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Exploration of Phosphate-Solubilizing Rhizobacteria Associated with Liberica Tungkal Composite Coffee (*Coffea liberica*) in Jambi, Indonesia

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Abstract

Phosphorus (P) is an essential macronutrient required for plant growth and productivity; however, most phosphorus in soil occurs in insoluble forms that are unavailable for plant uptake. Phosphate-solubilizing bacteria (PSB) play an important role in improving phosphorus availability by converting insoluble phosphate into soluble forms, thereby enhancing nutrient-use efficiency and reducing dependence on chemical fertilizers. This study aimed to explore phosphate-solubilizing rhizobacteria associated with Liberica Tungkal Composite coffee (*Coffea liberica*) cultivated in Tanjung Jabung Barat and Tanjung Jabung Timur Regencies, Jambi Province, Indonesia. Rhizosphere bacteria were isolated and screened for their phosphate-solubilizing ability on Pikovskaya agar medium. Phosphate-solubilizing activity was evaluated qualitatively based on the formation of clear halos surrounding bacterial colonies. A total of 30 rhizosphere bacterial isolates were successfully obtained. All isolates formed clear halos on Pikovskaya agar medium, with halo diameters ranging from 0.2 to 1.2 cm, indicating their ability to solubilize insoluble phosphate. The variation in halo diameter suggests differences in phosphate-solubilizing activity among the isolates. These findings demonstrate that the rhizosphere of Liberica Tungkal Composite coffee harbors diverse indigenous phosphate-solubilizing bacteria with potential for further characterization and development as biofertilizers to improve phosphorus availability and support sustainable Liberica coffee cultivation.

Keywords: biofertilizer; *Coffea liberica*; phosphate-solubilizing bacteria; Pikovskaya agar; rhizosphere bacteria.

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INTRODUCTION

Phosphorus (P) is an essential macronutrient that plays a vital role in plant growth and development. However, most phosphorus in soil exists in insoluble forms that are unavailable for plant uptake (1,2). In acidic soils, phosphorus is commonly fixed by iron (Fe) and aluminum (Al), resulting in low phosphorus availability and reduced fertilizer-use efficiency. Continuous application of chemical phosphate fertilizers is often ineffective because a large proportion of the applied phosphorus becomes immobilized in the soil and may negatively affect soil quality and the environment (3,4).

Phosphate-solubilizing bacteria (PSB) have emerged as an environmentally friendly alternative for improving phosphorus availability. These bacteria convert insoluble phosphate into soluble forms through the production of organic acids and phosphatase enzymes, thereby enhancing phosphorus availability and supporting sustainable crop production (5).

Liberica Tungkal Composite coffee (*Coffea liberica*) is an important coffee cultivar cultivated mainly in Tanjung Jabung Barat and Tanjung Jabung Timur Regencies, Jambi Province, Indonesia. This coffee is predominantly grown on peat soils, which are characterized by high acidity and limited phosphorus availability (6–8). Although phosphate-solubilizing bacteria have been widely reported in various agricultural crops, information regarding indigenous PSB associated with the rhizosphere of Liberica Tungkal Composite coffee remains limited (9,10).

Therefore, this study aimed to explore phosphate-solubilizing rhizobacteria associated with Liberica Tungkal Composite coffee cultivated in Jambi Province, Indonesia. The phosphate-solubilizing ability of the bacterial isolates was qualitatively evaluated on Pikovskaya agar medium based on the formation of clear halos surrounding bacterial colonies. The results of this study provide baseline information on indigenous phosphate-solubilizing bacteria with potential for further development as biofertilizers to support sustainable Liberica coffee cultivation.

METHODS

Study Area and Sample Collection

This study was conducted at the Plant Pathology Laboratory, Faculty of Agriculture, Universitas Jambi, Indonesia. Rhizosphere soil samples were collected from Liberica Tungkal Composite coffee (*Coffea liberica*) plantations located in Tanjung Jabung Barat and Tanjung Jabung Timur Regencies, Jambi Province, Indonesia. These areas represent the main production centers of Liberica coffee cultivated on tropical peatlands.

Rhizosphere soil was collected from the root zone of healthy coffee plants by carefully removing the upper soil layer and collecting soil adhering to the roots at a depth of approximately 10–20 cm. Approximately 500 g of rhizosphere soil was collected from each sampling point using sterile plastic bags and transported to the laboratory for bacterial isolation (11).

Isolation of Rhizosphere Bacteria

Rhizosphere bacteria were isolated using the serial dilution technique. Ten grams of rhizosphere soil were suspended in 90 mL of sterile distilled water and homogenized using an orbital shaker for 30 minutes. The suspension was serially diluted up to 10^{-6}. Subsequently,

0.1 mL of each dilution was spread onto Nutrient Agar (NA) medium using the spread plate method and incubated at 28 ± 2°C for 48 hours (11,12).

Individual colonies exhibiting distinct morphological characteristics, including colony shape, color, elevation, and margin, were selected and purified by repeated streaking on fresh Nutrient Agar medium until pure cultures were obtained. Each purified isolate was maintained on Nutrient Agar slants at 4°C for further analysis (13).

Screening of Phosphate-Solubilizing Bacteria

The phosphate-solubilizing ability of the bacterial isolates was evaluated qualitatively using Pikovskaya agar medium containing tricalcium phosphate [$Ca_3(PO_4)_2$] as the insoluble phosphate source.

Each bacterial isolate was inoculated onto the center of a Pikovskaya agar plate using a sterile inoculating loop and incubated at 28 ± 2°C for 5–7 days. Phosphate-solubilizing activity was determined by observing the formation of a clear halo surrounding the bacterial colony. The diameter of the clear halo was measured using a digital caliper and expressed in centimeters (cm).

The appearance of a clear halo around the colony was considered evidence of phosphate-solubilizing activity, indicating the ability of bacterial isolates to convert insoluble phosphate into soluble forms (5).

Data Analysis

The results were analyzed descriptively based on the presence and diameter of the clear halo produced by each isolate on Pikovskaya agar medium. The halo diameter was used as an indicator of phosphate-solubilizing ability. Data are presented in tabular form and discussed by comparing the observed phosphate-solubilizing activity among the isolates with findings reported in previous studies.

Because this study focused on the exploration and preliminary screening of phosphate-solubilizing rhizobacteria, no inferential statistical analysis was performed.

RESULTS

Isolation of Phosphate-Solubilizing Rhizobacteria

Rhizosphere soil samples collected from Liberica Tungkal Composite coffee plantations yielded a total of 30 bacterial isolates. The isolates were obtained from the rhizosphere of healthy coffee plants growing in peatland areas of Tanjung Jabung Barat and Tanjung Jabung Timur Regencies, Jambi Province. All purified isolates were successfully cultured on Nutrient Agar medium and subsequently screened for their phosphate-solubilizing ability using Pikovskaya agar medium.

Screening on Pikovskaya agar demonstrated that all bacterial isolates were capable of producing clear halos surrounding their colonies (Fig. 1). The formation of these halos indicates the ability of the isolates to solubilize insoluble phosphate present in the medium. The diameter

of the clear halos ranged from 0.2 to 1.2 cm, suggesting that all isolates possessed phosphate-solubilizing activity, although the extent of halo formation varied among isolates.

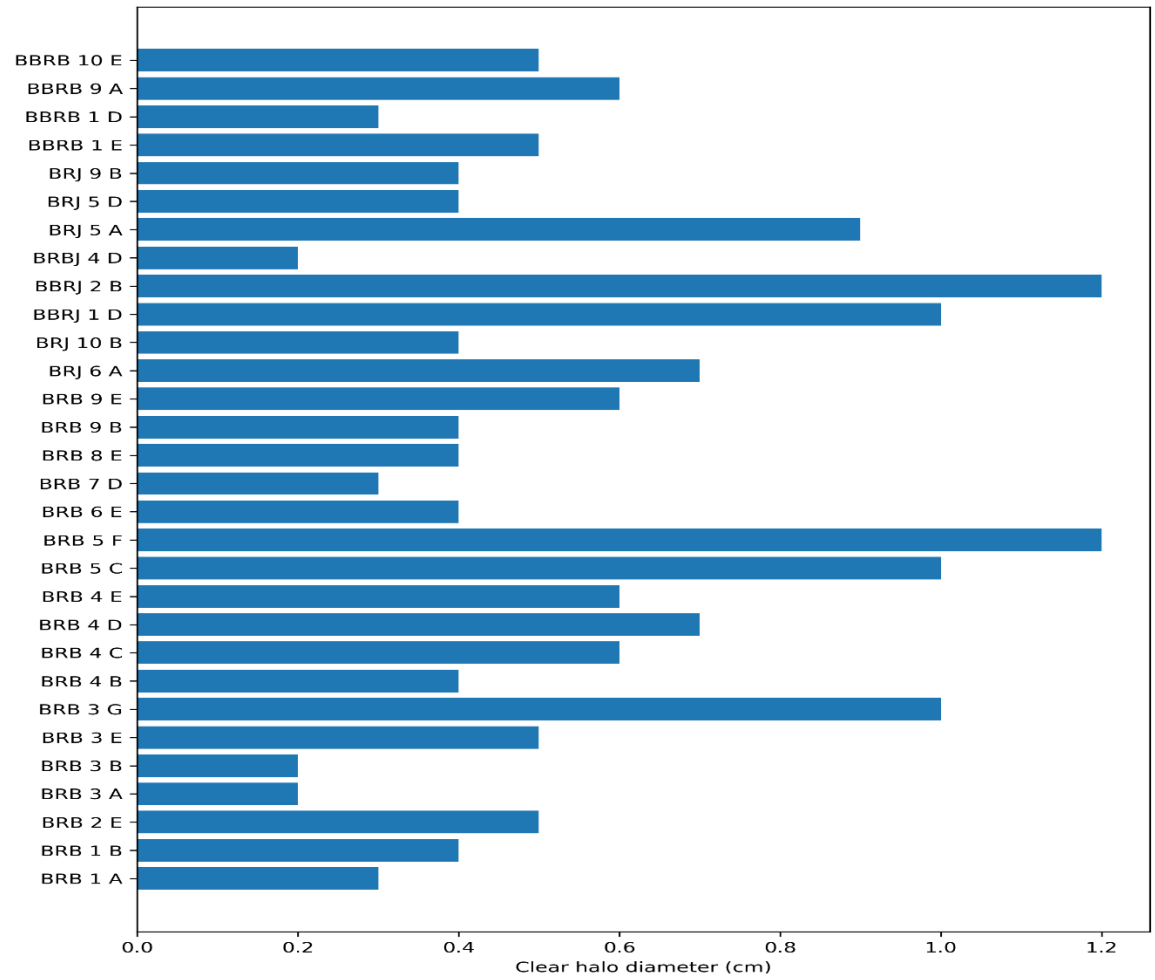

Figure 1. Phosphate-solubilizing activity of rhizosphere bacterial isolates on Pikovskaya agar medium.

The qualitative screening revealed that every isolate exhibited phosphate-solubilizing capability as evidenced by halo formation. This finding indicates that the rhizosphere of Liberica Tungkal Composite coffee harbors a diverse community of functional bacteria involved in phosphorus mobilization. The observed variation in halo diameter reflects differences in the metabolic capacity of each isolate to release compounds responsible for phosphate solubilization (Fig.2)

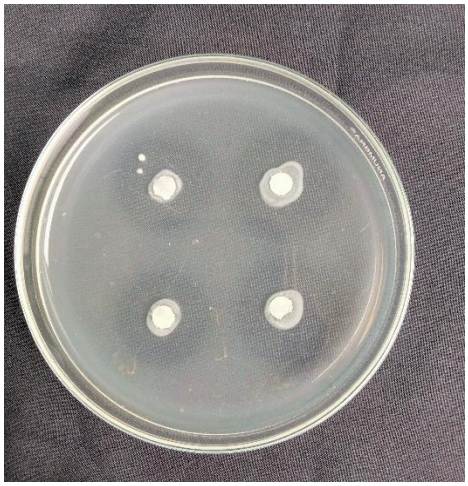

Figure 2. Representative clear halo formation produced by phosphate-solubilizing rhizobacteria on Pikovskaya agar medium.

DISCUSSION

The formation of clear halos on Pikovskaya agar is widely accepted as a qualitative indicator of phosphate-solubilizing activity (14). The transparent zone surrounding the bacterial colony results from the dissolution of insoluble tricalcium phosphate present in the medium, demonstrating the ability of the bacteria to convert unavailable phosphorus into soluble forms. Therefore, the presence of clear halos confirms that all isolates obtained in this study possess phosphate-solubilizing capability(15).

The observed variation in halo diameter (0.2–1.2 cm) suggests differences in the phosphate-solubilizing capacity among the isolates. Such variation is commonly reported in rhizosphere bacteria and is associated with differences in bacterial metabolism, growth rate, and the production of phosphate-solubilizing compounds. However, because this study represents a preliminary exploration, the observed halo diameter was used only as a qualitative indicator of phosphate-solubilizing activity rather than as a criterion for selecting superior isolates (16,17).

Phosphate solubilization by rhizobacteria generally occurs through the secretion of low-molecular-weight organic acids, including gluconic, citric, oxalic, malic, and succinic acids. These organic acids reduce the pH surrounding bacterial colonies and chelate calcium, iron, or aluminum ions that bind phosphate, thereby releasing soluble orthophosphate that can be absorbed by plants. In addition, several phosphate-solubilizing bacteria produce phosphatase enzymes that mineralize organic phosphorus compounds into inorganic phosphate. These mechanisms play an essential role in increasing phosphorus availability in the rhizosphere and improving plant nutrient acquisition (18,19).

The successful isolation of 30 phosphate-solubilizing bacterial isolates demonstrates that the rhizosphere of Liberica Tungkal Composite coffee supports a diverse community of functional microorganisms involved in phosphorus cycling. This finding is consistent with previous studies reporting that the rhizosphere is one of the most biologically active soil environments due to the continuous release of root exudates, which provide carbon sources and nutrients for microbial growth. Consequently, the rhizosphere serves as an important ecological niche

for beneficial microorganisms, including phosphate-solubilizing bacteria, that contribute to nutrient mobilization and plant growth promotion (20,21).

The occurrence of phosphate-solubilizing bacteria in the rhizosphere of Liberica coffee is particularly important because this crop is predominantly cultivated on peat soils, where phosphorus availability is frequently limited by soil acidity and nutrient fixation (7). Indigenous phosphate-solubilizing bacteria adapted to local environmental conditions may therefore contribute to improving phosphorus availability and reducing dependence on chemical phosphate fertilizers. Their adaptation to peatland ecosystems also increases their potential for survival and colonization when applied under field conditions (22).

Since this research was designed as an initial exploration, only qualitative screening was performed to confirm phosphate-solubilizing activity. Further studies are required to identify the isolates using molecular techniques, quantify phosphate solubilization under liquid culture conditions, characterize additional plant growth-promoting traits, and evaluate their effectiveness in promoting the growth of Liberica coffee under greenhouse and field conditions. Such investigations will facilitate the selection of promising indigenous bacterial strains for future biofertilizer development.

Overall, this study provides the first baseline information on phosphate-solubilizing rhizobacteria associated with Liberica Tungkal Composite coffee in Jambi Province. The successful isolation of 30 functional bacterial isolates expands the available microbial resources and provides a scientific basis for future studies aimed at developing indigenous biofertilizers to support sustainable Liberica coffee cultivation.

CONCLUSIONS

A total of 30 rhizosphere bacterial isolates were successfully isolated from the rhizosphere of Liberica Tungkal Composite coffee (*Coffea liberica*) cultivated in Jambi Province, Indonesia. All isolates exhibited phosphate-solubilizing activity as indicated by the formation of clear halos on Pikovskaya agar medium, with halo diameters ranging from 0.2 to 1.2 cm. These findings demonstrate that the rhizosphere of Liberica Tungkal Composite coffee harbors indigenous phosphate-solubilizing bacteria with the potential to improve phosphorus availability in the rhizosphere. This study provides baseline information for future research on the characterization and utilization of phosphate-solubilizing bacteria

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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DECLARATION OF ARTIFICIAL INTELLIGENCE USE

This study used artificial intelligence (AI) tools and methodologies in the capacities of **Manuscript writing support** that was employed to conduct language refinement (improving the grammar, sentence structure, and readability of the manuscript). *We confirm that AI-assisted processes were critically reviewed by the authors to ensure the integrity and*

reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors.

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