Identification of phosphate solubilizing fungi under Scaphium macropadum stands in Gunung Leuser National Park, North Sumatra

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Abstract. Scaphium macropadum is a wild tree species producing important timber and medicinal fruits. As a result of overexploitation, this species has decreased its population in the wild although categorized as least concern based on the IUCN red list of threatened species. Phosphate solubilizing fungi (PSF) are a group of soil microorganisms that can increase the availability and uptake of phosphorus (P) by plants. This study aims to isolate the phosphate solubilizing fungi from the soil under Scaphium macropadum stands following its morphological identification of species. Soil sampling was carried out randomly, with a depth of 0–20 cm under 12 stands of Scaphium macropadum in Gunung Leuser National Park, North Sumatra. Isolation of PSF was carried out using Pikovskaya medium with tricalcium phosphate (Ca$_3$(PO$_4$)$_2$) as a source of inorganic P then the solubilization activity was expressed in the solubilization index (SI). The isolates obtained were identified morphologically to the genus level. The results obtained 15 isolates of PSF with SI ranging from 0.96 to 1.16. Based on the morphological identification, all isolates were identified as members of Aspergillus.

1. Introduction

Soil is a media where plants growth and a habitat for the various organisms that live in it. Plants affect the existence of organisms in the soil and conversely, the activities of organisms in the soil also affected plant growth, which in turn will determine the productivity of the land where they live [1]. The presence of microorganisms plays a significant role in soil fertility, especially regarding the supply and absorption of nutrients for plants. One of them is phosphate solubilizing fungi (PSF). Phosphate solubilizing fungi are a group of soil microorganisms with the main role as phosphorus (P) solubilizers to plants [2-4]. Phosphorus is an essential macronutrient needed for plant growth, development and metabolisms. The other roles of soluble P or phosphate for plants i.e cell growth and metabolism, the formation of root hairs, floral initiation, the formation of fruit and seeds and disease resistance [1]. In the soil, the nutrient availability of phosphorus is generally low, thus limiting plant growth. Phosphate solubilizing fungi have the ability to solubilize inorganic P to alter its availability in the soil for absorption by plants [1,3-5].

Scaphium macropadum is locally known as kembang semangkok, tempayang, merpayang or malva nut which is a wild tree species known for its important non-timber forest products that support the local community living around the forest region [6,7]. In Vietnam, the fruit of S. macropadum was widely used as a medicinal remedy. Apart from fruit, other parts that have medicinal benefits are sap
and roots [8]. The overexploitation and improper harvesting management have threatened its population in the wild [8]. The utilization of microbes associated with plant roots has a very important role because in addition to increasing the availability of nutrients it also produces growth hormones, thereby helping to increase plant growth [9,10]. Thus it is necessary to conduct research to investigate the possible presence of PSF in the soil under the S. macropadum stands. The aim of this study was to isolate and identify PSF isolates under S. macropadum stands.

2. Materials and methods

2.1. Soil sampling
Soil samples were taken in a composite manner at a depth of 0-20 cm. Soil samples were taken under 12 stands of Scaphium macropadum in Gunung Leuser National Park, North Sumatra. Each composite of soil was inserted into a separate plastic bag and processed immediately in the laboratory. Soil samples were measured for its pH, organic C content, total N, total P, and cation exchange capacity.

2.2. Isolation of PSF
Phosphate solubilizing fungi were isolated based on previous studies [11,12]. Ten grams of soil was mixed into 90 mL of sterile physiological saline and shaken for 30 min. A serial dilution of soil suspension was prepared in which the 1 mL aliquot (10^3, 10^4, 10^5) were suspended into petri dishes. Then, 12 mL of Pikovskaya medium (+ 45°C) were poured into petri dishes and incubated for three days at 27–30°C. The presence of PSF was indicated by the presence of a clear zone around the fungal colonies. The colonies were purified and stored for further testing.

2.3. Phosphate solubilization capacity on Pikovskaya medium
The PSF was tested individually for its P solubilization on Pikovskaya agar medium supplemented with tricalcium phosphate (Ca_3(PO_4)_2) as an inorganic P source. The test medium was poured into a petri dish and allowed to solidify. Furthermore, pure culture was grown on the test medium. Isolates were incubated for five days. The dissolving activity of phosphorus by fungi was calculating was using the solubilizing index value, namely by dividing clear zone diameter by the colony diameter [13].

2.4. Morphological identification of PSF
After obtaining the PSF, morphological characterization was carried out at the level of genus identification. Fungal cultures were grown on Potato Dextrose Agar (PDA) medium and incubated for three days at 25–28°C. The macroscopic characteristics of the fungi colonies were observed including the growth of hyphae, colony color, and colony diameter and microscopic characteristics of hyphae, hyphae branching types, and conidia characteristics. The characteristics found from each function was then described and matched with the fungal identification reference [14,15].

3. Results and discussion

3.1. Isolation of PSF
Isolation was aimed to select microbes from their original environment to obtain a pure culture. A pure culture is needed to identify and obtain valid test results from the activity of only one type of microbe. The population of PSF obtained was 1.04 x 10^3 Colony Forming Unit (CFU)/m. This amount is small because the PSF population generally ranges 10^4 to 10^6 CFU/g in the soil [16,17].

The presence of PSF is related to the C organic content of the energy and carbon source. The result of soil analysis in our study revealed that the soil was classified as infertile with very acidic pH (4.4), low organic C content (1.7%), low total N (0.17%), very low available P (2.49 ppm), and medium cation exchange capacity (11.88 me/100 g). The low organic C content may reduce the growth
performance of soil microbes, including the PSF which explained their low population in the soil. Meanwhile, we successfully recovered 15 isolates of PSF indicated by the clear zone around their colonies after incubation.

3.2. **Phosphate solubilization capacity on Pikovskaya medium**

The PSF isolates were tested for their ability to release bound P using a source of inorganic P in the form of Ca$_3$(PO$_4$)$_2$. The data is presented in the solubilizing index (SI) in Table 1. Based on SI index, it can be seen that all PSF isolates have the varying capacities in phosphorus solubilization. The SI was ranged from 0.96 by isolate F10S to 1.16 by isolate F1S. The different capacity in phosphorus solubilization was explained due to the different types of organic acids produced by the isolates. Organic acids with a low molecular weight such as acetic acid, citric acid, formic acid, malic acid, and oxalic acid are some examples of the common acids produced by microbes from their metabolic processes. These organic acids will act as chelating agents for bound P complexes with aluminum, iron, and calcium, therefore releasing the phosphorus in the available form which can be uptaken by plants [18-20]. A high SI also correlates with the better capacity in phosphorus solubilization by the PSF isolates which can be formulated as biofertilizers in the future.

| Isolate code | Colony diameter (cm) | Clear zone diameter (cm) | Solubilizing index (SI) |
|--------------|----------------------|--------------------------|------------------------|
| F1S          | 3.62                 | 4.22                     | 1.16                   |
| F2S          | 4.87                 | 5.39                     | 1.11                   |
| F3S          | 5.25                 | 5.65                     | 1.08                   |
| F4S          | 5.02                 | 5.49                     | 1.09                   |
| F5S          | 3.86                 | 4.30                     | 1.11                   |
| F6S          | 4.51                 | 4.97                     | 1.10                   |
| F7S          | 5.14                 | 5.65                     | 1.10                   |
| F8S          | 3.52                 | 3.77                     | 1.07                   |
| F9S          | 3.67                 | 4.17                     | 1.13                   |
| F10S         | 3.94                 | 3.78                     | 0.96                   |
| F11S         | 1.66                 | 1.84                     | 1.10                   |
| F12S         | 4.16                 | 4.53                     | 1.09                   |
| F13S         | 4.08                 | 4.10                     | 1.00                   |
| F14S         | 3.98                 | 4.44                     | 1.12                   |
| F15S         | 6.45                 | 6.88                     | 1.07                   |

3.3. **Identification of PSF isolates**

Fifteen isolates with the potential to dissolve phosphorus were identified to the genus level. Based on macroscopic and microscopic observations, all isolates were included in the genus *Aspergillus*. This shows that apart from the small population, there are not many species diversity of PSF in the field. This condition may be related to the soil characteristics as a suitable habitat for PSF. The soil under *S. macropadum* is categorized as low in fertility so that the number and diversity of PSF is small. Sufficient nutrient availability is one of the growth requirements for soil microbial population.

Macroscopic characteristics of the genus *Aspergillus* are as the following: a thin black colony with a white base with a diameter 1 to 7 cm, the colony grows in a circular shape filling the petri dish, while microscopic features are round vesicles, round conidia, and brown black. The conidiophore stalk is clear, thick walled and striking [14] (Figure 1). The *Aspergillus* genus is has a wide life distribution, where this genus can be found in various habitats. The *Aspergillus* genus is a fungus that has good adaptability.
Aspergillus is classified as a mesophilic microbe with growth at a temperature optimum of 35° C to 37° C. The degree of acidity for growth is 2.0 to 8.5 but growth will be better in conditions of low acidity [14,15]. The result of previous studies indicated that of the various plant rhizosphere the phosphate solubilizing fungi found were the Aspergillus genus [3-5,9-11]. The genus Aspergillus is a type of fungus that has a high ability to release bound phosphate. The results then prove that several species of the Aspergillus genus such as A. niger, A. awamori, A. flavus possess a high ability to release phosphate bound to soil components [21,22]. According to [23] the genus Aspergillus is a group of PSF that is predominantly found in acid soils in Indonesia.

**Figure 1.** A typical Aspergillus microscopical characteristic. (A) Phialide, (B) Conidiophore, (C) Conidia. (Magnification at 400×)

### 4. Conclusions

There were 15 isolates phosphate solubilizing fungi successfully isolated from Scaphium macropadum stands. Based on the clear zone ratio or solubilization index (SI), all PSF isolates were the potential to dissolve phosphorus. Based on morphological identification, all PSF belong to *Aspergillus*.

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