

Diversity of potassium solubilizing microbes on andisol soil affected by the eruption of Mount Sinabung, North Sumatra, Indonesia

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Abstract. Sembiring M, Sabrina T. 2022. Diversity of potassium solubilizing microbes on andisol soil affected by the eruption of Mount Sinabung, North Sumatra, Indonesia. *Biodiversitas* 23: 1759-1764. Potassium (K) is one of the macronutrients needed by plants for seeds' growth, development, and quality. Andisols covered by volcanic ash from Mount Sinabung contain potassium ranging from 0.39-0.58 me/100g. The use of microbes is one of the alternatives methods to increase the availability of potassium in the soil that can be absorbed by plants. This study aimed to find environment-specific superior potassium-solvent microbes. Soil sampling was carried out in Kutarayut Village, Nama Teran Sub-district, Karo District, North Sumatra, Indonesia. The method used is random composite sampling by taking samples from 4 different locations. Sampling points were taken on Andisol soil that had been processed, covered with thin, medium, and thick ash. Sampling was carried out at a depth of 0-20 cm from the ground surface around the rhizosphere area. Isolation of bacteria and fungi was carried out by using multilevel dilutions. The microbes obtained after using the pour plate method were identified at molecular level by using PCR. Microbial potential test was carried out by using andisol soil which was incubated for 30 days. Parameters observed were soil pH, exchangeable potassium, soil organic carbon, and microbial population. 7 bacterial isolates and 3 potassium solubilizing fungi were isolated and identified during the present study. All bacterial and fungal isolates obtained were able to increase the availability of potassium soil exchange. The research results show that *Talaromyces pinophilus* can increase potassium soil exchange up to 102.94%.

Keywords: Andisol, microbial potential, potassium exchange, potassium solubilizing microbes, *Talaromyces pinophilus*

INTRODUCTION

Andisol soil is clay soil, which contains high concentration of potassium. The soil potassium is generally reduced due to the use of potassium by plants and soil erosion. As potassium plays an important role in plant growth, it can inhibit root growth, cause stunted plants, and lower production (Dasan 2012; Wang and Wu 2013; Zhang et al. 2013). Potassium is mostly bound in primary minerals or fixed in secondary minerals from clay minerals. Potassium is absorbed by plants in the form of K^+ ions. Around 2.3% of potassium found on earth, is mostly bound in primary minerals or fixed in secondary minerals and clays. Potassium in the soil available to plants is only around 2-10%, while 90-98% is in the form of minerals. The most common potassium carrier minerals are Kfeldspar, Leucite, Biotite, Phlogopite, and Glauconite as well as clay minerals. According to Mutscher (1995), there are several factors that affect the availability of potassium for plants including soil pH, texture, type and content of clay minerals, water content, soil CEC, and interactions with other cations such as Ca and Mg. The amount of potassium fixed in the soil depends on the particle size distribution, the type and amount of clay minerals, and the addition or reduction of potassium from these minerals. Meanwhile, the addition of potassium to the soil containing many interlayer potassium (vermiculite) sites results in high potassium adsorption. On the other hand, the

reduction of potassium in the soil solution due to absorption by plants and microbes or leaching can cause the potassium-fixed to be released into exchangeable or potassium-soluble. The fixed potassium-form and the structural potassium-form is the main potassium reservoir in the pedosphere or is often referred to as the non-exchangeable potassium-form (Dasan 2012; Yadegari et al. 2012; Gundala et al. 2013; Zhang et al. 2013).

Soil microbes play an important role in potassium cycle. The potassium solubilizing microbes present in the soil could provide an alternative technology to make potassium available to plants (Herdiyantoro et al. 2018). Different bacteria and fungi, namely *Pseudomonas*, *Burkholderia*, *Acidithiobacillus ferrooxidans*, *Bacillus mucilaginosus*, *Bacillus edaphicus*, *Bacillus circulans*, *Paenibacillus* sp, *Aspergillus niger* and *Aspergillus terreus* are microbes that can dissolve potassium in the soil (Sheng 2005; Liu et al. 2012). Potassium solubilizing bacteria are able to release potassium from a mineral by producing organic acids, such as acetate, citrate, oxalate, and so on. The organic acids produced by the potassium Solvent Bacteria will provide protons (H^+), where these protons replace potassium at the adsorption site so that potassium becomes available in solution. In addition, these organic acids also interact with other cations such as Ca, Al, and Fe to then form complex compounds (Shanware et al. 2014).

Organic and inorganic acids produced accompanied by an acidolysis exchange reaction or decrease in pH convert

insoluble potassium (mica, muscovite, biotite feldspar) into soluble forms of potassium (soil solution form), thereby increasing the availability of nutrients for plants. Different types of organic acids produced by potassium solubilizing bacteria are different in each organism (Uroz et al. 2009; Zhang and Kong 2014). The release of potassium from minerals is affected by availability of oxygen, pH and the strain of bacteria used. The dissolution efficiency of potassium by various microorganisms was found to vary according to the nature of the potassium-containing minerals and aerobic conditions (Uroz et al. 2009). potassium can be increased 84.8-127.9% after microbial application compared to soil without microbial application. Potassium solubilizing bacteria play a very important role in increasing soil fertility (Bagyalakshmi et al. 2012). Keeping in view the role of different bacteria and fungi in the availability of potassium in the soil, this study aimed to find environmentally specific superior potassium-solvent microbes.

MATERIALS AND METHODS

This research was carried out from June to December 2021. Soil sampling was carried out using a random composite sampling method from 4 different locations. At location I the soil had been processed, Andisol was covered with thin volcanic ash 2 cm (Location II), Andisol was covered in moderate volcanic ash. 2-5 cm (location III) and covered with volcanic ash 5 cm thick (location IV). The Aleksandrov media ($\text{Ca}_3(\text{PO}_4)_2$ 2 g, K_2HPO_4 3 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g, FeCl_3 0.1 g, CaCO_3 2 g, Glucose 5 g, Agar 20 g, aquadest 1L) was used as well as other chemicals used for analytical purposes in the laboratory. The tools used in this research were soil drill, autoclave, Petri dish, laminar airflow, weighing cup, plastic, styrofoam, aluminum foil, cotton, test tube, oven, plastic cling wrap, glass slides and other tools used during the research.

Isolation and identification of potassium solvent microbes

Total 3 g of soil was put into 27 mL of 0.85% NaCl physiological solution, was shaken in the incubator for 1 hour at a speed of 120 rpm and serial dilutions were made from 10^{-1} to 10^{-4} . The results of the 0.1 mL dilutions of 10^{-2} , 10^{-3} , and 10^{-4} were spread on Aleksandrov's medium and incubated at 28°C for 3-7 days (Prajapati and Modi 2012). The growth of potassium solubilizing bacteria and fungi was indicated by the presence of a clear zone around the colony. The obtained microbes were then identified at molecular level by using PCR. Selected microbes were identified by PCR-ITS primers for fungi and 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') are used to amplify the gene sequence of 16S rRNA through PCR.

Testing the potassium dissolving ability

Microbial potential test was carried out using 50 g of sterile andisol soil and then 1 mL of each microbe was inoculated (10^8) and then incubated for 30 days. After the incubation process was completed, the soil pH (Electrometry), C-organic (Walkley and Black) and potassium exchange were observed.

RESULT AND DISCUSSION

Andisol soil samples were collected from various study sites in Kutaray Village, Nama Teran Sub-district, Karo District, North Sumatra, Indonesia; were analyzed for soil temperature, humidity, pH, soil C-organic and K total. The results so obtained are presented in Table 1.

Isolation and identification of potassium solvent microbes

After analyses of the present study results, there were seven bacterial isolates (isolate codes K1, K2, K3, K4, K5, K6, and K7) and are 3 fungal isolates, namely KJ1, KJ2, and KJ3 codes were isolated (Table 2).

Table 1. Andisol soil analysis at soil sampling locations

Location	Parameter				
	Soil temperature (°C)	Humidity (%)	pH H_2O	C-organic (%)	K total (%)
I	18-21	80-81	5.62-5.89	6.21-6.49	0.61-0.72
II	19-21	76-78	5.01-5.11	2.65-3.34	0.46-0.60
III	21-22	77-78	4.07-4.21	1.36- 2.43	0.57-0.61
IV	22-23	67-68	3.27-4.12	1.17-1.54	0.46-0.55

Notes: Location I: already processed, Location II ash thickness (<2cm), Location III ash thickness (2-5 cm) and Location IV ash thickness (>5 cm).

Table 2. Microbial isolates and their microbial population at the study site

Location	Bacteria isolates	Fungal isolates	Microbial population (10^3)
I	K1	KJ1	54-55
II	K2, K3	KJ1, KJ3	38-44
III	K,3, K4, K5	KJ2, KJ3	23-31
IV	K6, K7	KJ2	15-17

Notes: Location I: already processed, Location II ash thickness (<2cm), Location III ash thickness (2-5 cm) and Location IV ash thickness (>5 cm).

Soil temperature at location I (already processed) has a lower temperature, ranging from 18-21°C, at location II (ash thickness <2cm) it is between 19-21°C lower than location III (ash thickness 2-5 cm) ranging from 21-22°C while location IV (ash thickness >5 cm) by 22-23°C this shows that the volcanic ash produced by Mount Sinabung causes the soil structure to become solid which also affects soil aggregates, which makes it difficult for water to enter the soil, thus affecting soil moisture so that the temperature in the soil is higher and the resulting soil moisture is lower. It was found that the pH at location I (already processed) was higher when compared to other locations. The pH at location I ranged from 5.62 to 6.49 while location IV was 3.27 to 4.12, this indicates that the thicker the volcanic ash covers the soil, the lower the pH. The results of the Sianturi et al. (2021); Zebua et al. (2020); Qadaryanty et al. (2020); Sembiring et al (2021) research show that the thicker the volcanic ash covers the soil, the higher the soil temperature, while the lower the pH of the soil. Soil organic carbon matter decreased with the thickness of volcanic ash covering the soil. At location I organic carbon ranged from 6.21-6.49% while location IV 1.17-1.54% based on the data obtained that volcanic ash had an effect on soil organic carbon, the thicker the ash, the lower the carbon organic of the soil. This is also supported by the research of Pakolo et al. (2018); Zebua et al. (2020); Qadaryanty et al. (2020) also stated that the organic carbon content of andisol soil is affected by the Sinabung eruption was 4.7%. Soil analysis was carried out to obtain data that soil temperature, soil moisture, soil pH and total potassium were different in each thickness. This also affects the activity of microorganisms in the soil. The total potassium at each research location was different at location I, the highest total potassium soil was 0.72% and the lowest was at location IV, which was 0.46%. This indicates that the thicker the ash covering the soil, the lower the total potassium value in the soil. From the data obtained, the thicker the ash covers the soil, the pH of the soil has decreased. This is because volcanic ash contains sulfur which can lower the pH. Sulfur is known to be oxidized to form sulfide acid and lower the pH of the soil.

Variation in microbial life ability of potassium solvent was observed in this study, seen, K1 was only found at location I, K2 was only found at location II, K3 was found at locations II and III, while at location IV bacteria coded K6 and K7 were found. Potassium solubilizing fungus isolate code KJ1 was found at locations I and II, Isolate Code KJ2 was found at Locations III and IV while KJ3 was found at locations II and III. The thicker the soil covered with volcanic ash, the lower the microbial population. At location I the highest microbial population was 55×10^3 while at location IV the lowest microbial population was 15×10^3 CFU/g. This shows that microbial growth in the soil is strongly influenced by environmental conditions, especially temperature, humidity, and soil pH which is strongly influenced by the level of ash thickness covering the soil surface. Microbial population in the soil is strongly influenced by pH, humidity and temperature (Prajapati and Modi 2012; Meena et al. 2014).

Molecular identification of potassium solubilizing fungi and bacteria

From the results of the isolation of fungi and bacteria solubilizing potassium on Andisol soil, it was observed that three fungi were identified based on the similarity of colony color, as fungal isolates with black colonies having moss green colonies surrounded by dark green and white (coded KJ1), dark green and yellowish and surrounded by white color (coded KJ2) and yellow colonies surrounded by light green and white (coded KJ3). There were 7 isolates of bacteria that were differentiated based on the similarity of colony shape with isolate code K1, K2, K3, K4, K5, K6 and K7. The results of molecular identification of bacteria are presented in Figure 1 while for fungi in Figure 2.

The bacterial identification results revealed the identity of K1, K2, K3, K4, K5, K6 and K7 as that *Bacillus subtilis*, *Bacillus amyloliquefaciens*, uncultured *Bacillus* sp, *Burkholderia vietnamensis*, *Enterobacter ludwigii*, *B. subtilis* and *Bacillus nealsonii* respectively. A large number of bacterial strains such as *Burkholderia* sp. (Zhang and Kong 2014), *Bacillus* sp. can dissolve available potassium in the soil (Liu et al. 2012; Bagyalakshmi et al. 2017). *B. subtilis* can dissolve potassium (Verma et al. 2016). *Enterobacter* sp. can increase K uptake and plant growth (Bakhshandeh et al. 2017). Some bacteria are able to dissolve potassium in the soil by producing organic acids (Sheng et al. 2008; Meena et al. 2014; Zhang and Kong, 2014). The mechanism of potassium dissolution by microbes is closely related to the organic acids produced which are able to dissolve potassium directly or chelate silicon ions so that potassium becomes soluble (Prajapati et al. 2013). The results of the identification of fungi identify KJ1 as *Aspergillus fumigatus*, KJ2 as *Gongronella hydei* and KJ3 as *Talaromyces pinophilus*. Potassium solubilizing fungi are able to increase the available potassium in the soil (Shelobolina et al. 2012; Prajapati et al. 2012). *Talaromyces pinophilus* can increase plant nutrient uptake (Sembiring et al. 2020a, 2020b).

Potential test of potassium solubilizing microbial in andisol soil

The results of the analysis and statistical tests of organic carbon values, pH, microbial population and potassium soil exchange after application of potassium solubilizing microbial in thin ash thickness with incubation for 30 days on sterile are presented in Table 3.

It was observed from the results presented in Table 3 that the microbial application of potassium solvent did not significantly affect the pH. The application of potassium solubilizing microbes can significantly increase soil organic carbon, microbial population, and exchangeable potassium in the soil. The application of potassium solubilizing bacteria *B. subtilis* can increase soil organic carbon by 5.17% which showed an increase in organic carbon of 40.48% higher when compared to the control. The increase of organic carbon with the application of *B. subtilis* was not significantly different from the treatment of *B. subtilis* which showed that the ability of these microbes to increase soil organic carbon was the same.

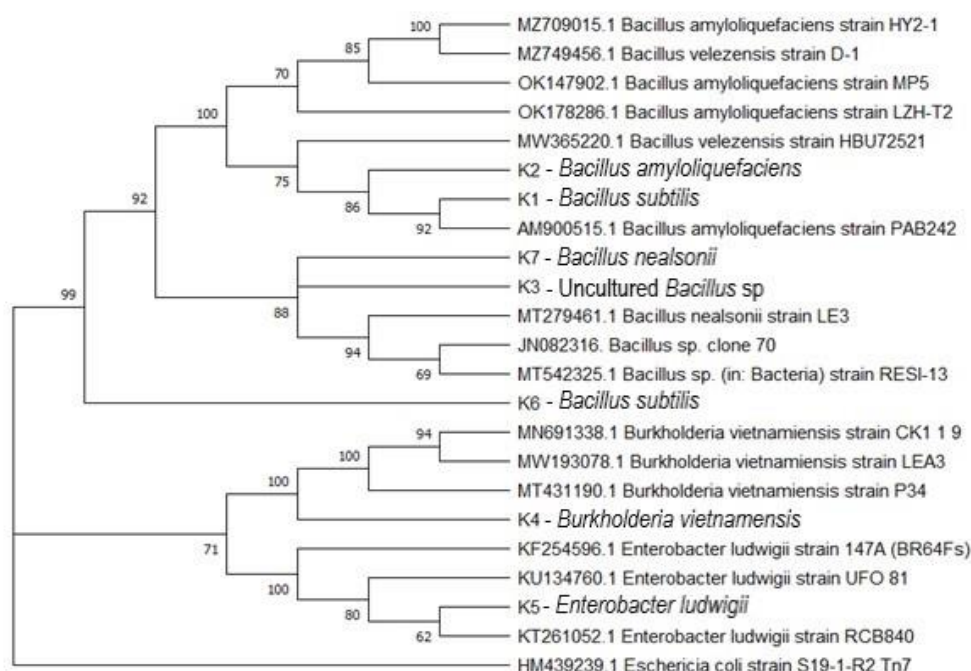


Figure 1. Phylogeny tree of phosphate solubilizing bacteria

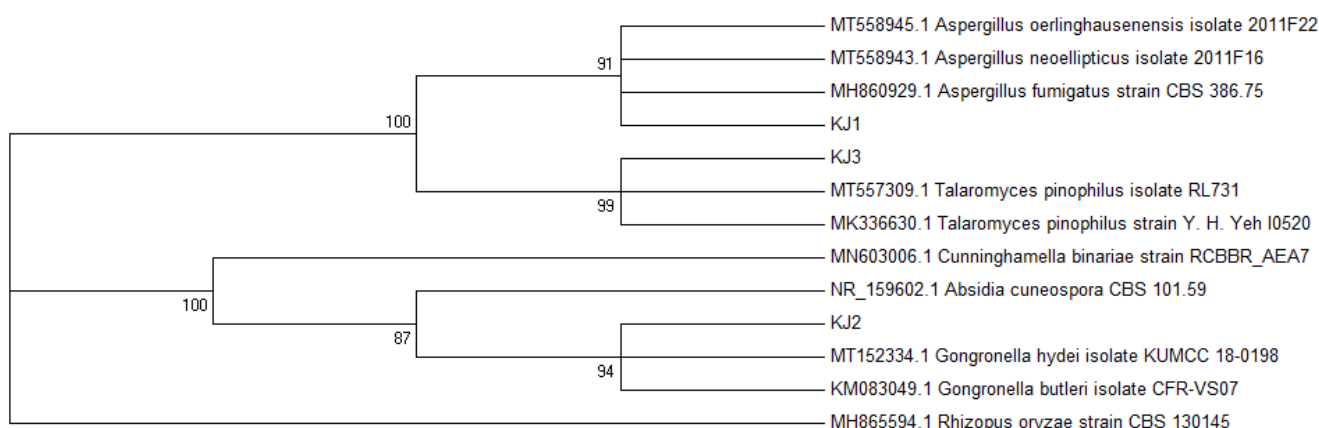


Figure 2. Phylogeny tree of phosphate solubilizing fungi

Table 3. Results of analysis of pH, organic carbon microbial population and exchangeable potassium after 30 days of incubation

Isolate	pH soil	Organic carbon (%)	Microbial population (10 ⁶)	Potassium exchange (me/100g)
No microbes	4.38	3.68a	0a	0.34a
<i>Bacillus subtilis</i>	4.43	5.17d	40.1b	0.38a
<i>Bacillus amyloliquefaciens</i>	4.41	4.84cd	38.2b	0.57b
Uncultured <i>Bacillus</i> sp.	4.44	4.66bcd	41b	0.37a
<i>Burkholderia vietnamensis</i>	4.46	5.14d	42b	0.40a
<i>Enterobacter ludwigii</i>	4.46	4.09ab	55b	0.40a
<i>Bacillus subtilis</i>	4.52	4.89cd	62.5b	0.38a
<i>Bacillus nealsonii</i>	4.41	4.88cd	56b	0.45a
<i>Aspergillus fumigatus</i>	4.32	4.11ab	153.5cd	0.67d
<i>Gongronella hydei</i>	4.41	5.16d	251e	0.62bc
<i>Talaromyces pinophilus</i>	4.45	5.32de	143c	0.69d

Note: the numbers followed by the same letter are not significantly different (5%) according to the DMRT test.

Organic carbon content with the application of potassium solvent fungus *T. pinophilus* had the highest organic carbon content of 5.32 which showed an increase of 44.56% higher soil organic carbon when compared to the control. The application of the fungus *T. pinophilus* had a higher organic content when compared to the application of the bacterium *Bacillus subtilis* although statistically, it was not significantly different. The organic carbon content in the soil is influenced by the activity of microorganisms. The increase in organic carbon with the application of potassium solubilizing fungi was higher when compared to the application of bacteria. This was related to a higher fungal population when compared to the population of bacteria in the soil which indicated that potassium solubilizing fungi were able to grow efficiently on Andisol soil affected by the eruption of Sinabung when compared to bacteria. The application of potassium solubilizing bacteria *B. amyloliquefaciens* had higher exchange potassium and was significantly different from other bacterial applications. The increase in exchangeable potassium with *B. amyloliquefaciens* treatment increased exchangeable potassium by 67.64% higher than the control and 50% higher when compared to *B. subtilis*. Treatment of potassium solvent fungus *T. pinophilus* had a potassium content of 0.69 which showed increased potassium of 102.94% higher when compared to the control and 21.05% higher when compared to the application of *B. amyloliquefaciens* bacteria. According to Zhang and Khong (2014) organic acids convert insoluble potassium into a soluble form of potassium that can be absorbed by plants. The ability of soil organisms to produce organic acids is different so their ability to dissolve potassium is also different (Archana et al. 2013). Potassium dissolution activity by potassium solvent organisms can occur at pH 4.5. This is in accordance with the results of the study that at pH < 5 potassium solvent microbes are able to increase the availability of potassium in the soil (Bagyalakshmi et al. 2017). Increasing the availability of potassium in the soil with the application of bacteria and fungi solubilizing potassium increase plant growth and reduce the use of inorganic fertilizers (Archana et al. 2013; Prajapati et al. 2013).

In conclusion, diversity of potassium solubilizing microbes at different research sites was observed in this study. Total seven bacterial isolates and 3 potassium solubilizing fungi were isolated and identified. All bacterial and fungal isolates obtained were able to increase the availability of potassium soil exchange. The isolates that had the most potential to increase potassium soil exchange were *B. amyloliquefaciens* isolates capable of increasing the potassium exchangeable up to 67.64% and *T. pinophilus* able to increase potassium soil exchange up to 102.94%.

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