Isolation and Identification of Phosphate Solubilising Bacteria from Potato Rhizosphere on Andisol

A E Marpaung1*, D N Susilowati2

1Indonesian Vegetables Research Institute, Berastagi, Indonesia
2Indonesian Center for Agricultural Biotechnology and Genetic Resources Research, Bogor, Indonesia

*agustinamarpaung81@gmail.com

Abstract. Andisol soil is a very strong soil that binds phosphate nutrients, because most of it is bound by allophane clay minerals which can retain P up to 97.8%. Therefore, P in the soil available for plants is very little found, while the total P in the soil is high. Where the P nutrient is one of the essential nutrients that plants need to grow and produce well. The availability of P in Andisols soil can be done by giving phosphate solubilising microbes to providing P nutrients to plants. The using of phosphate solubilising bacteria as the inoculants simultaneously increased the uptake of P by plants and increase production. Therefore, a study aimed to isolate, identify phosphate solubilising bacteria to the species level and to test the phosphate dissolution rate of potato plant rhizosphere on andisol soil in Tongkoh-Karo Regency. Isolation can be done by the spread plate method on solid pikovskaya media, the phosphate dissolving activity was tested qualitatively by looking at the appearance of clear zones around the colony, measuring the phosphate dissolution index and identifying the isolates using PCR 16S rRNA. Four phosphate solubilising bacterial isolates were successfully obtained from the potato plant rhizosphere from andisol soil in Tongkoh-Karo regency. Where each isolote are BPFT 1: Bacillus cereus, BPFT 2 : Bacillus pseudomycoides, BPFT 3 : Bacillus cereus and BPFT 4 : Bacillus amyloliquefaciens. BPFT 4 (Bacillus amyloliquefaciens) has the highest phosphate dissolubility index, which is 6,00.

1. Introduction

Andisol soil has the characteristic of having high phosphate retention, which causes P in the soil to be unavailable to plants and can reduce the yield of plants. Andisol soil develops from acidic parent material (fliparit), which has high aluminium (Al) content. Andisols are formed from volcanic ash which is dominated by amorphous minerals. One characteristic of soils containing amorphous minerals is high P retention (> 85%). This causes Andisol soil to have high retention of phosphate so that the availability of phosphate (P) in the soil decreases due to the presence of P uptake on the surface of soil colloids which causes P in the soil to be unavailable for plants [1].

Giving phosphate nutrients is very important to plant growth and yield. Phosphate nutrient (P) is the second essential element after N which an important in photosynthesis and root development. Deficiency of P can cause slow, weak, and stunted plant growth [2]. Therefore, andisol soil management needs to be directed to reduce the high absorbed P which causes the availability of P for plants to be very low. The availability of P in Andisol soil can be done by applying phosphate solubilising microbes and can be supplying P in plants. Phosphate solubilising microbes are bacteria and fungi. Strains from the genus Pseudomonas, Bacillus and Rhizobium have a large number of the most potential to solvents the phosphate. According to the research results of [3], obtained the genus Pseudomonas sp and Bacillus sp. has the potential to dissolve phosphate in peat soil. The principle of
the mineral phosphate dissolution mechanism is the production of organic acids, and the enzyme acid phosphatase is a major role in the mineralization of organic phosphate in soil [4]. Given the phosphate solubilising bacteria is important for soil fertility, so that isolation and identification of bacteria is necessary to obtain superior species. This study aims to isolate, identify phosphate solubilising bacteria to the species level and test the phosphate dissolution index of potato rhizosphere on andisol soil in Tongkoh-Karo Regency.

2. Method and procedure

2.1. Location and sampling
Soil samples were collected from the rhizosphere of potato plants and taken as a composite from a depth of 0 -15 cm in the Berastagi Experimental farm, Karo Regency with coordinate 03° 12’ 04” N dan 98° 32’ 26” E.

2.2. Source of rhizosphere bacteria isolate
The prepared Andisol soil sample was weighed 10 g and put in 90 ml of 0.85% physiological salt solution, then shaken for 30 minutes, at room temperature with agitation speed 120 rpm. Bacteria were isolated by the spread plate method on solid pikovskaya media. Incubation was carried out for 2-3 days at 28°C. The growth of phosphate solubilising bacteria is characterized by a clear zone around the colony. Bacterial colonies were re-purified until pure isolates were obtained.

2.3. Testing the ability of isolate as qualitative phosphate solubilising bacteria
The culture of bacteria aged 24 hours grown on NB media was taken as much as 5 μl inoculated on solid pikovskaya media and incubated for 4 days at room temperature. The clear zone formed is measured and calculated for the dissolution index (DI) with the following formula (Paul & Sinha, 2016).

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DI = \frac{\text{Clear zone diameter (mm)}}{\text{Colony diameter (mm)}} \tag{1}
\]

2.4. Identification of phosphate solubilising bacterial isolate
2.4.1. DNA extraction
Bacterial DNA was extracted from all rhizosphere bacterial isolates. Each isolate was grown in 1/10 liquid medium from Luria Bertani Broth consisting of 1% trypton, 0.5% yeast extract and 1% NaCl. The overnight cultures were centrifuged at 13,000 rpm for 2 minutes to pellet cells and remove the supernatant. The genomic DNA of each isolate was then extracted from the bacterial cell pellets using the witch DNA genome extraction kit (Promega). The quality and quantity of isolated DNA were examined using nanodrops and gel electrophoresis on 0.8% agarose gel.

2.4.2. Amplification and sequencing of the 16S rRNA gene
PCR amplification targeting the 16S rRNA gene was carried out on bacterial DNA samples using 16S-27F (5'AGTTTGATCCTGGCTCAG3') and 16S-1492R (5'GGTTACCTTGTTACGACTT3') primers. Phylogenetic affiliation and taxonomic hierarchies based on 16S rRNA were determined with 95% confidence using the CLASSIFIER tool (<http://rdp.cme.msu.edu>) from the RDP-II database [5].

3. Result and discussion

3.1. Isolation of phosphate solubilising bacteria from andisol soil
Andisol soil used as a source of phosphate solubilising bacterial isolates (PSB) was obtained from the Tongkoh area, Dolat Rayat District, Karo Regency, North Sumatra. Soil samples were taken at several points on the potato plant rhizosper which were taken composite.
The isolation results obtained four isolates which were then tested qualitatively for their ability to dissolve phosphate by measuring the solubility index of the phosphate based on the clear zone formed. Phosphate dissolving bacteria are identified by the presence of a clear zone around the colony when grown on pikovskaya agar. [6] stated that the formation of a clear zone around the colony showed that the isolate was able to produce organic acids which were able to bind with Ca ions to form Ca₃(PO₄)₂ compounds in pikovskaya agar media and liberate H₂PO₄ ions to form an area clearer color.

3.2. Ability of isolates as qualitative phosphate solubilising bacteria

The qualitative test results of the four isolates showed that the four isolates were able to dissolve phosphate which was indicated by the formation of a clear zone (Fig. 1). The wider the clear zone formed indicates the higher the ability of bacteria to dissolve phosphate.

The ability of each bacterial isolate to dissolve phosphate can be seen from the results of the dissolution index. The phosphate dissolution index in the phosphate solubilising bacteria, it can be seen that the BPFT 4 isolate has the highest phosphate solubility index, which is 6.00 and the lowest BPFT 3, which is 1.55 (Table 1). This shows that the isolate has phosphatase enzyme activity and produces the organic acids highest. Where the higher the enzyme activity produced by the phosphate solubilising bacteria, the larger the clear zone was produced [7]. The clear zone is formed due to the dissolving of the undissolved phosphate into a dissolved form by the phosphate solubilising bacteria. This happens because these bacteria produce the enzyme phosphatase. Phosphatase enzymes are a group of enzymes that catalyze enzymatic hydrolytic mineralization reactions by releasing undissolved phosphate to dissolve [3]. The change in the turbidity of the medium around the colony becomes clear, due to a decrease in pH in the medium [8].

| Isolate | Clear zone diameter (mm) | Colony diameter (mm) | Dissolution index |
|---------|--------------------------|----------------------|------------------|
| BPFT 1  | 1.83                     | 0.95                 | 1.92             |
| BPFT 2  | 1.75                     | 0.50                 | 3.50             |
| BPFT 3  | 1.55                     | 1.00                 | 1.55             |
| BPFT 4  | 1.50                     | 0.25                 | 6.00             |

Observations of the colony morphological characters of the four isolates showed that the isolates BPFT 1, BPFT 2, BPFT 3 and BPFT 4 had the same characters, namely round shape, irregular edges, and white color (Table 2). While the microscopic observations of the four isolate cells showed that the four phosphate solubilising bacterial cells were in the form of bacilli with gram-positive staining (Fig. 2), this indicates that it is suspected the four isolates belong to one genus.
3.3. Identification of phosphate solubilising bacterial isolates

The results of DNA sequencing analysis of the four isolates with phylogenetic affiliation and taxonomic hierarchy based on 16S rRNA were determined with 95% confidence using the CLASSIFIER tool, so that from the nucleotide sequence and from the phylogenetic tree (Fig. 3) of each phosphate solubilising bacteria identified the phosphate solubilising bacterial species were obtained, namely BPFT 1 isolate: *Bacillus cereus*, BPFT 2 isolate: *Bacillus pseudomycoides*, BPFT 3 isolate: *Bacillus cereus* and BPFT 4: *Bacillus amyloliquefaciens* (Table 3).

Bacteria *Bacillus sp.* is one of the phosphate solubilising bacteria species [9]. According to research by [10], the genus *Bacillus* has the ability to qualitatively dissolve phosphate. *Bacillus* has the potential to improve cultivated plants that are deficient in phosphate. Some *Bacillus* species that can increase the availability of phosphate nutrients in the soil are *B. subtilis*, *B. Amiloliquefaciens*, *B. pumilus* [11] and *B. mycoides* [12]. *B. Amiloliquefaciens* can increase the absorption of P and N in maize [13]. According to the research results of [14], *B. cereus* acts as a growth promoter for rhizobacteria, which is characterized by the growth of shallot tuber roots. In the research of [15], found that one of the bacterial species found in the roots of potato plants was *B. cereus*.
4. Conclusion

It was found 4 isolates of phosphate solubilising bacteria from potato plant rhizosphere from andisol soil in Tongkoh-Karo Regency. Identification based on 16S rRNA shows that each BPFT 1 isolate has a close relationship with Bacillus cereus, BPFT 2 isolate has a close relationship with Bacillus pseudomycoides, BPFT 3 isolate has a close relationship with Bacillus cereus and BPFT 4 isolate has a close relationship with Bacillus amyloliquefaciens. BPFT 4 (Bacillus amyloliquefaciens) has the highest phosphate solubility index, which is 6.00.

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