

Diversity of phosphate solubilizing bacteria and fungi from andisol soil affected by the eruption of Mount Sinabung, North Sumatra, Indonesia

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Abstract. Sembiring M, Sabrina T. 2022. Diversity of phosphate solubilizing bacteria and fungi from andisol soil affected by the eruption of Mount Sinabung, North Sumatra, Indonesia. *Biodiversitas* 23: 714-720. Phosphate solubilizing microbes are present in all locations with different population levels. The eruption of Mount Sinabung in 2013 resulted in changes in soil pH and the soil's microbial population, such as phosphate solubilizing microbes. This research aimed to obtain environmentally-specific phosphate solubilizing microbes derived from andisol soil impacted by the eruption of Mount Sinabung, which excelled in increasing the availability of phosphate. Sampling was conducted in Desa Kutarayut, Naman Teran Sub-district, Karo District, North Sumatra Province, Indonesia. Isolation of phosphate solubilizing microbes was carried out on pikovskaya media, and test of phosphate solubilizing microbes potential was conducted on solid and liquid pikovskaya media. Organic acid content was determined using high performance liquid chromatography (HPLC) and microbial identification was performed using PCR-sequencing. The result showed that 4 isolates of each phosphate-soluble fungi and bacteria were isolated from andisol soil affected by the eruption of Sinabung mountain. It was also observed that phosphate solubilizing microbes' ability to produce organic acids varied. The obtained fungi could dissolve P in some phosphate source either on solid media or liquid medium. *Talaromyces pinophilus* (J2) can increase the highest availability of P in andisol soil.

Keywords: Andisol, phosphate solubilizing fungi, pikovskaya media, P available, *Talaromyces pinophilus*

INTRODUCTION

The presence of phosphate solubilizing microorganisms varies greatly from one place to another. One of the factors that cause such diversity is its biological nature. These microorganisms have distinctive features and different optimal environmental conditions that affect its effectiveness (Sharma et al. 2013; Gupta et al. 2017). In acid soil, the activity of microorganisms is dominated by fungi because fungi grow optimally at 3-5.5 pH. Fungal growth decreases when pH increases. The pH level of andisol soil ranges from 4.00 to 5.33, which is acidic pH (Sinaga et al. 2015; Sembiring et al. 2016; Sembiring et al. 2017a, b). This may affect the number and type of phosphate solubilizing microbes capable of living under such soil conditions. The presence of phosphate solubilizing microbes in the soil varies greatly depending on the soil's physics-chemical characteristics (Khan et al. 2013; Bi et al. 2019).

Phosphate solubilizing microorganisms can be isolated from soils with low phosphate content, especially around plant roots. These microbes use phosphate in small quantities and can utilize phosphate that is not available for metabolic purposes. The ability of P solubilizing fungi in dissolving is different depending on the strain type. Insoluble P forms such as tricalcium phosphate (Ca_3PO_4), aluminum phosphate (AlPO_4), iron phosphate (FePO_4), etc., can be converted into soluble P by phosphate solubilizing microorganisms (Gupta et al. 2007; Khan et al.

2013; Sharma et al. 2013; Alfiah et al. 2018).

Phosphate solubilizing microorganisms can dissolve phosphate in soil through the excretion of organic acids so that the availability of P increases. The ability of microbes to increase the availability of P differs depending on the type of microbes and organic acids produced (Scervino et al. 2010). The organic acids produced by the phosphate solubilizing microbes can increase the availability of P in the soil through several mechanisms, among which (i) organic anions compete with orthophosphates on the surface of the positively charged colloidal smelter (ii) the release of orthophosphate from the metal bond P through the formation of organic metal complex (iii) modification of the tread sorption content by organic ligands.

Citric acid and oxalate are classified as highly effective in reducing P retention from kaolinite and gypsite, whereas malonic, tartaric and malate acids have moderate effectiveness, acetic acid and succinate are classified as less effective. In volcanic soils which is rich in alofan organic acids (benzoate, salicylate and phthalate) are not able to decrease P retention. Citric acid absorbs Fe much more than tartrate. Acetic acid is ineffective in lowering retention, because acetate is less powerful in forming complexes with Al or Fe (Dou et al. 2015). Therefore, the purpose of this study was to isolate the environment-specific phosphate solubilizing microbes that have the potential to increase P's availability with the resulting organic acid.

MATERIALS AND METHODS

Sampling was conducted in Kutaray Village, Naman Teran Sub-district, Karo District, North Sumatra Province, Indonesia. The present study was conducted from May to December 2020. The materials used in this research were soil samples taken from potato plant rhizosphere affected by eruption of Mt. Sinabung. Pikovskaya media was used for isolation and the composition per liter of aquadet was: (glucose 10 g; $\text{Ca}_3(\text{PO}_4)_2$ 5g; $(\text{NH}_4)_2\text{SO}_4$ 0.5 g; KCl 0.2g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1g; MnSO_4 0.002g; FeSO_4 0.002g; yeast extract 0.5g; gelatin 20g; aquades (Pikovskaya 1948), rock phosphate 5g, AlPO_4 5g and FePO_4 5g, and other chemicals used for laboratory analysis purposes.

The instruments used in this research were ground drill, autoclave, petri dish, laminar air flow, mask, bunsen burner, high performance liquid chromatography (HPLC), polymerase chain reaction machine (PCR).

Soil sampling

The soil samples were taken from the rhizosphere of potato crops affected by eruption of Mount Sinabung, Indonesia compositely at a depth of 0 - 20cm.

Isolation of phosphate solubilizing microbes

10 g of andisol soil was inserted into a 250 mL erlenmeyer flask containing 90 mL of sterile physiological solution (dilution 10^{-1}), then shaken for 30 minutes at a shaker. For a serial dilution, 1 mL was taken from a dilution of 10^{-1} and transferred to a reaction tube containing 9 mL of sterile physiological solution (dilution 10^{-2}) and stirred on a rotary mixer until homogeneous. The remaining desired dilutions (10^{-3} , 10^{-4} and 10^{-5}) were also made by the same procedure. Then pour 12 mL of pikovskaya medium (45-50°C) into a petri dish containing 1 mL of soil suspension. Then gently shake so that media evenly mix with the soil suspension and leave for solidification. After the media solidify, the petri dishes were kept inverted in incubator and incubated for 3 days at 28-30°C. After 3 days of incubation, observations of growth were made on the media. The presence of phosphate solubilizing microbes was shown by the formation of a clear (halozone) region surrounding the colony.

Identification of isolates

Fungi and bacteria which showed the best phosphate dissolving ability compared to the others were selected for molecular identification. Selected fungi were identified by PCR-ITS primers and to identify bacteria, universal primers of 63f (5'CAG GCC TAA CAC ATG CAA GTC 3'), Primer 1387r (5' GGG CGG WGT GTA CAA GGC 3') were used to amplify the gene sequence of 16S rRNA through PCR.

Organic acid content

The bacterial and fungal cells were inoculated as much as 8×10^9 in 10 mL medium pikovskaya phosphate source

was phosphate rock (PR), incubated for 3 and 7 days at room temperature at 100 rpm shaker. At the end of incubation, culture was centrifuged at 7500 rpm at a temperature of 25°C for 20 min. The obtained filtrate was used to determine the levels of organic acids: citric, oxalate, acetate, propionate and malate. The determination was performed with high performance liquid chromatography (HPLC) using Aminex® HPX-87H, 300 mm x 7.8 mm at 35°C.

Phosphate dissolving fungi and bacteria potential test

Selection on pikovskaya solid media

Phosphate solubilizing microbes were isolated, then tested on a petri dish containing sterile solid pikovskaya media with $\text{Ca}_3(\text{PO}_4)_2$, AlPO_4 , FePO_4 and Rock Phosphate (RP), as phosphate source. The test medium was inoculated in a petri dish and left for solidification. Furthermore, each isolate of fungi and bacteria was grown on test media with 2 replications to obtain average results and incubated for 7 days. The phosphate solubilizing microbes that form the fastest halozone with the largest diameter qualitatively around the colony show the magnitude of the potential of phosphate solvent fungi and bacteria to dissolve the P element of the non-soluble form. Then, the fungi potential was calculated using the value of dissolution index. The phosphate dissolution index and the efficiency of phosphate dissolution were calculated according to the formula of Premono et al. (1996).

$$\text{Phosphate Dissolution Index} = \frac{\text{Clear zone diameter} - \text{Colony diameter}}{\text{Colony diameter}}$$

Test on liquid pikovskaya media

A total of 50 mL of liquid pikovskaya media which has been administered with several phosphate sources, such as $\text{Ca}_3(\text{PO}_4)_2$, AlPO_4 , FePO_4 and Rock Phosphate (RP), was transferred to a 250 mL Erlenmeyer flask and then sterilized in an autoclave at 121°C with 1.5 atm for 30-40 minutes and cooled. In liquid medium, it was inoculated as much as 1 ose needle of phosphate solubilizing microbes and incubated for 7 days at room temperature. After the incubation, the culture of the liquid medium was centrifuged at 6000 rpm for 10 min until separation occurred between filtrate and microbial deposits of phosphate solvent. The filtrate was taken using a pipette to measure the content of P-available filtrate, by Bray-2 method and after that, pH of solution was measured.

Test on andisol soil

50 g of andisol soil was placed in a 250 mL erlenmeyer flask which was sterilized in an autoclave at 121°C with a pressure of 1.5 atm for 30-40 minutes and then cooled. 1 mL of phosphate solubilizing microbes (both bacteria and fungi) were inoculated. Each experiment used two replications, then incubated for 1 month under field capacity conditions. Then, the soil pH and P-availability were measured with Bray-2 method.

RESULTS AND DISCUSSION

A total of 4 fungal were grouped based on colony color similarity i.e., fungal isolates which have black color colony coded as J1, fungal isolate which has yellowish colony which is coded J2, isolate which has of green light color colony coded J3, and isolate that has a dark black color colony color coded as J4. Likewise, 4 bacterial isolates were classified with isolate codes B1, B2, B3 and B4 based on colony size similarity.

Identification of fungi and bacteria with PCR-sequencing method

Isolated phosphate soluble microbes were then identified at the molecular level, with fungi and bacteria representing Figure 1 and Figure 2, respectively.

The fungal identification results showed that J1 was identified as *Penicillium* sp 1TMS, J2 as *Talaromyces pinophilus*, J3 as *Aspergillus terreus* and J4 as *Aspergillus awamori* (Figure 1). Several researchers have reported shown that all identified fungi are phosphate-soluble fungi. *Aspergillus awamori* (Jain et al. 2012), *T. pinophilus* (Majumder et al. 2019; Beheshtia et al. 2021), *Aspergillus*

and *Penicillium* (Saxena et al. 2012) are able to solubilize phosphate and increase the availability of phosphate in the soil. The bacterial identification results showed that B1 identified as *Burkholderia cepacia*, B2 as *Bacillus subtilis*, B3 as *Burkholderia cenocepacia*, B4 as *Burkholderia seminalis* (Figure 2). Soil bacteria capable of dissolving phosphate include *Burkholderia* (Mamta et al. 2010; Zhao et al. 2014), *Bacillus* (Babalola and Glick 2012; Jahan et al. 2013).

Organic acid content

The result of organic acid analysis of phosphate dissolving fungi using HPLC shows Table 1.

Potency test on solid pikovskaya media at multiple P source

The ability of microbes to form a clear zone was correlated to the amount of P that can be dissolved qualitatively. The clear zone was measured by calculating the solubility index value of each isolate. The results of phosphate dissolution index measurement by phosphate dissolving fungi on solid Pikovskaya media with several P sources for 7 days can be seen in Figure 3.

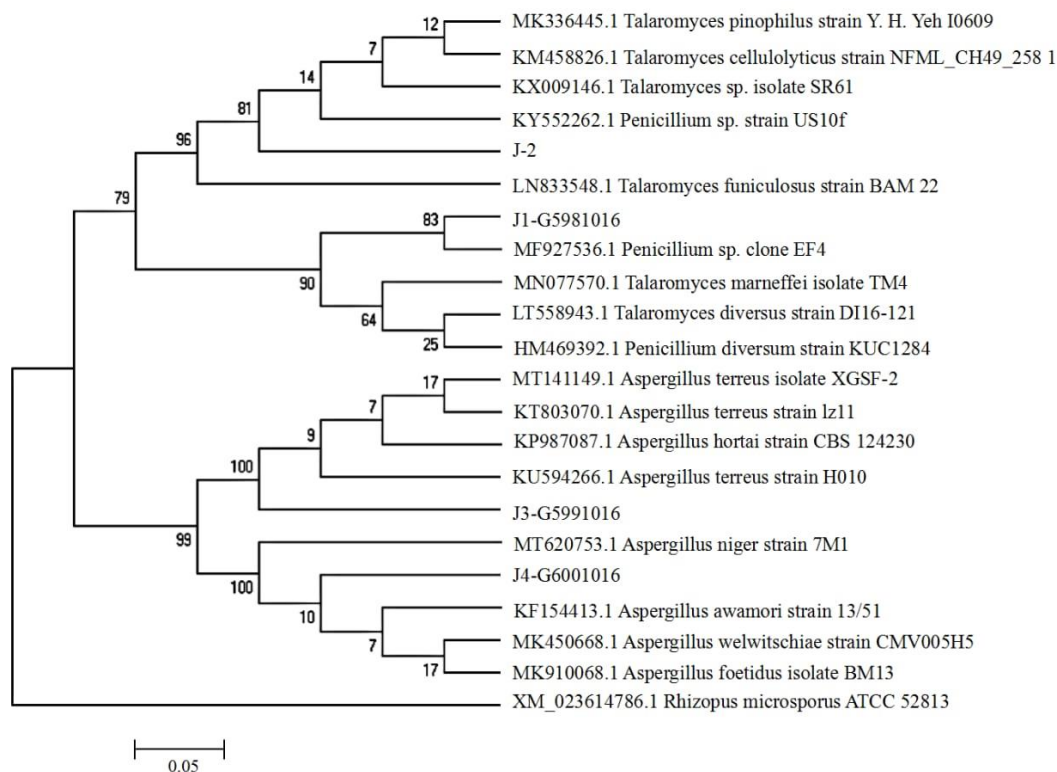


Figure1. Phylogeny tree of phosphate dissolving fungi

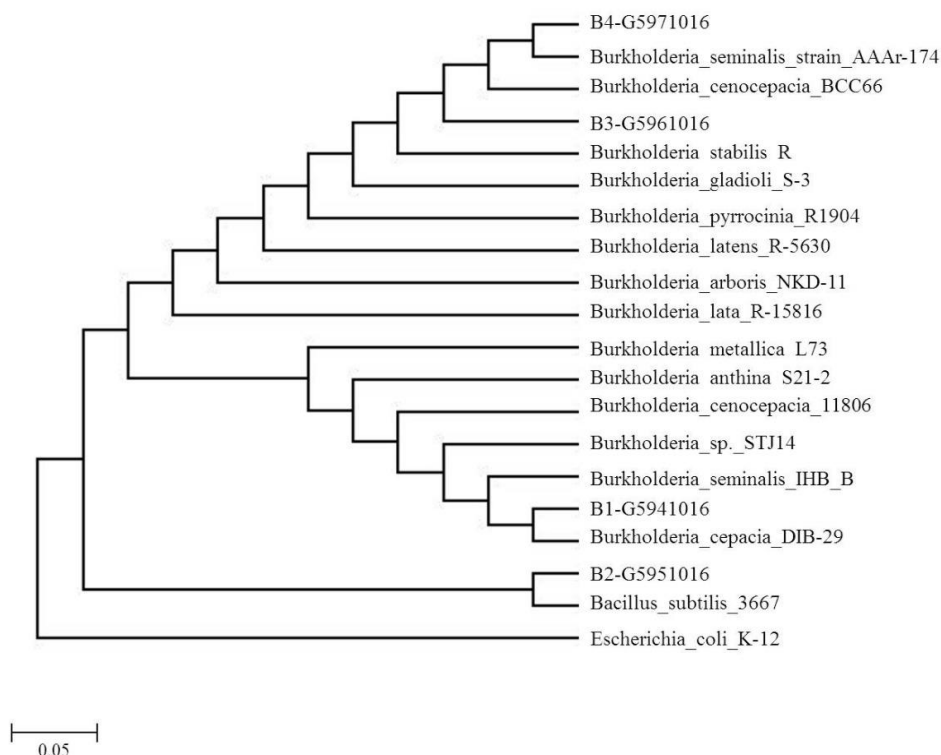


Figure 2. Phylogeny tree of phosphate dissolving bacteria

Table 1. Organic acid produced by phosphate dissolving fungi and bacteria

Isolates	Organic acid (ppm)					
	Oxalate	Citric	Lactate	Acetate	Malate	Propionate
Fungal isolate codes						
<i>Penicillium</i> sp 1TMS (J1)	Nd	21.25	Nd	42	51.39	Nd
<i>Talaromices pinophilus</i> (J2)	37	42	Nd	475	147.49	Nd
<i>Aspergillus terreus</i> (J3)	Nd	Nd	Nd	32.5	183.43	Nd
<i>Aspergillus awamori</i> (J4)	Nd	52.34	Nd	63	195.88	Nd
Bacteria isolate codes						
<i>Burkholderia cepacia</i> (B1)	24.5	11.3	Nd	98	75	Nd
<i>Bacillus subtilis</i> (B2)	10.01	Nd	Nd	24.5	174.2	Nd
<i>Burkholderia cenocepacia</i> (B3)	Nd	Nd	Nd	15	97.64	Nd
<i>Burkholderia seminalis</i> (B4)	Nd	Nd	Nd	42	37.49	Nd

Note: Nd: Not detected.

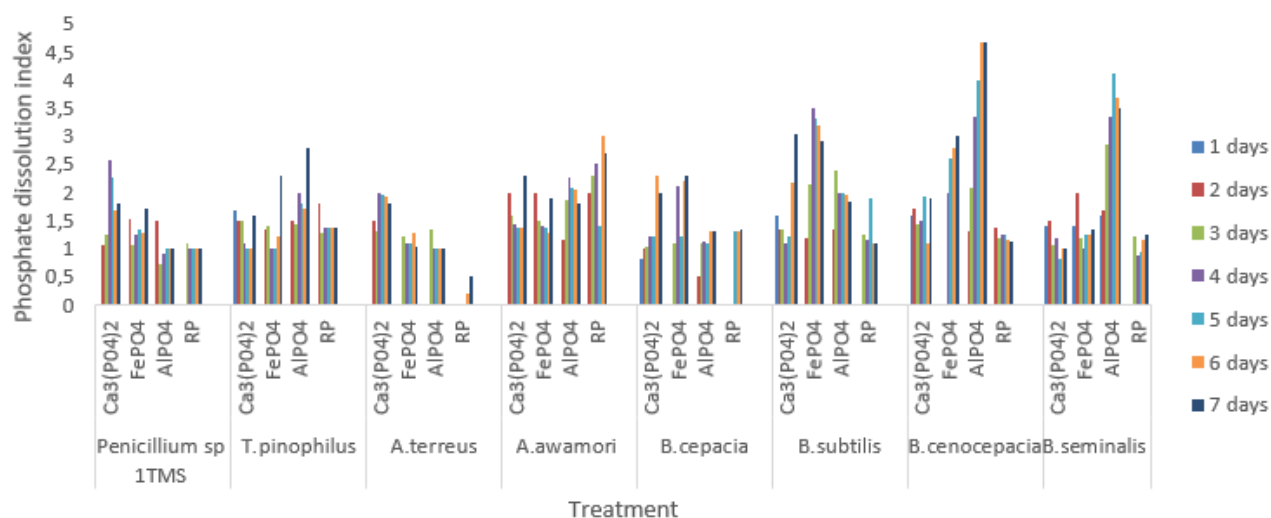


Figure 3. Phosphate dissolution index in solid pikovskaya medium at some P sources for 7 days incubation

The ability of phosphate solubilizing microbes to dissolve P in several P sources varies depending on the type of microbe. *Penicillium* sp. 1TMS (J1) and *A. terreus* (J3) had the highest (1.8 each) P dissolution index in $\text{Ca}_3(\text{PO}_4)_2$ with a phosphate solubilization efficiency of 180% each. *Talaromices pinophilus* (J2) showed the highest P solubilization index in AlPO_4 was 2.8. *Aspergillus awamori* (J4) had the highest (2.7) P dissolution index at a source P of RP. Whereas *B. cepacia* (B1), *B. cenocepacia* (B3) and *B. seminalis* (B4) exhibited the highest P dissolution index in AlPO_4 was 3.33, 3.59 and 3.50 respectively. *Bacillus subtilis* (B2) had the highest (3.04) P dissolution index in the P source of $\text{Ca}_3(\text{PO}_4)_2$. This indicated that all tested isolates were able to dissolve P in solid pikovskaya media in several P sources but had different abilities. The difference in the ability of each isolate to form a clear zone is thought to be due to their different ability to produce organic acids and phosphatase enzymes (Zhu et al. 2011; Sharma et al. 2013). This is in accordance with the results of Fatmala et al. (2015) who

tested the ability of phosphate solubilizing fungi on pikovskaya solid media, resulting in *Aspergillus* sp. and *Penicillium* sp. showed a phosphate solubility index of 1.14 and 1.57, respectively with a phosphate solubility efficiency of 157%.

Potency test on liquid pikovskaya media on several P sources

The results showed that pH level was decreased in the media, allegedly due to the release of a number of organic acids by phosphate solubilizing bacterial. Organic acids produced by phosphate solvent bacteria as a result of metabolism include citric acid, glutamate, succinate, lactate, oxalate, glyoxalate, malate, fumarate, tartaric, and α -ketobutyrate (Seshachala and Tallapragada 2012; Zhao et al. 2014). The change of pH media in the application of phosphate dissolving fungi on liquid pikovskaya medium with several P sources for 7 days incubation can be seen in Figures 4 and 5.

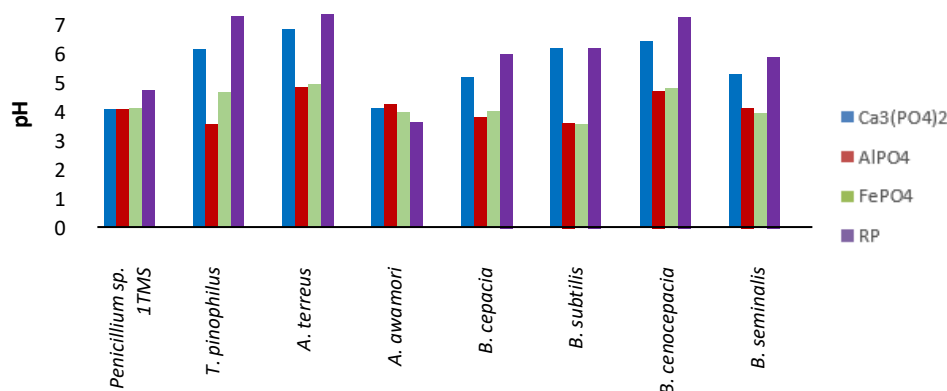


Figure 4. Media pH from various P sources after 7 days incubation of pikovskaya liquid media test

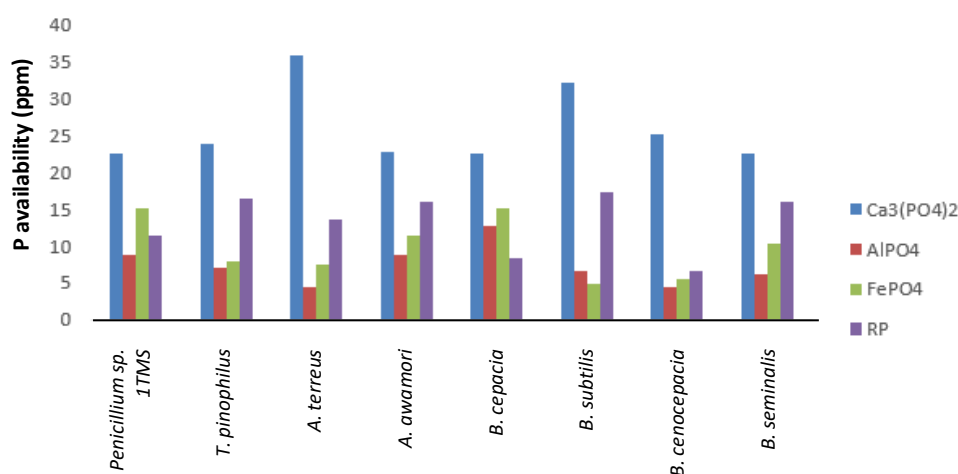


Figure 5. P availability from various P sources after 7 days incubation of pikovskaya liquid media test

Table 2. The ability of bacteria and fungi phosphate solvents in increasing P availability in andisol soils

Isolate codes	pH	P available (ppm)	Criteria
<i>Burkholderia cepacia</i> (B1)	5.08	16.10	Medium
<i>Bacillus subtilis</i> (B2)	5.13	11.00	Low
<i>Burkholderia cenocepacia</i> (B3)	5.06	12.10	Low
<i>Burkholderia seminalis</i> (B4)	5.01	9.85	Low
<i>Penicillium</i> sp. 1TMS (J1)	5.08	16.35	Medium
<i>Talaromyces pinophilus</i> (J2)	5.12	18.50	Medium
<i>Aspergillus terreus</i> (J3)	5.10	10.9	Low
<i>Aspergillus awamori</i> (J4)	5.16	14.25	Low
Control (No microbes)	5.15	8.78	Low

Note: Source of criteria: Soil-Bogor Research and BPTP-Medan

Application of phosphate dissolving fungi can decrease the pH of liquid medium in some phosphate sources this may be because the phosphate dissolving fungi can produce organic acid which can lower the pH (Figure 4 and 5). The decrease in pH of the solution was not the same because of the ability of each isolate to produce different organic acids. Dissolution of P by microbes is related to pH (Seshachala and Tallapragada 2012; Zhao et al. 2014). Application of *Penicillium* sp 1TMS isolate had the highest (26.63 ppm) P availability value on the liquid medium with P source of $\text{Ca}_3(\text{PO}_4)_2$ and the lowest (8.75 ppm) value was on media with a P source of AlPO_4 . *T. pinophilus* had the highest (922.8 ppm) P-availability value on liquid medium with P source in the form of $\text{Ca}_3(\text{PO}_4)_2$ and the lowest (6.91 ppm) was in medium with P source of AlPO_4 . *Aspergillus terreus* showed the highest P-availability value on liquid medium with P source in the form of $\text{Ca}_3(\text{PO}_4)_2$ which was 36 ppm and the lowest in medium with P source of AlPO_4 with a value of 4.32 ppm. *Aspergillus awamori* had the highest P-availability value on liquid medium with P source of $\text{Ca}_3(\text{PO}_4)_2$ with a value of 22.78 ppm and the lowest on media with P source of AlPO_4 with a value 8.77 ppm. *Burkholderia cepacia* (B1) showed the highest (32.01 ppm) P availability in $\text{Ca}_3(\text{PO}_4)_2$ media and the lowest (6.04 ppm) was in FePO_4 media. *Bacillus subtilis* (B2) had the highest P availability value in $\text{Ca}_3(\text{PO}_4)_2$, which was 32.1 ppm (high) and the lowest in FePO_4 was 4.82 ppm. *Burkholderia cenocepacia* (B3) showed the highest (25.24 ppm) P availability value in $\text{Ca}_3(\text{PO}_4)_2$ and the lowest (4.36 ppm) in AlPO_4 media. *Burkholderia seminalis* (B4) showed the highest P availability was in $\text{Ca}_3(\text{PO}_4)_2$ media, which was 22.64 ppm and the lowest in AlPO_4 media, which was 8.21 ppm. The ability of microbes to dissolve phosphate from $\text{Ca}_3(\text{PO}_4)_2$ source was much greater than that of AlPO_4 , FePO_4 and RP sources. This may be because the sources of AlPO_4 , FePO_4 and RP are more difficult to dissolve or require longer time to dissolve than $\text{Ca}_3(\text{PO}_4)_2$ sources. This shows that phosphate solubilizing microbes selectivity dissolve P from different P sources. The insoluble P form such as tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$), aluminum phosphate, iron phosphate, etc. can be converted into soluble P and available by phosphate solubilizing

microbes in different ecosystems (Gupta et al. 2007; Khan et al. 2013; Sharma et al. 2013).

Potency test on andisol soil

Phosphate solubilizing bacteria and fungi tested on solid and liquid pikovskaya media were retested for their ability to dissolve phosphate in andisol soil affected by the eruption of Mount Sinabung. This test was carried out to determine the best ability of the isolate in dissolving phosphate. The available P in the initial analysis was 8.8 ppm. The results of the analysis of pH and available P by the Bray II method with an incubation period of 1 month are shown in Table 2.

The application of phosphate solubilizing microbes to andisol soil can increase P availability and decrease soil pH. The results showed that decrease in pH with the application of phosphate solubilizing microbes did not affect microbial activity in increasing the availability of P in andisol soil. This may be due to phosphate solubilizing microbes used are microbes sourced from andisol soil affected by the Sinabung eruption that have acidic pH. The pH level of andisol soil ranges from 4.00 to 5.33 which is an acidic pH (Sinaga et al. 2015; Sembiring et al. 2016). This is because phosphate solubilizing microbes are able to live at which pH. Dissolution of phosphate by microbes can be followed by a decrease in soil pH (Zhao et al. 2014). *Burkholderia cepacia* could increase the available P to 16.10 ppm, the increase in available P of soil was 83.37% when compared to control. *Bacillus subtilis* (B2) can increase the availability of P by 25.28% than control. *Burkholderia cenocepacia* (B3) can increase the availability of P to 12.10 ppm, an increase of 37.81% when compared to control. *Burkholderia seminalis* (B4) can increase P availability by 12.18% than control. The application of phosphate solubilizing fungi can increase the availability of P in the soil, this can be seen from the results of the analysis showing that *Penicillium* sp 1TMS (J1) can increase the availability of P to 16.35 ppm, the increase in P availability was 86.21%. The highest (18.5 ppm) increase in P availability was shown by isolate *T. pinophilus* (J2) with a 110.7% increase in P availability compared to control. While *A. terreus* (J3) and *A. awamori* (J4) were able to increase availability of P by 24.14% and 61.36%, respectively when compared to control. The results showed that the ability of phosphate solubilizing bacteria and fungi to increase the availability of P in andisol soils was not the same depending on the type of microbe, the ability to adapt to the environment and produce organic acids and enzymes (Sharma et al. 2013; Zhao et al. 2014; Gupta et al. 2017).

In conclusion, the results showed that 4 isolates of each phosphate dissolving fungi and bacteria were isolated from andisol affected by eruption of Sinabung Mountain. The ability of phosphate solubilizing microbes to produce organic acids was different from one to another and they were capable of dissolving P in several sources of phosphate both in solid and liquid media. *Talaromyces pinophilus* (J2) isolate, exhibited the highest availability of P in andisol, it can increase available P as much as 110.7%.

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