase the available-P in soil. Therefore, giving mix inoculant of PSB and PSF was better than giving inoculant independently. Mix inoculant of PSB and PSF also supported the growth of maize roots significantly up to 18,18% compared with control.

Based on Table 2, giving fertilizer P with 100% dose recommendation can increase P-available for 64,25% compared to controls, while the P fertilizer with a dose of 25%, 50%, and 75% of recommendations can increase the available-P up to 52.24%, 54,61 %, and 58,71% respectively compared to controls. However, the results of the statistical analysis showed no noticeable difference between the P fertilizer treatment doses with 100 % recommendations compared to fertilizer P dose of 25%, 50%, and 75% of recommendations. This showed the role of PSM in improving the efficiency of P fertilizer so that a fertilizer under the undertaking recommendation is still able to provide P equals to available P with 100 % recommendation. The results of Kaleeswari et al.(2007) research showed that bacterial inoculation and phosphate fertilizer application with 50 kg ha⁻¹ dose and also organic matter increase soil available-P up to 23.4%.

Soil Phosphatase

Soil phosphatase activity play a role in the process of mineralized P in soil. Phosphatase can be produced by plants and soil microbia extracellularly. The effect of PSM inoculant and P fertilizer dose towards soil phosphatase can be seen in table 3. Based on soil analysis results in end vegetative phase toward phosphatase on maize showed the application with multi level doses of fertilizer P and the various PSM didnt give significant effect. It is expected to be due to the high rainfall during the research that can lead the fertilizer and incoulant leached. In addition, giving the treatment of inoculant once in this researched led to the unoptimum activity of PSM effecting the content of soil phosphatase

Table 3. The effect of PSM inoculant and P fertilizer on soil phosphatase

Treatment	Phosphatase ($\mu\text{g pNP g}^{-1} \text{jam}^{-1}$)
Inoculant of PSM	
Control	0.32
PSB (<i>Pseudomonas maleii</i> + <i>P. cepacea</i>)	0.33
PSF (<i>Penicillium sp</i> + <i>Aspergillus sp</i>)	0.28
Mix inoculant (PSB+PSF)	0.24
Fertilizer P	
0 % recommendation	0.31
25 % recommendation	0.26
50 % recommendation	0.28
75 % recommendation	0.35
100 % recommendation	0.26

Results of statistical analyze showed giving various doses of fertilizer P had no real effect against soil phosphatase content in greenhouse experiment. However it is known that giving 25% recommendation dose of fertilizer P provide a better influence on the activity of phosphatase in soil. whereas granting of fertilizer P with a dose of 100% recommendation gives a lower value of phosphatase. This shows that granting a high P fertilizer may inhibit the phosphatase activity. This is in line with the results of Fitriatin et al. (2007b) research that reported the granting of inorganic P cause phosphatase lowers on a liquid medium. It is supported by Sarapatka (2003) and the George et al. (2002) that explaining the activity of phosphatase will be hampered by the existence of high P.

Yields of Maize

Results of experiment in the field showed that application of PSM can increase yield of maize (weight of cornhusk), showed in Table 4. Application with mix inoculant of PSB and PSF turns out to have a better potential in increasing maize yields up to 20.02% compared to controls. While, application with PSB and PSF independently increase the weights of cornhusk up to 11,96% and 12,61% respectively.

Mix inoculant of PSB and PSF is able to provide optimal P for plants that are able to support the establishment of grains on maize. Elfiati (2005) describes the roles of phosphate which is important for cell growth, roots and root hairs development, strengthens the straw so that the plants are not easily fall, improve the quality of the plants, flowers development, fruit and seeds as well as to strengthen the resistance against disease.

Application of mix inoculant of PSB and PSF was able to increase the weight of cornhusk due between *Pseudomonas* sp. and *Penicillium* sp. will support each other in providing supply of nutrients especially P for their life, because of *Pseudomonas* SP. and *Penicillium* sp.. work synergistically producing phosphatase enzymes in the process of mineralizing and immobilizing to transform organic P into inorganic P, so that the growth of both can still be optimal from vegetative stage to harvesting of the plant. The existence of this synergism, assist in providing P for maize until harvest, especially in filling of maize grains, which ultimately increase the results of cornhusk weight (Fitriatin et al., 2013).

Table 4. The effect of PSM inoculant and P fertilizer on yields of maize

Treatment	Maize yields (g plot ⁻¹)
Inoculant of PSM	
Control	229,49 a
PSB (<i>Pseudomonas maleii</i> + <i>P. cepacea</i>)	260,68 b
PSF (<i>Penicillium</i> sp. + <i>Aspergillus</i> sp.)	262,62 b
Mix inoculant (PSB+PSF)	286,94 b
Fertilizer P	
0 % recommendation	259,75
25 % recommendation	246,58
50 % recommendation	272,43
75 % recommendation	260,52
100 % recommendation	260,37

Description: average value followed by the same letter does not differ markedly according to Multiple Range Test Duncan at 5% level.

Conclusion

Applications of PSM significantly improves yield of maize on Ultisol from Jatinangor, Indonesia but has no real effect towards potential-P, available-P, phosphatase and P uptake of plants. Application of PSM which is a mix inoculant of PSB and PSF gave better effects on available-P in soil and yield of maize. The P fertilizer with a dose of 25%, 50%, 75% and 100% recommendation increase soil available-P significantly. Increasing doses of P fertilizer can inhibit the activity of phosphatase. Phosphate fertilizer with 50% recommendations dose gave better effects on potential-P in soil and maize yields.

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