

Optimum Population Size of Indigenous P-solubilizing Bacteria to Correct P Availability in Acid Soils

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ABSTRACT

Indonesian acid soils were used to determine an optimum population size of indigenous P-solubilizing bacteria (PSB) for solubilizing fixed P. The experiment consisted of two sub-experiments. Sub-experiment I was to isolate the indigenous PSB from Ultisols, Fresh-water lowland Inceptisols, and tidal-swamp Inceptisols. Sub-experiment II was to study the capacity of the isolated PSB to correct P availability in acid soils by inoculating the isolated PSB into the tested soils at 0, 10⁵, 10¹⁰, and 10¹⁵ cells. The population of the indigenous PSB in the tested soils increased as a result of the inoculation. Both Al-P and Fe-P content in the three tested soils decreased as compared with the initial content. The increases of available P were significantly correlated with the decreases both in Al-P ($r^2 = 0.68$ for the Ultisols; $r^2 = 0.51$ for the fresh-water Inceptisols; and $r^2 = 0.35$ for the tidal-swamp Inceptisols) and in Fe-P ($r^2 = 0.91$ for the Ultisols; $r^2 = 0.45$ for the fresh-water lowland Inceptisols; and $r^2 = 0.78$ for the tidal-swamp Inceptisols). The increases of available P were significantly correlated with the increases of the population of the PSB ($r^2 = 0.60$ for the Ultisols; $r^2 = 0.55$ for the fresh-water lowland Inceptisols; and $r^2 = 0.69$ for the tidal-swamp Inceptisols). The available P in the three tested soils sharply increased if the population size of the PSB was about 1×10^9 cfu g⁻¹ of soil.

Keywords: Al-P, Fe-P, fresh-water lowland, isolated, Pikovskaya medium, tidal swamp

INTRODUCTION

The form of P most readily accessed by plants is Pi (in-organic P), the concentration of which rarely exceeds 10 μ m in soil solutions (Bielecki 1973; Paul and Clark 1989). Therefore, in many agricultural systems, application of P fertilizers to the soil is necessary to ensure plant growth and productivity. However, more than 80% of the added P becomes immobile in acid soils and unavailable for plant uptake because of the strong fixation into unavailable complexes (Rajan and Watkinson 1976; Mehadi and Taylor 1988; Holford 1997; Rodríguez and Fraga 1999). Various soil properties have been reported to be closely related to the P retention capacity of soils (Bertrand *et al.* 2003; Daly *et al.* 2001; Leclerc *et al.* 2001). Furthermore Burt *et al.* (2002), Borling *et al.* (2001) and Ige *et al.* (2003) suggested the combination of Fe_{ox} and Al_{ox} for the prediction of soil P sorption capacity in non-calcareous soils.

Numerous microorganisms, especially those associated with roots, have the ability to increase plant growth and productivity (Glick 1995; Gyaneshwar *et al.* 1998). In few cases, this effect has been suggested to involve solubilization of otherwise unavailable mineral nutrients. This group of bacteria has been termed 'plant growth promoting rhizobacteria' or PGPR. Among of this group is phosphate-solubilizing bacteria (PSB), which is already used as commercial biofertilizers for agricultural improvement (Suba Rao 1993; Rodríguez and Fraga 1999).

Although PSB occur in soil, usually their numbers are not high enough to compete with other bacteria commonly established in the rhizosphere. Therefore, for agronomic purposes, inoculation at a much higher number than those normally found in soil is necessary to take advantage of their beneficial properties for plant yield enhancement (Igal *et al.* 2001) because the efficiency of biological solubilization of insoluble P is also affected by environmental factors (Taiwo and Ogundiya 2008).

Acid Ultisols of South Sumatra has been reported to have a population size of the indigenous

PSB ranging from 0.1 to 0.2×10^7 colony forming unit (cfu) per gram of soil (Sabaruddin 2004). Such population size was not capable of correcting P availability in the investigated soils, due to low population size and low potential P reserve in the soils. Therefore, the inoculation of PSB into the soils may alleviate this problem. Current paper reported results of a study on the attempt to determine the optimum population size of the indigenous PSB for optimum solubilization of P in acid soils.

MATERIALS AND METHODS

Soil Sampling

Soils were collected from three field sites to represent three different acid soils widely found in South Sumatra, Indonesia. The first field site, Sembawa Village, Banyuasin District, South Sumatra Province, was represented by Ultisols, while the other two were represented by Inceptisols. The Inceptisols was taken from two lowland areas, fresh-water lowland and tidal swamp. The fresh-water lowland Inceptisol was collected from Agro Techno Park, Gelumbang, Muara Enim District, South Sumatra Province, and the tidal-lowland Inceptisol sample was collected from Telang II Village, Banyuasin District, South Sumatra Province.

Three disturbed soil samples were collected from the depth of 0 to 30 cm of each field site. To avoid contamination, sampling tools were sterilized using alcohol (70% v/v) prior to sampling. The soil samples were kept in a cooler box and transferred into a refrigerator unless directly characterized. The Ultisols characterized by high acidity (pH 4.60), very low content of available P ($2.95 \mu\text{g g}^{-1}$ of soil), and high and very high content of Al-P ($71.25 \mu\text{g g}^{-1}$ of soil) and Fe-P ($306.17 \mu\text{g g}^{-1}$ of soil), consecutively. The fresh-water lowland soil was characterized by high acidity (pH 4.85), moderate content of available P ($10.15 \mu\text{g g}^{-1}$ of soil) and low content of Al-P ($12.75 \mu\text{g g}^{-1}$ of soil) but very high content of Fe-P ($262.46 \mu\text{g g}^{-1}$ of soil). The tidal-swamp soil was characterized by high acidity (pH 4.69), high content of available P ($18.41 \mu\text{g g}^{-1}$ of soil) but moderate content of Al-P ($41.25 \mu\text{g g}^{-1}$ of soil) and very high content of Fe-P ($378.49 \mu\text{g g}^{-1}$ of soil).

Experimental Outline

Current experiment consisted of two sub-experiments. Sub-experiment I was meant to obtain indigenous PSB isolates from each soil. Sub-experiment II was to investigate the ability of PSB

isolated in the Sub-experiment I in correcting P availability of soils. Both Sub-experiment I and Sub-experiment II were conducted at the Laboratory of Soil Chemistry, Biology and Fertility of Soil Science Department, Faculty of Agriculture, Sriwijaya University, Indralaya, South Sumatra.

PSB were isolated using modified Pikovskaya's agar medium. Instead of using $\text{Ca}_3(\text{PO}_4)_2$, current experiment used Al-P as a source of P in the growth medium to get PSB isolates, which were well adapted to high solubility of Al in the tested soils. In addition, Bromophenol Blue 0.4% (Gupta *et al.* 1994) was also added as a dyeing agent to enhance PSB colony identification.

Prior to isolation, soils were sieved (1.00 mm aperture) to separate debris. The soils (10 g) were transferred into a 250-mL erlenmeyer containing 90 mL of sterilized physiological solution ($8.5 \text{ g NaCl L}^{-1} \text{ H}_2\text{O}$) to obtain 10^{-1} -soil suspension. The suspension was shaken reciprocally for 20 minutes, and 1 mL of the suspension was pipetted into test tubes containing 9 mL of sterilized physiological solution to obtain 10^{-2} -soil suspension. These steps were repeated up to 10^{-7} -dilution level.

PSB were isolated by transferring 1 mL of soil suspension into sterilized petri dishes containing sterilized Pikovskaya's medium (10 mL per petridish). The petridish was then swirled to homogenize the soil suspension and the growth medium, and incubated in an incubator for 4 days at $30^\circ\text{C} \pm 1^\circ\text{C}$. PSB colony was characterized by clear zones on the medium. Only petridishes resulting 30 to 300 colonies were included in the colony counting.

Sub-experiment II consisted of 2 stages. The first stage was to propagate sufficient number of PSB isolates for the subsequent P availability experiment. Propagation was carried out in liquid Pikovskaya's medium. The inoculation rate tested in the Sub-experiment II were 0 (no inoculation), 10^5 , 10^{10} , and 10^{15} cells of PSB ml^{-1} of inoculant.

Correction on P availability by PSB was investigated by inoculation and incubation methods. Prior to inoculation, the soils were sieved (1.00 mm aperture) to separate debris. The three tested soils were saturated with P by adding 0.65 g, 0.37 g, and 0.43 g SP-36 into the Ultisols, the two types of Inceptisols, consecutively, and then equilibrated for one week. Soil water content was maintained at field capacity during the equilibrium period (Marsi and Sabaruddin 2006). The P-saturated soils (200 g) were transferred into plastic containers. The soils were then inoculated with 0 (no inoculation), 10^5 , 10^{10} , and 10^{15} cells ml^{-1} of PSB inoculant. Two milliliter of PSB inoculant was pipetted into the

soils; and two milliliter of regular water was used for the uninoculated soils. The soils were incubated at 30°C ± 1°C for 60 days.

Measurement

Measurements were made on the population density of PSB at 0 (prior to inoculation), 7, 15, 30, and 60 days after incubation, and available P and fixed P (Al-P and Fe-P) at 7, 15, 30, and 60 days after incubation.

Statistical Analysis

Population density of PSB was averaged over 5-petridish readings. ANOVA was performed to test the effects of treatments on PSB variables using General Linear model (GLM) of Costat Version 2.0. Relationships among variables were analysed using regression and correlation analysis.

RESULTS AND DISCUSION

PSB Population

Table 1 presents the population of PSB during the experiment. Data on 0d was the population of PSB at the start of the experiment (immediately prior to inoculation). In relation to the dynamics of PSB’s population in Table 1, there are two important findings of current experiment. First, inoculation increased the population of PSB in all tested soils. Second, the population of PSB in all tested soils fluctuated over time.

One of basic and important indicators to estimate the success of inoculating microbes into

the soils is the population density of the introduced microbes some times after the inoculation (van Veen *et al.* 1997). Current experiment showed that increases in inoculant density were followed by constant increases in population density of PSB in the three tested soils (Tabel 1). Similar results were also reported by Reyes *et al.* (2002) who observed a significant increase of total rhizospheric bacterial community and PSB population in soil as a result of introducing nonindigenous strain of PSB. Capability of the introduced bacteria of responding external stimulant, such as soil pH, nutrient availability, soil moisture, is an important characteristic. Such capability reflects adaptability of the bacteria to their new environment. The increasing population density of PSB in the soils of current experiment demonstrated positive response of the introduced PSB to the growing factors in the soils, such as sufficient availability of Al- and Fe-P in soils and favorable soil moisture and temperature during the experiment. In addition, the increases in population density of PSB observed in current study as well in previous study by Reyes *et al.* (2002) confirmed that the introduced PSB successfully colonized the soil and interacted with the indigenous PSB.

The population density of PSB in the soils was fluctuating over time (Table 1). The declining population density of PSB in the uninoculated soils occurred because the soil-born PSB could not survive the growth condition (nutrient deficiency and unfavorable soil micro conditions), and this constraint is known as a ‘microbiostatic’ phenomenon (Ho and Ko 1985). Because the

Table 1. Dynamics of PSB population in tested soils

Soils	Inoculant density ----- cells ml ⁻¹ of inoculant -----	PSB population at				
		0d	7d	15d	30d	60d
		----- ×10 ⁹ cfu g ⁻¹ of soil -----				
Ultisols	0	0.000057	0.00005	0.00049	0.000047	0.00044
	0.12 × 10 ⁵	0.000057	0.055	5.46	0.0054	0.0068
	0.12 × 10 ¹⁰	0.000057	4.73	6.69	0.05	0.06
	0.12 × 10 ¹⁵	0.000057	5.37	7.71	0.44	0.53
Freshwater- lowland Inceptisols	0	0.000045	0.00017	0.0002	0.00018	0.00021
	0.72 × 10 ⁵	0.000045	0.12	0.20	0.17	0.21
	0.72 × 10 ¹⁰	0.000045	14.75	17.50	16.04	20.00
Tidal swamp Inceptisols	0	0.000045	17.81	20.06	19.98	21.45
	0	0.000028	0.00027	0.0003	0.00024	0.0003
	2.29 × 10 ⁵	0.000028	0.26	0.25	0.21	0.26
	2.29 × 10 ¹⁰	0.000028	25.22	23.83	17.86	21.33
	2.29 × 10 ¹⁵	0.000028	26.92	27.92	21.22	29.58

availability of nutrients, mainly P, in the soils used in current study was corrected prior to the inoculation of PSB, the decline in the population of PSB in the uninoculated soils was caused by the failure of the soil-born PSB to adapt to the controlled growth condition. In addition, it was also an evidence for an ecological selection process occurring in the soils. Hence, it is believed that the counted PSB in the uninoculated soils in current study belonged to PSB, which managed to overcome the 'microbiostatic' constraints.

The population of PSB in the inoculated soils showed similar pattern of dynamics. The population density increased up to 15 days after incubation, and declined subsequently. The population decline occurred through several mechanisms (Elliot *et al.* 1980; van Elsas *et al.* 1991). Soil characteristics, such as texture, pH, temperature, and moisture content, exert direct effects on microbial population in soils because these factors determine cell performance (Elliot *et al.* 1980; van Elsas *et al.* 1991). Because these factors were kept uniform during the experiment, the decline in the PSB's population observed in current study was not driven by factors as described by Elliot *et al.* (1980) and van Elsas *et al.* (1991). In stead, it was caused by indirect effects of the abiotic factors, stimulating competitions between PSB and any other microbes in the soils as explained by Paul and Clark (1989) and Berry and Hagedorn (1991).

In addition to the abiotic factors, biotic factors are also contributing factors to the declining population of PSB in current study. Protozoa living in the soils can function as a regulator of the survival of introduced bacteria because they graze on the inoculated bacteria (Danso and Alexander 1975; Habte and Alexander 1975; Heijnen *et al.* 1988; Wright *et al.* 1993; Wright *et al.* 1995). The decline in the population of the introduced bacteria was significantly correlated with the increases in protozoa activity in the soils (Heijnen *et al.* 1988; Wright *et al.* 1995). Once the soils were sterilized prior to inoculation, the decline was not observed (Heijnen *et al.* 1988).

Additionally, population decline was also driven by competition over substrat and space between the indigenous microbes in the soils and the inoculated PSB. As shown in the previous experiment, strelized soils were inoculated with consortium of bacteria, protozoa, and combination of bacteria and protozoa. The soils receiving those treatments were then inoculated with *Rhizobium leguminosarum* bv. trifolii. The results showed that population of the rhizobia were not affected by single inoculation, either bacteria alone or protozoa

alone. Significant decline in the population of the introduced rhizobia was observed only in soils receiving dual inoculations of bacteria and protozoa (Postma *et al.* 1990).

Current experiment also found significantly different population density of PSB among the three tested soils (Table 1). The highest population density of PSB during the experiment was consistently observed in the tidal-swamp Inceptisols. In a field experiment using genetically modified bacteria, it was showed that under the same climate condition, the introduced *Pseudomonas* had better survival rate in fine-textured soils (clay) as compared with those in coarse-textured soils (van Elsas *et al.* 1986). It happened because clay could physically protect the introduced *Pseudomonas* from predation by the protozoa (Marshall 1975). However, such argument failed to describe why the population density of PSB in the tidal-swamp soil of current study was higher than that in the other two soils in spite of the fact that the tidal-swamp Inceptisols had the lowest content of clay fraction (13.8%), while the Ultisols and the fresh-water lowland Inceptisols had 24.4% and 35.2%, consecutively.

Chemically, the three tested soils were acidic with pH of 4.60 in Ultisols, 4.85 in fresh-water lowland soil, and 4.69 in tidal-swamp soil. Hence, soil pH could not either be regarded as an important driving force that caused the differences in population density of PSB in the three soils. In seems that, higher amount of fixed P (Al- and Fe-P) and organic matter in the tidal-swamp soil than those in the other two soils appeared to be an important factor for the survival and growth of PSB.

Population Density of PSB and Available P

Amount of available P in the three soils shows steady increases with time (Figure 1). The increases were exceptionally already evident at 7d of incubation and highest in the Ultiosols. To further investigate the main source of P in current experiment, both Al- and Fe-P complexes were also measured, as presented in Figure 2. The increases in available P in the three tested soils were significantly correlated with the decreases in both Al- and Fe-P (Figure 3) and with the increases in PSB population (Figure 4).

Soil microbes have been recognized as an important component linking soil system with plant, mainly through their roles in biogeochemical cycle of soil nutrients (Jeffries and Barea 1994; Oberson *et al.* 1993). About 20% of soil microbes are contributing to the solubilization of unavailable inorganic P, such as Al- and Fe-P, and known as

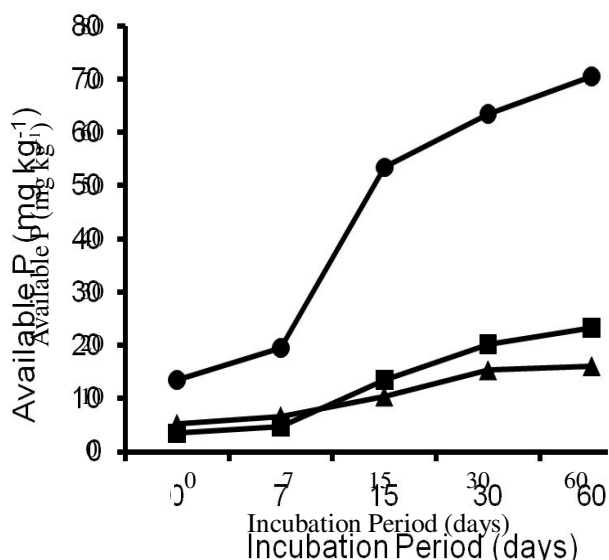


Figure 1. Changing in available P in Ultisols (●), fresh-water lowland Inceptisols (■), and tidal-swamp Inceptisols (▲)

PSB (Goldstein 1986; Cerezine *et al.* 1988; Illmer *et al.* 1995; Gyaneshwar *et al.* 1998).

Two main sources of available P in the current study (Figure 1) include decomposition of organic P and solubilization of unavailable inorganic P. Decomposition of organic P was not measured in the current experiment. However, the decreases in the Al-P and Fe-P (Figure 2) indicate that these two pools of the unavailable P contributed to the increases of the available P in soils. The decline of Fe-P in the three tested soils showed a similar pattern (Figure 2a), while Al-P in the tidal-swamp Inceptisols showed a sharp decline during the first week of incubation and showed a similar patten for the rest of incubation period of the three soils (Figure 2b). This argument was supported by a significant relationship between the decreases of the fixed P and the increases of the available P in the three tested soils, as shown by Figure 3.

Figure 4 depicted the relationship between the population density of PSB and the available P in the three tested soils. Figure 4 clearly shows that the increases of the available were significantly correlated with the increases of the PSB population. About 60%, 55%, and 69% of the increases in the available P in the Ultisols, fresh-water lowland Inceptisols, and tidal-swamp Inceptisols, were mediated by the activity of PSB. In a previous experiment it was shown that inoculating calcareous soils with 10^9 cells of PSB g^{-1} of soils increased the available P as much as $60 \mu g g^{-1}$ of soil as compared with the uninoculated control (Egamberdiyeva *et al.* 2004). However, acid soils used in the current

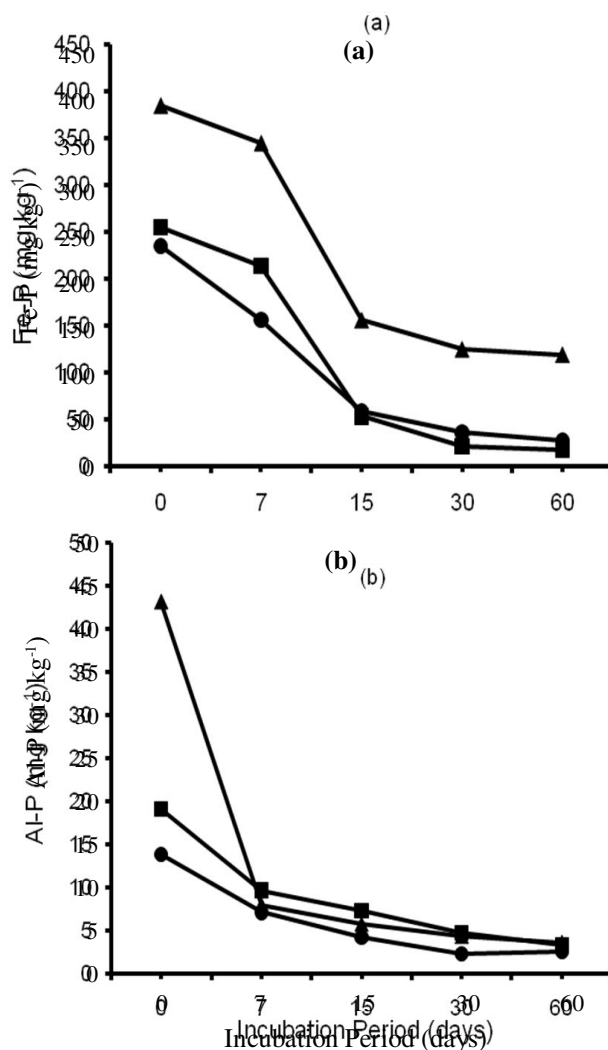


Figure 2. Changing in fixed P in Ultisols (●), fresh-water lowland Inceptisols (■), and tidal swamp Inceptisols (▲).

study showed different pattern of response. The highest available P contents were $72.6 \mu g g^{-1}$ in the Ultisols, $29.40 \mu g g^{-1}$ in the fresh-water Inceptisols, and $17.55 \mu g g^{-1}$ in the tidal-lowland Inceptisols. At the same time, the population density of PSB was 7.71×10^9 cells g^{-1} in the Ultisols, 19.98×10^9 cells g^{-1} in the fresh-water Inceptisols, and 21.22×10^9 cells g^{-1} in the tidal-swamp Inceptisols, consecutively. Such differences were likely due to the inherent differences of characteristics among the three tested soils, which were were important in determining whether P released through biological dissolution by the PSB would be adsorbed by soil components or readily available for plant uptake (Bertrand *et al.* 2003; Daly *et al.* 2001; Leclerc *et al.* 2001). Zhang *et al.* (2005) further explained that the significant correlation observed between P sorption capacity and clay content was due to the significant indirect influences of Al_{ox} and Fe_{ox} .

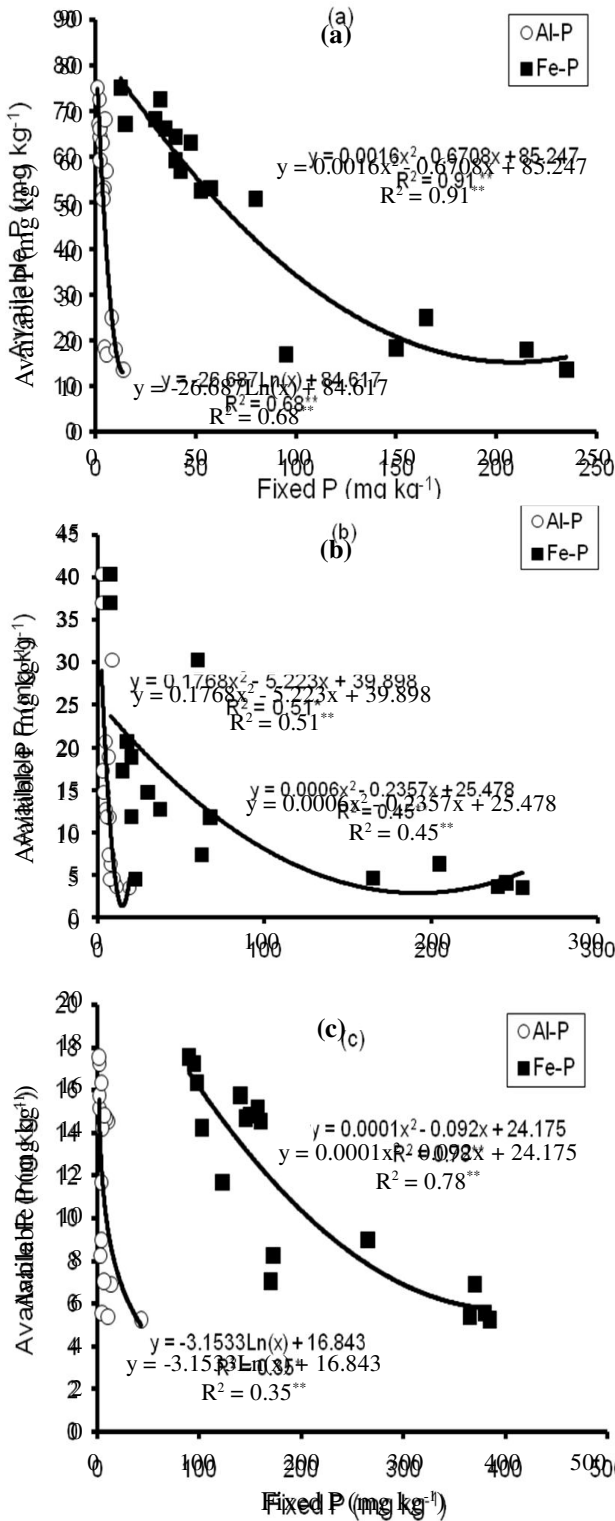


Figure 3. Relationship between fixed P and available P in Ultisols (a), fresh-water lowland Inceptisols (b), and tidal-swamp Inceptisols (c). ■ = Al-P, ○ = Fe-P.

Figure 4 also shows that the threshold population size of PSB to significantly increase the available P in the acid soils of Indonesia must be at least 0.055×10^9 cfu g^{-1} of soil in the Ultisols, 0.17×10^9 cfu g^{-1} of soil in the fresh-water Inceptisols, and 0.21×10^9 cfu g^{-1} of soil in the tidal-swamp

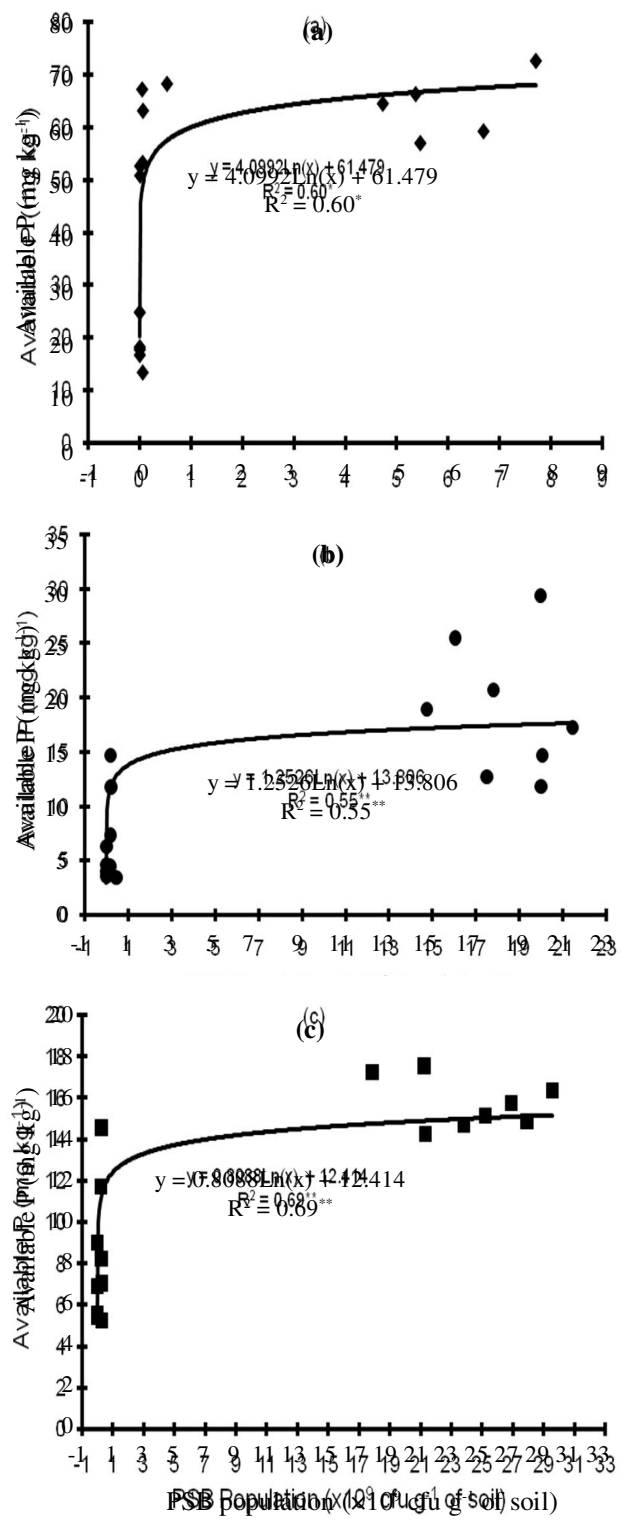


Figure 4. Relationship between available P and population density of PSB in Ultisols (a), fresh-water lowland Inceptisols (b), and tidal-swamp Inceptisols (c).

Inceptisols. Although it has been widely known that biological solubilization of fixed P in soil is through soil acidification, current experiment revealed that such mechanisms was not occurring. Instead of decreasing, the pH of soils used in current experiment increased with time, from pH of 5.03

in the Ultisols, 4.78 in the fresh-water Inceptisol, and 4.04 in the tidal-swamp Inceptisols at the start of the experiment to pH of 5.61 in the Ultisols, 5.17 in the fresh-water Inceptisols, and 4.48 in the tidal-swamp Inceptisol at the end of the experiment. A similar finding has also been reported earlier that inoculating soils with PSB did not necessarily result in any increases of organic acids and decreases of soil pH in spite of the increases in available P in soils (Asea *et al.* 1988; Illmer and Schiner 1995; Altomare *et al.* 1999). Therefore, it is concluded that the increases in the available P observed in current study were resulted in by chelation of both Al and Fe, as shown by the significant decreases of Al- and Fe-P omplexes in Figure 2 (He *et al.* 1990; Violante *et al.* 1991; He *et al.* 1992; Altomare *et al.* 1999; He *et al.* 2002).

CONCLUSION

Based on the results obtained it can be concluded that the availability of P in soils of current experiment was significantly correlated with the population size of PSB. The PSB population size determined the availability of P in Ultisols, fresh-water lowland Inceptisols, and tidal-swamp Inceptisol as much as 60%, 55%, and 69%, consecutively. To obtain significant correction of the available P in the Ultisols, fresh-water lowland Inceptisols, and tidal-swamp Inceptisols, the population of PSB must be at least 0.53×10^9 cfu g⁻¹ of soil, 2.94×10^{10} cfu g⁻¹ of soil, and 1.73×10^{10} cfu g⁻¹ of soil, cosecutively.

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