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Isolation of phosphate-solubilizing rhizosphere fungi from jabon merah (*Neolamarckia macrophylla*) stand of Sidrap Provenance, South Sulawesi

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Abstract. Phosphate is an essential macro element that has a necessary function as a constituent of ATP and DNA in plants. However, the availability of dissolved phosphate in the soil is minimal because it tends to bind with soil minerals to form phosphate complexes. With rhizosphere fungi, the low available phosphate in the soil can be overcome. This study aimed to determine the character and potential of fungi capable of dissolving phosphate. The source of the isolates used was a collection of rhizosphere fungus isolates under the red jabon stand. Purification was carried out using the point method on PDA media. The phosphate dissolving ability test was done using the standard method using liquid pikovskaya media and then analyzed descriptively and quantitatively. Eighteen rhizosphere fungus isolates were observed, two of which could dissolve phosphate, respectively, obtained from isolates JCS16 with a concentration value of 10.48 ppm, JCS 13 with a concentration value of 10.06 ppm.

1. Introduction

Phosphate is an essential macro element that has a vital function as a constituent of ATP and DNA in plants. Plants utilize only 10-30% of the phosphate fertilizer given, meaning that 70-90% of the phosphate fertilizer remains in the soil. Naturally, phosphate in the soil is in the form of organic or inorganic compounds. Soil with low organic content has varying organic phosphate content depending on the type of soil. Still, the availability of dissolved phosphate in the soil is minimal because it tends to bind with soil minerals to form complex phosphates. One alternative to overcome the low availability of phosphate in the soil is to use rhizosphere fungi associated with a plant stand. The fungus can dissolve phosphate that is difficult to dissolve into soluble to be absorbed by plants [1].

Fungi can secrete organic acid substances in the early stages of growth. The resulting organic acids can break down calcium phosphate compounds in Pikovskaya medium, converting phosphate from insoluble to soluble. A high concentration of solubilizing phosphate can be used in oxidative respiration activity, thereby increasing growth. [2,3]. Some fungi, such as *Aspergillus niger* and *Penicillium*, can dissolve phosphate by producing large organic acid compounds. *Aspergillus niger* produces phosphate-soluble oxalic, gluconic, and citric acids [4,5].

Neolamarckia macrophylla (Roxb.) The Bosser tree is a member of the Rubiaceae family and is a significant reforestation and timber tree in Southeast Asia. Due to its rapid growth rate and adaptability, it has been prioritized for planting in forestation and agroforestry initiatives. Its wood is suitable for making plywood, boards, crates, and paper [6–8]. Exploration of trees and identification of rhizosphere microbes in some community forest stands in South Sulawesi needs be conducted. Given the role of phosphate-solubilizing fungi in soil fertility stand Jabon merah, it is crucial to know the character and potential of fungi capable of dissolving phosphate.

2. Materials and methods

This research was conducted at the Laboratory of Biotechnology and Tree Breeding, Faculty of Forestry, Universitas Hasanuddin. The research materials used included rhizosphere soil samples, PDA (Potatoes Dextrose Agar) media, PDB (Potatoes Dextrose Broth), chloramphenicol, sterile distilled water, pikovskaya solution, 70% alcohol, agar, heat-resistant plastic, and aluminum foil, and other materials.

2.1. Purification of soil fungus collection

The rhizosphere fungus isolate was derived from fungal isolates of the Laboratory of Biotechnology and Tree Breeding, Faculty of Forestry, Universitas Hasanuddin. The isolates were taken from soil samples obtained from the red Jabon stand area, located in Pitu Riase District, Sidrap Regency, South Sulawesi. Purification of fungal isolates was done by moving the fungus using the point method on the media [9].

2.2. Testing the ability of isolates as phosphate-solubilizing fungi

This test was conducted using the standard method, namely pikovskaya media [10]. 30 ml of the isolated culture was cultured on Pikovskaya media for seven days and in a shaker. The culture was filtered using filter paper (Whatman 41) and centrifuged at 1000 rpm for 15 minutes. 5 ml of supernatant was taken, and 0.5 ml of concentrated reagent was added. The absorbance was measured using a spectrophotometer at a wavelength of 693 nm and the blank using aquadest. The phosphate concentration of the sample was calculated based on the standard curve with the PO_4 standard.

3. Result and discussion

3.1 The character of Rhizosphere fungus

The rhizosphere soil was used as a source of exploration of phosphate-solubilizing fungi isolates. The results of macroscopic isolation showed that in the rhizosphere of the red jabon plant, 18 isolates of the fungus were found. The results of the morphological characterization of the 18 isolates of the fungus included color, texture, and surface elevation and microscopically referred to Domsch et al. (1980) [11]; Barnet and Hunter (1998); Kubicek and Harman (2002). The morphological characterization of 18 fungal isolates showed variations in color, texture and surface elevation of the fungus. The colony color was dominated by green and white, while the texture of the colony was dominated by velvet and cotton.

3.2 The ability of fungal isolates in dissolving phosphate

In this study, the isolated fungal isolates were grown in Pikovskaya medium for seven days. The culture results were then filtered and centrifuged to separate the pellet and supernatant. The supernatant obtained was then added with a concentrated reagent solution. The observations showed color differences in some of the observed fungal isolates (Figure 1). The color difference in the Pikovskaya broth medium indicated phosphate solubilization activity by the fungus isolate. Some isolates look lighter in color than other isolates. Phosphate dissolving activity can occur due to the breakdown of tricalcium phosphate compounds in the media into dissolved phosphate compounds [12]. The fungal isolate surrounded by a light-colored zone indicated that the fungus could dissolve phosphate compounds [13–15].

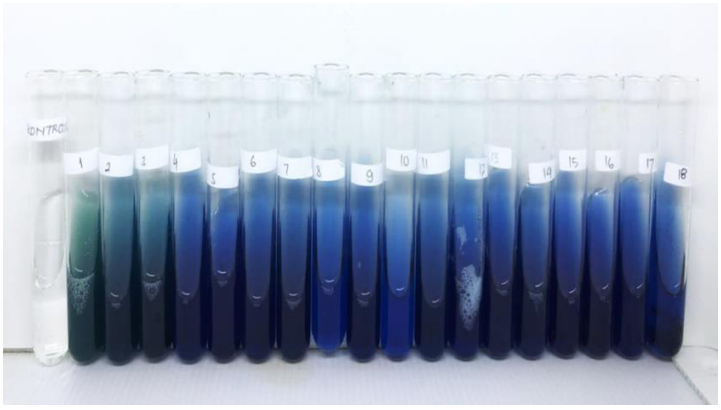

Figure 1. Color differences in fungal isolates

The 18 isolates that had been successfully purified were then tested quantitatively using a spectrophotometer to determine the ability to dissolve phosphate based on the absorbance results. The quantitative test results showed that all isolates could dissolve phosphate with a concentration value ranging from 5-10 ppm. The high value of phosphate concentration indicates the strength of the isolate in dissolving phosphate. High phosphate solubilization ability was obtained successively from isolate JCS16 with a concentration value of 10.48 ppm, followed by JCS 13 with a concentration value of 10.06 ppm (Table 1).

Table 1. Results of Phosphate-Solubilizing Fungi

Isolate Code	Absorbance (nm)	Concentration (ppm)
JCS 1	1.824	9.30
JCS 2	1.414	7.15
JCS 3	1.706	8.68
JCS 4	1.566	7.95
JCS 5	1.797	9.16
JCS 6	1.561	7.92
JCS 7	1.672	8.50
JCS 8	0.782	3.84
JCS 9	1.677	8.53
JCS 10	1.03	5.14
JCS 11	1.523	7.72
JCS 12	1.566	7.95
JCS 13	1.969	10.06
JCS 14	1.57	7.97
JCS 15	1.657	8.42
JCS 16	2.049	10.48
JCS 17	1.634	8.30
JCS 18	1.469	7.44

In this study, the concentration of phosphate-solubilizing by the JCS16 fungus isolate was higher than the highest concentration of phosphate-solubilizing by the mahogany rhizosphere fungus in Maros Regency, which was 6.65 ppm . The results obtained were also higher than the highest concentration of phosphate-solubilizing by peat fungus isolates, which was 7.87 ppm [16]. However, the results of this study were lower than the highest concentration of phosphate solubilization by peat soil bacteria, which was 24.81 ppm [12]. The results of this study were also lower than the highest concentration of dissolved phosphate by *Azotobacter* bacterial isolates, which was 37.93 ppm [17]. Several other studies on the dissolution of phosphate by microbes stated that the bacteria *Bacillus cereus* was able to dissolve phosphate with a phosphate solubilizing index value of 2.52; actinomycetes can dissolve phosphate with a phosphate solvent index value of 21 mg-P/L; and soybean plant rhizosphere bacteria can dissolve phosphate with a phosphate solvent index value of 1.5 [18,19] It shows that several types of bacteria and fungi have different levels of ability to dissolve phosphate. Differences in the ability of fungi to dissolve phosphate are influenced by several factors, including the type of microbe, source of the inoculum, type of medium, pH of the medium, and incubation period.

Phosphate dissolving activity by isolates through organic acid secretion caused an increase in dissolved phosphate concentration. The isolates utilized dissolved phosphate in the medium for cell metabolism and energy formation. The isolates can grow and divide properly, increasing the number of cells and producing organic acids, which causes the concentration of dissolved phosphate to be higher. Microbes use inorganic phosphate for activity and new cells.

The type of solid medium to the liquid medium can affect the growth and viability of microbes even though the medium used is the same. In a solid medium, microbial growth is attached to the surface of the medium (attached growth); thus, only the bottom of the colony is in contact with the medium. In contrast, in the liquid medium, the growth type resembles a soluble suspension (suspended growth) [20]

3.3. Characteristics of phosphate-solubilizing Rhizosphere fungi

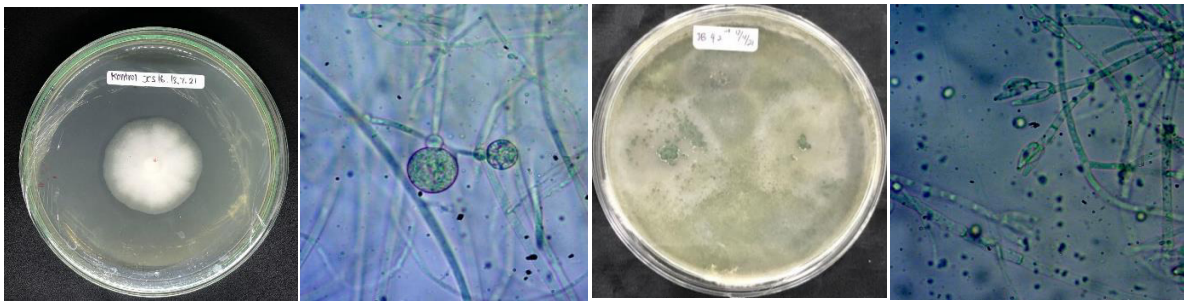

Figure 2. The macroscopic and microscopic morphology of the selected phosphate-solubilizing fungi isolates were: (a)(b) JCS 16 isolate and (c)(d) JCS 13 isolate.

Isolated JCS 16 macroscopic observation showed isolate were white and had a fine cotton texture. The isolates of JCS 13 were white and had green spores, textured like velvet. The results of microscopic observations observed under a microscope with 400x magnification of the colony, JCS 16 isolate was seen to have unbranched hyphae, maturing sporangia: 1, small round Apophysate; 2, Globose large round sporangia (compound microscope images). JCS 13 isolate colonies were seen to have branched hyphae and many spherical spores (Figure 2).

Identification of rhizosphere fungi based on macroscopic and microscopic characteristics often does not provide certainty of the identity of an isolate because the morphology of the fungus can change. Thus, the identity can be known through the PCR technique followed by DNA sequence analysis. Identification

of rhizosphere fungi using PCR techniques and DNA analysis has a relatively high and accurate sensitivity. Therefore, this research will continue with identifying a molecular approach to identify the identity of rhizosphere fungi that can be easily and quickly known to the species level based on BLASTN analysis on DNA sequences.

4. Conclusion

Eighteen isolates of rhizosphere fungus from red Jabon (*Neolamarckia macrophylla*) stand of Sidrap Provenance, South Sulawesi with phosphate solvents were observed, two isolates of which have the potential to dissolve phosphate were obtained from isolates JCS16 with a concentration value of 10.48 ppm and JCS 13 with a concentration value of 10.06 ppm.

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