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Effect of phosphate solubilizing bacteria and phosphate fertilizer on soil bacterial population and some soil chemical properties

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Abstract. The agricultural waste from the agricultural processing industry can generate environmental pollution. On the other hand, the organic waste, especially from the Empty Oil Palm Fruit Bunches (EFB) and Pineapple Rhizomes (PR), can be used as bio fertilizer, such as phosphate solubilizing bacteria (PSB). This study examined the effect of applying PSB and the type of phosphate fertilizer (PF) on the soil bacterial population and some soil chemical properties. The study was designed in a 4×3 factorial in a randomized block design (RBD) with three replications. The first factor was the type of PSB (without PSB, PSB from EFB, PSB from PR, and a mixture of PSB from EFB+PR). The second factor was the type of phosphate fertilizer (without PF, SP-36 PF, and Moroccan Natural Rock Phosphate (NRP) Fertilizer. The research was conducted in two parallel experiments, one on incubated soil without plants and one on soil planted with cucumbers. The results showed that the PSB population in the PSB PR treatment gave the best results in soil conditions for incubated soil without plants. Soil incubation with PSB and PF had a higher soil pH and organic C percentage than the control treatment. The PSB population in the PSB PR treatment was higher than in the PSB EFB treatment and the PSB EFB+PR mixture on cucumber-planted soil. The PSB populations, available P, and soil organic C with PSB gave the best results on cucumber-planted soil. PSB population was significantly correlated with the number of cucumber fruit, and soil available P was significantly correlated with root dry weight. Therefore, agro-industry organic waste, especially FEB and PR, can be used as bio fertilizers to improve soil fertility.

Keywords: Agricultural waste, soil bacteria, soil properties

1. Introduction

The plantation industry of coconut palm and pineapple in Lampung Province produces waste in empty fruit of oil palm bunches (EFB) and pineapple rhizome (PR). This waste has the potential



to pollute the environment and can host several insects and pathogens. EFB can be reused as organic fertilizer and green mulch [1]. Additionally, PR contains the essential nutrition required for the plant growth [2]. However, the waste of EFB and PR contain cellulose and lignin, which possess high fiber strength, rendering the composition challenging [3, 4]. It is necessary to explore alternative uses for this plantation industry waste, one of which is as a source of biological fertilizers including indigenous microorganisms.

Some researchers have studied agricultural waste utilization as local microorganisms (LOM) [5, 6]. However, there has yet to be a report about utilizing LOM from EFB and PR waste on the total soil bacterial population and some soil chemical properties. Conversely, the finding indicated that the suspensions of EFB and PR contained bacteria capable of dissolving phosphate (Phosphate solubilizing bacteria, PSB) [7]. The presence of PSB is expected to dissolve phosphate in the form of Al-P and Fe-P bound in the soil.

Tropical soils generally have low organic matter content, and soil colloid adsorption processes of phosphorus are relatively high [8]. On the other hand, phosphorus's role is crucial in cell division, photosynthesis, and root system growth. Phosphorus is absorbed by plants in the form of H₂PO₄⁻; however, in soils with high acidity, the availability of P decreases because it is adsorbed by Al and Fe so that it becomes Al-P and Fe-P, which are difficult to dissolve [9]. Therefore, phosphate fertilizers are continuously applied to fulfill the need for phosphate in agricultural soil. However, phosphate fertilizers applied to agricultural land have reasonably low availability, only about 10 to 30 percent of which plants can utilize [10]. The rest will become residue in the soil because it is bound by colloidal clay soil.

Soil microorganisms and soil chemical properties influence plant phosphate availability [11]. Some bacteria can convert phosphate adsorbed in soil colloids, phosphate from natural phosphate rock, and phosphate from inorganic fertilizers into phosphate available to plants [12]. Bacteria confirmed as *Bacillus magisterium* var. Phosphaticum is the isolate that dissolves phosphate [13].

The total population of bacteria in the soil is determined by organic matter factors, soil pH, and soil depth, so soil properties affect the role of bacteria in nutrient availability [14]. The increased activity of PSB can be seen from the ability of these bacteria to produce organic acids, enzymes, and hormones, which is influenced by a favorable environment for PSB [15]. Several studies [16, 17] found bacteria can dissolve phosphates. It has been reported that bacteria from bintaro fruit (*Carbera manghas* L.) could act as phosphate solvents [18]. Dermiyati et al [7] successfully isolated eight isolates from local microorganism suspensions made from EFB and PR waste. However, the potential of these isolates to improve soil properties has yet to be determined. Therefore, the current study aimed to investigate the ability of these PSMs to improve soil chemical properties and the amount of soil bacterial population.

2. Material and Methods

2.1 Place and time study

The research was conducted in the Integrated Field Laboratory, Faculty of Agriculture, University of Lampung, Bandar Lampung, Indonesia. The research location at coordinate UTM of 554766.0785902005 (X) and 9353352.614392031 (Y) or 5°36'90" SL and 105°24'34.1" EL. The research was done from May to December, 2022.

2.2 Research design

The research was conducted in the greenhouse and consisted of two parallel trials. The first trial used soil applied with PSB and incubated without growing plants. The second trial was

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conducted on soil applied with PSB and planted with cucumbers. Factorial treatments 4 x 3 were applied with three replications in a randomized block design. The first factor was Phosphate Solubilizing Bacteria (PSB) with four levels, namely: M0 (Without PSB), M1 (PSB EFB), M2 (PSB PR), and M3 (PSB EFB+PR). The second factor was the Phosphate fertilizer (PF) type with three levels, namely P0 (without PF), P1 (SP-36 fertilizer), and P2 (Rock Phosphate).

2.3 Preparation of phosphate solubilizing bacterial suspension

Eight isolates of PSB used in this study were collection of Dermiyati *et al* [7] (Table 1). The isolates were prepared by inoculating each PSB isolate from PPGA-slanted media into 10 mL of distilled water in a test tube. Next, the suspension was homogenized using a vortex mixer. After being homogeneous, 40 mL of each PSB isolate was put into an Erlenmeyer containing sterile distilled water. Eight were combined with PSB EFB+PR suspension (a consortium combination of 4 PSB EFB isolates and 4 PSB PR isolates) (with the same volume ratio). After being homogeneous, 5 mL of each PSB isolate was put into an Erlenmeyer containing sterile distilled water.

Table 1. Code isolates of PSB from oil palm empty fruit bunches (EFB) and pineapple rhizome (PR)

Isolate code	Source*	
AS (1).50.10PKR	LOM isolation from EFB	
US (3).50.8PKR	LOM isolation from EFB	
SS (2).50.8PKR	LOM isolation from EFB	
SS (1).50.8PKR	LOM isolation from EFB	
AN (1).100.10PKR	LOM isolation from PR	
SN(3).50.12P	LOM isolation from PR	
SN(2).50.12P	LOM isolation from PR	
SN(3).50.12PKR	LOM isolation from PR	

*LOM: Local Microorganism; EFB: Oil Palm Empty Fruit Bunches; PR: Pineapple Rhizome

2.4 Incubated soil without plant

The soil from the field at a 0-20 cm depth was taken, air-dried, and passed through a 5 mm sieve. Then, 1 kg of soil was put in a polybag and mixed with manure for each treatment. Manure was given as essential fertilizer as much as 5 t ha⁻¹. After 7 days, the bacterial suspension was applied as much as 35 mL per polybag and carried out four times at seven days' intervals. Urea fertilizer 225 kg ha⁻¹, KCl 200 kg ha⁻¹, SP-36 150 kg ha⁻¹, and Moroccan NRP 244.12 kg ha⁻¹ were applied. The treatment was repeated three times. Then, the polybags were tied up (not perforated), arranged according to the layout, and incubated for 70 days in the greenhouse. At the end of the incubation period, the soil was taken to analyze soil pH (Electrometric method), available P (Bray I method), and organic C (Walkley and Black method). The total bacterial population distinguished between PSB and non-PSB was also calculated.

2.5 Soil planted with cucumbers

In this parallel experiment, the soil preparation and treatments were similar to the experiment of incubated soil without the plant above. However, the soil used in this experiment was 10 kg. A total of 2 cucumber seeds (F1 Mercy) were planted at a depth of 3 cm from the soil surface. Urea fertilizer 225 kg ha⁻¹, KCl 200 kg ha⁻¹, SP-36 150 kg ha⁻¹, and Moroccan NRP 244.12 kg ha⁻¹ were added seven days after planting [19]. A 350 mL of PSB suspension (ten times higher dose than incubated soil without plant experiment because this experiment used 10 kg soil) was applied to the soil surface seven days after planting. PSB suspension was also added four times with an interval of 7 days. At harvesting, the fruits and cucumber plants were taken to observe the number of fruits, fruit weight, dry weight of stover, dry weight of roots, and fruit weight.

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However, these data are only used as supporting data to test the correlation with soil properties data. The soil in the pot was then taken for soil pH, available P, and organic C analysis, and the total bacterial population was also observed.

Table 2. Initial chemical properties of the experimental soil

Variable	Value	Analysis Method	Criteria *	
Available P (mg kg ⁻¹)	2.48	Bray I	Very Low	
Organic C (%)	1.58	Walkley & Black	Low	
pH (H ₂ O)	6.09	Electrometric	Slightly Acid	

*Criteria based on Eviati et al. [20]

2.6 Isolation of Phosphate Solubilizing Bacteria

Bacteria were isolated using a dilution series and a spread plate technique using a Yeast Peptone Agar (YPA) medium. 10 g of soil samples were put into sterile distilled water (10⁻¹). The dilution series was carried out on 10⁻²-10⁻⁸, and 1 mL of suspension from the 10⁻¹ dilution series was transferred into a test tube containing sterile distilled water until the 10⁻⁸ dilution series stage. The spreading technique was carried out to isolate bacteria from diluted samples using a Drigalski tool. Bacterial colonies growing on YPA media were then inoculated in Pikovskaya media [21]. Transparent edges around the colonies characterize bacteria that can dissolve phosphate in Pikovskaya media [22].

2.7 Data analysis

Homogeneity data was tested using Bartlett's test, and additivity was tested using Tukey's test, followed by an analysis of variance (ANOVA) to evaluate the treatment effect. Comparisons between variables were carried out using the Duncan Multiple Range Test (DMRT).

3 Results and discussion

3.1 Effect of phosphate solubilizing bacteria (PSB) and type of phosphate fertilizer on bacterial populations

The experiments calculated the total bacterial population that contained PSB and non-PSB populations. The analysis of variance showed that the PSB treatment and the type of phosphate fertilizer had no significant effect on PSB and non-PSB populations on the incubated soil without plant experiment. However, the PSB treatment and the type of phosphate fertilizer significantly affected the PSB population on the soil planted with cucumbers experiment. However, it did not significantly affect the non-PSB population.

The 5% DMRT test results on the incubated soil showed that PSB and non-PSB populations did not differ in the PSB treatment (Table 3). Furthermore, on the polybag planted with cucumbers, the PSB population in the PR PSB treatment was higher than without PSB; however, it was not different from the EFB PSB treatment and the EFB+PR PSB mixed treatment. In contrast, the non-PSB population did not differ in the PSB treatment (Table 3).

The PSB population in the PR PSB treatment was higher than other treatments, although no different from the EFB PSB and EFB+PR mixed PSB treatment, presumably because PR PSB survived compared to EFB PSB. In addition, it is suspected that there was a competition between PSB EFB and PSB PR in the soil, so PSB EFB and PSB PR could not be

placed simultaneously in one treatment. It has been found that *P. fluorescens* and *A. subtilis* cannot be placed in a consortium because these bacteria are mutually antagonistic [23]. There is competition between the two bacteria to colonize the roots of tomato plants. Competition can occur when two or more microorganisms need the same substrate in the form of nutrients, space, or oxygen.

Coil treatment	Bacterial population (Log CFU g ⁻¹ soil)			
Son treatment	PSB population Non-PSB population			
Soil incubation				
Without PSB (M ₀)	9.43 a	9.37 a		
PSB EFB(M ₁)	9.46 a	9.39 a		
PSB PR (M ₂)	9.47 a	9.41 a		
PSB EFB+PR (M ₃)	9.42 a	9.35 a		
Soil planted with cucumber				
Without PSB (M ₀)	9.45 b	9.34 a		
PSB EFB(M ₁)	9.60 ab	9.51 a		
PSB PR (M2)	9.72 a	9.43 a		
PSB EFB+PR (M ₃)	9.53 ab	9.50 a		

Table 3. Effect of PSB application on bacterial populations

Note: Numbers in one column followed by the same letter are not significantly different based on the DMRT test at a 5% significance level.

The abundance of bacteria in the cucumber-planted soil was generally higher than incubated soil without plants. The PSB treatment affected the total soil bacterial population in the cucumber-planted soil. It was reported that plant roots affect soil organic matter [24]. Dead and decaying plant roots contribute to soil organic matter. The increase in soil organic matter increases the organic C content needed by soil microorganisms to grow and develop.

The PSB PR treatment produced the highest PSB population compared to other treatments in the incubated soil without plant and cucumber-planted soil (Table 3). It is suspected that this happened due to the raw material used to make LOM, which affected the ability and abundance of bacteria in LOM suspension. Pineapple rhizome is part of the plant that is closest to the root area, which is the PSB habitat in the soil [25]; so, the ability of bacteria to survive is more potent when compared to PSB derived from EFB. Plant roots directly influence the rhizosphere. The roots are associated with microorganisms to carry out their metabolic processes so the plants can produce compounds such as amino acids and sugars used by bacteria [26].

Moreover, the PSB and non-PSB populations in the incubated soil without plants and the non-PSB population in the cucumber-plated soil did not differ (Table 4). In the soil planted with cucumbers, PSB populations in soils treated with SP-36 and RP fertilizers were significantly higher than without fertilizer (control) (Table 4).

Phosphorus from chemical fertilizers and natural rocks phosphate increases PSB populations. PSB in the soil increases if the environmental conditions where the bacteria live can provide the needed nutrients so the bacterial population can increase and develop properly [27]. The PSB population in the cucumber-planted soil tended to be higher than in incubated soil without plants. These results are influenced by the roots of cucumber plants, which produce exudate through root metabolic activity in the soil. It is reported that the

metabolic activity released by plants through the roots is a factor that significantly determines the presence of soil microorganisms in the root area [28].

Table 4. Effect of application of phosphate fertilizer types on bacterial populations in incubated soilconditions and soil planted with cucumbers.

	Bacterial population (Log CFU g ⁻¹ soil)			
Soil treatment	PSB population	Non-PSB population		
Soil incubation				
Without P fertilizer (P ₀)	9.36 a	9.36 a		
SP-36 fertilizer (P ₁)	9.42 a	9.56 a		
NRP fertilizer (P ₂)	9.56 a	9.42 a		
Soil planted with cucumber				
Without P fertilizer (P ₀)	9.45 b	9.42 a		
SP-36 fertilizer (P ₁)	9.65 a	9.44 a		
NRP fertilizer (P ₂)	9.63 a	9.48 a		

Note: Numbers in one column followed by the same letter are not significantly different based on the DMRT test at a 5% significance level.

The PSB population increased by adding SP-36 phosphate fertilizer to the cucumberplanted soil. The high PSB population in the SP-36 fertilizer treatment is due to the ability of PSB to dissolve P. Consequently; they can also utilize P in the soil. Research showed that SP 36 fertilizer treatment + MPF biological fertilizer inoculation increased the PSB population from 1.2×10^{-11} to 2.0×10^{-11} [29]. The role of biological agents in the soil cannot replace the role of chemical fertilizers in the soil and plants. However, combining biological agents and chemical fertilizers is the best approach.

3.2 The effect of phosphate solubilizing bacteria (PSB) and the type of phosphate fertilizer on some soil properties

Soil reaction (soil pH) treated with PSB did not differ in the incubated soil without plants. However, the pH of the soil-applied with PSB PR was significantly lower than that of other PSB treatments and did not differ from that of the PSB mixed with EFB+PR on the cucumber-planted soil. The pH values in the incubated soil without plant ranged from 6.10 to 6.20, while the cucumber-planted soil ranged from 5.89 to 6.23 (Table 5).

The soil reaction (pH) applied with phosphate fertilizer (SP-36 and NRP) was significantly lower than without phosphate fertilizer but did not differ between SP-36 and NRP fertilizer in incubated soil without plants. The type of fertilizer treatment did not affect the pH of the cucumber-planted soil (Table 5).

The decrease in soil pH was thought to be due to the application of PSB [30, 31]. PSB produces some citric, gluconic, lactic, succinic, and propionic acids [32], which can dissolve phosphate through the mechanism of organic acid production, acidification of the pH of the medium as a result of H+ excretion by bacteria, then through the enzyme phosphatase produced by bacteria [33]. An increase in organic acids in the soil was followed by a decrease in pH in the soil media applied by phosphate-solubilizing bacteria [15]. PSB activity can be seen from the amount of enzyme phosphatase excreted; acid phosphatase is determined at pH < 7, and alkaline phosphatase is determined at a pH above neutral or > 7 [8]. Rhizosphere soils have a lower soil pH than non-rhizosphere soils, with differences in pH values between 0.07-0.65 pH units due to

H⁺ ions relesed by plant roots to maintain mass and charge balance around plant roots [34]. Ilmiasari [35] discovered that the isolated bacteria in PSB PR are *Stenotrophomonas malthophilia* and *Bacillus* sp. While Dermiyati *et al* [36] discovered that isolated bacteria in PSB EFB are *B. velezensis, B. paramycoides, and B. tequilensis.*

Table 5. The effect of applying phosphate solubilizing bacteria (PSB) and the type of phosphate fertilizeron soil pH and soil Organic C

	So	Soil pH		Soil organic C (%)	
Treatment	Incubated	Cucumbers-	Incubated	Cucumbers-planted	
	Soil	planted soil	Soil	soil	
PSB					
Without PSB (M ₀)	6.20a	6.23a	0.88b	1.39a	
PSB EFB (M ₁)	6.10a	6.13ab	1.03b	1.23a	
PSB PR (M ₂)	6,13a	5.89c	1.45a	0.82a	
PSB EFB+PR (M ₃)	6.14a	6.02bc	1.20ab	0.75a	
Types of Phosphate					
Without P fertilizer (P ₀)	6.21a	6.10a	0.89a	0.92a	
SP-36 fertilizer (P ₁)	6.13b	5.98a	1.27a	1.16ab	
NRP fertilizer (P ₂)	6.09b	6.09a	1.25a	1.40b	

Note: Numbers in one column followed by the same letter are not significantly different based on the DMRT test at a 5% significance level.

Soil organic C given PSB PR application was higher than other PSB treatments and was not different from the PSB application mixed with EFB+PR in incubated soil without plant; however, it was no different in the cucumber-planted soil (Table 5).

In the incubated soil without plants, soil organic C in all types of fertilizer treatment did not differ. However, in the cucumber-planted soil, soil organic C treated with NRP was higher than without fertilizer and was not different from that given with SP-36 fertilizer (Table 5).

The percentage of soil organic C at harvesting decreased compared to before treatment application (initial value). This is suspected to have happened because bacteria widely used the carbon in the soil as a source of energy, which was matched by adding organic matter. Carbon is the main factor for high microbial density and activity [37]. It is supported by Sagervanshi [38], who reported that soil microorganisms can utilize carbon contained in organic matter as an energy source.

The results showed an interaction between PSB and the type of phosphate fertilizer on soil available P in the cucumber-planted soil. In various PSB treatments, soil available P significantly increased with the application of NRP fertilizer compared to other phosphate fertilizer treatments. On the other hand, the soil available P in the various types of fertilizer treatments did not differ, except in the application of SP-36; the soil available P in the PSB PR treatment was higher than the PSB EFB+PR treatment. Interaction between PSB EFB+PR and NRP fertilizer significantly increased the soil available P by 32% compared to soil without fertilizer phosphate with various PSB treatments (Table 6).

The application of SP-36 affects the availability of phosphate compounds that are more readily used by bacteria during cell formation. The element phosphate in fertilizers is needed by plants primarily as a source of cellular energy (ATP) in cell metabolism [28, 13].

Table 6. The effect of applying PSB and the type of phosphate fertilizer on available P (mg kg⁻¹) in the cucumbers-planted soil

		Available P (mg kg ⁻¹)		
P Fertilizer Type	Without	PSB EFB	PSB PR	PSB EFB + PR (M ₃)
	$PSB(M_0)$	(M ₁)	(M ₂)	
Without P fertilizer (P ₀)	9.29 a	29.00 a	31.40 a	29.81 a
	С	В	В	В
SP-36 fertilizer (P1)	40.28 ab	35.06 ab	53.68 a	30.49 b
	В	В	А	В
NRP fertilizer (P ₂)	59.38 a	64.12 a	56.82 a	93.20 a
	А	А	А	А

Note: Numbers in one column followed by the same letter are not significantly different based on the DMRT test at a 5% significance level; lowercase letters are read horizontally, and capital letters are read vertically.

The presence of NRP was likely to experience a residual effect where the fertilizer given was not used up but remained in the soil. According to Sukaryorini [39], the process of phosphate mineralization increases with the increasing organic C in the soil. The availability of phosphate in the soil shows the activity and role of bacteria in the soil treated with PSB. The bacterial activity supports the continuity of the nutrient cycle process in the soil because the still-alive PSB can accelerate the decomposition process of dead bacterial bodies. Decomposition of organic matter using dead microbial cells as a source of nutrients for plants and substrates for microorganisms still alive [9]. It follows the research that adding NRP affects soil-available P compared to SP-36 fertilizer [10]. Moreover, NRP at doses of 30, 60, and 90 kg P ha⁻¹ increased soil available P by 247%, 356%, and 592% compared to treatment without NRP, respectively [13]. The increase in available P is likely due to the low levels of soil available P in the initial soil samples (Table 1) and the solubility of the natural phosphate used in the experiment. According to Noor [13], soil acidity significantly affects the solubility of natural phosphate in the soil. It is reported that the use of PSB (Bacillus megaterium var. Phosphaticum) combined with rock phosphate fertilization increased the availability of P by the roots of pepper (*Capsicum annum* L.) and cucumber (Cucumis sativus L.) [14].

3.3 Correlation between soil and cucumber yield variables.

The PSB population had a significantly positive correlation (r=0.89) with fruit number but not with fruit weight, stover dry weight, and root dry weight. The higher the BPF population, the more fruits are produced by plants. Soil pH did not correlate with fruit number, fruit weight, stover dry weight, and root dry weight. Soil available P had a significant positive correlation (r=0.94) with plant root dry weight but did not correlate with the number of fruit, fruit weight, and stover dry weight. Soil-available P correlates positively with the dry weight of cucumber roots; the higher the soil-available P-values, the more plant root dry weights increase. Soil organic C did not correlate with fruit number, fruit weight, stover dry weight, and root dry weight. Applying PSB isolates provides the phosphate plants need [40]. Phosphates are building blocks for cell nuclei (nucleic acids), fats, and proteins that affect photosynthesis, plant growth, and development [41]

4 Conclusions

The total population of bacteria (PSB and non-PSB) in the incubated soil without plant and non-PSB in the cucumber-planted soil were similar between various types of PSB and various types of phosphate fertilizers applications. However, in the cucumber-planted soil, the total PSB population with PSB PR application was high, between the PSB EFB and the PSB EFB+PR application was similar, and the total PSB population applied by SP-36 and RP fertilizer was high.

In the incubated soil without the plant, soil pH applied by SP-36 fertilizer and NRP decreased; the soil pH with the PSB PR application decreased; while, the soil organic C with the application of PSB PR increased. In the cucumber-planted soil, soil organic C with NRP increased compare to the control but similar to the application of SP-36. The soil available P with NRP and various PSB treatments were higher than that of other fertilizer treatments.

These findings show that applying PSB EFB and PSB PR with NRP could improve biological and some chemical properties of the soil.

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