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(Research Article)

Effect of application of phosphate solubilizing bacteria and organic fertilizers on soil phosphorus solubility and growth of maize (*Zea mays* var. *saccharata* Sturt L.) on andisols

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Abstract

Phosphate solubilizing microbes are a group of soil microbes that have the ability to extract P from bonds with Al, Fe, Ca, and Mg, so that they can dissolve P that is not available to become available for plants. This is because these microbes release organic acids which can form stable complexes with P-binding cations in the soil. The aim of this experiment was to study application of phosphate solubilizing bacteria and organic fertilizer to increase solubility of soil P through mineralization process of organic P to inorganic P and also solubility of P which adsorbed so that increase P available of Andisols and also increase of plant growth of maize. Design experiment of Randomized Block Design (RBD) was used in green house experiment, consisted of 12 treatments and three replications. The treatment consisted of three isolates (based on selection result) i.e. control (without both isolate and organic manure), *Bacillus mycoides, B macerans, and Pseudomonas pseudoalcaligenes*, respectively, combined with organic manure (cow manure and compost derived from bamboo leaf) and applied on Andisols soil. The result of experiment revealed that application of PSB and organic manure significantly increased of phosphatase activity and available P, decreased of soil organic P, but did not significant effect growth of maize on Andisols from Lembang. Inoculation of P. pseudoalcaligenes combined with compost gave significant effect on increase of available P and decrease of organic P.

Keywords: Compost; Cow manure; Mineralization; Phosphatasel; Organic acid

1. Introduction

Andisols include large areas of acidic soil that are generally black in color, where this soil was previously called Andosols [1](Eswaran and Reich, 2022). The main obstacles to plant growth in acid mineral soils, especially Lembang Andisols, include low pH, high organic matter and very high total P, but low P available to plants and Al, Fe, S, Zn and Mn toxicity [2]. The lack of P nutrients is caused by the element being strongly bound to soil colloids such as clay minerals and the presence of allophane [3], there are also iron and aluminum oxides forming Al-P and Fe-P so that they become unavailable. for plants. This event can be caused by retention of P which binds P where this element can still be extracted with dilute acid so that the form of P can be available large enough for plant growth [4].

A common problem faced by P in soil is that not all soil P is immediately available to plants. In this case, it really depends on the nature and characteristics of the land and the management of the land itself by humans [5]. Phosphorus in tropical soils is mostly found in organic form. The forms of organic P are very diverse, complex and largely uncharacterized [6]. Mengel et al. [7]stated that between 15 – 80% of P in soil is found in organic. Reusser et al. [8] added that the average organic P content in soil ranges between 5 – 50% of total P. The results of research by Borie and Rubio [9] reported that the percentage of organic P to total P in volcanic soils in Chile ranges between 50 – 65 %. The

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high organic P content of the soil is a potential source of P availability for plants [10]. However, P in organic form cannot be immediately used by plants, but needs to be transformed first into inorganic P through a mineralization process catalyzed by soil enzymes [11], [12].

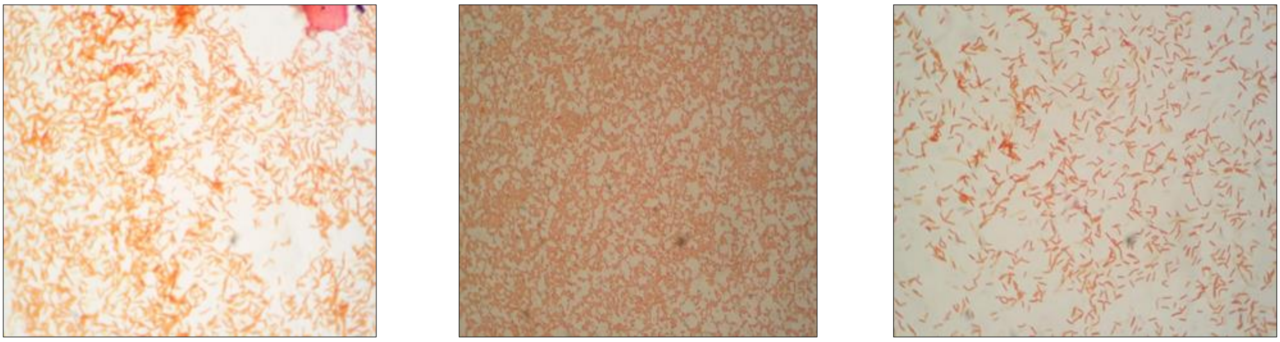
Soil enzymes that play a role in the process of mineralizing organic P compounds into inorganic P are a group of enzymes known as phosphatases [13],[14]. These phosphatase enzymes are included in the group of hydrolase enzymes, namely enzymes that can hydrolyze organic phosphorus compounds (phosphoric ester hydrolysis) into inorganic phosphorus compounds [15]. The results of this hydrolysis are used to meet the inorganic P needs of microbes and can contribute inorganic P to the soil which can be utilized by plants [16].

To overcome the problem of constraints on the availability of phosphorus nutrients in Andisols, the use of soil microbes that are superior in dissolving P, especially in mineralizing soil organic P into inorganic P that is available for plants, can overcome the problem of P deficiency in Andisols soil and enhancing plant growth.

2. Materials and methods

Isolation of phosphate solubilizing bacteria from Sanggabuana Forest, West Java was carried out on Pikovskaya agar medium and the parameters were qualitatively based on the diameter of the highest clear zone. Analysis of the phosphatase content of the isolate was determined by administering the substrate p-nitrophenylphosphate. The p-nitrophenol compound formed due to enzyme activity is then colored by sodium hydroxide solution to produce a yellow color that can be detected with a 400 nm spectrophotometer [17].

Based on the results of phosphatase enzyme analysis of the isolated and selected, three isolates with the highest phosphatase activity were selected (Figure 1).



PSB 1 *Bacillus mycoides* | PSB 2 *Bacillus macerans* | PSB 3 *Pseudomonas pseudoalcaligenes*

Figure 1 Selected Phosphate-Solubilizing Bacterial Isolates

Viability testing is aimed at testing the viability of the selected isolate type on the carrier material. In this research, two types of carrier materials were tested, namely peat and husk charcoal. Changes in the PSB population density of each isolate in the carrier material were observed at time intervals of 10, 20 and 30 days after incubation (DAI).

Based on the results of testing the viability of isolates in the carrier material, it shows that the PSB population in peat is generally higher than in husk charcoal, so for this research the next carrier material used is peat.

Testing of the ability of selected isolates to increase the solubility of Andisols soil P and its effect on maize plant growth was carried out in the greenhouse of the Faculty of Agriculture, Universitas Padjadjaran at Jatinangor, Sumedang District, West Java, Indonesia. In this experiment, three selected isolates (*Bacillus mycoides, Bacillus macerans* and *Pseudomonas pseudoalcaligenes*) were tested for mineralizing organic P from different organic material sources (namely cow manure and bamboo leaf compost).

Application of inoculant and organic fertilizer into polybags (incubation for 14 days), maintained at field capacity conditions. The treatment consisted of 12 treatments which were repeated 3 times. The treatments tested consisted of control, isolate type combined with organic fertilizer (cow manure and bamboo leaf compost).

3. Results and discussion

3.1. Soil Phosphatase Enzyme

The results of the statistical analysis of the influence of P-solubilizing bacteria and organic fertilizer on phosphatase activity can be seen in Table 1.

Table 1 Effect of PSB isolate and organic fertilizer on phosphatase enzyme activity (4 and 8 weeks after planting/WAP)

Treatments	**Phosphatase (μ NP/g/h)**	
	4 WAP	**8 WAP**
control	0.10 a	0.45 abc
Cow manure	0.19 ab	0.23 a
Bamboo leaf compost	0.38 ab	0.45 abc
B. mycoides	0.45 ab	0.71 bc
B. mycoides + cow manure	0.27 ab	0.41 abc
B. mycoides + bamboo leaf compost	0.57 b	0.52 abc
B. macerans	0.35 ab	0.73 bc
B. macerans + cow manure	0.24 ab	0.43 abc
B. macerans + bamboo leaf compost	0.35 ab	0.40 abc
P. pseudoalcaligenes	0.36 ab	0.84 c
P. pseudoalcaligenes + cow manure.	0.29 ab	0.33 ab
P. pseudoalcaligenes + bamboo leaf compost	0.29 ab	0.33 ab

Note: The average values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at a significance level of 5%.

Based on Table 1, it can be seen that inoculation of three PSB isolates and application of organic fertilizer to Andisols had a significant effect on phosphatase activity at both 4 WAP and 8 WAP. Phosphatase activity at 4 WAP increased by almost 50% in the combination treatment of *B. mycoides* and bamboo leaf compost compared to the control. Meanwhile, at 8 WAP, the increase in phosphatase activity reached 54% in the combination treatment of *P. pseudoalcaligenes* with leaf compost compared to the control. This shows that the application of PSB isolate and leaf compost organic fertilizer was able to increase Andisols soil phosphatase activity. In general, the application of leaf compost fertilizer is able to increase phosphatase activity higher than cow manure, this is thought to be because the nutrient content and C/N of leaf compost fertilizer is higher so it can provide a better substrate for microbial growth which in turn can increase enzyme activity.

Fitriatin et al. [18] reported that *Burkholderia* sp. isolate increased soil phosphatase activity by 142.7% compared to the control. Futhermore, soil phosphatase was increased by 147.9% when *Burkholderia* sp. isolate and *Penicillium* sp. were combined,

3.2. Soil P availability

The results of statistical analysis showed that there was a real effect due to inoculation of PSB isolates and application of organic fertilizer on soil available P at both 4 WAP and 8 WAP. Based on Table 2, application of PSB inoculant combined with organic fertilizer (cow manure and bamboo leaf compost) increased the availability of P in the soil.

Table 2 Effect of PSB isolate and organic fertilizer on soil P-available

Treatments	P available (ppm)	
	4 WAP	**8 WAP**
control	6.00 a	7.33 a
Cow manure	8.00 a	11.00 ab
Bamboo leaf compost	8.00 a	12.33 bc
B. mycoides	14.00 bc	16.00 cd
B. mycoides + cow manure	15.67 cd	16.67 cd
B. mycoides + bamboo leaf compost	15.67 cd	17.00 cd
B. macerans	19.67 d	17.33 cd
B. macerans + cow manure	14.67 bcd	18.67 d
B. macerans + bamboo leaf compost	10.00 ab	16.33 cd
P. pseudoalcaligenes	14.33 bcd	17.67 cd
P. pseudoalcaligenes + cow manure.	16.67 cd	16.00 cd
P. pseudoalcaligenes + bamboo leaf compost	16.00 cd	24.67 e

Note: The average values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at a significance level of 5%.

P. pseudoalcaligenes isolate combined with bamboo leaf compost apparently provided the highest available P compared to other treatments. This is thought to be due to a real correlation between P dissolution and the quality of organic matter added to the soil. The results of the analysis of the nutrient content of bamboo leaf compost were higher than cow manure.

In general, the soil available P content was higher at 8 WAP compared to 4 WAP. This is in line with the results of analysis of phosphatase content which showed that phosphatase activity is higher at 8 WAP compared to 4 WAP. Thus, there is a correlation between phosphatase activity and soil P solubility. The ability of phosphatase to hydrolyze phosphate esters can increase dissolved P in the soil. In relation to the availability of P for plants, the form of inorganic P that plays an important role in P availability is the secondary form of inorganic P in the form of Al, Fe and Ca phosphate compounds which are unstable in nature [19]. This inorganic P will become P available for plants when environmental conditions change in the soil.

3.3. Soil Organic P

The statistical test results (Table 3) show that there was a significant effect of PSB isolate and organic fertilizer on organic P both at 4 WAP and 8 WAP. The experimental results showed that the application of PSB inoculant included in a peat carrier combined with organic fertilizer had a significant effect on changes in organic P both at 4 WAP and 8 WAP.

Based on the results of this experiment, as can be seen in Table 3, the highest organic P was found in the treatment without isolate and without organic fertilizer. This shows that the isolate given to the soil is able to increase the organic P mineralization process due to an increase in soil enzymes, especially phostase and phytase. This can be seen from the decrease in organic P in the soil treated with PSB isolate. There is a strong relationship between organic P mineralization and soil microbial activity, in this case soil microbes synthesize enzymes that act as biocatalysts in the organic P hydrolysis reaction to produce inorganic P by phosphatase enzyme [20].

Table 3 Effect of PSB isolate and organic fertilizer on soil organic P

Treatments	Soi organic P (mg kg^{-1})	
	4 WAP	**8 WAP**
control	65.33 c	46.00 f
Cow manure	27.33 b	24.33 e
Bamboo leaf compost	19.67 a	16.33 a
B. mycoides	29.00 b	24.33 e
B. mycoides + cow manure	29.00 b	23.33 e
B. mycoides + bamboo leaf compost	23.67 ab	17.00 ab
B. macerans	29.00 b	19.67 bc
B. macerans + cow manure	23.00 ab	20.00 bcd
B. macerans + bamboo leaf compost	18.33 a	16.00 a
P. pseudoalcaligenes	23.00 ab	17.00 ab
P. pseudoalcaligenes + cow manure.	26.67 b	22.67 de
P. pseudoalcaligenes + bamboo leaf compost	24.33 ab	23.00 e

Note: The average values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at a significance level of 5%.

3.4. Plant P-uptake

The results of the statistical analysis of the effect of PSB isolate and organic fertilizer on P uptake are presented in Table 4. Based on the results of the statistical analysis, it shows that at 4 WAP the application of PSB inoculant and organic fertilizer did not have a significant effect on P uptake of corn plants, however, observations of plant P uptake at 8 WAP, treatment of *B. mycoides* isolates and bamboo leaf compost significantly increased P uptake, reaching 3.83 mg plant $^{-1}$. This shows that leaf compost is of better quality in increasing plant P levels. This was in line with the higher availability of soil P in soil treated with bamboo leaf compost.

Table 4 Effect of PSB isolate and organic fertilizer on plant P-uptake

Treatments	P-uptake (mg plant $^{-1}$)			
	4 WAP		**8 WAP**	
control	1.04	a	1.45	a
Cow manure	1.50	a	1.47	a
Bamboo leaf compost	1.67	a	2.74	ab
B. mycoides	1.95	a	2.87	ab
B. mycoides + cow manure	3.06	a	1.78	ab
B. mycoides + bamboo leaf compost	3.36	a	3.82	b
B. macerans	1.31	a	3.04	ab
B. macerans + cow manure	1.65	a	3.35	ab
B. macerans + bamboo leaf compost	1.69	a	3.03	ab
P. pseudoalcaligenes	2.08	a	2.00	ab
P. pseudoalcaligenes + cow manure.	2.85	a	3.24	ab
P. pseudoalcaligenes + bamboo leaf compost	3.32	a	1.46	a

Note: The average values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at a significance level of 5%.

3.5. Plant Growth

Plant height growth in the second week after planting was significantly influenced by the addition of leaf compost. However, the treatment of PSB isolate and organic fertilizer did not have a significant effect on plant height growth at 8 WAP.

Table 5 Effect of PSB isolate and organic fertilizer on plant height

Treatments	Plant height (cm)					
	2 WAP		4 WAP		6 WAP	
control	24.30	abc	50.33	ab	73.83	a
Cow manure	19.43	a	39.93	ab	69.67	a
Bamboo leaf compost	24.30	abc	54.00	b	73.33	a
B. mycoides	20.30	ab	41.43	ab	67.33	a
B. mycoides + cow manure	25.13	abc	49.27	ab	79.50	a
B. mycoides + bamboo leaf compost	24.73	abc	50.60	ab	78.17	a
B. macerans	25.03	abc	50.17	ab	76.50	a
B. macerans + cow manure	24.50	abc	49.83	ab	76.50	a
B. macerans + bamboo leaf compost	27.20	c	51.50	ab	73.83	a
P. pseudoalcaligenes	24.93	abc	51.70	ab	73.67	a
P. pseudoalcaligenes + cow manure.	25.83	bc	51.47	ab	79.83	a
P. pseudoalcaligenes + bamboo leaf compost	26.37	c	51.83	ab	67.83	a

Note: The average values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at a significance level of 5%.

According to preliminary analysis results, these Andisols are acidic soils (pH H_2O 5.2 and pH KCl 4.9). Both the problem of acidity and the disruption of the availability, absorption and disruption of nutrients to plants growing on acidic soil are focused on the presence of Al as the main cause (Al = 392) indicating that Al is toxic in conditions of pH 4.5 and furthermore it is also stated that it tends to responsible for plant root growth are the results of Al ion hydrolysis. Soil acidity is caused by a high concentration of H^+ ions, apart from that the source of soil acidity usually comes from humus or organic material, clay minerals, Al silicate, Fe and Al hydroxides [21].

In addition, organic materials encourage plant growth by providing the auxin they contain and are useful in increasing nutrient uptake [22]. The direct positive influence of organic materials on plant growth occurs through their decomposition products in the form of organic acids, where these organic acids function as chelating agents for Zn^{2+} and Fe^{3+} [23]. The formation of these chelates increases the transport of these elements, as well as their absorption by plant. However, sometimes in certain circumstances, namely in a reductive atmosphere, the organic acids formed are toxic to plants, for example certain phenolic acids, such as butyrate, lactate and methane gas, so that plant growth becomes poor.

4. Conclusion

The application of phosphate-solubilizing bacteria and organic fertilizer has a significant effect on increasing phosphatase activity and soil available P, reducing soil organic P, but has no significant effect on corn growth in Andisols. *P. pseudoalcaligenes* inoculation combined with bamboo leaf compost had a significant effect on increasing available P, as well as having a significant effect on reducing organic P. In addition, the decrease soil organic P indicates the mineralization of organic P into inorganic P, this causes an increase in soil P solubility.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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