

Isolation, Purification and Potential Test of Actinomycetes in Increasing Phosphate Availability

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Abstract. High phosphate retention in andisol soils causes low P-Availability in the soil. Nutrient phosphate has a very important role in plant growth. Actinomycetes can be used to dissolve phosphates that are bound to the soil. This research aims to obtain Actinomycetes and tests their effect on increasing the availability of phosphate. This research was carried out in the Soil Biology Laboratory with 2 stages, namely: 1. Isolation and Purification and 2. Test potential of Actinomycetes on Pykovskaya media with several phosphate sources ($\text{Ca}_3(\text{PO}_4)_2$; AlPO_4 and FePO_4). The results obtained showed that there were 17 Actinomycetes isolates isolated from andisol soil at various locations. Some Actinomycetes obtained have the ability to dissolve P-not available into P available on solid pykovskaya media with various phosphate sources ($\text{Ca}_3(\text{PO}_4)_2$; AlPO_4 and FePO_4) but with different abilities.

Keywords: Actinomycetes, Andisol and P-Available

1 Introduction

Andisols are fertile soils that have suitable physical and chemical characteristics for growing agricultural crops [1]. However, high phosphate retention in Andisols (> 85%) causes most of the P in Andisols not available to be used by plants [2,3,4,5].

Although Andisols is said to be fertile, the main problem found in Andisols is to have high P retention and low P-Availability. Andisols can be formed from volcanic ash which is dominated by amorphous minerals [6]. One special feature of soils containing amorphous mineral materials is high P retention (> 85%). This causes Andisols soil has a high retention of phosphate hence the availability of phosphorus (P) in the soil is reduced due to P adsorption on the surface of the soil colloid which causes P nutrients in the soil not available to plants.

The element of phosphorus is an essential macronutrient that has a very important role for the process of plant growth, such as photosynthesis, assimilation and respiration [7]. Not all phosphorus can be absorbed by plants, plants absorb phosphorus available in the form of orthophosphate ions (H_2PO_4^-) and secondary orthophosphate ions (HPO_4^{2-}). The nature of phosphorus is mobile or easily move between plant tissues [8].

Actinomycetes are soil microorganisms that are commonly found in various types of soil. Actinomycetes live as saprofit and actively decompose organic matter, hence it can increase soil fertility and life in plant rhizosphere [9].

Actinomycetes can dissolve phosphates that are bound in the soil, but not all Actinomycetes species can dissolve phosphates in the soil. Actinomycetes of the genus *Micromonospora* sp., *Nocardia* sp., *Actinomadura* sp., *Rhodococcus* sp., *Actinoplanes* sp., *Microbispora* sp., and *Streptosporangium* sp., can produce the phosphatase enzyme hence it can dissolve the phosphorus that is bound in the acidic or basic soil conditions [10].

2 Research Method

2.1 Materials. This research used Andisols from Kutarayay Village, Namanteran District at different sample locations. The research was conducted from May - October 2019 at the Faculty of Agriculture Soil Biology Laboratory – Universitas Sumatera Utara. The material used in this research was Starch Casein Agar Medium (10 g of Starch solubel, 2 g K_2HPO_4 , 2 g KNO_3 , 0.3 g Casein, 0,05 g $MgSO_4 \cdot 7 H_2O$, 0.02 g, $CaCO_3$, 0,01 g $FeSO_4 \cdot 7 H_2O$, 2 g NaCl, 18 g *agar*, and aquadest 1000 mL) for isolation. Actinomycetes were tested for potency on pikovskaya media (10 g glucose, 5 g $Ca_3(PO_4)_2/AlPO_4/FePO_4$, 0.1 g $MgSO_4 \cdot 2 H_2O$, 0.5 g $(NH_4)_2SO_4$, 0.1 g $FeSO_4$, 0.1 g $MnSO_4$, 0.5 g yeast extract, 15g agar and 1000 mL aquadest).

2.2 Methods. This research was carried out in 2 stages: Stage 1. Isolation and Purification. At this stage, Actinomycetes was isolated from sample soils that were in the rhizosphere of plants or around a homogeneous rhizosphere on Starch Casein Agar (SCA) media by diluting 10 g of soil into 90 mL of Physiological NaCl solution (10^{-1}), then taken 1 mL of the solution before it and put into 9 mL of physiological NaCl solution (10^{-2}) is carried out again until a dilution of 10^{-3} , 10^{-4} and 10^{-5} is obtained and the result of dilution is put into the growth medium. After incubating for 3-5 days at 30°C, purification was carried out by separating the colonies on sterile SCA media in order to purify the isolates. After obtaining pure Actinomycetes then proceed to the next stage. Stage 2. Actinomycetes Potential Test on solid pykovskaya media. At this stage Actinomycetes isolates obtained will be grown on pykovskaya media (source of P: $Ca_3(PO_4)_2$, $FePO_4$ dan $AlPO_4$) to test the ability of Actinomycetes in dissolving phosphate based on clear zones. The calculation of the phosphate dissolving index can be seen in formula (1).

$$PSI = \frac{a-b}{b} \quad (1)$$

Description:

PSI : Phosphate Solubilization Index
a : Clear Zone Diameter + Colony Diameter
b : Colony Diameter

3 Result and Discussion

Sampling of andisols and litter above andisol soil surface from 5 coordinate points can be seen on the soil sampling map. Look at Figure 1.

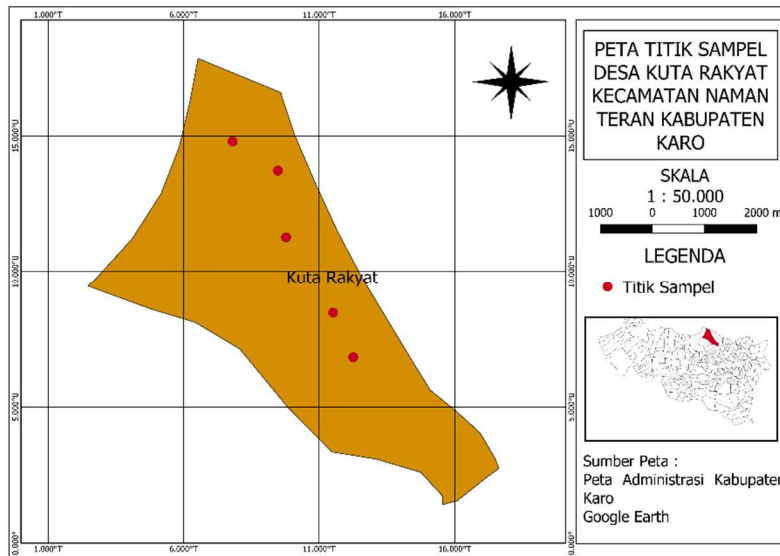


Fig. 1.Maps of soil type and andisol soil sampling

Based on the research results, it was obtained the results of Actinomycetes isolation which was isolated from Andisol soil or litter that was above Andisol soil from several soil sampling points that were believed to be able to dissolve phosphate.

Table 1. Isolation and purification of Actinomycetes from Andisol and Litter Above Andisol

Isolate	Coordinate	Code of Save Isolate	Description
1	98.3621° S 3.2659 ° E	A132	Vegetation: Cabbage, Corn, Chili
2	98.3700 °S 3.2613 ° E	A468	Forest Plants, Litter
3	98.3796 °S 3.2369 ° E	A458	Forest Plants, Litter
4	98.3714 °S 3.2497 ° E	A471	The dominant vegetation is coffee plants
5	98.3796 °S	A459	Forest Plants, Litter

Isolate	Coordinate	Code of Save Isolate	Description
	3.2369 °E		
6	98.3621° S 3.2659 ° E	A156	Forest Plants, Litter
7	98.3714 °S 3.2497 °E	A623	The dominant vegetation is coffee plants
8	98.3796 °S 3.2369 °E	A425	Forest Plants, Litter
9	98.3831° S 3.2288 ° E	A322	Roots of hibiscus plants
10	98.3831 °S 3.2288 °E	A231	Roots of hibiscus plants
11	98.3700 °S 3.2613 ° E	A241	Forest Plants, Litter
12	98.3831 °S 3.2288° E	A321	Roots of hibiscus plants
13	98.3621° S 3.2659 ° E	A356	Vegetation: Cabbage, Corn, Chili
14	98.3714 °S 3.2497 °E	A321	The dominant vegetation is coffee plants
15	98.3714 °S 3.2497 °E	A331	The dominant vegetation is coffee plants
16	98.3700 °S 3.2613 ° E	A652	Forest Plants, Litter
17	98.3700 °S 3.2613 ° E	A363	Forest Plants, Litter

Based on Table 1, it can be seen that there are 17 Actinomycetes isolates from 5 different locations. Isolates were given isolate codes from each sampling point. Location 1 (98.3621° S, 3.2659 ° E) obtained 3 different isolates with isolate codes and code of save isolate (1, A132; 6, A156 and 13, A356); location 2 (98.3796 °S, 3.2369 °E) obtained 3 different isolates with isolate codes and code of save isolate (3, A458; 5, A459 and 8, A425); location 3 (98.3714 °S, 3.2497 °E) obtained 4 different isolates with the isolate codes and code of save isolate (4, A471; 7, A623; 14, A361 and 15, A331); location 4 (98.3700 °S, 3.2613 ° E) obtained 4 different isolates with isolate codes and code of save isolate (2, A468; 11, A241, 16, A652 and 17, A363); location 5 (98.3831° S, 3.2288° E) obtained 3 different isolates with isolate codes and code of save isolate (9, A322; 10, A231 and 12, A321).

Based on the results seen in Table 1, various isolates were obtained as well as different growth of each isolate. Visually, the appearance of Actinomycetes is different and varied hence to distinguish isolates visually can be done by a glance without the aid of a microscope. Colony characterization of several isolates of Actinomycetes isolated can be characterized by their diverse and pleimorphic cell morphology, irregular rod shape, branched filaments which form the mycelium structure, non-motile, gram-positive and facultative anaerobic test that has been done before.

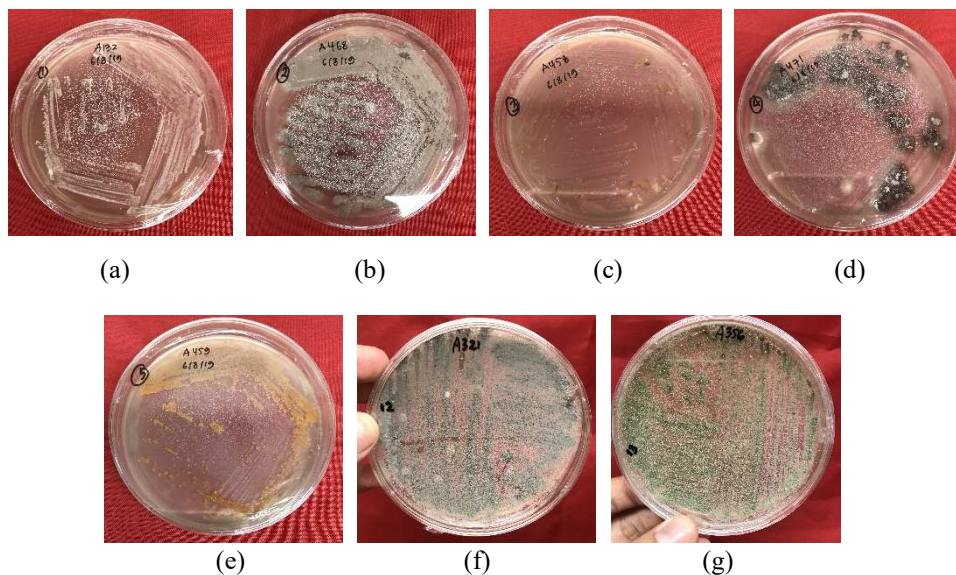


Fig. 2. Visual Actinomycetes Appearance (a) A132; (b) A468; (c) A458; (d) A471; (e) A459; (f) A321; (g) A356.

Some of these Actinomycetes will be tested for phosphate solubility activity on pikovskaya media by looking at the phosphate dissolving index with several phosphate sources such as $\text{Ca}_3(\text{PO}_4)_2/\text{FePO}_4/\text{AlPO}_4$. Some Actinomycetes have the potential to dissolve phosphate with different phosphate sources.

Based on the testing of the phosphate solubilization index on Actinomycetes in solid Pikovskaya media with various sources of P: $\text{Ca}_3(\text{PO}_4)_2$, FePO_4 , AlPO_4 for 8 days of incubation can be seen from Table 2 below.

Table 2. Phosphate Solubilization Index on Actinomycetes in solid Pikovskaya media with various sources of P: $\text{Ca}_3(\text{PO}_4)_2$, FePO_4 , AlPO_4

Isolate	Sources of Phosphate	Phosphate Solubilization Index / Observation Day			
		2	4	6	8
1	$\text{Ca}_3(\text{PO}_4)_2$	0	0	0	0
	AlPO_4	1.56	2.00	1.67	1.96
	FePO_4	11.33	4.10	2.88	2.73
2	$\text{Ca}_3(\text{PO}_4)_2$	1.87	1.03	1.38	1.52
	AlPO_4	0	0	0	0
	FePO_4	1.56	1.51	2.63	2.65
3	$\text{Ca}_3(\text{PO}_4)_2$	0	0	0	0
	AlPO_4	2.50	1.43	2.32	2.56
	FePO_4	0	0	0	0
4	$\text{Ca}_3(\text{PO}_4)_2$	0	0	0	0

Isolate	Sources of Phosphate	Phosphate Solubilization Index / Observation Day			
		2	4	6	8
5	AlPO ₄	3.25	2.81	2.80	3.00
	FePO ₄	0	0	0	0
	Ca ₃ (PO ₄) ₂	0	0	0	0
6	AlPO ₄	2.64	1.72	2.58	3.07
	FePO ₄	0	0	0	0
	Ca ₃ (PO ₄) ₂	0	0	0	0
7	AlPO ₄	0	0	0	0
	Ca ₃ (PO ₄) ₂	2.00	2.76	2.98	2.78
	FePO ₄	1.08	1.62	1.79	1.79
8	AlPO ₄	0	0	0	0
	Ca ₃ (PO ₄) ₂	0	0	0	0
	FePO ₄	0	0	0	0
9	AlPO ₄	0.97	2.93	2.40	2.10
	Ca ₃ (PO ₄) ₂	0	0	0	0
	FePO ₄	0	0	0	0
10	AlPO ₄	3.10	2.89	3.36	2.56
	Ca ₃ (PO ₄) ₂	0	0	0	0
	FePO ₄	0	0	0	0
11	AlPO ₄	4.80	4.47	3.36	2.73
	Ca ₃ (PO ₄) ₂	0	0	0	0
	FePO ₄	0	0	0	0
12	AlPO ₄	0	0	0	0
	Ca ₃ (PO ₄) ₂	0	0	0	0
	FePO ₄	1.65	0.98	1.07	1.03
13	AlPO ₄	0	0	0	0
	Ca ₃ (PO ₄) ₂	0	0	0	0
	FePO ₄	3.67	1.04	1.67	2.16
14	AlPO ₄	0	0	0	0
	Ca ₃ (PO ₄) ₂	0	0	0	0
	FePO ₄	0	0	0	0
15	AlPO ₄	1.88	1.40	1.73	1.71
	Ca ₃ (PO ₄) ₂	0	0	0	0
	FePO ₄	3.68	1.85	2.09	2.07
16	AlPO ₄	0	0	0	0
	Ca ₃ (PO ₄) ₂	0	0	0	0
	FePO ₄	0	0	0	0
17	AlPO ₄	0.91	1.61	1.61	1.54
	Ca ₃ (PO ₄) ₂	0	0	0	0
	FePO ₄	0	0	0	0

Observation was done on solid pikovskaya media of various P sources for 8 days, it was found that the largest efficiency of the phosphate solvent was in isolate 5 with AlPO₄ phosphate

source of 307% and the smallest efficiency of the phosphate solvent was in isolate 11 with FePO_4 phosphate source of 103%. This showed that isolate 5 application has more potential in releasing P bound to an available form of 307%. The ability of microbial phosphate solvents to grow and dissolve phosphate varies from the area of the clear zone and incubation time.

Actinomycetes that were able to dissolve phosphate with the highest phosphate solubility index was in isolate 7 with a phosphate source of $\text{Ca}_3(\text{PO}_4)_2$ of 278% and the lowest phosphate dissolving index was in isolate 2 of 152% and several Actinomycetes isolates were unable to dissolve the Ca phosphate source, namely isolate 1, 3, 4, 5, 6, 8, 9, 11, 12, 13, 14, 15 and 17. Not all Actinomycetes are able to dissolve phosphate, Actinomycetes from *Micromonospora* sp., *Nocardia* sp., *Actinomadura* sp., *Rhodococcus* sp., *Actinoplanes* sp., *Microbispora* sp., and *Streptosporangium* sp. genus were able to produce the phosphatase enzyme hence it can dissolve phosphorus that is bound in the soil in acidic or basic conditions [10].

The highest phosphate solubility index with AlPO_4 phosphate source was in isolate 5 of 307% and the lowest phosphate solubility index was in isolate 15 of 161% and some Actinomycetes isolates which were unable to dissolve at Al phosphate sources namely isolate 2, 6, 10, 11, 14, 16 and 17.

The ability of Actinomycetes in dissolving phosphate with Fe phosphate sources had the highest phosphate solubility index in isolate 1 of 273% and the lowest phosphate solubility index in isolate 11 by 103% as well as some Actinomycetes isolates which were unable to dissolve in Fe phosphate sources that is isolate 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 15, 16 and 17.

Several Actinomycetes isolates that cannot dissolve phosphate at the source of phosphate $\text{Ca}_3(\text{PO}_4)_2$, FePO_4 , AlPO_4 namely isolates 6, 14 and 17. This is because the ability of these isolates to dissolve phosphate is not found, the Actinomycetes cannot remove organic acids or enzymes which functions in dissolving phosphates such as the enzyme phosphatase. An enzyme that can dissolve phosphate can be either a phosphatase enzyme or a phosphomonoesterase enzyme [8].

4 Conclusion

Several Actinomycetes obtained have the ability to dissolve P-not available into P available on solid pykovskaya media with various phosphate sources ($\text{Ca}_3(\text{PO}_4)_2$; AlPO_4 and FePO_4) with different abilities.

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