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Isolation, Population, and Selection of Phosphate Solubilizing Bacteria from Acid Soils of Institut Teknologi Sumatera's Region, Lampung

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Abstract. Ultisol, which is acidic soil, is widely distributed in Indonesia, especially Sumatera Island. The characteristics of acidic ultisol make the presence of phosphate available in the soil deficient. This condition is caused by soil pH, which is very influential in phosphate availability in the soil. In acidic conditions, phosphate (P) binds to Fe or Al. Plants highly need the availability of phosphate in the soil. This condition is because phosphate is a nutrient that is needed by plants. Phosphate solubilizing bacteria are soil microbes that can improve P's availability in acid soils by dissolving P that is bound to other metals. This study aims to obtain indigenous phosphate solubilizing bacteria from acid soils located in the Institut Teknologi Sumatera (ITERA). The study was conducted from April to June 2019, which included isolation of phosphate solubilizing bacteria from the soil, a test of the ability of phosphate dissolution on Pikovskaya medium, and pathogenicity test on potato tubers. The results showed that there were 35-74 x 10⁵ cfu/g of soil samples. Seventeen isolates were obtained, which were able to use phosphate in the medium. Of the seventeen isolates, there were four potential isolates with the highest phosphate solubility index, namely EF.NAP 1 (1.2143), EF.NA3 (1,100), EF.NAP 4 (0.8616) and EF.NAP 9 (0.7188). Isolates have the best phosphate solubilizing index on the 6th day to the 7th day. The four potential isolates were not pathogenic in pathogenicity testing using potato tubers. These four isolates can be used to isolate candidates to improve the availability of phosphorus in the soil needed by plants in the ITERA region.

1. Introduction

Acidic soils such as ultisol and oxysol are prevalent in Indonesia, especially on Sumatra and Kalimantan [1]. Its wide distribution causes this type of soil to be used as an agricultural area. A common problem found in Ultisol soils is the low P available due to high phosphate fixation by several Fe, Al and Ca minerals. The binding of phosphate by some minerals causes the phosphate in the soil not to be absorbed by plants because it is in the form of phosphate bound [2]. This condition causes the effectiveness of inorganic phosphate



fertilization on ultisol soil is very low, and plants can absorb only 10-30%. The remaining 70-90% fertilizer cannot be absorbed by plants and settles into the soil. This situation causes the soil to become infertile. This soil type is the primary condition in the area of the Institut Teknologi Sumatera (ITERA). Acidic soil conditions in ITERA are also supported because ITERA land is a former rubber plantation that has undergone years of fertilization so that inorganic phosphate buildup is very high.

Another thing is also due to the condition of the ITERA soils originating from rock weathering, and former rubber plantation land has limited nutrient availability. It has a very acidic soil pH [3]. This factor is the cause of the condition of plants in the ITERA region to become infertile. Plants are very dependent on plant nutrients, mostly taken from the soil, one of which is phosphorus [4]. Phosphorus (P) is the main element of macronutrients needed for plant growth and development. Soil generally contains much phosphorus, but only a little is available for plants. Plants are only able to absorb mono or phosphate-based, organic phosphate or a form of phosphate that is not dissolved must be mineralized or dissolved by microorganisms [5], if the soil does not contain phosphate solubilizing bacteria, only a small amount of phosphate can be absorbed by the soil or plants, so that causing the soil to become infertile [6]. There needs to be an ecological treatment or manipulation to increase the growth of other plants, one of them with phosphate solubilizing bacteria.

Phosphate solubilizing bacteria are known to dissolve P by releasing P compounds through the mechanism of chelating formation, exchange reactions, and organic acid production [7]. The effect of phosphate solubilizing microorganisms on plants is due to their ability to increase P's availability and their ability to produce growth regulators, especially by microorganisms that live on the root surface [4] called by *plant growth-promoting rhizobacteria*. Some groups of bacteria, such as *Pseudomonas*, *Bacillus*, and *Rhizobium*, are the most potential phosphate solubilizing bacteria and can be found on the soils. The potential use of indigenous soil microbes from acid soils can be carried out to support the greening program carried out by ITERA. This method is environmentally friendly rather than the method often used so far in fertilization that is not environmentally friendly [1], as the use of chemical fertilizers.

Bacteria that can dissolve phosphate will experience inhibition of its activity if introduced into habitats that are not native. Besides, the acidity factor (pH) of an environment is a limiting factor for phosphate solubilizing bacteria activity [8]. Therefore, the search for indigenous phosphate solubilizing bacteria isolates from acid soils is very useful for obtaining suitable potential bacteria applied on ultisol land in ITERA to improve plants' uptake nutrients. This study aims to obtain indigenous phosphate solubilizing bacteria from acid soils from ITERA's region that can dissolve phosphate, which can later be developed as biological fertilizer to improve soil fertility ITERA regions.

2. Methods

The research was conducted at the ITERA Biology Laboratory in April to June 2019 in four steps consist of:

2.1. Soil Sampling

Soil sampling is done using a purposive random sampling method. Soil samples were taken randomly from embankment F at a depth of 0-15 cm at 17.00 WIB. The sample is put in a plastic clip and stored at room temperature before used.

2.2. Isolation and Characterization of Phosphate Solubilizing Bacteria from Soil

Soil samples were grown on nutrient broth medium enriched with 5% $\text{Ca}_3(\text{PO}_4)_2$ and incubated in a shaking incubator at a speed of 120 rpm for 24 hours at room temperature. The soil sample is diluted and then grown on the surface of the Pikovskaya's medium for 3-7 days at room temperature. The growth of P solubilizing bacterial colonies is characterized by the formation of clear zones around the colony. The colonies were purified to obtain a single colony.

2.3. Testing the ability of bacteria in dissolving P

Pure culture of P solubilizing bacterial isolates was regenerated on solid Pikovskaya medium and incubated for seven days at room temperature. The clear zone formed around the colony is measured. The P dissolving index (IP) is calculated to determine the bacterial degradation ability of P. Testing the ability of bacteria to dissolve phosphate is carried out using pure bacterial isolates, grown on a solid Pikovskaya medium containing (10 g $\text{C}_6\text{H}_{12}\text{O}_6$, 5 g $\text{Ca}_3(\text{PO}_4)_2$, 0.5 g $(\text{NH}_4)_2\text{SO}_4$, 0.2 g KCl, 0.1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.002 g $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.002 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g NaCl, 0.5 g yeast extract, 20 g to be dissolved in 1000 ml aquadest with a pH of 7.0), with dot inoculation and incubated for seven days at 30 °C. The growth of P solubilizing bacterial colonies is characterized by the formation of clear zones around the colony. The clear zone formed around the colony is measured, and the P dissolving index (IP) of each colony is calculated to determine the degradation ability of bacteria against P by the following equation [9].

2.4. Pathogenicity Test

Bacterial isolates with the highest phosphate solubilizing index were tested for simple pathogenicity using potato tubers. This method was modified by Asril and Leksikowati [10] based on Lelliot and Stead method [11]. This test aims to see the ability of bacterial isolates pectinolytic. Pectinolytic activity is an early indication of bacterial isolates capable of damaging pectin (in the form of lesions on potato tubers) found in plant cell walls. This potato spoilage test is done by inoculating bacteria by being pricked in the middle of a potato tuber washed with running water. Potato bacteria that have been inoculated by bacteria are incubated in a petri dish which has been given filter paper and sterile water to keep it moist. The pectinolytic activity was observed for two days and incubated at room temperature. The isolate's positive reaction was demonstrated by the presence of decay in the middle of the potato tuber inoculated with test bacteria. The positive reaction indicates that the isolate is a pathogen in plants and cannot be used as a potential isolate candidate.

3. Results and Discussion

3.1. Isolation and Total Population of Phosphate Solubilizing Bacteria from Soil

The isolation of phosphate solubilizing bacteria from soil samples at Embankment F Location amounted to $35\text{-}74 \times 10^5$ cfu/g of soil samples. Asril & Lisafitri [3] reported that the population of phosphate solubilizing bacteria at the same sampling point location only amounted to 14.2×10^4 cfu/g. The population of phosphate bacteria in the soil is influenced by soil pH. The soil pH of the sample of 4.09 was the main factor causing the number of isolates of phosphate solubilizing bacteria from the sample to be limited. The acidity factor of an environment is a limiting factor for phosphate-solubilizing bacteria activity [8]. The initial treatment also caused the increase in the population of phosphate solubilizing bacteria from previous studies in phosphate addition in the initial isolation medium before it was grown on the pikovskaya medium. The addition of a phosphate ($\text{Ca}_3(\text{PO}_4)_2$) stimulates phosphatase formation by bacteria in soil samples.

Based on the total population obtained 17 isolates of phosphate solubilizing bacteria that have diverse abilities. The ability of the seventeen bacteria to dissolve phosphate is shown by forming a clear zone on the Pikovskaya medium (Figure 1). The clear zone shows that the bacteria can dissolve phosphate. The formation of a clear zone around the colony shows that the isolate can produce organic acids that can bind with Ca ions to form $\text{Ca}_3(\text{PO}_4)_2$ compounds on Pikovskaya medium and free H_2PO_4 ions to form clearer coloured areas [12]. The greater the clear zone around the colony, the higher the ability of bacteria to dissolve phosphate in the media. Bacteria that show the highest clear zone will be used as a potential isolate to proceed to the next step.

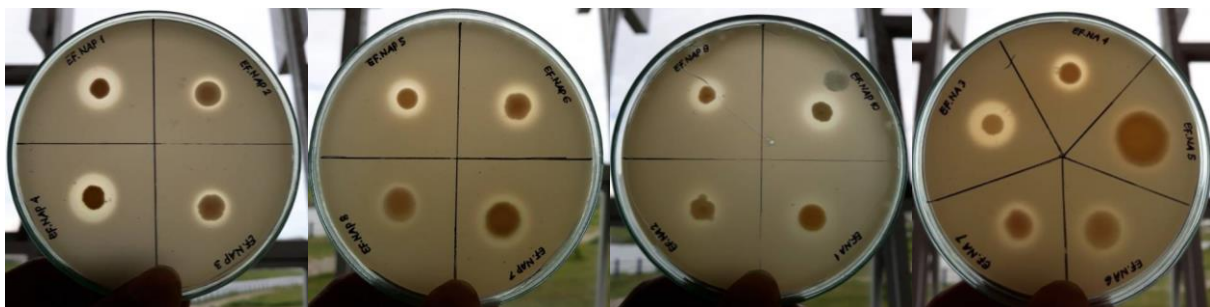


Figure 1. Ability of phosphate solubilizing bacteria on Pikovskaya medium

3.2. The ability of bacteria in dissolving P

Bacterial isolates that had the highest phosphate solubility index in pikovskaya media were EF.NAP 1 (1.2143), EF.NA 3 (1.100), EF.NAP 4 (0.8616) and EF.NAP 9 (0.7188) (Figure 2). In general, *Pseudomonas* is a type of bacteria that is known as the best phosphate solubilizing besides *Bacillus* and *Rhizobium*. The *Pseudomonas* group's existence on acid soils and because of this genus can adapt to various environmental conditions. This bacterium can live in acid soils such as *Pseudomonas* sp. DSMZ strain 13134 [13]. *Pseudomonas* bacteria are widely reported as bacteria with the highest phosphate dissolution concentration in the rhizosphere [14]. Even one of the species of *Pseudomonas* FBJ6 is the most efficient phosphate-solubilizing as biological fertilizer

[15,16]. The use of *Pseudomonas* such as *P. fluorescens* in agricultural soils is very high. It can be a plant growth-promoting rhizobacteria to be developed as a biofertilizer and bioinoculant for plants [17]. Plant growth-promoting rhizobacteria can increase plant growth with two main mechanisms, namely directly and indirectly. The ability of phosphate solubilizing bacteria is one of the direct mechanisms besides producing phytohormone, nitrogen-fixing, and producing hydrolytic enzymes such as chitinase, glucanase, and protease.

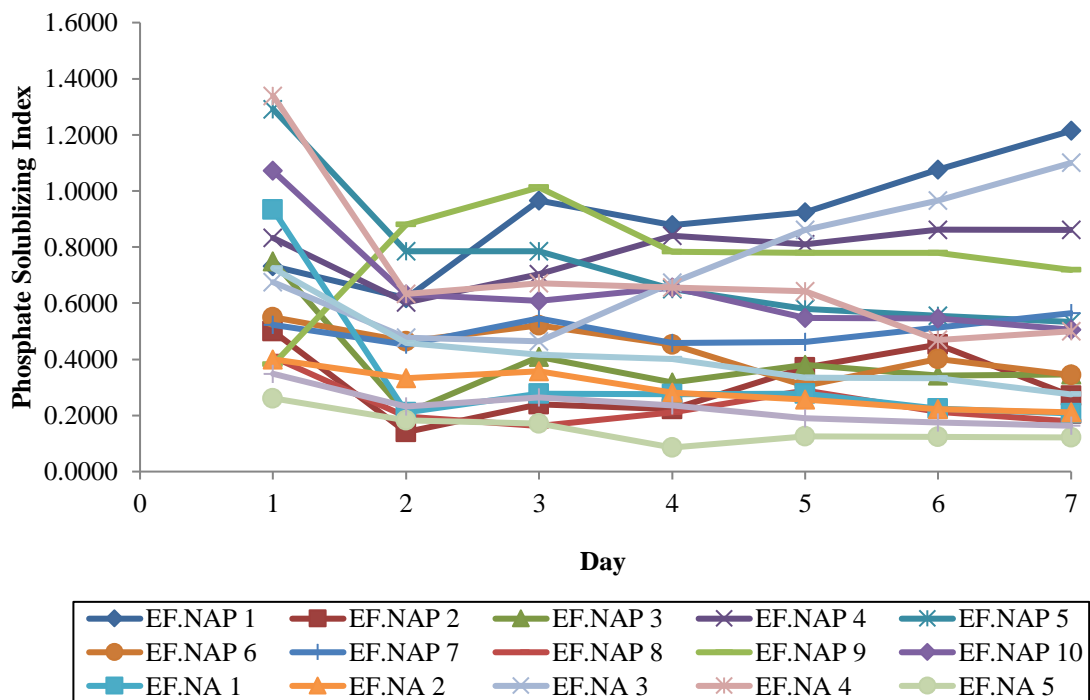


Figure 2. The ability of phosphate solubilizing bacteria in 7 days incubation

Besides, the phosphate solubilizing index of the four isolates varied depending on the incubation period. All four isolates had the highest phosphate solubility index on day 2 to day 7. Based on the incubation period, bacterial isolates with the highest phosphate dissolving index were EF.NAP 1, EF.NA 3, EF.NAP 9 and EF.NAP 4. The formation of clear zones of each isolate began to form from day 2 to 7 [18]. The ability to form a clear zone depends on the bacterial self-division during the logarithmic phase. Cell division in the logarithmic phase is related to bacterial phosphatase activity produced at the stage of cell division. The activity of the enzyme phosphatase in dissolving phosphate is metabolic that is produced in the logarithmic phase. The ability of bacteria to dissolve phosphate enzymatically is strongly influenced by the type of bacteria and environmental factors such as pH, temperature, the substrate (phosphate composition in the medium).

Table 2. The incubation time of isolates with the highest phosphate solubilizing index.

Isolate Code	Phosphate Solubilizing Index	Incubation Time (Day)
EF.NAP 1	1.2143	7 th
EF.NA 3	1.1000	7 th
EF.NAP 9	0.8807	2 nd
EF.NAP 4	0.8622	6 th

3.3. Pathogenicity of Potential Isolates

The pathogenicity of a potential isolate is very important to know. A simple test can be done using a potato spoilage test (pectinolytic test). Four isolates (EF.NAP 1, EF.NA 3, EF.NAP 9, and EF.NAP 4), which had the highest phosphate solubility index, showed negative results in potato spoilage (Table 3). This test aims to see the bacterial pectinolytic activity used can damage the pectin found in potato tubers. Potato tubers become one of the sample models for testing pectin's degradation, which is usually found in plant cell walls. Bacterial indications are classified as plant pathogens, namely the occurrence of lesions in tested potatoes [13]. The use of potatoes as a model host because potatoes are an alternative host that can provide information on the pathogenicity of a test bacterium [19]. Usually, the purpose is related to bacteria's pathogenicity in plants using tobacco plants called hypersensitivity testing. However, testing with potatoes can provide representative results in the pathogenicity of bacteria in plants. Bacteria that cause soft rot in plants and are pathogenic are classified into soft rot bacteria [20]. This condition is caused by the bacteria being able to soften potatoes as a substrate to be referred to as soft rot. These results show that the four potential bacterial isolates are not pathogenic to plants when applied to soil and plants.

Table 3. Patogeneicity test of potential isolates on tuber of *Solanum tuberosum*

Isolate Code	Results
EF.NAP 1	Negative
EF.NA 3	Negative
EF.NAP 9	Negative
EF.NAP 4	Negative

4. Conclusion

Seventeen phosphate solubilizing bacterial isolates were successfully obtained from acid soils from and around the embankment F, a former rubber plantation land. The four isolates that had the highest phosphate (IP) were EF.NAP 1 (1.2143), EF.NA 3 (1.100), EF.NAP 4 (0.8616) and EF.NAP 9 (0.7188). The variation of phosphate dissolution index depends on the incubation period of isolates. The incubation period with the best phosphate dissolving index is days until the sixth and seventh days. The four potential isolates could not decompose potatoes as a simple test of the pathogenicity of an **isolate**. This potential isolate can be used as a candidate for bacteria that can improve the availability of acidic soil nutrients, especially the available phosphate needed by plants.

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References

- [1] Lubis A, Ani N, and Sofian A 2016. *Agrium*. **20**(2), 96-100
- [2] Asril M, and Lisafitri Y 2020. *Jurnal Teknologi Lingkungan*. **21**(1), 40-48
- [3] Asril M, and Lisafitri Y 2019. *IOP Conference Series: Earth and Environmental Science*, **258**, DOI: 10.1088/1755-1315/258/1/012026
- [4] Roni NG, Witariadi NM, Candraasih KN and Siti NW 2013. *Pastura*. **3**(1), 13-16
- [5] Ramaekers L, Remans R, Rao IM, Blair MW and Vanderleyden J 2010. *Field Crops Research*, **117**, 167-176
- [6] Ilham, Darmayasa IBG, Nurjaya IGMO, and Kawuri R 2014. *Jurnal Simbiosis II*, **2**(1), 173-183
- [7] Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, and Young CC 2006. *Applied Soil and Ecology*, **34**(1), 33-41
- [8] Setiawati MR, and Pranoto E 2015. *Jurnal Penelitian Teh dan Kina*, **18**(2), 159-164
- [9] Mursyida E, Mubarik NR, and Tjahjoleksono A 2015. *Research Journal of Microbiology*, 10(6), 270-279
- [10] Asril M, and Leksikowati SS 2019. *Elkawnie Journal of Islamic Science and Technology*, **5**(2), 1-14
- [11] Lelliot, Stead. (1987). Oxford: Blackwell Sci. Pub.
- [12] Sagervanshi A, Kumari P, nd AN, and Kumar A 2012. *International Journal of Life Science & Pharma Research*, 2(3), 245-255
- [13] Sumarni A, Aiyen and Panggeso J 2015. *J Agrotekbs*, 3(3), 338-344
- [14] Reyes VA, and Valduz Z 2006. *Plant Soil*, 287, 69-75.
- [15] Kumar A, Kumar A, Devi S, Patil S, Payal C, and Negi S 2012. *Recent Research in Science and Technology*, 4(1), 1-5
- [16] Parani K, and Saha BK 2012. *European Journal of Biological Sciences*, 4(2), 40-44
- [17] Noori MSS, and Saud HM 2012. *Journal of Plant Pathology and Microbiology*, 3(2), 1-4
- [18] Purwaningsih S 2012. *Jurnal Teknologi Lingkungan*, **13**(1), 101-108
- [19] Supriadi, Nildar I, and Taryono 2002. *Jurnal LITTRI (Penelitian Tanaman Industri)*, **8**(2), 45-48
- [20] Oviana T, Aeny TN, and Prasetyo J 2015. *Jurnal Agrotek Tropika*, **3**(2), 220-225