Morphological identification of phosphate solubilizing and cellulolytic fungi from mangrove soil under Rhizophora stylosa stands

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Abstract. Mangrove forest is one of the forest ecosystems that can be found in tidal areas. Mangrove forest have ecological, socio-economic benefits for organisms that live in the sea. One of the microorganism that live in mangrove forest is fungus. Therefore, this study aimed to isolate and identify phosphate solubilizing and cellulolytic fungi from mangrove soil. Soil samples were collected from a stands of Rhizophora stylosa in Lubuk Kertang Village, West Brandan District, Langkat Regency, North Sumatra. Soil samples were taken randomly with a dept of 0-20 cm. Isolation of phosphate solubilizing fungi was carried out using Pikovskaya medium and cellulolytic fungi using carboxyl methyl cellulose (CMC) medium. To determine the effectiveness of fungi qualitatively, the phosphate solubility and cellulolytic index were calculated. All isolates obtained were identified morphologically by observing colonies macroscopically and microscopically. This study obtained 12 isolates of phosphate solubilizing and 8 isolates of cellulolytic fungi. Phosphate solubility index ranging from 2.06-2.87 and cellulolytic index of 0.33-3.00. Morphological identifications showed that all isolates of phosphate solubilizing fungi belonged to the genus Aspergillus, while all isolates of cellulolytic fungi belonged to the genus Chaetomium.

1. Introduction
The mangrove ecosystem is a tropical coastal vegetation community which is dominated by various species of mangrove trees, that is species can grow and develop in muddy coastal tidal areas. This tree vegetation can adapt physiologically to salinity and they are affected by tides. The most common types are Avicenia sp, Bruguera sp, and Rhizophora sp [1, 2]. Mangrove forest is a potential ecosystem because it is a combination of physical and biological elements of land and sea, thus creating a complex ecosystem. Mangrove forest is a very important ecosystem economically and ecologically [3, 4].

Mangrove forest is a unique ecosystem, the soil is always wet, contains salt, little oxygen and it is rich in organic matter [3, 5]. Apart from flora and fauna, various types of microbes are important component in this ecosystem community. Organic matter produce by mangrove litter, apart from being a source of nutrients for mangrove plants also play a role as a source of energy and carbon for microbes around mangroves. These microbes play an important role in various nutrient cycles such as the carbon,
sulfur, nitrogen and phosphorus cycles which then control the chemical environment in the mangrove ecosystem [2,5,6-8].

Phosphate solubilizing fungi are involved in the phosphorus nutrient cycle [9]. This fungus helps increase the availability of phosphorus (P) in the soil because it is bound to soil components. In acidic soils, P is bound by aluminum and iron, while in alkaline soils, P is bound by calcium. This condition results in low availability of P nutrients in the soil and it is not available to plants [10-13]. Deficiency of P can interfere with plant growth, because P is one of the essential macro nutrients for plant [14]. Fungi from the genera *Aspergillus*, *Penicillium* and *Fusarium* have the ability to assist the availability of P for plants [15,16].

Cellulolytic fungi are involved in the carbon cycle by decompose organic matter into nutrients that can be taken up by plants. Organic matter mainly contains nitrogen, phosphorus, sulfur, basic cations and some micro-nutrients [9]. This fungus is able to produce cellulase enzymes that play a role in decomposing organic matter [17,18]. Several fungi that produce cellulase enzymes belong to the genus *Aspergillus*, *Penicillium*, *Trichoderma*, *Acremonium*, *Rhizopus*, *Chaetomium* and *Curvularia* [19,20]. Given the importance of the presence of phosphate solubilizing and cellulolytic fungi in the soil, it is necessary to explore to obtain these two types of fungi that can help plant growth. Thus, the aim of this study was to isolate and identify the type of phosphate solubilizing and cellulolytic fungi from mangrove soils under *Rhizophora stylosa* stands.

2. Materials and Methods

2.1. Soil sampling
Soil samples were carried out from Lubuk Kertang Village, West Brandan District, Langkat Regency, North Sumatra. Composite soil sample were taken randomly at a depth 0-20 cm under *Rhizophora stylosa* stands. Composite soil sample were put into a plastic bag and brought to the laboratory for further analysis.

2.2. Isolation of phosphate solubilizing and cellulolytic fungi
Phosphate solubilizing and cellulolytic fungi were isolated following the method used by [21, 22] and [20] respectively. Ten g of soil was input in to Erlenmeyer flask containing 90 mL sterile NaCl (0.85%) solution then shaked for 30 minutes. Serial dilution was made until reaching the concentration of 10^{-5}. One mL of resultant solution was pipetted out from each of 10^{-3}, 10^{-4}, and 10^{-5} serial dilution, poured out in sterilized petri dish containing 12 mL agar medium (Pikovskaya medium for phosphate solubilizing and Carboxyl Methyl Cellulose with 1% Congo red medium for cellulolytic fungi). After the medium solidified, the petri dish was incubated for 3 days at 28°C. The presence of phosphate solubilizing and cellulolytic fungi was indicated by the formation of clear zone surrounding the fungal colony. The colonies were purified and stored for further process.

2.3. Qualitative testing of fungal isolates
All isolates of phosphate solubilizing and cellulolytic fungi were tested for their effectiveness qualitatively by calculating their dissolution index. The phosphate solubility index was calculated by dividing the diameter of the colony plus the diameter of clear zone by the diameter of the colony [23,24]. The cellulolytic index was calculated by dividing the diameter of clear zone minus the colony diameter by the colony diameter [25].

2.4. Morphological identification of fungal isolates
Phosphate solubilizing and cellulolytic fungal isolates obtained were identified morphologically to the genus level. Fungal culture were grown on potato dextrose agar (PDA) medium and incubated for 3 days. Fungal colonies were observed for macroscopic characteristic that is the growth characteristic of colony, colony color, and colony diameter, and microscopic characteristic that is hyphae characteristic,
hyphae branching type, and conidia characteristic. The characteristic found from each fungus are then described and matched with the fungal identification book [26,27].

3. Results and Discussion

3.1. Isolation of phosphate solubilizing and cellulolytic fungi
The population of phosphate solubilizing and cellulolytic fungi were 13.4 \times 10^4 colony forming units (CFU)/mL and 27.5 \times 10^4 CFU/mL, respectively. The presence of fungi in the soil is influence by environmental factors in their habitat, where fungi are more dominant in acid soil (pH 5.5). The content of organic matter also affects the presence of fungi in the soil. Organic matter is a source of energy and carbon for fungi that are heterotroph. In this study, the soil pH was slightly acidic (5.76) with high organic C content (3.98%). Organic material produced by mangroves is a source of nutrition for organisms that exist around the mangrove ecosystem. The presence of cellulolytic fungi in the mangrove ecosystem has an important role in decomposing the remains of mangrove plants into organic matter as a source of nutrition for plant and other organisms that live in mangrove forests. The presence of phosphate solubilizing fungi helps increase the availability of phosphate nutrients for plant growth.

The result of the isolation of phosphate solubilizing and cellulolytic fungi were found to be 12 and 8 isolates, respectively. The isolates were characterized by the formation of clear zone around the colony. The formation of clear zone indicates that phosphate dissolution has occurrence by phosphate solubilizing fungi or decomposition of cellulose by cellulolytic fungi.

3.2. Qualitative testing of fungal isolates
All isolates of phosphate solubilizing and cellulolytic fungi were tested for their effectiveness qualitatively by calculating their dissolution index (Table 1 and Table 2). In general, the dissolution index expresses their ability or each isolate to act according to its function. The higher the dissolution index of each isolate, the higher the effectiveness of the isolate in carrying out its function.

The phosphate solubilization index produced by each isolate was not the same, the values obtained varied from 2.06-2.87 (Table 1). FPRS11 is the isolate with the highest solubilization index. This shows that the effectiveness of each isolate in releasing P is different. The difference in effectiveness was related to the ability of each isolate to produce organic acids in their metabolic processes. These organic acids with low molecular weight will form chelate with soil components so that P will be free to solution [13,28]. Thus, all isolates were potential to be used as phosphate solubilizing biofertilizers.

Table 1. Phosphate solubilization index

| No | Isolate code | Colony diameter (cm) | Clear zone diameter (cm) | Solubilization index |
|----|--------------|----------------------|--------------------------|----------------------|
| 1  | FPRS1        | 3.0                  | 3.3                      | 2.10                 |
| 2  | FPRS2        | 2.9                  | 3.1                      | 2.07                 |
| 3  | FPRS3        | 2.8                  | 3.4                      | 2.21                 |
| 4  | FPRS4        | 1.4                  | 2.4                      | 2.71                 |
| 5  | FPRS5        | 1.5                  | 2.4                      | 2.78                 |
| 6  | FPRS6        | 3.5                  | 3.8                      | 2.08                 |
| 7  | FPRS7        | 3.6                  | 3.9                      | 2.08                 |
| 8  | FPRS8        | 3.1                  | 3.3                      | 2.06                 |
| 9  | FPRS9        | 3.3                  | 3.8                      | 2.15                 |
| 10 | FPRS10       | 2.2                  | 3.4                      | 2.54                 |
| 11 | FPRS11       | 1.5                  | 2.8                      | 2.87                 |
| 12 | FPRS12       | 3.3                  | 3.5                      | 2.06                 |

The cellulolytic index produced by each isolate varied between 0.33-3.00 (Table 2). FCRS5 is the isolate with highest cellulolytic index. This difference indicates that there are differences from each
isolate in producing cellulase enzymes to hydrolyze cellulose in CMC medium. The higher value indicates the higher the ability of the isolate to degrade cellulose. Based on the cellulolytic index values, there were 2 isolates with cellulolytic index values <1 (including low criteria), there were 5 isolates with moderate criteria (cellulolytic index values between 1-2), and only one isolate included high criteria (cellulolytic index value >2) [20]. According to [29], fungal isolate with index values >3 showed the highest potential to produce cellulase.

| No | Isolate code | Colony diameter (cm) | Clear zone diameter (cm) | Cellulolytic index |
|----|--------------|----------------------|--------------------------|--------------------|
| 1  | FCRS1        | 0.50                 | 1.00                     | 1.00               |
| 2  | FCRS2        | 0.50                 | 1.00                     | 1.00               |
| 3  | FCRS3        | 0.50                 | 1.50                     | 2.00               |
| 4  | FCRS4        | 0.75                 | 1.00                     | 0.33               |
| 5  | FCRS5        | 0.25                 | 1.00                     | 3.00               |
| 6  | FCRS6        | 0.50                 | 1.00                     | 1.00               |
| 7  | FCRS7        | 0.50                 | 0.80                     | 0.60               |
| 8  | FCRS8        | 0.50                 | 1.00                     | 1.00               |

3.3. Morphological identification of fungal isolates
All fungal isolates obtained were identified to the genus level. Based on morphological identification, as many as 12 isolates of phosphate solubilizing fungi belonged to the genus *Aspergillus* (Figure 1a), while as many as 8 isolates of cellulolytic fungi belonged to the genus *Chaetomium* (Figure 1b).

![a](image1a.png) ![b](image1b.png)

**Figure 1.** Morphology of soil fungi isolated from mangrove soil (a). *Aspergillus*, (b). *Chaetomium*

3.3.1. *Aspergillus*. Aspergillus as a fungus that belongs to the Ascomycetes class that can be found in various ecosystems. Aspergillus is a eukaryotic organism, it is forms long branching filaments, and in the culture medium forms mycelia and conidiophores. Aspergillus reproduces by the formation of hyphae or buds and produces spore-forming conidiophores [27,28]. In this study, there are 4 types Aspergillus based on macroscopic and microscopic differences, namely Aspergillus sp1 (3 isolates), Aspergillus sp2 (5 isolates), Aspergillus sp3 (2 isolates) and Aspergillus sp4 (2 isolates). Aspergillus sp1 has a colony diameter of 3.0-3.3 cm, the shape of the colonies is semi-round and yellowish green, conidia are round and yellowish green. Aspergillus sp2 has a colony diameter of 1.5-5.5 cm, the shape of the colonies is semi-round and yellow, the conidia are round and brownish yellow. Aspergillus sp3 has a colony diameter of 1.4-1.5 cm, the shape of colonies is semi-round and white, the conidia are semispherical and light green. Aspergillus sp4 has a colony diameter of 2.2-3.6 cm, colonies form and spread and it is dark green, conidia are round and brownish green.
The genus Aspergillus has a wide distribution of life, where this genus can be found in the soil under stands of various types of plant. This genus has a good adaptability and it is one type of fungus that has a high ability to release bound phosphate [30-32].

3.3.2. Chaetomium. Chaetomium belongs to the black fungus group or the Dematiaceae group because it has hyphal pigmentation and a reproductive structure that is dark brown to black in color. The genus Chaetomium is easily recognize from the shape of the ascoma which is round or semi-round and dark brown to black in color and covered with lateral and terminal hairs. Chaetomium is a fungus that has rather thick and dense colonies [26,27]. There are 2 types of Chaetomium fungi based on macroscopic and microscopic observations in this study. Chaetomium sp1 (3 isolates) forms pink colonies, the surface texture of the colonies is like cotton with flat edges. It has an oval to semi-round greenish ascoma an there are ascoma hairs. Chaetomium sp2 (5 isolates) forms dark brown colonies, it has a thick green ascoma layer.

Chaetomium is a fungus that has very strong keratinolytic and cellulolytic activity so that it is able to decompose organic compounds especially keratin and cellulose in a short time [29,33].

4. Conclusions
There were 12 isolates of phosphate solubilizing fungi and 8 isolates of cellulolytic fungi isolated from mangrove soil under Rhizophora stylosa stands. Based on morphological identification, all isolates of phosphate solubilizing fungi belonged to the genus Aspergillus while all isolates of cellulolytic fungi belonged to the genus Chaetomium.

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