Characterization of phosphate solubilizing and cellulolytic fungi isolated from soil under *Eurycoma longifolia* stands

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Abstract. *Eurycoma longifolia* is one of the potential medicinal plants and has a high economic value if developed. The bioactive compound produced by *E. longifolia* has potential as medicine, thus causing increased exploitation of their habitat. This condition resulted in the decreasing population of *E. longifolia* in nature. The distribution of *E. longifolia* is influenced by soil fertility conditions. The presence of phosphate solubilizing fungi and cellulolytic fungi in the soil can support the growth of *E. longifolia* in nature. The purpose of this study was to calculate the population of phosphate solubilizing and cellulolytic fungi and to identify the types of phosphate solubilizing and cellulolytic fungi found in the soil under *E. longifolia* stands. Soil samples were taken randomly at a depth of 0–20 cm in a natural forest of Papaso Village, Lubu Sutam District, Padang Lawas Regency, North Sumatra. Population and isolation of phosphate solubilizing and cellulolytic fungi were obtained by dilution methods. The isolates obtained were identified morphologically to the genus level by macroscopic and microscopic observation of colonies. The results showed that the population of phosphate solubilizing and cellulolytic fungi were \(6.64 \times 10^4\) CFU/mL and \(9.31 \times 10^3\) CFU/mL, respectively. The isolation results in 11 isolates of phosphate solubilizing and 10 isolates of cellulolytic fungi. Based on the morphological identification of phosphate solubilizing fungi isolates consisted of 5 genera, namely Aspergillus, Penicillium, Rhizopus, Fusarium, and Mucor, while isolates of cellulolytic fungi also included 5 genera, namely Aspergillus, Cladosporium, Penicillium, Rhizopus, and Mucor.

1. Introduction

*Eurycoma longifolia* (known as *pasak bumi* in Indonesia) is one of the biological natural resources that has a growing distribution in Southeast Asia. Its distribution includes Indonesia, Peninsular Malaysia, Cambodia, Laos, and Vietnam \([1,2]\). *E. longifolia* is one of the potential medicinal plants and has high economic value if developed. *E. longifolia* roots contain several bioactive compounds that have medicinal properties \([3–5]\). The presence of bioactive compounds in *E. longifolia* that have potential as medicines can have an impact on increasing harvesting *E. longifolia* in its natural habitat. If exploitation is not balanced with cultivation, it can lead to scarcity \([6,7]\).

The distribution of *E. longifolia* is influenced by soil conditions or edaphic factors. Soil is a medium for plant growth and development. The soil condition that directly affects the plant is soil fertility. Indicators of soil fertility are seen from the content of humus or organic matter, nutrients, soil texture, and structure, as well as the availability of water in the soil pores. Soil fertility can be
increased by the presence of soil microorganisms, especially in the rhizosphere, because microorganisms play an important role in the process decomposition of organic matter and nutrient cycles in the soil so that nutrients become available to plants [8]. Rhizosphere microorganisms also play a role in the soil formation process [9], plant growth [10] as well as biological control against root pathogens [11].

Fungi are one of the microorganisms in the soil that play an important role in soil fertility and plant growth. Phosphate solubilizing fungi play a role in the phosphorus nutrient cycle and help increase the availability of phosphorus (P) nutrients in the soil. Nutrient P is an essential macronutrient whose availability is low because it is bound to soil components [8]. Several fungi from the genera Aspergillus, Penicillium, and Fusarium are known to play a role in increasing the availability of P [10,12–15]. Cellulolytic fungi play a role in decomposing organic matter by producing cellulase enzymes. The result of the composition in the form of nutrients nitrogen, phosphorus, sulfur, and some micronutrients can be absorbed by plants to meet their nutrient needs [8]. Fungi from the genera Aspergillus, Chaetomium, Penicillium, Trichoderma, Rhizopus, and Acremonium have the ability to produce cellulase enzymes and play a role in the decomposition of organic matter [16,17]. Based on the role of phosphate solubilizing and cellulolytic fungi, therefore, a study was conducted to determine the presence of phosphate solubilizing and cellulolytic fungi under E. longifolia stands in the natural forest of Papaso Village, Batang Lubu Sutam District, Padang Lawas Regency. The purpose of the study was to calculate the population and identify the types of phosphate solubilizing and cellulolytic fungi found in the soil under E. longifolia stands.

2. Materials and methods

2.1. Study site and soil sampling
Soil samples were taken in the natural forest area of Papaso Village, Batang Lubu Sutam Distric, Padang Lawas Regency, North Sumatra. The altitude ranges from 250-700 m, and the temperature is 27-30°C. Soil samples were taken randomly under E. longifolia stands at a depth of 0-20 cm. Soil samples were put in plastic bags and brought to the laboratory for further analysis. The soil was analyzed for pH, organic C content, available P and cation exchange capacity.

2.2. Isolation of fungi
Isolation of phosphate solubilizing and cellulolytic fungi using the dilution method [18,19]. Ten g of soil was put into an Erlenmeyer flask containing 90 mL of physiological solution (NaCl 0.85%) and shaken for 30 minutes. The dilution was made to 10^-5. The 10^-3, 10^-4, and 10^-5 dilutions were pipetted 1 mL each and put into a petri dish. Isolation of phosphate solubilizing fungi using Pikovskaya medium, while cellulolytic fungi using carboxyl methylcellulose (CMC) medium with 0.1% Congo red. Phosphate solubilizing and cellulolytic fungi will form a clear zone around their colonies. Prior to purification, the fungal population was calculated, the pure isolates were stored for identification.

2.3. Isolates effectiveness test
The effectiveness was tested qualitatively by calculating the ratio of clear zone obtained by dividing the diameter of the clear zone by the diameter of the colony [20].

2.4. Identification of fungal isolates
The pure fungal isolates were identified morphologically by observing the colonies macroscopically and microscopically. Macroscopically observed colony color and colony diameter while microscopically observed hyphae, hyphae branching types, and the characteristic of conidia. Identification was carried out to the genus level. The characteristics found from each fungus are then described and matched with the fungal identification book [21,22].
3. Results and discussion

3.1. Soil analysis and isolation of fungi

The soil under *E. longifolia* stands very acidic (pH 4.4), has low organic C content (1.95%), very low available P (8.96 ppm), and low cation exchange capacity (7.38 me/100g). Soil pH affects the availability of nutrients and the presence of microorganisms in the soil. Nutrients are generally available at pH 6.0-7.0, while the presence of microorganisms depends on the species. Bacteria and actinomycetes were generally dominant at pH 6.5-7.5, while fungi were dominant at acidic pH (pH <5.5) [8]. Organic matter is a source of energy and carbon for heterotrophic microorganisms. The number and types of microorganisms are directly proportional to the content of organic matter. If the organic matter content is high, the number and types of microorganisms are also high [10]. Cation exchange capacity (CEC) is the ability of the soil to adsorb and exchange cations. The low CEC value indicates that the availability of exchangeable bases in the soil is also low. It is also associated with low organic matter content. Humus in the soil is the colloid or active ingredient where cation exchange occurs [8]. The presence of phosphate solubilizing and cellulolytic fungi help increase the availability of nutrients in the soil.

The population of phosphate solubilizing and cellulolytic fungi are 6.64x10^4 colony forming units (CFU)/mL (medium category) and 9.31x10^3 CFU/mL (low category), respectively [23]. The soil under *E. longifolia* stands infertile because of its low organic matter, low nutrient content, and very acidic pH. This causes the fungal population to decrease. The presence of *E. longifolia* trees in natural forests and the litter their produce maintains the microbial population.

Isolation of phosphate solubilizing and cellulolytic fungi obtained 11 and 10 isolates, respectively. All the isolates formed a clear zone around the colony. The clear zone indicates the dissolution of Ca_3(PO_4)_2 compounds in Pikovskaya medium by phosphate solubilizing fungi or degradation of cellulose contained in CMC medium by cellulolytic fungi.

3.2. Isolate effectiveness test

Clear zone ratio calculation of all isolates of phosphate solubilizing and cellulolytic fungi is presented in Table 1 and Table 2. Based on Table 1, the clear zone ratio produced by phosphate solubilizing fungi isolates ranged from 1.19-2.42. The clear zone ratio shows the ability of the isolates qualitatively to release the bound P. The highest clear zone ratio value was found in isolate FELP11. Variations in the clear zone ratio value indicated that there were differences in the effectiveness of each isolate in releasing P. The phosphate dissolution mechanisms were related to the amount and type of organic acid produced by each isolate. Organic acid such as citric acid, oxalic acid, and other low molecular weight organic acids was produced by fungi in their metabolic processes. This organic acid will form compounds with P-binding components so that P is release [13].

The clear zone ratio produced by cellulolytic fungal isolates ranged from 1.25-1.68 (Table 2). FELC10 is the isolate with the highest clear zone ratio value. This shows that the effectiveness of each isolate qualitatively in releasing the cellulase enzyme is not the same.

| Table 1. Clear zone ratio and genus name of phosphate solubilizing fungi |
|--------------------------|--------------------------|
| Isolate code | Clear zone ratio | Genus |
|---------------|------------------|-------|
| FELP1         | 1.19             | Aspergillus |
| FELP2         | 1.23             | Penicillium |
| FELP3         | 1.27             | Aspergillus |
| FELP4         | 1.34             | Rhizopus |
| FELP5         | 1.42             | Fusarium |
| FELP6         | 1.44             | Rhizopus |
| FELP7         | 1.58             | Penicillium |
| FELP8         | 1.58             | Rhizopus |
| FELP9         | 1.67             | Mucor |

| Isolate code | Clear zone ratio | Genus     |
|--------------|------------------|-----------|
| FELP10       | 1.92             | Mucor     |
| FELP11       | 2.42             | Rhizopus  |

Table 2. Clear zone ratio and genus name of cellulolytic fungi

| Isolate code | Clear zone ratio | Genus     |
|--------------|------------------|-----------|
| FELC1        | 1.25             | Cladosporium |
| FELC2        | 1.32             | Aspergillus |
| FELC3        | 1.37             | Aspergillus |
| FELC4        | 1.38             | Aspergillus |
| FELC5        | 1.40             | Mucor     |
| FELC6        | 1.41             | Cladosporium |
| FELC7        | 1.43             | Aspergillus |
| FELC8        | 1.46             | Cladosporium |
| FELC9        | 1.61             | Penicillium |
| FELC10       | 1.68             | Rhizopus  |

3.3. Morphological identification of fungal isolates

Based on morphological colony observations, isolates of phosphate solubilizing fungi were included in 5 genera (Table 1), namely the genus *Aspergillus* (2 isolates) (Figure 1a), *Penicillium* (2 isolates) (Figure 1b), *Fusarium* (1 isolate) (Figure 1c), *Rhizopus* (4 isolates) (Figure 1d) and *Mucor* (2 isolates) (Figure 1e). Isolates of cellulolytic fungi were included in 5 genera (Table 2), namely *Aspergillus* (4 isolates, *Cladosporium* (3 isolates) (Figure 1f), *Mucor* (1 isolate), *Rhizopus* (1 isolate), and *Penicillium* (1 isolate).

![Figure 1](image_url)

**Figure 1.** Morphology of soil fungi isolated from soil under *E. longifolia* stands (a). *Aspergillus*, (b). *Penicillium*, (c). *Fusarium*, (d). *Rhizopus*, (e) *Mucor*, (f). *Cladosporium*

3.3.1. *Aspergillus*. In this study, there were 2 isolates of phosphate solubilizing fungi identified as *Aspergillus*. The characteristics of *Aspergillus* are as follow: Colony diameter 1.5-2.5 cm, dark green, conidia oval in shape, and grayish-black in color. There were 4 isolates of cellulolytic fungi, including *Aspergillus*, and 2 types of colonies with different characteristics. The characteristics are as follow (1) colonies with a diameter 2.0-3.5 cm, grayish-white to dark green, conidia oval in shape, and grayish brown to brownish-black in color, (2) colonies 3.0-3.5 cm in diameter, dark green, conidia semi-round and grayish black.
3.3.2. *Penicillium*. There were 2 isolates of phosphate solubilizing fungi, which were identified as *Penicillium* with the following characteristics: colony diameter 1.7-2.2 cm, green in color, conidia were semi-spherical and grayish-brown in color. There was one isolate of cellulolytic fungi identified as *Penicillium* with the following characteristics: colony diameter 2.0-3.2 cm, yellow color, conidia pseudo-round, and greyish brown in color.

3.3.3. *Fusarium*. There was one isolate of phosphate solubilizing fungi identified as *Fusarium* with the following characteristics: colonies 1.5-2.0 cm in diameter, light green in color, conidia were spherical in shape and grayish brown in color.

3.3.4. *Rhizopus*. There were 4 isolates of phosphate solubilizing fungi identified as *Rhizopus* and 2 types of colonies with different characteristics. The characteristics were as follows: (1) colony diameter 0.8-1.5 cm, green, conidia round in shape and brown, (2) colonies 1.5-2.0 cm in diameter, green in color, conidia oval in shape, and black. There is 1 isolate of cellulolytic fungi belonging to the genus *Rhizopus* with the following characteristics: colonies 1.0-2.0 cm in diameter, light green in color, conidia oval in shape, and brownish-black in color.

3.3.5. *Mucor*. There were 2 isolates of phosphate solubilizing fungi belonging to the genus *Mucor*. The characteristics are as follows: (1) colonies with a diameter 2.5-3.0 cm, green, conidia oval in shape and brown, (2) colonies with a diameter of 2.0-2.5 cm, green color, conidia semi-round in shape, and dark brown in color. The characteristics of cellulolytic fungi belonging to the genus *Mucor*: colonies 1-2 cm in diameter, colonies are green, conidia are semi-spherical, and brownish-black.

3.3.6. *Cladosporium*. Only isolates of cellulolytic fungi belonging to the genus *Cladosporium*. There were 2 types of *Cladosporium* with the following characteristics, namely: (1) colony with a diameter of 2.5-3.5 cm, colonies grayish-green, conidia round in shape and brownish-gray in color, (2) colony with a diameter of 2-3.5 cm, colonies dark green, oval-shaped conidia, and black.

4. Conclusions

The population of phosphate solubilizing and cellulolytic fungi under *E. longifolia* stands was 6.64x10⁴ and 9.31x10³ CFU/mL, respectively. The identification result showed that the phosphate solubilizing fungi isolates belonged to 5 genera, namely *Aspergillus, Penicillium, Fusarium, Mucor, and Rhizopus*. The isolate of cellulolytic fungi are also included 5 genera, namely *Aspergillus, Penicillium, Cladosporium, Mucor, and Rhizopus*.

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