The exploration of phosphate solubilizing bacteria in mangrove forest at Teluk Naga, Banten

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Abstract. The aim of this study was to explore phosphate solubilizing bacteria in mangrove forest at Teluk Naga, Tangerang district, Banten. To collect samples, random sampling was applied. Bacteria were isolated by Pikovskaya media, using pour plate method. Nineteen isolates collected from soil demonstrated a potency to solubilize phosphate. Six isolates showed high phosphate solubilization activity from calculation of dissolution index. They were identified morpho-physiologically and biochemically as *Pseudomonas* (isolate R22 and A17), *Klebsiella* (A11, A14, A18, and A38), *Agromyces* (A13), *Bacillus* (A33 and A37), *Azomonas* (A15), and *Enterobacter* (A31 and A32). These bacteria were found in the rhizosphere of mangrove forests which consist of *Avicennia* and *Rhizophora* mangroves. These bacteria are known to have the ability to increase the availability of phosphate in the soil. This ability is one of the capabilities of the potential growth of promoting bacteria (PGPR or Plant Growth Promoting Rhizobacteria).

Key Words: Mangrove forest, Rhizobacteria, Phosphate solubilization bacteria, PGPR.

1. Introduction

Teluk Naga mangrove ecosystem is located in the Banten Bay region, Muara Village, Teluk Naga District, Tangerang Regency, Banten. Teluk Naga mangrove forests is one of mangrove ecosystems in Banten which have many potentials in various fields, such as eco-tourism, fisheries and agriculture. The potential of mangrove forests in agriculture is inseparable from bacteria that support plant growth. [1] and [2], explored epyphitic cyanobacteria isolated from pneumatophore of *Avicennia marina* and planktonic cyanobacteria isolated from brackish water in mangrove forest of Cagar Alam Pulau Dua, Serang, Banten. Some of these cyanobacteria known to have an activity in solubilizing phosphate. Meanwhile, no research has been reported on the exploration of phosphate solubilizing bacteria in the Teluk Naga mangrove forest.

[3] conducted a research about phosphate solubilizing microorganisms associated with Chollangi Mangrove Soil in East Coast of India. They isolated 23 bacterial strains from the soil of Chollangi mangrove environment. Seven isolates showed phosphate solubilization activity. Two isolates out of these bacteria were identified morphophysio logically and biochemically as *Bacillus subtilis*, three
isolates as fluorescent *Pseudomonas* and two isolates as *Azotobacter sp*. Phosphate solubilizing bacteria play an important role in soil enrichment, because of their capability of dissolving phosphate by excreting a number of low molecular weight organic acids such as oxalate, succinate, fumarate, and malate. These organic acids will react with phosphate binders, such as $\text{Al}^{3+}$, $\text{Fe}^{3+}$, $\text{Ca}^{2+}$, or $\text{Mg}^{2+}$ to form stable organic chelates so that they can free phosphate ions bound and be utilized by plants [4].

In this study, we aimed to explore rhizobacteria from Teluk Naga mangrove forest which have an ability to solubilize phosphate. The bacterial isolates found can be further applied as biofertilizer that utilize to promote plant growth in agricultural fields.

2. Methods
The research was conducted in two main locations, namely Teluk Naga Mangrove Forest, Tangerang as the location of rhizobacteria sampling and Biology Education Laboratory of Sultan Ageng Tirtayasa University for isolation, selection, and morphological characterization of rhizobacteria. Teluk Naga Mangrove Forest is located in Tanjung Pasir Bay, Muara Village, Teluk Naga District, Tangerang Regency, Banten. Total area of Teluk Naga Mangrove Forest is 270 Ha.

The method sampling used in this study was random sampling. Each transect was made by pulling a 300 m transect line from the sea to the land direction perpendicular to the coastline. The transect was determined based on the zone of mangrove vegetation. Rhizosphere soil samples were taken at low tide. The soil was taken using pipes as deep as 10-20 cm and the roots of the plants were cut 3 cm long. Then stored in a sterile plastic.

As much as 1 g of soil was aseptically weighed and diluted in 0.85% NaCl and put into a sterile container containing 99 ml sterile solution. Soil samples were diluted to $10^{-4}$ and poured in sterile petri dishes. Serial dilution methods were carried out following [5]. One milliliter of each dilution was spread on Pikovskaya media [6], then incubated at 28°C for 7 days. Colonies that formed clear zones (halo zones) were purified and subcultured on pikovskaya media. The characteristics of each colony were observed morphologically and physiologically following [7]. Isolation of phosphate solubilizing bacteria was carried out using Pikovskaya media, with a composition (in g / L): 5 g CaCO₃, 10 g Glucose, 0.5 g ($\text{NH}_4$)$_2$SO₄, 0.2 g NaCl, 0.2 g MgSO₄, 0.5 g yeast extract, 0.004 g MnSO₄·4H₂O, 0.002 g FeSO₄·7H₂O, and 15 g agar (media pH 7.5) [8].

Qualitative measurements of phosphate solvents were carried out using the dot method using puncture needles on the Pikovskaya medium and incubated at room temperature for 7 days. Solubility index (SI) is measured by the formula of clear zone diameter minus the colony diameter and the results are divided into colony diameter [9]. Bacterial identification is carried out through characterization of bacterial morphology which includes observation of colonies (color, shape, edges, and elevation), gram staining, observation of cell morphology (shape), motility, catalase test and other physiological tests. Gram staining was done to find out whether bacterial cells belong to the Gram Positive or Gram Negative group. The overall observations were adjusted to the Bergey’s Manual of Determinative Identification Book.

3. Results and Disscusion
There were 19 isolates that have an ability to dissolve phosphate. Selection of phosphate solubilizing bacteria was carried out by growing the isolates on Pikovskaya media. The ability of bacteria to dissolve phosphate is measured qualitatively by measuring the phosphate dissolution index (IP). This index value is obtained by calculating the ratio between the clear zone diameter difference (DZ) minus the colony diameter (DK) and the diameter of the colony ((DZ-DK) / DK). Clear zones are seen when isolates are grown in Pikovskaya media (Figure 1).
Figure 1. A clear zone on Pikovskaya media resulted by isolate A18

It is assumed that the higher index value, the higher the ability of bacteria to dissolve phosphate. In Table 1, it is seen that isolates which have the highest IP values are A18 and A38. These rhizobacteria were isolated from "Avicennia" mangrove forest, respectively at stations 1 and 3.

Table 1. The phosphate dissolubilizing index produced by rhizobacteria at mangrove forest Teluk Naga

| Isolat | Diameter |       | Indeks Pelarutan Fosfat ((DZ-DK)/DK) |
|--------|----------|-------|-------------------------------------|
|        | zona bening (DZ) | koloni (DK) |                                  |
| A18    | 1.10     | 0.10  | 10.000                              |
| A38    | 2.00     | 0.20  | 9.000                               |
| A14    | 0.10     | 0.02  | 4.000                               |
| A13    | 0.10     | 0.02  | 4.000                               |
| A15    | 0.90     | 0.20  | 3.500                               |
| A37    | 0.40     | 0.10  | 3.000                               |
| A12    | 0.60     | 0.20  | 2.000                               |
| R21    | 0.20     | 0.10  | 1.000                               |
| R22    | 0.20     | 0.10  | 1.000                               |
| A33    | 0.60     | 0.30  | 1.000                               |
| A34    | 0.80     | 0.30  | 1.670                               |
| A31    | 0.90     | 0.50  | 0.800                               |
| A32    | 0.60     | 0.40  | 0.500                               |
| A36    | 0.70     | 0.50  | 0.400                               |
| R24    | 0.40     | 0.30  | 0.330                               |
| A11    | 0.05     | 0.04  | 0.250                               |
| A17    | 0.50     | 0.40  | 0.250                               |
| A35    | 0.50     | 0.40  | 0.250                               |
| A16    | 0.90     | 0.80  | 0.125                               |
Based on the calculation of dissolution index, six isolates showed high phosphate solubilizing activity. According to Bergey’s Manual of Systematic Bacteriology, they were identified morphologically and biochemically as a member of genus Klebsiella, Bacillus, Pseudomonas, and agromyces, and azomonas.

1. Klebsiella
Isolate A11, A14, A18, and isolate A38 showed a rounded colony, flat elevation, shiny colony surface, flat colony edge and cream coloured. Based on microscopic observations, isolates A11 and A38 have basid cell forms and are Gram negative (Figure 2). Both isolates demonstrated positive reaction on the catalase test, motile, and aerobic for A38 and anaerobic for A11. According to Bergey’s Manual of Determinative Bacteriology 7th edition, the characteristics of isolate A11 and A38 mostly are owned by Klebsiella, which are bacillus, Gram negative, long or short shaped, and facultative anaerobes. However, Klebsiella are non motile. These bacteria measure 0.5-1.5 × 1-2 microns and have a sheath which is 2-3 times the size of germs [10]. They can produce nitrite from nitrate, grows well on ordinary culture media. These organisms can be isolated from various animals and soils, and are often found in the respiratory, intestinal and urogenital tracts of human. Klebsiella are also a soil bacteria which known to produce IAA hormones and have a potency to be PGPR [11].

![Figure 2. Bacterial isolate A11 (100x magnification)](image)

2. Bacillus
Colonies characteristics of round, flat elevation, shiny surface, flat edge, and creamy white were shown on isolates A33 and A37. These isolates have basid cell form and are Gram positive (Figure 3). Characteristic observation on the biochemical activity test showed that the isolates could produce catalase enzyme (positive reaction). They are motile and aerobic. The results obtained showed that isolates A33 and A37 belong to the Bacillus genus [10]. Bacillus have trunk-shaped characteristics, and can be found in soil and water including sea water. Some of the bacteria produce extracellular enzymes that hydrolyze proteins and complex polysaccharides. Bacillus form an endospore, Gram positive, move by erythricus flagellum, catalase positive, and either aerobic or facultative anaerobic [12].

Bacillus are a true marine occupant that have been investigated by experts in marine research and proven to have several capabilities. Some of these bacteria able to produce antibiotics that can fight pathogenic bacteria Vibrio cholerae. Bacillus circulans known as oil breaking bacteria, produce glucan-breaking enzymes which can decompose crude oil and other hydrocarbons. Enzymes produced by Bacillus have been produced on an industrial scale, including alanine and formicate, α-amylase, isoamilase, β-amylase, glucoamylase, chitinase, and cholesterol oxidase [13]. The Bacillus are widely used as a biocontrol agent, producing antimicrobial substances in the form of bacteriocin, which is an
antimicrobial polypeptide or protein produced by bactericidal microorganisms. Bacteriocin kills its target cells by entering the target membrane and causing cell membrane’s function to become unstable, causing cell lysis [14]. [15] found that Bacillus are also able to dissolve phosphate in soil samples from the cultivation of cayenne.

Figure 3. Bacterial isolate A33 (100x magnification)

3. Pseudomonas
Based on macroscopic observations, isolates R22 and A17 showed irregular colonies, flat elevations, dull or non-glossy colonies on R22 isolates but shiny on A17 isolates, the edge of the colony was wavy with milky white. Microscopic observations showed that isolates R22 and A17 have basil cell forms and are Gram negative (Figure 4). Characteristic observations on biochemical activity tests showed that isolate R22 and A17 have a positive reaction on catalase test, motile, and aerotolerant. According to their characteristics, it is assumed that isolate R22 and A17 belong to Pseudomonas group. [10] explain that Pseudomonas are Gram negative, have long or short shaped, and aerobic. These bacteria measure 0.5-1.0 × 1.5 - 5.0 microns, and do not have a resting phase. In some cases, Pseudomonas can use nitrates as alternative electron receptors. Some are oxidase positive, but some are negative. Most of them are spread in nature, some pathogenic species in humans, animals and or plants. According [16], Pseudomonas has high activity and production of enzyme phosphatase than Basilus.

Figure 4. Bacterial isolate A17 (100x magnification)

4. Agromyces
Isolate A13 is assumed to be Agromyces. The isolate showed irregular colonies, thick or convex elevations, shiny colony surfaces, flat colonies with white color colonies. Under microscope, isolate A13 have filament cell forms and Gram positive (Figure 5). Their biochemical characteristics are catalase negative, non motile, and facultative anaerobes. According to Bergey’s Manual of Determinative Bacteriology 7th edition, Agromyces are Gram positive, cells are composed of
branched, slender filamentous element 0.3-1.0 microns and irregular cells in older culture. Widely distributed in soil, where it is very numerous [10]. Strengthened by [17] *Agromyces soli* sp. nov., isolated from farm soil.

**Figure 5.** Bacterial isolate A13 (100x magnification)

5. *Azomonas*
Isolate A15 showed irregular colony, convex elevation, shiny colony surface, flat colony edge and cream coloured. Based on microscopic observations, isolates A14 have coccus cell forms and are Gram negative (Figure 6). The isolate demonstrated positive reaction on the catalase test, motile. According to Bergey’s Manual of Determinative Bacteriology 7th edition, the characteristics of isolate A15 mostly is owned by *Azomonas* because of A15 have many characteristics same with *Azomonas*. The characteristics of *Azomonas* are cell generally stain Gram negative, motility occurs by peritrichous or polar flagella, aerobic, but can also grow under decreased oxygen tensions. Nitrogen fixers, and catalase positive. They occur in soil and water [10]. Based on the research of [18] *Azomonas agilis* released large amounts of organic acids to solubilize phosphate to increase soil fertility and enhance plant production.

**Figure 6.** Bacterial isolate A15 (100x magnification)

6. *Enterobacter*
Based on macroscopic observations, A31 and A32 isolates showed round colonies, thick or convex elevations, shiny colony surfaces, flat colonies with yellow color in A32 isolates and pink color colonies in A31 isolates. Microscopic observations, isolates A31 and A32 have long basil cell forms
and are Gram negative. Characteristic observations on biochemical activity tests of A31 and A32 isolates showed that the catalase test was positive, motile, and facultative anaerobes. The results obtained were the possibility of A31 and A32 isolates belonging to Enterobacter Genus as identified in the Bergey’s Manual of Determinative Bacteriology 7th edition. Enterobacter genus has features including Gram negative, long or short shaped facultative anaerobes. This bacterium measures 0.6-1.0 × 1.2-3.0 microns. Pair or line. Most are spread in nature; in fresh water, soil, swamps, plants, vegetables, animals and human faeces. According to [19], Enterobacter has been isolated in Sediments from Shallow Eutrophic Lake and a Wetland; also were identified molecularly as bacteria which were phosphorus release determination ability.

![Image](image.png)

**Figure 7**. The isolate bacteria A31 (100x magnification)

Phosphorus (P) is one of the major constituents in energy metabolism and biosynthesis of nucleic acids and cell membranes with an important role in regulation of a number of enzymes. Soil phosphorous is an important macronutrient for plant growth. Macronutrient means that the element needed in large quantities. Total soil P occurs in either organic or in organic form. The major form of organic P soil is not readily available to plant as a source of P [20].

Phosphorus is absorbed by the roots and leads to young leaves first and then transferred to older leaves. Many P elements are found in phloem tissue so they have a function for plant nutrient translocation. The P element is absorbed by plants in the form of ions, both positive and negative ions, for example H$_2$PO$_4^-$. After an H$_2$PO$_4^-$ ion enter into plant, it will be directly esterified through C chain hydroxy groups to high-energy phosphates, such as ATP [21].

In soil, some microorganisms are known to be able to convert non-soluble forms of phosphorus to dissolved form through acidification, secretion of organic acids or protons [22]. Phosphate solvent bacteria have been reported to be found more in mangrove samples than in the sea and brackish water. However, the abundance of phosphate solvent bacteria is correlated with the level (content) of phosphorus in the soil. This is possible due to the fact that the active bacteria converts insoluble phosphorus compounds into dissolved forms, which are easily transferred from the soil to the waters or have been used by plants and microbes. The mechanism of phosphate dissolution is a cumulative action that is influenced by many factors, including pH, oxygen concentration, wind speed, waves, water flow, the content of organic compounds, and carbohydrate levels [8].

4. Conclusion

Mangrove forest at Teluk Naga, Banten is a potential ecosystem, which vary in rhizobacteria. The result of this study found 19 bacterial isolates demonstrated phosphate solubilization activity. Six isolates with high phosphate dissolution index (IP) were identified morpho-physiologically and biochemically, as Pseudomonas (isolate A14), Klebsiella (A11, A14, A18 and A38), Agromyces
(A13), Bacillus (A37), Azomonas (A15), and Enterobacter (A31 and A32). For further study, these potential rhizobacteria can be applied as biofertilizer which promote plant growth in agricultural field.

5. References
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