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The mechanism of phosphate bacteria in increasing the solubility of phosphorus in Indonesian Andisols

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Abstract

The purpose of this research was to determine: 1) phosphate bacteria (PB) mechanisms responsible for enhancing the soil's soluble phosphorus (P), using the processes of inorganic P solubilisation, organic P mineralization, and blocking of soil colloidal adsorption site, and 2) to investigate the factors contributing to this increase.

Phosphate bacteria (PB) was inoculated into sterile Andisols in three separate compositions, termed 1 kg P·kg^{-1} (2.82 g phosphate rock, 0.5 g $Ca_3(PO4)_2$, 0.4 g $Al_3(PO4)_2$, or 0.4 g $Fe_3(PO4)_2$), organic P (0.5 cm^3 *para*-Nitrophenylphosphate (pNPP) or 0.5 g Na-phytate), and 1 kg P (KH_2PO_4)·kg^{-1}, in order to analyse inorganic P solubilisation, organic P mineralization, and evaluate blocking soil colloidal site for adsorption P, respectively. Furthermore, spectrophotometry technique was applied to determine the amount of dissolved P. The PB showed an improvement in inorganic P solubilisation from 147.66 to 194.61 mg P·kg^{-1}, and also in organic P from 63.6 to 91.7 mg P·kg^{-1}, compared to control, (31.06 mg P·kg^{-1}) and (23.7 mg P·kg^{-1}), respectively. Meanwhile, the micro-organisms were known to decrease P adsorption by 13.43%, beyond the restraint set at 85.34%. Therefore, increased soluble P in Andisols is possibly expressed, using the equation as follows: soluble P (mg P·kg^{-1}) = 1201.96 + 1.18 inorganic P solubilisation (mg P·kg^{-1}) + 1.09 organic P mineralization (mg P·kg^{-1}) – 0.92 adsorption P (mg P·kg^{-1}) (R^2 = 0.99).

Key words: *adsorption, Andisols, mineralization, phosphate bacteria, solubilisation*

INTRODUCTION

Over 40% of soils across the world are known to suffer phosphorus (P) deficiency [VANCE *et al*. 2003], and the need to increase the mineral availability appears very significant, in order to ensure plant growth, using P fertilizers. However, high application of stimulants does not necessarily guarantee absolute usefulness to plants, due to low solubility of approximately 10–20%. This minimal provision is associated with P fixation by Fe and Al oxide/hydroxide to form crystalline. Andisols are soils with relatively low P solubility, attributed to P fixation by allophane of specific surface area within 100–800 m^2·g^{-1}, CEC 5–350 cmol(+)·kg^{-1}, Si/Al ratio of about 0.5 and is also very reactive. These soils contribute immensely to Indonesia's land mass covering 5.4 mln ha, with intensive agricultural potentials [FIANTIS *et al*. 2005]. Therefore, significant increase in the P availability, specifically in Andisols, is very essential.

Phosphate fertilizers are sources of phosphorus supply to soils [GAJ *et al.* 2018], and the solubility tends to incline by PB inoculation. However, P biofertilizer are supplements and/or alternatives to chemical composts for the purpose of developing sustainable agriculture [HAJJAM, CHERKAOUI 2017]. In addition, PB is well known to release adsorbed P and potentially enhances soil available P by inorganic P solubilisation, organic P mineralization, and blocking soil colloid adsorption site [CASTAGNO *et al.* 2011]. Therefore, the inoculation of micro-organisms presents a potentially important strategy towards increasing P availability in soils [SPOHN *et al.* 2013].

Furthermore, PB shows the capacity to solubilize inorganic P by releasing citric, malic, fumaric, glutamic, succinic, lactic, oxalic, glycooksalic, and β-ketobutyric acids, where most are secreted into the soil [CASTAGNO *et al.* 2011]. Subsequently, organic acid released by phosphate microorganisms tends to greatly improve P content in soil solutions, through chelation and exchange reactions [JAMAL *et al.* 2018]. Also, the influence of these acids on P depends on the acid type and the ratio of organic acid ligands to P anions [WEI *et al.* 2009].

The average quantities of soil P produced by entire organic acids increased in sequence of mono-carboxylic acid, di-carboxylic, and the tri-carboxylic acid groups. Similarly, tri-carboxylic citric acid showed significant improvement in soil solution P, compared to di-carboxylic oxalic acid, probably due to rapid degradation of di-carboxylic oxalic acids in soils. Based on the equilibration of soils with citric or oxalic acids, the liberation of P tends to depreciate, but generated an enhance response time of solution P equilibration [MENEZES-BLACKBURN *et al.* 2016]. This discharged P was negatively correlated to the equilibrium soil pH, but reflected positively for soil Ca [KPOMBLEKOU, TABATABAI 2003].

In general, phosphate bacteria are able to mineralize organic P, due to the secretion of phosphatase and phytase, both referred as catalysts. The phosphatase hydrolyses organic P to form inorganic phosphate/orthophosphorusic acid (–2 and –1), while phytase influences phytic acid, glucose 6-phosphate and glycerol 1-phosphate, resulting to inositol and ortho phosphorusic acid [AHMED *et al.* 2008]. These chemical agents are synthesized by micro-organisms in response to nutrient or energy limitation [KONIETZNY, GREINER 2004]. Based on optima pH, phosphatases are divided into acid and alkaline. Typically, the acid group predominates in acid soils, while alkaline reagents are more abundant in neutral and alkaline soils [MUKHAMETZYANOVA *et al*. 2012].

Organic P availability (inositol phosphate) requires mineralization (hydrolysis) of substrates by phosphatase and phytase enzymes. In addition, up to 60% of the total volume are probably hydrolysed by phosphatase, with highest quantities released by phytase known as monoester phosphatase active on phytate disintegration [RICHARDSON, SIMPSON 2011]. These phytates are strong chelating agents, and are responsible for binding the bivalent metal cations, as well as peptides and low-molecular metabolites into resilient poorly degradable compounds [SHARMA *et al.* 2013]. Also, the capacities of organic P mineralizing micro-organisms range from 8.2 to 17.8 μg $P \cdot cm^{-3}$ [TAO *et al.* 2008].

The P fixation in soil colloidal is generally attributed to phosphate adsorption, and therefore, is not readily available to plants. Micro-organisms tend to increase plant mineral availability by facilitating the transformation and distribution of separate P pools. However, under pure culture conditions, particular species have been shown to transform specific adsorbed P on certain soil samples, with greater capability, compared to others [HE *et al.* 2002].

The PB secrete organic acids into the soil to compete with phosphate ions for adsorption sites, resulting to less available minerals. Meanwhile, the introduction of PB leads to a decline in maximum soil adsorption capacity and adsorption constant, but increases the maximum desorption capacity and the average desorption rate of soil P [SHI *et al.* 2017]. These bacteria also demonstrate the ability to decrease P adsorption by soil colloidal, due to relative surface charge of PB cells (r/e cation and r/e anion). Also, the organic acid anions are able to compete with phosphate in order to occupy the soil colloidal adsorption site, leading to the formation of complex inner-sphere bonding [AHMED *et al.* 2008]. However, minimum P-adsorption capacities of Australian soils for organic acid and phosphate were estimated at 357 and 500 μg $P \cdot g^{-1}$, while buffering abilities were evaluated at 71.7 and 93.7 μg $P \cdot g^{-1}$, respectively [AHMED *et al.* 2008].

Phosphate bacteria potentially increases soil P dissolution by applying the processes of inorganic P solubilisation, organic P mineralization and released P from soil colloidal adsorption sites. However, the mechanisms and contributions involved require further investigations. These conditions for enhancing soil P solubility are described as the fundamental topics on present study, and the results formed the bases for incremental efforts. The objectives of this research were: 1) to determine dissolved P mechanisms and 2) to investigate the contribution of the system towards increasing soil soluble P. Furthermore, the formulated research hypothesis assumed the intentions for this experiment were achieved through the aforementioned processes.

MATERIALS AND METHODS

INORGANIC PHOSPHORUS SOLUBILISATION BY PHOSPHATE BACTERIA

Phosphate bacteria (PB) species applied as inoculants, are a consortium of *Pseudomonas trivialis*, *P. putida* and *P. fluorescens* with 10^8 colony forming unit (cfu) per cm^3 population. The PB were isolated from Ajibarang, Banyumas, Central Java, Indonesia's phosphate rock site, and were cultured in Pikovskaya media for 5 days (stationary phase) at 25°C. Subsequently, 10 cm^3 per dm^3 or per kg^{-1} of the specimen was injected into the soil sample and incubated for a month at 25°C. Furthermore, liquid PB inoculant carrier was comprised of 20% (v) sterile molasses and 5 g soybean extract per dm^3, while the solid portion was a mixture of 20% (100 mesh) sterile husk ash, 20% rice bran and 20% tapioca waste, 3.0% acetic acid, 2.0% $CuSO_4 \cdot 5H_2O$, 5% zeolite, and 30% H_2O [TAMAD, MARYANTO 2010].

Andisols were obtained at a depth between 0–40 cm from Gandatapa, Banyumas, Central Java, Indonesia, located on the eastern slope of Mount Slamet 3,432 m a.s.l. at an altitude around 600–1,200 m a.s.l. (Tab. 1). Also, atmospheric temperature ranged from 8 to 22°C during rainy season and from 4 to 12°C at dry period. Furthermore, the ratio SiO/AlO of sample soils was estimated at 0.26, indicating a positive reactivity to phosphate [ELSHEIKH *et al.* 2009]. Prior to commencement, these materials were sterilized, using co-irradiation at a dose of 25 kGy (2.5 Mrd) [NNEA/BATAN 2010].

Table 1. The characteristics of Gandatapa Andisols

Characteristics	Value	Criterium
Texture	40.5% sand; 37.2% silt; 22.3% clay	loam
Bulk density	0.87 g·cm^{-3}	
Total exchangeable bases	17.38 cmol(+)·kg^{-1}	
Soil exchangeable cations	31.04 cmol(+)·kg^{-1}	high
Base saturation	56%	medium
$pH_{(H2O)}$	6.1	slightly acid
$pH_{(NaF)}$	10.7	
Organic matter	10.54%	high
Total P	15.6% P_2O_5	low
Adsorption P	86.3%	
Available P	2.4 mg P·kg^{-1}	very low

Source: TAMAD [2012].

Phosphate rock was acquired from Ajibarang, Banyumas, Central Java, Indonesia (Tab. 2). According to ISO 02-3776-2005, the rock sample qualified as a natural P fertilizer of grade C.

Table 2. The characteristics of Ajibarang phosphate rock

Characteristics	Value
pH	7.2
$CaCO_3$ equality	16.95%
Total P	16.23% P_2O_5
2% citric acid soluble P	3.42% P_2O_5
Water soluble P	1.98–2.22% P_2O_5

Source: TAMAD [2012].

Organic acids were identified using HPLC (Hitachi, column OOF4250-CO/10 µm LaChrom Ultra C 18 (2 m) 100 A 150 × 4.60 mm 10 m KPOW 490065-1 Phenomenex), and also quantified by comparing retention times and peak areas with the acid standards [WEIMIN *et al.* 2016]. Dissolved P, phosphatase and phytase activities were assessed using ammonium vanado molybdate stains and UV-VIS-1240 Shimadzu spectrophotometer readings at 413 nm wavelength. These enzyme activities were expressed in mg P·cm^{-3} culture·h^{-1} incubation [VILLAMIZAR *et al.* 2019].

The laboratory experiment on inorganic P solubilisation in Andisols was conducted in a complete randomized design (CRD) of three factors, termed inoculant type, P sources, and PB inoculation time, with three replications. First trigger involves a type of PB inoculant (*Pseudomonas trivialis*, *P. putida* and *P. fluorescens*), comprised of control (no inoculation) and treatments with 8 separate PB inoculant, including liquid carrier (C0), liquid one isolate (C1), liquid two isolates (C2), liquid three isolates (C3), solid carrier (P0), solid one isolate (P1), solid two isolates (P2), and solid three isolates (P3). Also, 10 cm^3 liquid carrier or 10 g solid inoculant (10^8 cfu PB·g^{-1} solid or 10^8 cfu PB·cm^{-3} liquid) was injected into 100 g steril Andisols. Subsequently, the sample was incubated at room temperature for two weeks and maintained at field capacity during the incubation period. The second factor referred to P sources, consisted of control (no P_2O_5) and 400 kg P_2O_5·ha^{-1}, while the third was PB inoculation time, specified at two weeks. Furthermore, soluble P (NVM staining), P solubilizing effectivity (calculation), pH (pH metre), total acidity (titration), organic acids (HPLC) and PB population (total plate count) were also measured.

ORGANIC PHOSPHORUS MINERALIZATION BY PHOSPHATE BACTERIA

The laboratory experiment on organic P mineralization in Andisols involved complete randomized design (CRD) of three factors, termed PB inoculant, organic matter dose, and inoculation time, with three replications. First factor was described as PB inoculant (*Pseudomonas trivialis*, *P. putida* and *P. fluorescens*, comprised of control (no inoculation) and 8 varied PB inoculants, including liquid carrier (C0), liquid one isolate (C1), liquid two isolates (C2), liquid three isolates (C3), solid carrier (P0), solid one isolate (P1), solid two isolates (P2), and solid three isolates (P3). Also, 10 cm^3 liquid carrier or 10 g PB solid inoculant (10^8 cfu PB·g^{-1} solid or 10^8 cfu PB·cm^{-3} liquid) was injected into 100 g steril Andisols of two weeks incubation period at room incubated temperature and maintained at field capacity. The second consisted of (compost 2.6% P_2O_5) control (no compost) and 17.4 Mg compost per hectare (1%), while the third factor denoted a PB inoculant duration of two weeks. Furthermore, organic P mineralization, organic P mineralization efficiency, phosphatase and phytase activities (Tabatabai and Bremner method) [TABATABAI, BREMNER 1969], and PB population were measured.

SOIL ADSORPTION SITE BLOCKING BY PHOSPHATE BACTERIA

The laboratory experiment on PB capacity to block soil colloidal adsorption site was conducted in CRD of two factors, termed type and timing of PB inoculants (*Pseudomonas trivialis*, *P. putida* and *P. fluorescens*) with three replications in 100 g steril Andisols. First trigger consisted of control (no inoculation) and 8 separate PB inoculants, including liquid carrier (C0), liquid one isolate (C1), liquid two isolates (C2), liquid three isolates (C3), solid carrier (P0), solid one isolate (P1), solid two isolates (P2), and solid three isolates (P3), with three specified citric acid concentration (10 cm^3 of 2.5, 5.0 and 10 mmol) in original soil pH. Also, 10 cm^3 liquid inoculant or 10 g solid inoculant (10^8 cfu PB·g^{-1} solid or 10^8 cfu PB·cm^{-3} liquid) was injected into 100 g steril Andisols of two weeks incubation period at room incubated temperature and at field capacity. The second factor comprised of PB inoculation for two weeks. Subsequently, each experimental unit was added 60 cm^{3}of 10 mmol KCl solution containing 1 kg P (KH_2PO_4)·kg^{-1} with two weeks incubation period at room incubated temperature at field capacity. Maximum P adsorption (P adsorption (%) = P (100%) of the solution (1000 ppm) – P (%) in the filtrate). Furthermore, organic acid secretions, relative cell surface charge (optical density), and PB population were measured.

STATISTICAL ANALYSIS

The following data, termed soluble P, pH, total acidity, organic acids, P mineralization, phosphatase and phytase activities, maximum P adsorption, relative cell surface

charge, and PB population were analysed, using *F*-test with α = 5%. Treatment mechanisms were separated by Duncan multiple range test at 5% probability level. The soluble P equation of solubilisation of inorganic P, organic P mineralization, and P adsorption in Andisols was determined, using regression analysis (CoStat ver. 6311-Statistics Software).

RESULTS

INORGANIC PHOSPHORUS SOLUBILISATION BY PHOSPHATE BACTERIA

The interaction effect between inoculant types, P source, and PB inoculation duration on variables was not significant, but was very substantial for single factor influence. In addition, PB inoculation considerably increased inorganic P solubilisation, similar to an improvement in the bacteria population. However, Andisols pH declined from 6.20 to 5.94, and arrived at 5.70, as soil total acidity appreciated (Tab. 3). Subsequently, the soluble P of soil sample with PB, was also known to increase 5–7 times, compared to control (Tab. 3).

Table 3. Soluble P, phosphate bacteria population, pH and total acidity of Andisols influenced by phosphate bacteria inoculation

PB inoculant	Water soluble P (mg P·kg^{-1})	ESP (%)	Log PB population (cfu PB·cm^{-3})	Soil pH	Total acidity (me·100 g^{-1})
Control	31.06 f	100.00 f	0.00 d	6.20 a	1.19 c
C0	33.78 f	108.76 f	0.00 d	5.94 b	1.15 c
C1	152.11 d	489.73 d	12.98 c	5.74 c	1.97 b
C2	147.66 d	475.40 d	13.90 b	5.75 c	2.20 a
C3	166.25 c	535.25 c	14.21 a	5.78 c	1.88 b
P0	41.09 e	132.29 e	0.00 d	5.79 c	1.11 c
P1	188.99 a	608.47 a	13.04 c	5.74 c	2.43 a
P2	194.61 a	626.56 a	12.95 c	5.70 c	1.90 b
P3	178.84 b	575.79 b	13.49 bc	5.77 c	2.38 a

Numbers in column followed by the same letter are not different at DMRT (α = 5%). ESP = effectivity of soluble P, C = liquid PB inoculant, P = solid PB inoculant (0, 1, 2, 3 = number of PB species consortium)
Source: own study.

The PB inoculant of *Pseudomonas trivialis*, *P. putida* and *P. fluroscens* consortium effectively enhanced the soluble P, where the highest liberated organic acid was citric, while the lowest was acetic (Fig. 1, 2). Moreover, PB

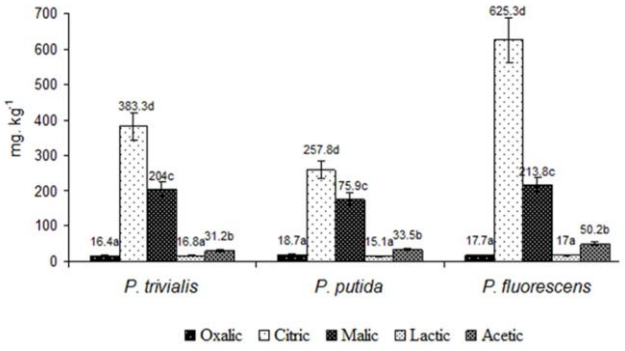

Fig. 1. Phosphate bacteria (PB) organic acids secreted in Andisols (4 week incubation, at 25°C temperature); source: own study

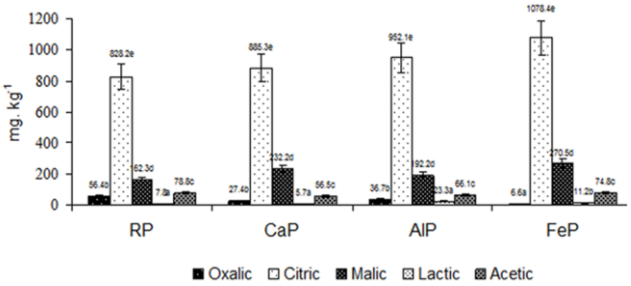

Fig. 2. Effects of P sources on phosphate bacteria (PB) organic acids secreted grown on Pikovskaya (5-day incubation, at 25°C temperature); source: own study

intensity in Andisols was more significant on calcium phosphorus, compared to other forms of P (Fig. 3), and the two weeks inoculation prior to P application, increased in soluble P, in line with PB population growth (Fig. 3, 4). However, PB ability to dissolve P was influenced by the species, phosphorus compound, released organic acids (Fig. 1, 2), and PB population (Fig. 3).

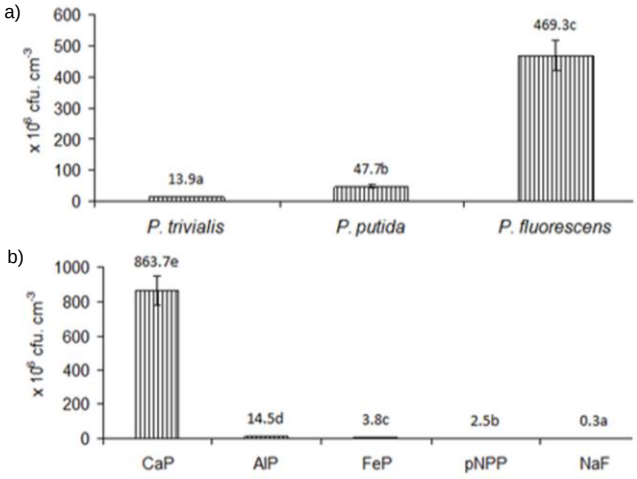

Fig. 3. PB population effects of PB type (a) and P source (b) at 25°C temperature incubated; source: own study

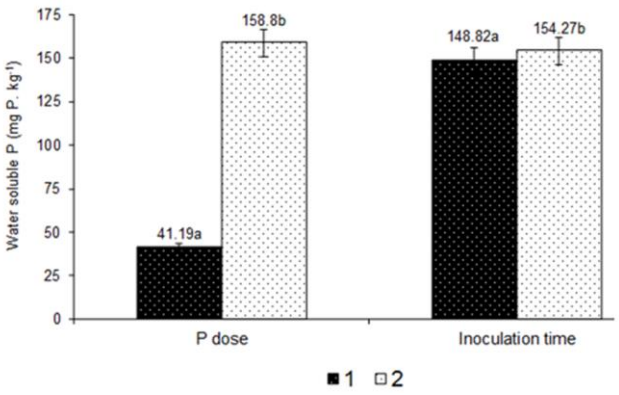

Fig. 4. Andisols P soluble influence by P dose and PB inoculation time (P dose: 1 = 0 kg P_2O_5·ha^{-1}, 2 = 400 kg P_2O_5·ha^{-1}; PB inoculation time: *1* = concurrent application of P, *2* = 2 weeks before P application); source: own study

ORGANIC P MINERALIZATION BY PHOSPHATE BACTERIA

The interaction effect between inoculants types, organic matter dose, and PB inoculation time on variables was not significant, but was very substantial for single factor influence. In addition, PB capability to mineralize organic P was dependent on phosphatase and phytase activities. The result showed an increase in the bacteria population and enzyme conditions (Tab. 4). Soluble P treated with PB was enhanced by 3–4 times, compared to control (Tab. 4).

Table 4. Organic P mineralization (OPM), phosphate bacteria population and phosphatase (Afo) and phytase (Afi) activity influence by phosphate bacteria inoculation

PB inoculant	OPM ws (mg P·kg^{-1})	OPM effectivity (%)	Log PB population (cfu PB·cm^{-3})	Afo	Afi
				mg PO_4^{3-}·dm^{-3}·h^{-1}	
Control	23.67 e	100.00 e	0.00 d	0.2 d	0.2 f
C0	23.85 e	100.76 e	0.00 d	0.4 d	0.3 f
C1	73.89 c	312.17 c	12.98 c	24.9 ab	26.8 c
C2	63.60 d	268.69 d	13.90 b	23.3 b	33.0 a
C3	79.67 b	336.59 b	14.21 a	24.0 ab	30.9 b
P0	23.67 e	100.00 e	0.00 d	0.3 d	0.6 f
P1	75.53 c	319.10 c	13.04 c	21.5 c	25.5 cd
P2	78.26 bc	330.63 bc	12.95 c	23.3 b	24.7 d
P3	91.69 a	387.37 a	13.49 bc	30.0 a	22.1 e

Numbers in column followed by the same letter are not different at DMRT (α = 5%); ws = water soluble, C = liquid PB inoculant, P = solid PB inoculant (0, 1, 2, 3 = number of PB isolates)
Source: own study.

Phosphate bacteria are effective to organic P mineralization between 6 and 16 mg P·kg^{-1} (Tab. 4). Also, enzyme activity of *Pseudomonas trivialis*, *P. putida* and *P. fluorescens* were relatively higher and estimated at 5 mg PO_4^{3-}·dm^{-3}·h^{-1}. In Andisols, the inoculation expanded PB population by $10^{13–14}$ cfu·cm^{-3} (Tab. 4) and the addition of organic matter tend enhanced organic P mineralization to 7 mg P·kg^{-1} (Fig. 5). PB inoculation of two weeks prior to additional P boosted organic P mineralization to 5 mg P·kg^{-1} and the PB population to 10 times (Tab. 4).

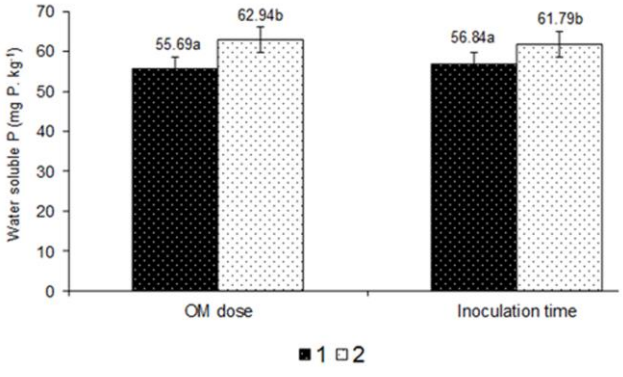

Fig. 5. Andisols organic P mineralisation influence by phosphate bacteria dose and time inoculation (OM = organic matter dose: 1 = 0, 2 = 17.4 Mg·ha^{-1}; inoculation time: *1* = concurrent application of OM, *2* = 2 weeks before OM application); source: own study

SOIL ADSORPTION SITE BLOCKING BY PHOSPHATE BACTERIA

The interaction effect between type and timing of PB inoculants on variables was not significant, but was very substantial for single factor influence. Gandatapa Andisols was able to adsorb P up to 85% of the total quantity and PB was known to decrease the mineral in line with increasing PB population (Tab. 5). Moreover, PB inoculation significantly released P from adsorption site by 3–6 times, compared to control (Tab. 5).

Table 5. Soluble P, adsorbed P, adsorption P and phosphate bacteria population in Andisols influence by phosphate bacteria inoculation

PB inoculant	ws P (mg P·kg^{-1})	Adsorbed P		APE (%)	Log PB (cfu PB·cm^{-3})
		mg P·kg^{-1}	%		
Control	52.11 a	947.89 e	85.34 e	100.00 e	0.00 a
C_0	53.69 a	946.31 e	85.20 e	99.84 e	0.00 a
C_1	644.83 c	355.17 c	35.52 c	41.62 c	16.42 c
C_2	641.30 c	358.70 c	35.87 c	42.03 c	15.95 bc
C_3	742.00 d	258.00 b	25.80 b	30.23 b	14.60 b
P_0	57.08 a	942.93 e	84.89 e	99.47 e	0.00 a
P_1	759.67 d	240.33 b	24.03 b	28.16 b	14.64 b
P_2	848.00 e	152.00 a	15.20 a	17.81 a	16.53 c
P_3	865.67 e	134.33 a	13.43 a	15.70 a	17.03 e
2.5 mM CA	538.83 b	461.17 d	46.12 d	54.04 d	0.00 a
5 mM CA	538.83 b	461.17 d	46.12 d	54.04 d	0.00 a
10 mM CA	591.83 b	408.17 d	40.82 d	47.83 d	0.00 a

Numbers in column followed by the same letter are not different at DMRT (α = 5%) (Andisols water soluble P is 94.40 mg P·kg^{-1}); ws = water soluble, AEP = adsorption effectivity P, CA = citric acid).
Source: own study.

Andisols were able to adsorbed P up to 85.34% (of 1 kg P·kg^{-1}) (Tab. 5). Also, PB inoculation decreased P adsorption from 36 to 13%. Three species of solid PB inoculant were relatively more effective to reduce the process, due to an increase in PB population (log cfu PB·cm^{-3} = 17.03), and the PB surface charge (69%), consisting of surface charge anion of 25% and surface charge cation of 30–40% (Tab. 5).

The increase in soluble P and the reduction of adsorbed P by adsorption site were related to an expanding PB population (Tab. 5). In addition, the adsorbed P was estimated at 14.9% for the first week, but decreased to 10.99% after two weeks of incubation (Fig. 6). Also, adsorbed P was

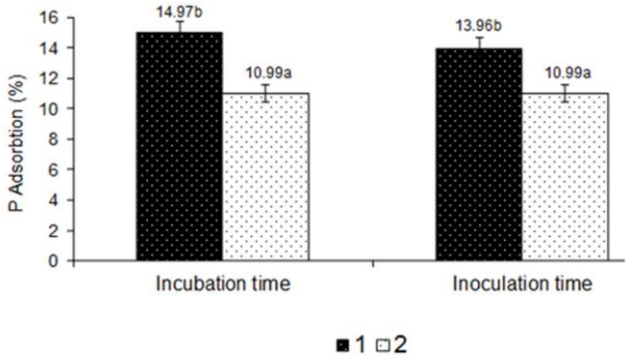

Fig. 6. Andisols P adsorbed influence of phosphate bacteria incubation and inoculation time (incubation: 1 = 1 week, *2* = 2 week; inoculation time: *1* = concurrent application of P, 2 = weeks before P application); source: own study

achieved at 10.99% as PB was inoculated two weeks prior to P application and 13.96% at similar occurrence as P application (Fig. 6).

The PB inoculation increased the solubilisation of inorganic P and organic P mineralization, and decreased P adsorption in Andisols. Soluble P is expressed as follows: soluble P (mg P·kg^{-1}) = 1201.96 + 1.18 inorganic P solubilisation (mg P·kg^{-1}) + 1.09 organic P mineralization (mg P·kg^{-1}) – 0.92 adsorption P (mg P·kg^{-1}) (R^2 = 0.99).

DISCUSSION

The soil pH reduction and multiplicationin PB population greatly contributed to the increase in soluble P [DELFIM *et al.* 2018]. Also, the influence of PB population on soluble P is related to microbial growth and the activities [HAJJAM, CHERKAOUI 2017]. CASTAGNO *et al.* [2011] reported the existence of a positive correlation between PB population and soluble P. In addition, the mineral was negatively correlated (–0.80) with the medium pH and the production of organic acids by PB (–0.68), but positively associated (0.42) with PB population [CASTAGNO *et al.* 2011].

Furthermore, PB showed the ability to solubilize phosphate complexes by acidification, chelation, and exchange reactions (LI *et al.* 2017). The secreted organic acids, including citric (tricarboxylic), oxalic, malic, tartaric, fumaric, malonic and gluconate (dicarboxylic) tend to dissolve P [LACOBAZZI *et al.* 2009].

Phosphatase and phytase were capable of mineralizing organic P. The phosphatase enzyme was responsible for the hydrolysis of organic phosphorus into inorganic phosphate/ orthophosphorusic acid (–2 and –1), although the specie with extra cellular phosphatase was dominant at acidic pH, while for intra cellular, alkaline pH appeared more prevailing. Meanwhile, phytase described as phytic acid hydrolysis catalyst, was responsible for converting glucose 6-phosphate and glycerol 1-phosphate to inositol and orthophosphorusic acid. Phosphatase activity was determined by hydrolysis pNPP (paraNitrophenylphos-phate), while for phytase, hydrolysis of phytate was involved [NAMLI *et al.* 2017].

The PB phosphatase activity as an indicator of organic P mineralization ranged from 21.5 to 30.0 mg PO_4^{3-}·dm^{-3}·h^{-1}, while phytase was from 22.1 to 43.0 mg PO_4^{3-}·dm^{-3}·h^{-1} [MOTAMEDI *et al.* 2016]. NAMLI *et al.* [2017] stated the PB phosphatase activity ranged from 14.4 to 35.5 mg PO_4^{3-}·dm^{-3}·h^{-1} and for PB phytase, the interval was from 4.8 to 24.9 mg PO_4^{3-}·dm^{-3}·h^{-1}.

The decrease in P adsorption by colloidal sites of Andisols is a function of relative PB surface charge known to bind the soil colloidal [JAIN *et al.* 2007]. Relative surface charge anion was evaluated at 25%, and for the cation, the range was between 30 and 40%. However, PB cationic charge appeared relatively higher, compared to anionic. This shows the PB is more reactive to negative charge nano molecule [JAIN *et al.* 2007].

Gram negative bacteria cell wall comprises peptidoglycan, phospholipids and lipopolysaccharide. Lipopolysaccharide consists of O-polysaccharides, lipoproteins and fatty acids as a source of surface charge cell [JAIN *et al.* 2007]. Relative surface charge r/e anion of bacteria was between 1 and 85%, while r/e cation ranged from 0.2 to 30%, with a surface area within 30–973 mm^2·10^{-6} cells [JAIN *et al.* 2007]. Anions of organic acids were able to successfully compete with phosphate for soil colloidal adsorption site [JAIN *et al.* 2007]. As a result, anions decreased P adsorption by (1) competition with phosphate for soil adsorption site and (2) chelate metal ions, with no bound to phosphate [AHMED *et al.* 2008].

CONCLUSIONS

Phosphate bacteria (PB) inoculation significantly increased inorganic phosphorus (P) solubilisation and organic P mineralization, but drastically decreased the adsorption P in Andisols. The PB ability to dissolve P was influenced by the species, phosphorus compound, released organic acids, and PB population. Furthermore, PB capability to mineralize organic P was dependent on phosphatase and phytase activities. However, the bacteria were known to substantially decline the adsorbed P, with increasing population.

The PB contribution in enhancing soil soluble P is known to vary. Andisols soluble P is described by the equation as follows: soluble P (mg P·kg^{-1}) = 1201.96 + 1.18 inorganic P solubilisation (mg P·kg^{-1}) + 1.09 organic P mineralization (mg P kg^{-1}) – 0,92 P adsorption (mg P·kg^{-1}) (R^2 = 0.99).

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