

Increasing Soil P and Yield of Upland Rice through Application Phosphate Solubilizing Microbes

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DOI: [10.36348/sjls.2021.v06i07.007](https://doi.org/10.36348/sjls.2021.v06i07.007)

| Received: 22.06.2021 | Accepted: 25.07.2021 | Published: 29.07.2021

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Abstract

Phosphate solubilizing microbes (PSM) are beneficial soil microbes that they have the ability to dissolve unavailable soil P into available and can mineralize organic P into inorganic P. The experiment was conducted to study the effect of phosphate solubilizing microbes on soil P, yield of upland rice plants and the efficiency of P fertilizer on marginal soil. 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 two levels i.e without and with PSM (a mixture of *Pseudomonas* sp. and *Penicillium* sp.). While the second factor was P fertilizer consists four levels ((0, 50, 75 and 100 kg P₂O₅ ha⁻¹). The result of experiment showed that PSM improved soil phosphatase activity and yield of upland rice on marginal soil. Phosphate solubilizing microbe increased soil organic P mineralization was characterized by a decrease in organic P. Inoculation of PSM with phosphate fertilizer dose of 75 kg P₂O₅ ha⁻¹ (75% recommended dose) gave best effect to soil phosphatase activity, soil P status and yield of upland rice in marginal soil.

Key words: Marginal soil, mineralize, *Pseudomonas*, *Penicillium* and organic P.

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INTRODUCTION

Upland rice cultivation is carried out on dry land, while dry land in Indonesia is dominated by marginal soil types such as Ultisol. This soil has problems in its utilization, namely the high level of soil acidity and low available P and high Al and Fe content. This low availability of P is due to the high P fixation by Al and Fe minerals making it difficult for plants to absorb [1].

Inorganic phosphate fertilization on Ultisol soil has a major problem, the low effectiveness of P fertilizer, which is 10% to 30%, so that 70% to 90% of P fertilizer remains in the soil and is difficult for plants to absorb [2]. Stated that low fertilization efficiency causes the amount of P fertilizer given by farmers to increase so that it has the potential to reduce land productivity, especially on acid soils so that its use needs to be reduced by utilizing biofertilizers.

The soil beneficial microbes have the ability to produce extracellular enzymes, namely a group of phosphatase enzymes that can mineralize organic P into inorganic P so as to provide high P for plants [3, 4].

This phosphatase enzyme belongs to the group of hydrolase enzymes, namely enzymes that can hydrolyze organic phosphorus compounds (phosphoric ester hydrolysis) into inorganic phosphorus compounds [5, 6].

Organic P content in the soil can reach between 20-80% of the total soil P [7]. The organic P content in the soil, which is more than 20%, is a potential source of P availability for plants [8]. However, P in this organic form cannot be used by plants, but needs to be first transformed into inorganic P forms through a mineralization process catalyzed by soil enzymes [9]. The process of organic P mineralization directly determines the availability of P to plants. Organic P mineralization begins with the destruction of plant litter by soil fauna, followed by the stage of changing the form of organic P to inorganic P [10].

The efficiency of P fertilizer can be increased by using phosphate solubilizing microbes. These microbes are not only able to produce phosphatase enzymes but can also secrete organic acids [11]. These organic acids are: citric acid, glutamate, succinic,

tartates, formic, acetic, propionic, lactonic, glyconic and fumaric acids [12]. These organic acids will react with FePO_4 , which can form a chelate (stable complex) with P-binding cations in the soil such as Fe^{3+} [13]. As a result, it can reduce the reactivity of the ions and cause effective dissolution so that the fixed P can be made available to plants. However, further research is needed to determine the phosphate solubilizing microbes for increasing the availability of soil P through the dissolution of fixed P and through mineralization of organic P to inorganic P, so as to increase upland rice production which further results in efficiency of P fertilization on marginal soils such as Ultisols.

MATERIALS AND METHOD

Phosphate solubilizing microbes used was *Pseudomonas* sp. and *Penicillium* sp. which has been tested has the ability to dissolve P and produce phosphatase enzymes. The experiment was carried out in the experimental field of the Faculty of Agriculture, Universitas Padjadjaran in Jatinangor, Kab. Sumedang, West Java Province with an altitude of about 700 m above sea levels.

The experimental design used a factorial randomized block design with factorial pattern which was repeated three times. The treatments in this study as the first factor were PSM inoculant (without and with PSM inoculant) and the second factor were dosage of P fertilizer (0,50,75 and 100 $\text{g P}_2\text{O}_5 \text{ ha}^{-1}$).

The upland rice seeds used were Situ Bagendit cultivars. Five seeds were planted in each planting hole with a spacing of 20 x 30 cm. PSM inoculants in solid form as much as 100 g were dissolved with 3 liters of water for each plot. Parameters taken in the end of vegetative period include the activity of the phosphatase enzyme which determined based on the Eivzy and Tabatai method [14], soil organic P content based on Olsen and Sommers method using 1 M sulfuric acid reagent [15], P available soil (Determination by extractor Bray I). Upland rice yields were determined at at harvest.

The land used in this research is soil with the order Ultisols in Jatinangor. Experimental plots were made with a size of 2 x 3 meters for each plot. The soil was given sheep manure as basic fertilizer and incubated for 2 weeks before planting. The basic fertilizer used Urea at a dose of 200 kg N ha^{-1} , KCl at a dose of 50 $\text{K}_2\text{O kg ha}^{-1}$, given at planting time (urea was given in three stages).

PSM inoculant propagation

The two isolates (*Pseudomonas* sp. and *Penicillium* sp.) were transplanted into agar slanted \. After being incubated for 48 hours, 10 ml of physiological NaCl was added. After the microbial isolate was dissolved, 50 ml (10%) was put into an Erlenmeyer containing 500 ml of Pikovskaya liquid

medium (the composition of the media in 1 L was: 5 g $\text{Ca}_3(\text{PO}_4)_2$, 10 g glucose, 0.2 g NaCl, 0, 2 g KCl, 0.0025 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0025 g FeSO_4 , 0.5 yeast extract) enriched with phytic acid and shaken at 125 rpm for 24 hours. The PSM population was calculated in this liquid medium before being used.

Preparation of inoculants in the carrier

The carrier material was used a mixture of peat, compost and husk charcoal with a ratio of 1: 1: 1. Phosphate solubilizing microbes in liquid form (bacterial density $22.2 \times 10^8 \text{ CFU ml}^{-1}$ and fungal density $90.4 \times 10^5 \text{ CFU ml}^{-1}$) were given as much as 10% (10 ml into 100 g of sterilized carrier).

RESULTS AND DISCUSSION

Characteristics of Jatinangor Ultisol Soil

The soil acidity in this experiment was classified as acid soil (pH 5.11) which was supported by a fairly high Fe content (34.69 mg kg^{-1}). Furthermore, P-available was moderate soil (16.9 mg kg^{-1}) due to P fixation by Fe so that P was not widely available for plants [16].

The base saturation of Ultisol Jatinangor is low at 25.7%, indicating that this soil is classified as marginal which has undergone intensive leaching so that the fertility status is very low. The content of macro nutrients such as K-total is relatively low at $16.4 \text{ mg } 100 \text{ g}^{-1}$ and K-dd at 0.1 cmol kg^{-1} . The low K element content occurs because the soil in Jatinangor is a soil with advanced weathering levels so that only a few minerals are the source of K. Meanwhile, the total N is in the moderate range of 0.31%. The low total N content in the soil is caused by the highly mobile characteristic of N in the soil [17].

Soil Phosphatase

Phosphate solubilizing microbes have an important role in dissolving phosphate so that it can be available to plants through phosphatase activity. In acid soils, acid phosphatase activity is more dominant than alkaline phosphatase activity which is generally active in alkaline soils [18]. The results showed that the application of PSM (*Penicillium* sp. and *Pseudomonas* sp.) increased phosphatase activity significantly

This PSM inoculant was able to increase soil phosphatase up to 173.27%. This indicated that the inoculated isolates could excrete phosphatase extracellularly thereby increasing the soil phosphatase content. This increased phosphatase activity indicates an increase in microbial growth in the soil which can further increase P transformation in the soil.

Table-1: The effect of PSM inoculant and P fertilizers on soil phosphatase

Treatments	Soil phosphatase ($\mu\text{g pNP g}^{-1} \text{hour}^{-1}$)
PSM inoculants:	
- no inoculant	78,40 a
- with inoculant	214,24 b
P fertilizers	
- 0 kg ha ⁻¹	66,01 a
- 50 kg ha ⁻¹	170,04 ab
- 75 kg ha ⁻¹	202,84 b
- 100 kg ha ⁻¹	146,40 ab

Remarks: The average score followed by the same letter is not significantly different according to the Duncan Test at the 5% level

The application of P fertilizer on Ultisols soil with a dose of 75 kg P₂O₅ ha⁻¹ was able to significantly increase soil phosphatase. In Table 1 it can be seen that increasing the dose of P fertilizer from 75 to 100 kg P₂O₅ ha⁻¹ tends to reduce soil phosphatase. The results of this experiment showed that phosphatase activity was

strongly influenced by the presence of P in the soil [19] stated that the concentration of P is very influential on phosphatase activity. The results of the research by [20] showed that increasing the concentration of KH₂PO₄ from 10 to 30 mg L⁻¹ decreased the phosphatase activity of *Pseudomonas mallei* in vitro from 2.52 to 0.64 g pNP ml⁻¹ hour⁻¹.

Soil P-available and Organic P

The experimental results showed that PSM inoculants had not been able to significantly increase the available P content of Ultisols soil in the late vegetative phase. Although the soil phosphatase increased sharply, this condition was not sufficient to increase the available P content of the soil. This is thought to be due to the strong P fixation by Al and Fe in Ultisol soils. P fixation by Al which is widely dissolved in pH < 5 makes the availability of P for plants to be low. The stability of P bound to Al immobilizes the availability of P and the level of P uptake by plants [21].

Table-2: The effect of PSM inoculant and P fertilizers on soil P-available and organic P

Treatments	P-available (mg kg ⁻¹)	Organic P (mg kg ⁻¹)
PSM inoculants:		
- no inoculant	32,84 a	10,50 b
- with inoculant	33,03 a	8,68 a
P fertilizers		
- 0 kg ha ⁻¹	27.52 a	8,42 a
- 50 kg ha ⁻¹	31.86 b	9,11 ab
- 75 kg ha ⁻¹	36.46 c	10,84 b
- 100 kg ha ⁻¹	35.92 bc	9,98 ab

Remarks: The average score followed by the same letter is not significantly different according to the Duncan Test at the 5% level

Based on the results of the analysis of the available P content of the soil, it showed that the application of P fertilizer was able to significantly increase the available P content of the soil. P fertilizer application of 75 kg P₂O₅ ha⁻¹ can increase the available P of soil by 32.49%. Meanwhile, increasing the dose up to 100 kg P₂O₅ ha⁻¹, increased the available soil P by 30.05%. This shows that the application of 75 kg P₂O₅ ha⁻¹ fertilizer can have a better effect on soil P nutrient status.

The experimental results showed a decrease in the organic P content of the soil after being treated with phosphate solubilizing microbial inoculants (Table 2). The decrease in organic P content caused by the inoculation of phosphatase-producing bacteria indicated that organic P mineralization had taken place. The mineralization of organic P was indicated by a decrease in soil organic P content. This is supported by the results of observations on soil phosphatase activity which increased significantly in the experimental plots inoculated with PSM [22].

A decrease in soil organic P and an increase in soil inorganic P content is an indicator of the process of soil organic P mineralization [23]. Research by [22] explained that mineralization of organic P was 28.66% higher in unsterilized soils than sterile soils in all incubation periods. The results of this study showed that the application of P fertilizer increased soil organic P (Table 2). Observation of the final vegetative phase showed that the application of P fertilizer at a dose of 75 kg P ha⁻¹ increased organic P by 28.74%. Increasing the dose of P fertilizer to 100 kg P ha⁻¹ increased the organic P content by 18.53%, [24] reported that when inorganic P (in the form of fertilizers, residues of plant and animal components) is applied to soil, most of the P is transformed into organic form as a result of microbial activity.

Upland Rice Yield

The application of PSM inoculants increased the yield of upland rice significantly by 16% (Table 3). This indicates a significant contribution of this PSM inoculant in increasing crop yields. *Pseudomonas* sp. isolates. and *Penicillium* sp. used in this study can

provide a supply of P nutrients, due to *Pseudomonas* sp. and *Penicillium* sp. synergistically to secrete phosphatase enzymes in the mineralization and immobilization process to convert organic P to inorganic P, so that both growth can still be optimal during plant growth and until harvest. In addition, *Pseudomonas* sp. and *Penicillium* sp. secrete organic acids which function to release P from Fe fixation. The existence of this synergy helps in providing P for upland rice plants until harvest, especially in filling rice grains, which in the end the results of milled dry grain can increase.

Table-3: The effect of PSM inoculant and P fertilizers on yield of upland rice

Treatments	Yield (kg plot ⁻¹)
PSM inoculants:	
- no inoculant	1,97 a
- with inoculant	2,29 b
P fertilizers	
- 0 kg ha ⁻¹	1,65 a
- 50 kg ha ⁻¹	2,04 b
- 75 kg ha ⁻¹	2,62 c
- 100 kg ha ⁻¹	2,22 b

Remarks: The average score followed by the same letter is not significantly different according to the Duncan Test at the 5% level

Application of P fertilizer with a dose of 75 kg P₂O₅ ha⁻¹ increased the yield of upland rice by 58%. This sharp increase with a dose of 75% of the recommended dose is very significant in an effort to increase the efficiency of P fertilization on marginal land. The addition of a P fertilizer dose of more than 75 kg P₂O₅ ha⁻¹ to 100 kg P₂O₅ ha⁻¹ did not increase the yield of upland rice. Fertilization of P in high doses will cause deficiency of micro nutrients such as Zn, Fe, Bo, and Mn so that the nutrients become unbalanced and consequently will interfere with root activity to absorb nutrients (Dhaliwal *et al.* 2019). The higher the soil nutrient content resulting from fertilization, the smaller the response of plants to fertilization.

CONCLUSION

Based on the experimental results it can be concluded that the application of phosphate solubilizing microbes increased soil phosphatase activity and upland rice yields on Ultisol. PSM inoculation can increase soil organic P mineralization characterized by a decrease in organic P. Inoculation of phosphate solubilizing microbes with P fertilizer at a dose of 75 kg P₂O₅ ha⁻¹ (75% recommended dose) was able to give the best effect on soil phosphatase activity, soil P status and upland rice yields on Ultisol.

ACKNOWLEDGMENT

This research was supported by grants received from the Directorate General of Higher Education Ministry of Research and Technology Indonesia. We

are also thankful to our student Lucky Pramudita Suhartono for supporting us during experiment at field.

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