Ilmu Pertanian (Agricultural Science)
http://journal.ugm.ac.id/jip
Vol. 8 No. 3 December, 2023: 169–174 | DOI: doi.org/10.22146/ipas.77785

Utilization of Actinomycetes to increase phosphate availability at different soil moisture conditions in Andisols Namanteran, North Sumatera

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Article Info

Received : 16th September 2022
Revised : 16th November 2023
Accepted: 29th November 2023

Keywords:
Actinomycetes, Andisol, phosphate, moisture content, available P

Abstract

The high phosphate retention in Andisols causes the availability of P to be low, can not be absorbed by plants. Actinomycetes are capable of solubilizing bound phosphates. This research aimed to identify Actinomycetes in Andisols Namanteran, assess their ability to increase phosphate availability, and understand how they work to increase phosphate availability in this soil. The research design used a factorial randomized block design with 2 factors; factor 1 was Actinomycetes isolate, consisting of A_o = No Inoculant, A_1 = Inoculant A_{132} (vegetable crops; 32×10^8 CFU mL^{-1}), $A_2 = A_{468}$ (forest plants; 41×10^8 CFU mL^{-1}), $A_3 = A_{458}$ (forest plant; 58×10^8 CFUmL^{-1}), $A_4 = A_{471}$ (coffee plant; 35×10^8 CFU mL^{-1}), $A_5 = A_{459}$ (forest plant; 63×10^8 CFU mL^{-1}), $A_6 = A_{321}$ (hibiscus plant; 37×10^8 CFU mL^{-1}), and $A_7 = A_{356}$ (vegetable plant; 33×10^8 CFU mL^{-1}), and factor 2 was soil water content, consisting of K_1 = 50%, K_2 = 75% and K_3 = 100% of field capacity. The results showed that the availability of P in Andisols increased due to the application of *Actinomycetes* from 42.46 ppm to 159.20−266.60 ppm. The population of Actinomycetes in *Actinomycetes* treatment ranged from 27.33−31.58 × 10^8 CFU mL^{-1}), with a soil pH of 4.41. Water content of 100% was the best in increasing soil pH and *Actinomycetes* population, but not having significant effects on the available P of the soil. The results of molecular identification of *Actinomycetes* that have the best potential in dissolving P include $A_3>A_5>A_2>A_4>A_1$.

INTRODUCTION

Phosphorus (P) is an essential mineral macronutrient required for plant growth and development (Alori et al., 2017). Most of soil P in insoluble form cannot be absorbed by plants. In acidic soils, the predominance of Aluminum (Al) and iron (Fe) oxides in both crystalline and amorphous forms reduces the solubility of soil inorganic P through fixation on positively charged surfaces and formation of insoluble Al and Fe precipitates (Johan et al., 2021). Andisols is generally deficient in phosphorus because it has very high phosphorus fixation capacity, and lack of phosphorus will inhibit plant growth (Ajidirman, 2010).

The soil typically has low P availability, resulting in a shortage of absorbable phosphate elements for plants. Fertilization containing element P is usually needed to maintain plant production. However, P fertilizer that is applied to the soil is rapidly deposited into an insoluble form of $CaHPO_4$, $Ca_3(PO_4)^{2-}$, FePO4 and $AlPO_4$, so it cannot be absorbed by plants (Wahbi, 2016).

Actinomycetes can solubilize phosphate bound in the soil, but not all *Actinomycetes* species can solubilize phosphate in the soil. *Actinomycetes* from the genus *Actinoplanes* sp., *Actinomadura* sp., *Micromonospora sp.*, *Nocardia* sp., *Streptosporangium* sp., *Rhodococcus* sp., and *Microbispora* sp. can produce organic acid so

How to cite: Alfikri, M.R., Sabrina, T., and Sahar, A. (2023). Utilization of Actinomycetes to increase phosphate availability at different soil moisture conditions in Andisols Namanteran, North Sumatera. *Ilmu Pertanian (Agricultural Science)*, 8(3), pp. 169–174.

ISSN 0126-4214 (print) ISSN 2527-7162 (online)

that they can solubilize phosphorus bound in the soil under acidic or alkaline conditions (Bhatti et al., 2017). Actinomycetes isolated from the soil on Waigeo Island were able to solubilize calcium phosphate into an orthophosphate form (Siddiqui and Akhtar, 2017). Microorganisms isolated on Pb contaminated land were *Bacillus* sp., *Pseudomonas* sp., and *Actinomycetes* sp. with phosphate dissolution index, namely 2.98, 2.43, and 1.87, respectively. Despite *Actinomycetes* sp. having a low phosphate solubilization index, it remains in the potential category because of its capability to solubilize phosphates (Susilowati and Syekhfani, 2014). Research conducted on Iranian soil obtained 70 isolates of *Actinomycetes* from the genus *Streptomyces* spp. identified based on their morphology, but only 31% were able to solubilize phosphate rock (Biglari et al., 2016).

Actinomycetes can be responsible for protecting the environment, because they do not pollute the environment. On the contrary, *Actinomycetes* can help maintain the biotic balance of the soil. In addition, by bioremediating contaminated soil, actinomycetes can breakdown pesticides, which plays a significant role in increasing the availability of P in soil (Bhatti et al., 2017).

Actinomycetes are still not widely used as biological fertilizers, but many studies are developing the use of *Actinomycetes* as decomposers. This is a way that *Actinomycetes* can be used to increase soil fertility (Javed et al., 2021).

This study is a continuation of previous research by Alfikri et al. (2019), which aimed to obtain *Actinomycetes* and determine their effects on increasing the availability of phosphate at different soil moisture conditions.

MATERIALS AND METHODS

This study used 7 *Actinomycetes* isolates from the research of Alfikri et al. (2019). Isolates were obtained from various plant rhizospheres, namely vegetable plant rhizosphere, forest plant rhizosphere, coffee plant rhizosphere and hibiscus plant in Namanteran District, Karo Regency, North Sumatra Province.

This study used a randomized block design with two factors and two replications. Factor I was the selected *Actinomycetes* inoculants, consisting of A_0 = No Inoculant (without *Actinomycetes* application), A_1 = Inoculant A_{132} (vegetable plants; 32×10^8 CFU mL^{-1}), A_2 = Inoculant A_{468} (forest plants; 41×10^8 CFU mL^{-1}), A_3 = Inoculant A_{458} (forest plant; 58×10^8 CFU mL^{-1}), A_4 = Inoculant A_{471} (coffee plant; 35×10^8 CFU mL^{-1}), A_5 = Inoculant A_{459} (forest plant; 63×10^8 CFU mL^{-1}), A_6 = Inoculant A_{321} (hibiscus plant; 37×10^8 CFU mL^{-1}), and A_7 = Inoculant A_{356} (vegetable plant; 33×10^8 CFU mL^{-1}). Factor 2 was the condition of soil moisture content, consisting of K_1 = 50% × field capacity, K_2 = 75% × field capacity, K_3 = 100% × field capacity. The treated soil was incubated for 40 days in the laboratory using Andisols at several points and then homogenized. Soil incubation was carried out under anaerobic conditions at a temperature of 32°C. Variables observed included soil pH using the H_2O method, Actinomycetes population using the colony counting method, and available P using the Bray II method. Statistical analysis was performed at the level of 5% according to the Duncan Multiple Range Test using SPSS.

RESULTS AND DISCUSSION

Applying *Actinomycetes* to soil incubated for 40 days can affect soil pH, *Actinomycetes* population, and available P. Changes in soil pH is due to organic acids produced by *Actinomycetes*, in which the activity of *Actinomycetes* can produce organic acids that can decrease in pH (soil pH before treatment = 4.70).

From the observation data, it can be seen that soil given inoculants A_{458} (A_2) had the lowest soil pH. Each inoculant application had an effect on decreasing soil pH. This is due to the administration of inoculants or different types of organisms that will produce different amounts and types of organic acids. Differences in the amount and types of organic acids produced by organisms will affect the increase and decrease in soil pH. Organic acids can contribute to the acidification of soil by increasing the concentration of H^+ ions in the soil solution. However, the impact of organic acids on soil pH can vary depending on several factors (Zuo et al., 2022).

Soil pH in treatment A_3 (4.41) had a significant effect on A_2, A_6, A_1 and A_0, but had no significant effect on A_5. This is due to the influence of the organic acids produced causing a decrease in pH changes. This is in accordance with the research of Marbun et al. (2015), stating that P-solvent microbes will produce organic acids, including citric acid, glutamate, succinate, lactate, and oxalate. The increase in organic acids is usually followed by a decrease in pH.

The condition of water content in andisol soil has a significant effect on soil pH. The higher the water

Table 1. Andisols pH after incubation for 40 days

Isolate of Actinomycetes	Soil water content			Average
	K_1 (50%)	K_2 (75%)	K_3 (100%)	
A_0 (Control)	5.31	5.50	5.50	5.44 a
A_1 (Isolate A_{132})	5.05	5.43	5.38	5.28 b
A_2 (Isolate A_{468})	4.56	4.67	4.73	4.65 ef
A_3 (Isolate A_{458})	4.38	4.49	4.37	4.41 h
A_4 (Isolate A_{471})	4.44	4.59	5.17	4.73 de
A_5 (Isolate A_{459})	4.43	4.61	4.73	4.59 gh
A_6 (Isolate A_{321})	4.64	4.65	5.15	4.81 cd
A_7 (Isolate A_{356})	4.92	4.88	4.83	4.87 c
Average	4.71 c	4.85 b	4.98 a	

Remarks: Means followed by the same letters in the same column or row are not significantly different at the level of 5% according to the Duncan Multiple Range Test.

Table 2. Population of Actinomycetes ($\times 10^8$ CFU ml^{-1})

Isolate of Actinomycetes	Soil water content			Average
	K_1 (50%)	K_2 (75%)	K_3 (100%)	
A_0 (Control)	00.00	00.00	00.00	00.00 h
A_1 (Isolate A_{132})	27.50	26.50	28.00	27.33 bcdefg
A_2 (Isolate A_{468})	24.75	28.00	34.00	28.92 b
A_3 (Isolate A_{458})	26.25	32.00	36.50	31.58 a
A_4 (Isolate A_{471})	24.00	29.75	31.25	28.33 bcd
A_5 (Isolate A_{459})	25.25	33.25	27.50	28.67 bc
A_6 (Isolate A_{321})	25.75	29.25	27.50	27.50 bcdef
A_7 (Isolate A_{356})	25.75	29.75	27.50	27.67 bcde
Average	22.41 c	26.06 ab	26.53 a	

Remarks: Means followed by the same letters in the same column or row are not significantly different at the level of 5% according to the Duncan Multiple Range Test.

content of the soil, the higher the pH of the soil. The water content of 100%, 75%, and 50% showed pH value of 4.98, 4.85, and 4.71, respectively. This is caused by the water content in the form of H_20, so providing neutral water will affect the soil pH. Water indirectly affects soil pH by influencing the availability of ions in the soil solution. Dry soil increases the concentration of H^+ ions, making it more acidic, while wet soil decreases the concentration of H^+ ions, making it less acidic. Water can dissolve mineral salts in the soil, releasing ions that affect soil pH. Water also affects the activity of soil microorganisms, which can influence pH regulation. Therefore, water plays a significant role in regulating soil pH.

Actinomycetes can have an effect on the population of *Actinomycetes*, the incubated soil is sterilized so that the organisms in the soil are lost or dead, and the increase in the population of *Actinomycetes* is influenced by the type of isolate. Different isolates in different applications will affect the population in the soil, where the growth and reproduction of an organism different for each species and strain. The application of *Actinomycetes* has a significant effect on the *Actinomycetes* population. Application of *Actinomycetes* can increase the number of *Actinomycetes* population to 108 (Table 2).

The highest *Actinomycetes* population was isolate A_{458} (A_3) with a total population of 31.58 × 108 CFU mL^{-1}, and isolate A_{132} (A_1) showed the lowest population (2.73 × 109 CFU mL^{-1}), while the control did not have *Actinomycetes* or other microorganisms in the soil due to no application. This proves that the type of isolate or strain of an organism has the ability to develop or grow different, and environmental conditions can also affect the ability of an organism to survive, one of which is the humidity factor. Soil moisture is very large in relation to conditions of water content in the soil, where in Table 2, it can be seen that 100% water content treatment can increase the population of *Actinomycetes*. The ability of an organism or soil microorganism to survive is affected certain environmental conditions such as soil pH, humidity,

and soil temperature. Actinomycetes thrive in well-drained soils with a pH range of 6.5−8.0. They prefer neutral to slightly alkaline soils and can tolerate moderately acidic soils. Actinomycetes can also grow and adapt to a wide range of temperatures, from as low as 0°C to as high as 70°C, although their optimal temperature range is between 25°C to 35°C (Kavitha and Doble, 2014).

The application of *Actinomycetes* in K_3 treatment (100% water content) showed the highest population (26.53 × 108 CFU mL^{-1}) and the lowest (22.41 × 108 CFU mL^{-1}) in K_1 treatment (50% water content). The K_3 treatment had a significant effect on the K_2 and K_1 treatments. The higher the humidity of soil, the more the population of microbes or *Actinomycetes* in the soil is due to the optimal living place for breeding in the soil and carrying out their activities in Andisols (Table 2).

Actinomycetes application was able to increase P availability from 59.7 ppm in control (without *Actinomycetes* application) to 159.20−266.60 ppm significantly compared to other treatments. The increase in available P was more than 527% when compared to available P in the soil before *Actinomycetes* application, which was seen in the results of the initial analysis of Andisols (Total P of 0.21% and Available P of 42.46 ppm). This is due to the ability of Actinomycetes to assist the process of availability of P in the soil to produce organic acids and phosphatase enzymes. The condition of soil water content did not affect the statistically significant changes in available soil P. However, two isolates (Isolates A_{458} and A_{459}) at 50% groundwater conditions of field capacity were able to increase available P higher than field capacity conditions. The process of organic acids or phosphatase enzymes can increase the availability of phosphorus (P) in soil. Organic acids can solubilize phosphorus compounds in soil particles, making them more accessible to plants. Phosphatase enzymes, on the other hand, can break down organic phosphorus compounds into inorganic forms that plants can absorb. This increases the amount of available P in the soil, which can promote plant growth and productivity. Overall, organic acids and phosphatase enzymes play important roles in the cycling of P in the soil, contributing to the nutrient supply and productivity of agricultural systems (Li et al., 2021).

The ability of an isolate to increase available P is related to the number of *Actinomycetes* populations in the soil, where large number of *Actinomycetes*

Table 3. Available P in Andisols after 40 days incubation (ppm)

Isolate of Actinomycetes	Soil water content			Average
	K_1 (50%)	K_2 (75%)	K_3 (100%)	
A_0 (Control)	63.45	52.80	62.95	59.73 h
A_1 (Isolate A_{132})	165.95	167.60	154.65	162.73 f
A_2 (Isolate A_{468})	224.05	256.75	228.95	236.58 c
A_3 (Isolate A_{458})	280.95	268.60	250.25	266.60 a
A_4 (Isolate A_{471})	223.75	237.30	246.60	235.88 cd
A_5 (Isolate A_{459})	259.70	257.20	231.60	249.50 b
A_6 (Isolate A_{321})	172.70	179.70	200.05	184.15 e
A_7 (Isolate A_{356})	156.75	151.65	169.20	159.20 fg
Average	193.41	196.45	193.03	

Remarks: Means followed by the same letters in the same column or row are not significantly different at the level of 5% according to the Duncan Multiple Range Test.

Table 4. Characteristics of isolates and naming of *Actinomycetes*

Treatment	Color of Air Mycelium	Color of Mycelium Vegetative	Color of Diffused Pigmen	Gram Stain	Species Named of *Actinomycetes*
A_1 (Isolate A_{132})	White	White	-	Positive	*Nocardia sp. KK-F5*
A_2 (Isolate A_{468})	White Gray	Gray	-	Positive	*Streptomyces collinus Tu 365*
A_3 (Isolate A_{458})	Orange	Orange	-	Positive	*Actinoplanes cibodasensis strain LIPI11-2-Ac042*
A_4 (Isolate A_{471})	Black Brown	Black	Black	Positive	*Micromonospora sp. 2602HV4*
A_5 (Isolate A_{459})	Orange	Orange	-	Positive	*Actinoplanes sp. TD028 16S*

populations will be directly proportional to the amount of *Actinomycetes* activity in changing the form of unavailable P to available P in Andisols. This can be seen with the correlation value between the available P and the population of +0.853.

The treatment of water content in the soil did not affect the available P of the Andisols, but the application of *Actinomycetes* could increase the available P of the Andisols, so that the P retention in the Andisols could decrease due to the dissolution of phosphate that was adsorbed on the colloidal surface of the soil. The increase in available P in the soil is due to the activity of *Actinomycetes* in producing organic acids and phosphatase enzymes. Several organic acids known to be produced by *Actinomycetes* can be malic, acetic, citric and oxalic. *Actinomycetes* can be known to be able to solubilize bound phosphate in the soil.

Based on Table 4, the color grouping observations obtained 5 isolates, each of which had a different color appearance from the air mycelium and vegetative mycelium, but not all of the pigment colors diffused on the agarose were formed.

The data that have been assembled and subjected to BLAST with genomic data registered with the NCBI (National Center for Biotechnology Information) state that isolate A_{132} has the species name *Nocardia sp. KK-F5*; Isolate A_{468} has the species name *Streptomyces collinus Tu 365*; Isolate A_{458} has the species name *Actinoplanes cibodasensis strain LIPI11-2-Ac042*; isolate A_{471} has the species name *Micromonospora sp. 2602HV4*; and Isolate A_{459} has the species name *Actinoplanes sp. TD028 16S*.

Based on the results of observations made in stage 2 and stage 3, it is known that isolates with identified species names such as *Nocardia sp. KK-F5*; *Streptomyces collinus Tu 365*; *Actinoplanes cibodasensis strain LIPI11-2-Ac042*; *Micromonospora sp. 2602HV4*; and *Actinoplanes sp. TD028 16S* can solubilize phosphate in Andisols because it can produce organic acids and phosphatase enzymes to solubilize phosphate in soil. The different types of *Actinomycetes* can affect the availability of phosphate in the soil; this is because *Actinomycetes* produce organic acids to convert phosphorus bound by allophane so that it becomes available phosphorus. Further research needs to be carried out to see how much organic acid is produced to see the extent of the differences caused by the application of different types of *Actinomycetes*.

CONCLUSIONS

The application of *Actinomycetes* to Andisols incubated for 40 days had a significant effect on soil pH, increasing the *Actinomycetes* population, and available P. Water content 100% of field capacity was the best in increasing soil pH and *Actinomycetes* population. The interaction between *Actinomycetes* and soil water content did not have a significant effect. Selected *Actinomycetes* identified with the species name of *Actinoplanes cibodasensis strain LIPI11-2-Ac042* was very effective in increasing the availability of phosphate.

ACKNOWLEDGEMENTS

We would like to thank the Directorate of Research and Community Service (DRPM) of the Ministry of Research, Technology and Higher Education for funding this research through the “Skema Penelitian Tesis Magister” Program.

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