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SCREENING PHOSPHATE-SOLUBILIZING BACTERIA FROM RHIZOSPHERE SOIL UNDER DURIAN (*DURIO ZIBETHINUS* L.) CANOPIES IN THAILAND

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Abstract

Phosphate-solubilizing bacteria (PSB) play a crucial role in enhancing phosphorus availability in agricultural soils. This study aimed to isolate and screen efficient PSB from rhizosphere soils under durian (*Durio zibethinus* L.) orchards in eastern and southern Thailand. A total of 40 bacterial isolates were obtained and evaluated for phosphate-solubilizing activity using Czapek's and Pikovskaya's medium. Three isolates (R24, J33 and J35) exhibited the highest solubilization efficiency. Isolate R24 produced the largest clear zone on Czapek's medium (1.30 ± 0.33 cm), followed by J33 (1.10 ± 0.03 cm); meanwhile, J35 showed the highest activity on Pikovskaya's medium (0.60 ± 0.14 cm). Molecular identification based on 16S rRNA gene sequencing revealed that isolates R24 and J35 were closely related to *Pseudomonas orientalis*, whereas J33 was identified as *Massilia norwichensis*. These findings highlight the potential application of selected PSB as biofertilizers to improve phosphorus availability and support sustainable durian cultivation in Thailand.

Keywords: phosphate-solubilizing bacteria, durian, Thailand

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1. INTRODUCTION

Durian (*Durio zibethinus* L.) is a high-value export fruit with rapidly increasing market demand, particularly in the eastern and southern regions of Thailand. In 2023, total durian production reached 1.09 million tons (1). To achieve high yields for export, farmers rely heavily on chemical fertilizers, especially compound formulations such as 15-15-15 and 16-16-16, which are often applied at high rates. However, excessive fertilizer application leads to the accumulation of phosphorus (P) in forms that are not readily available to plants, resulting in low nutrient use efficiency and increased production costs (2). In highly weathered soils, phosphorus is rapidly fixed through reactions with iron (Fe^{3+}), aluminum (Al^{3+}) and calcium (Ca^{2+}), forming stable and insoluble complexes that limit its bioavailability (3). Consequently, excessive fertilizer use not only increases production costs but also contributes to environmental problems, such as nutrient imbalance and eutrophication of water bodies (4). In this context, biological approaches have gained increasing attention as sustainable alternatives to improve phosphorus use efficiency in agricultural systems. Phosphate-solubilizing microorganisms (PSMs), particularly phosphate-solubilizing bacteria (PSB), play a crucial role in enhancing phosphorus availability by converting insoluble phosphorus into plant-available forms through various biochemical mechanisms (5,6). One of the primary mechanisms involves the secretion of low-molecular-weight organic acids, which reduce soil pH and promote the dissolution of mineral phosphates through acidification and chelation processes (3,7). In addition, proton (H^+) release during microbial metabolism further enhances rhizosphere acidification, thereby improving phosphorus solubilization (6). Beyond inorganic phosphorus solubilization, PSMs also contribute to the mineralization of organic phosphorus through the production of extracellular enzymes, such as phosphatases and phytases, which release inorganic phosphate from organic compounds (8). These processes increase the concentration of soluble phosphate ions (H_2PO_4^- and HPO_4^{2-}) in the soil solution, thereby enhancing phosphorus availability and nutrient use efficiency (2). Furthermore, PSMs exhibit additional plant growth-promoting traits, including phytohormone production, stimulation of root development, and improvement of soil structure, which collectively support plant growth (9). Although numerous studies have investigated PSMs, most have been conducted under control laboratory or greenhouse conditions, and their effectiveness

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under field conditions remains inconsistent due to variations in soil properties, environmental factors, and microbial interactions (5). This limitation is particularly relevant in perennial cropping systems such as durian orchards, where long-term soil management and spatial variability strongly influence nutrient dynamics. In addition, limited information is available on the effects of PSM application on soil phosphorus fractions and their relationship with plant phosphorus uptake in tropical fruit systems. Therefore, this study aimed to evaluate the effects of phosphate-solubilizing microorganisms on phosphorus availability and selected soil chemical properties under the durian canopy. In addition, the study assessed the potential of PSMs to enhance phosphorus uptake and accumulation in durian leaves, with the goal of developing sustainable nutrient management strategies for durian production systems.

2. METHODS

This study was conducted in durian cultivation areas located in Khlong Phlu, Khao Khitchakut District, Chanthaburi (UTM Zone 48N: 176225 m E, 1427694 m N), Tham Sing, Mueang Chumphon District, Chumphon (UTM Zone 47N: 508036 m E, 1148369 m N). All sample analyses were performed at the Advanced Soil and Fertilizer Laboratory, Faculty of Agricultural Production, Maejo University. Soil samples were collected at a depth of 0-10 cm from the rhizosphere zone and were subjected to serial dilution. The diluted samples were then plated onto Nutrient agar (NA) and incubated at $28 \pm 2^\circ\text{C}$ for 3–7 days, from which a total of 40 bacterial isolates were obtained and classified based on morphological characteristics (10). All isolates were subsequently purified using the streak plate method and were prepared for further evaluation of their phosphate-solubilizing efficiency.

2.1 Testing Phosphate-Solubilizing Efficiency

All 40 microbial isolates were evaluated for their phosphate-solubilizing efficiency. A sterilized forceps was used to pick a circular agar disc, (0.6 cm in diameter) from the microbial colonies to be tested. The agar discs were subsequently transferred to Czapek’s medium (10) and Pikovskaya’s medium (11) for phosphate-solubilization. The cultures were incubated at room temperature, and observations were recorded on days 3 and 7 with 3 replications. The formation of a clear halo zone surrounding the bacterial colonies on the culture media indicated phosphate-

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solubilizing activity, whereas the absence of a clear zone indicated a lack of phosphate-solubilizing ability. In addition, the morphological characteristics of the purified isolates (8).

2.2 Identification of Microorganisms Using DNA Sequencing

The three isolates with the highest phosphate-solubilizing ability were cultured on Nutrient agar (NA) and maintained in the logarithmic phase. Pure bacterial colonies were collected, and genomic DNA was extracted using a genomic DNA extraction kit. Polymerase chain reaction (PCR) was performed to amplify the 16S rDNA region using universal primers 27F and 1522R. The purified PCR products were subsequently submitted for DNA sequencing. The obtained DNA sequences were compared with those available in the GenBank database using the BLASTn program, and bacterial identification was determined based on sequence similarity.

3. RESULTS AND DISCUSSION

3.1 Morphological Characteristics of Bacterial Isolates

A total of 40 phosphate-solubilizing bacterial isolates were obtained from rhizosphere soils collected from durian orchards located in the eastern and southern regions of Thailand. Among these, 16 isolates were collected from the eastern region, 15 isolates from the southern region, and 9 isolates from rhizoplane. Colony morphology analysis revealed variations in colony shape, elevation, margin, color, and surface texture among the isolates. Most isolates exhibited irregular colony morphology with convex elevation, entire margins, milky coloration, and moist surface texture (Table 1).

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Table 1: Colony Characteristics of Bacteria under Durian Canopies

Isolate	Elevation	Margin	Color	Texture	Shape
1	Flat	Entire	Milky	Moist	Round
2	Flat	Entire	Milky	Moist	Irregular
3	Flat	Undulate	Milky	Moist	Irregular
4	Flat	Entire	Milky	Moist	Round
5	Flat	Entire	Milky	Moist	Irregular
6	Flat	Entire	Milky	Moist	Round
7	Flat	Entire	Milky	Moist	Round
8	Flat	Entire	Milky	Moist	Round
9	Flat	Curled	Milky	Moist	Irregular
10	Flat	Entire	Milky	Moist	Irregular
11	Flat	Entire	Milky	Moist	Irregular
12	Flat	Entire	Yellow	Moist	Irregular
13	Flat	Entire	Milky	Moist	Irregular
14	Flat	Entire	Milky	Moist	Irregular
15	Flat	Entire	Milky	Moist	Irregular
16	Convex	Entire	Milky	Moist	Irregular
17	Convex	Entire	Milky	Moist	Irregular
18	Convex	Entire	Milky	Moist	Irregular
19	Convex	Entire	Milky	Moist	Irregular
20	Convex	Entire	Milky	Moist	Irregular
21	Convex	Entire	Milky	Moist	Irregular
22	Convex	Entire	Milky	Moist	Irregular
23	Convex	Curled	Milky	Moist	Irregular
24	Convex	Curled	Milky	Moist	Irregular
25	Convex	Entire	Milky	Moist	Round
26	Convex	Entire	Milky	Moist	Round
27	Convex	Entire	Milky	Moist	Round
28	Convex	Entire	Milky	Moist	Round
29	Flat	Entire	Milky	Moist	Round
30	Convex	Entire	Milky	Moist	Round
31	Convex	Entire	Milky	Moist	Round
32	Convex	Curled	Milky	Moist	Irregular
33	Convex	Entire	Milky	Moist	Round

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34	Convex	Entire	Orange	Matte	Punctiform
35	Convex	Entire	Orange	Moist	Irregular
36	Convex	Entire	Milky	Moist	Irregular
37	Convex	Entire	Milky	Moist	Punctiform
38	Convex	Entire	Milky	Moist	Round
39	Convex	Curled	Yellow	Moist	Irregular
40	Flat	Entire	Milky	Moist	Irregular

ND = Non-detect, Elevation = Colony elevation, Margin = Colony margin, Color = Colony color, Texture = Colony texture, Shape = Colony shape

3.2 Phosphate-Solubilizing Efficiency

Screening of phosphate-solubilizing bacteria (PSB) from durian orchards in Chanthaburi and Chumphon Provinces, which differed in soil available phosphorus levels, resulted in the identification of three effective isolates: R24, J33 and J35. These three isolates, selected from a total of 40 isolates, exhibited different phosphate-solubilizing capacities when cultured on Czapek’s medium and evaluated after 3 and 7 days of incubation. Notably, isolate R24 exhibited convex elevation, curled margins, and milky colony coloration and showed a phosphate-solubilization zone diameter of 1.30 ± 0.33 cm on day 7 (Table 2). These findings are consistent with those reported by (12), who isolated 135 phosphate-solubilizing bacterial isolates from longan orchards in Chiang Mai Province and selected five promising isolates (B01, B03, B04, B017, and B024) for further testing. Among the selected isolates, isolate B04 exhibited the highest phosphate-solubilization zone diameter of 4.31 cm on day 7, followed by isolate B01 at 3.94 cm.

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Table 2: Bacteria Cultured on Solid Media and the Phosphate Solubilization Index of Bacteria

Isolate	Czapek's medium (cm)	Pikovskaya medium (cm)	Site
1	0.4	ND	CP
2	0.3	ND	CP
3	0.2	ND	CP
4	0.4	ND	CP
5	0.2	ND	CP
6	0.1	ND	CP
7	0.1	0.4	CP
8	0.5	ND	CP
9	ND	ND	CP
10	0.2	ND	CP
11	0.1	0.1	CP
12	0.2	ND	CP
13	0.6	ND	CP
14	ND	ND	CP
15	ND	ND	CP
16	ND	0.4	R
17	0.2	ND	R
18	ND	ND	R
19	ND	ND	R
20	ND	ND	R
21	ND	ND	R
22	ND	ND	R
23	0.8	0.2	R
24	1.3	ND	R
25	ND	ND	J
26	ND	ND	J
27	ND	0.1	J

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28	0.7	ND	J
29	0.4	ND	J
30	ND	ND	J
31	0.6	ND	J
32	0.5	ND	J
33	1.1	ND	J
34	0.9	0.2	J
35	0.1	0.6	J
36	ND	0.1	J
37	ND	ND	J
38	0.2	ND	J
39	ND	ND	J
40	1.0	ND	J

*ND = Non-detect, J = Durian orchard in Chanthaburi province, CP = Durian orchard in Chumphon province,
R = around plant roots (rhizoplane)



Figure 1: Clear zone of phosphate solubilization of bacterial isolate R24 on Czapek's medium after 7 days of incubation.



Figure 2: Clear zone of phosphate solubilization of bacterial isolate J35 on Pikovskaya medium after 7 days of incubation.

3.3 Identification of Microorganisms Using DNA sequencing

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Isolate code	Species	Identity (%)	Gram
R24	<i>Pseudomonas orientalis</i>	99.79%	Negative
J33	<i>Massilia norwichensis</i>	99.00%	Negative
J35	<i>Pseudomonas orientalis</i>	99.86%	Negative

The three most efficient isolates (R24, J33 and J35) were selected for molecular identification based on their phosphate-solubilizing performance. Sequence analysis of the 16S rRNA gene revealed that isolates R24 and J35 showed high similarity to *Pseudomonas orientalis* (99.79% and 99.00% identity) respectively, while isolate J33 was identified as *Massilia norwichensis* (99.00% identity) based on comparison with sequences in the GenBank database using the BLASTn program. These findings are consistent with previous studies reporting that species within the genera *Pseudomonas* and *Massilia* play important roles in nutrient solubilization and plant growth promotion, particularly in enhancing phosphorus availability in soil (7).

Table 3: Selection of the Top 3 Most Efficient Microorganisms for DNA Analysis

4. CONCLUSIONS

A total of 40 phosphate-solubilizing bacterial isolates were successfully isolated from soil and rhizoplane collected from durian plantations in Chanthaburi and Chumphon provinces and subsequently screened for phosphate-solubilizing ability. Specifically, 16 isolates were obtained from soil in Chanthaburi, 15 isolates from soils in Chumphon, and 9 isolates from rhizoplane collected in Chumphon. Among these, three isolates exhibiting the highest phosphate-solubilizing capacities, designated as R24, J33 and J35, were selected for further evaluation. Notably, isolate R24 (*Pseudomonas orientalis*) demonstrated the greatest phosphate-

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solubilization zone, with a diameter of 1.30 ± 0.33 cm. Further field evaluation of these isolates under durian cultivation conditions will be conducted in Chanthaburi and Chumphon provinces.

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