Characterization of phosphate-solubilizing bacteria isolated from acidic Ultisol soil, South Borneo

G I Jaya, S N H Utami, J Widada and W A Yusuf

1Department of Soil Science, 2Department of Microbiology, Faculty of Agriculture, Universitas Gadjah Mada, Indonesia
3Research Centre For Swamp Land, Agriculture Research and Development, Indonesia

*Corresponding author’s e-mail address: galangindrajaya@gmail.com

Abstract. Borneo Island is an area crossed by the equator, which is characterized by high rainfall and stable warm temperatures condition. In these condition, the soil experiences advance weathering so that the content of Fe$^{2+}$ and Al$^{3+}$ in the soil is very high. This causes many problems, such as high fixation of P by poisonous Fe and Al so that P elements become unavailable for plant. Plant Growth Promoting Rhizobacteria (PGPR) can support the growth of plants by the Al and Fe chelation mechanism so that the plants are not poisoned by the toxic elements and P nutrients can be available for plants. The purpose of this study was to isolate Phosphate Solubilizing Bacteria (PSB) from acidic ultisol soil. The results showed that among isolated PSB, PL1 isolates and PL2 isolates had the highest value of P solubilizing index. DNA of these 2 potential isolates were extracted and then amplified gDNA showed 1330-1500 bp.

Keywords: PSB, PGPR, Acidic Ultisol Soil, Colony Morphology, DNA Amplification

1. Introduction

Indonesia has high rainfall throughout the year causing rapid soil development. On soil with advance development, low clay activity found. Clay is the most subtle fraction in the soil with low activity, for example kaolinite clay minerals. Soils that contain this type of clay are often found in Indonesia with various types of land formation. Recommendations from the International Committee on Classification of Low Activity Clays (ICOMLAC) classified as low-activity clays are soils with the order Ultisol and Alfisol [1]. The main characteristic of these soil types is having Basic Saturation (KB) of less than 35% and having argillic horizon and only has few organic matter.

Ultisol and Alfisol are potential soil types in Indonesia, considering that they cover 25% of the total land area in Indonesia. Borneo Island has the widest area in Indonesia, which is 21,938,000 ha [2]. Borneo Island has the most extensive distribution for these soil type, with a problem of P-absorption by Fe up to 90% [3].

Soil microorganisms have their respective roles. One of them is Phosphate Solubilizing Microorganisms (PSM), which play a role in dissolving P from insoluble to soluble forms so that it can be used by plants. The microorganisms of the bacterial group that have the ability to dissolve P are ranging from Azotobacter [4], Bacillus [5], and Pseudomonas and Leclercia [6], whereas the fungal group is Aspergillus [7], and Penicillium oxalicum [8]. This study examined the potential of Phosphate Soluble Bacteria (PSB) isolates from ultisol soil in South Borneo Province.
2. Materials and Methods

2.1. Enumeration of total colonies of isolates from soil samples

The soil sample was taken from Tanjung Raja Village, Tambang district, Laut land, South Borneo Province, with confirmed soil type as developed soil [9]. The measured soil parameter data included pH and available P. Soil sample was weighed 10 grams and dissolved in 90 ml of aquades to 10^-1 dilution, then pipetted 1 ml and added to 9 ml of distilled water to 10^-2 dilution. This step was repeated up to 10^-6, 10^-7 and 10^-8 dilution. Dilution results of 10^-6, 10^-7 and 10^-8 were grown on the NBRIP (National Botanical Research Institute's Phosphate) composing of glucose medium 10 g L^-1, KCl 0.20 g L^-1, MgSO_4.7H_2O 0, 25 g L^-1, MgCl_2.6H_2O 5 g L^-1, (NH_4)_2SO_4 0.10 g L^-1, agar 15 g L^-1 and (Ca_3(PO_4)) 5 g L^-1 as element P source. Inoculants were then incubated for 7 days until a clear zone was produced [10].

2.2. Observation of macroscopic morphology

Bacterial isolates were poured in the media and incubated for 7 days at 25 °C. The observation conducted were morphology of the bacterial colonies (shape, edges, elevation, and color of the colonies) and Gram testing.

2.3. Assay For Solubilizing Index

Potential PSB from soil was tested to its ability to dissolve phosphate in NBRIP media for 7 days. On each day size of clear zone and colony formation were measured. The existence of a clear zone represent the ability of bacteria to dissolve P. The next step was measuring the dissolution index P, as a ratio of diameter of clear zone to diameter of bacterial colonies [10].

2.4. DNA Extraction and Amplification

The method used in microbial DNA extraction was based on the protocol made by Geneaid, Presto™ Mini gDNA Bacteria Kit. The PCR process or called DNA doubling was used for biological analysis, by taking DNA template of 0.5 µl, 1 µl 27f (5'-AGAGTTTGATCCTGGCTCAG-3'), 1 µl 1492r (5'-GGCTACCTTGTTACGACTT-3'), 4 µl PCR mix and 4.5 µl ddH_2O making the total volume of 12 µl. The process were Predenaturation at 94 °C for 5 minutes, Denaturation at 94 °C for 30 seconds, Annealing at 55 °C for 30 seconds, Elongation at 72°C for 1 minute with a cycle of 20-30 times, Final Extension at 72 °C for 7 minutes and Finishing at 4 °C.

Test was carried out at 100 V voltage for 22 minutes. After the process, the gel was removed from the mold and soaked in a solution of concentrated ethidium bromide for 15 minutes. Then the gel was transferred to water injection for washing. During the washing process, the water container was waved slowly so that the washing process occur. DNA bands formed in the gel were observed under UV light using UV Transilluminator.

3. Results and Discussion

3.1. Enumeration and Colony Morphology of Bacteria Solubilizing Phosphate

Based on observation of the PSB colonies formed on 3rd day after isolation, bacteria which can dissolve phosphate have characteristics to form a clear zone around the colonies [11].

| Table 1. Total Colony from Soil Sample |
|----------------------------------------|
| Abundance of PSB (Dilution of 10^-8)   | Number of PSB (CFU per g dry soil*) |
| 30                                     | 3.5 10^7                            |
In Table 1, it is known that a $10^{-8}$ dilution was the most effective to count bacterial colony density. The results were in accordance with research by [12]. Dilution of PSB colonies can be important step for calculating colony density. In this result $10^{-8}$ dilution was easily calculated, 30 colonies have potential to solubilize phosphate.

### 3.2. Identification of Colonies

**Table 2. Colony Morphology of Bacterial Isolate**

| No | Isolate name | Colony morphology (Color, Elevation, Edge, Overall) | Gram |
|----|--------------|---------------------------------------------------|------|
| 1  | PL1          | Cream, Flat, Erose, Irregular                     | Positive |
| 2  | PL2          | Cream, Plateu, Smooth, Round                      | Negative |

Two selected isolates have a broad clear zone (Figure 1) which indicates that bacteria have a high ability to dissolve phosphate [13]. The bacteria are regenerated on solid NBRIP media to be tested qualitatively for phosphate dissolution.

**Figure 1.** Phosphate Solubilizing Bacteria (PSB). (A) Isolation from dilution 108; (B) Purification of PSB

### 3.3. Assay For Solubilizing Index

The land is a complex system composed of minerals and organic matter, the soil is also a place of residence for soil microorganisms. Phosphate Solubilizing Bacteria (PSB) which have various types ranging from *Pseudomonas* and *Bacillus* Spp. [14], [15]. Bacteria can solubilize phosphate by the mechanism of organic acid reduction.
The phosphate solubilizing index (Figure 2) is the first step to determine the efficiency of bacteria in solubilizing phosphate. The clear zone (Figure 3) formed is an indication that the bacteria has the ability to solubilize phosphate. Research conducted by [16] showed that *Burkholderia thailandensis jeinis*, *Sphingomonas pituitosa* and *Burkholderia seminalis* in addition to increasing phosphate solubility, were also able to reduce Al\(^{3+}\) solubility by reducing organic acids such as oxalic, citric and malic acids through chelating processes. Al\(^{3+}\) is an element that can bind P and in excess amounts has the potential to poison plants so that by decreasing the solubility of these elements can increase the growth of rice plants.

**Figure 2.** Phosphate solubilization index on solid NBRIP media

**Figure 3.** P solubilization test in solid NBRIP media. (A) PL1 & (B) Isolates PL2 Isolates.

3.4. 16S DNA Amplification For Identification Step

Electrophoresis is the stage used to separate bacterial DNA based on different molecular weight. The results of electrophoresis were observed with UV Transilluminator so that the band is formed.
Figure 4. Visualization of 16S DNA. M; 1 KB marker, PL1 & PL2 isolates amplified at 1330-1500 bp.

The visualization results showed that DNA of 2 selected bacteria that had the ability to dissolve phosphates were amplified with 16sDNA primers (27f and 1492r) in the range 1,380-1,500 bp means that 2 of these bacteria had about 1,380-1,500 base pairs (Figure 4). This is consistent with research conducted by [17] and [18] that Bacillus DNA was amplified in the range of 1500 bp. Besides, [19] revealed that Pseudomonas putida, Serratia marcescens and Burkholderia cepacia were amplified in the range of 1,500 bp. These bacteria are considered as Plant Growth Promoting Rhizobacter (PGPR) which is often used as a phosphate solubilizing agent to support plant growth. In Table 2, it is known that based on gram testing, PL1 and PL2 are gram (-)and gram (+) isolates, respectively. The example of gram (-) bacteria is Bacillus, while gram (+) is Pseudomonas.

4. Conclusion
There were potential isolates as phosphate solubilizing bacteria isolated from acid soils in South Borneo. Both isolates were amplified at 1,380-1,500 bp. PL2 gram (-) isolate qualitatively can dissolve phosphate higher with a ratio of 2.78 compared to PL1 gram (+) with a ratio of 1.17.

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