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Exploration of Phosphate Soluting Bacteries Located Near Rubber Plant (*Hevea brasiliensis*) Field on Different Topography

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ABSTRACT

The exploration of phosphate-solubilizing bacteria in the root-soil of rubber plants was conducted to determine their beneficial effects. This research aimed to identify the sampling locations of rubber plantations where phosphate solubilizing bacteria are suspected to be present, isolate and identify colonies of these bacteria, assess their ability to break down P elements from Pikovskaya media and determine their genus. The research took place at the INSTIPER Yogyakarta Central Laboratory, Kec. Maguwoharjo, Kab. Sleman from February to April 2022. A descriptive method was employed, involving surveying rubber plants in different topographies, sterilizing tools and materials, collecting soil samples from rubber plants, and isolating and identifying bacteria. The research analysis included macroscopic and microscopic observations and measuring the diameter of the transparent zone. Phosphate solubilizing bacteria were found in the sampling locations of Karanggondang Village and Popongan Village. Macroscopic observations revealed 17 isolates with bacterial color morphology ranging from milky white, transparent white, to yellow. The average diameter of the transparent zone, from highest to lowest, was as follows: B5(4)2A (18.3 mm), A1(4)1B (14.3 mm), A1(4)2A (14 mm), and C1(3)1 (14 mm). The phosphate solubilizing bacteria isolate A1(4)2A was identified as the Escherichia Genus while isolating C1(3)1 was identified as the Genus Acetobacter.

Keywords: *exploration, rubber plant, topography, identification, phosphate solubilizing bacteria*

1. INTRODUCTION

Rubber, scientifically known as Hevea brasiliensis, plays a significant role in Indonesia's economic activities as one of the country's key plantation commodities. Indonesia holds the title of being the world's largest producer and exporter of rubber, according to the BPS (2023). The root soil of rubber plants serves as a habitat for various microorganisms, including bacteria. Among these bacteria, phosphate-solubilizing bacteria are particularly beneficial for the growth of rubber plants. Despite high phosphate concentrations in the soil, only a small fraction, approximately 0.1% of total P, is available to the plants due to its poor solubility and fixation ability. This is attributed to the interaction of phosphate with other elements in the soil, such as calcium (Ca), aluminium (Al), and iron (Fe), resulting in the formation of calcium phosphate, aluminium phosphate, and iron phosphate.

Consequently, the phosphate becomes unavailable for plant uptake. However, phosphate-solubilizing bacteria have the ability to enhance the availability of phosphorus for plants by converting inorganic phosphate into forms that can be readily absorbed (Asril et al., 2023). Previous exploratory research has focused on studying phosphate-solubilizing bacteria in the rhizosphere of various plants, including banana nipah (Marista et al., 2013), oil palm (Nugraha et al., 2019), corn (Panjaitan et al., 2020), and sugar palm (Syarwani et al., 2022). However, there has been relatively limited exploration of phosphate-solubilizing bacteria in the root-soil of rubber plants. Therefore, this research aims to identify potential sampling locations within rubber plantations where phosphate-solubilizing bacteria are likely to be present. Additionally, this study aims to assess the abundance of suspected phosphate-solubilizing bacteria on the roots of rubber plants across different topographies. Furthermore, the research seeks to evaluate the bacteria's capability to break down phosphorus elements in Pikovskaya media and determine the genus of the phosphate-solubilizing bacteria.

2. MATERIAL AND METHODS

Research Time and Place

The study was conducted at the Central Laboratory of the Instiper campus, located in Maguwoharjo, Sleman, Yogyakarta. Various topographies in the Semarang Regency area were selected as the sampling sites for rubber plant soil. The research took place between February and April 2022.

Research Tool and Material

The plastic clips, markers, knives, notes, electric stove, aluminium foil, ruler, thermohygrometer, pH meter, pH stick, Lux meter, camera (HP), autoclave, analytical balance, measuring cup, Laminar Air Flow (LAF) Cabinet, bunsen, tweezers, pipette, beaker glass, erlenmeyer, petri dish, stirrer, microscope, tube needle, cover glass, slide, pipette, and refrigerator are the tools utilized. The material employed consists of soil from the roots of rubber plants acquired from rubber plantations in various topographies within the Semarang Regency area. Nutrient Agar and Pikovskaya media, distilled water, 70% alcohol, label paper, tissue paper, and Gram staining solution are utilized.

Research Design

The research uses descriptive methods. The illustrative method is carried out through surveys in the field and the identification of bacteria in the laboratory.

Research Implementation

The implementation of this research was carried out in several stages:

1. Sterilizating Tools and Materials

Tools and materials are sterilized by wrapping them in aluminium foil and brown umbrella paper, then putting them in an autoclave at a temperature of 121˚C with a pressure of 15 psi (per square inch)

for 60 minutes. 2. Taking soil samples at the roots of rubber plants

Soil samples at the roots of rubber plants were taken at different topography. At each location, five different plants were selected from different points. The soil from each plant was wrapped in aluminium foil and put into a chiller box, then taken to the laboratory.

3. Isolating and Identifying Bacteria

Isolation is carried out from the ground part of the rubber plant. Isolation of bacteria was carried out by dilution: weighing 5 g of rubber plant soil, putting the soil in a beaker, mixing it with 1 liter of distilled water, and then stirring it until homogeneous. The soil diluted to 10-5 is then poured into a cup containing Nutrient Agar media. After that, it was incubated at 28°C for five days. Bacteria that grew after inoculation were observed macroscopically. Then, the bacteria were inoculated again on Pikovskaya media to observe the transparent zone. After observing the transparent zone, microscopic observation was carried out. The research flow diagram can be seen below.

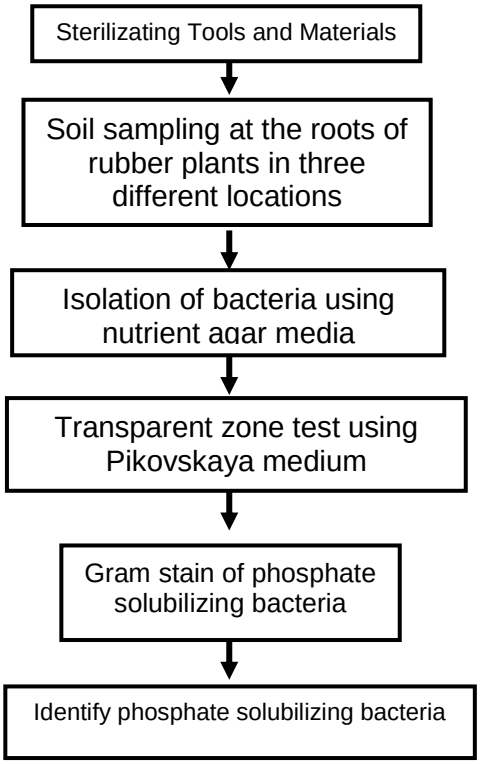

Figure 1. Research flow diagram

Data Analysis

Data were analyzed descriptively. Macroscopic observations include colony color, colony edge shape, colony surface type, colony elevation, colony size and transparent zone diameter. Microscopic observations include the gram staining results and the bacteria's shape.

3. RESULT AND DISCUSSION

3.1 Sampling locations for rubber

plantations where phosphate solubilizing bacteria are suspected.

Rubber plantation sampling locations were carried out in three different locations. The first location is in Karanggondang Village, Kec. Pabelan, Kab. Semarang. The second location is at SEAT (STIPER Edu Agro Tourism) in Lemahireng Village, Kec. Bergas, Kab.

Semarang. The third location is in Popongan Village, District. Bringin, Kab. Semarang. Observation data on the environmental conditions of rubber plantations at three different locations can be presented in Table 1.

Table 1. Observation data on environmental conditions of rubber plantations at three different locations

Location	Height of Site (m dpl)	Temp. (^{0} C)	Moisture (%)	Lighting Intensity (Lux)	Soil pH
Karanggondang Village	508	27,8	79,4	256	5,6
SEAT	507	28	76,8	649	5,3
Popongan Village	582	28,6	77,2	1.316	5,5

Note: the weather at the time of data collection was cloudy

3.2. Determination of Bacterial Colonies Suspected of Phosphate Solubilizing Bacteria

The results of sampling taken from Karanggondang Village amounted to 6 colonies. Macroscopic observations from sampling Karanggondang Village can be seen in Figures 1 – 6.

Figure 2. Isolate from Karanggondang Village, 2 samples, 1 replication, 1 colony purification.

Figure 3. Isolate from Karanggondang Village, 2 samples, 2 replicates, 2 colony purification.

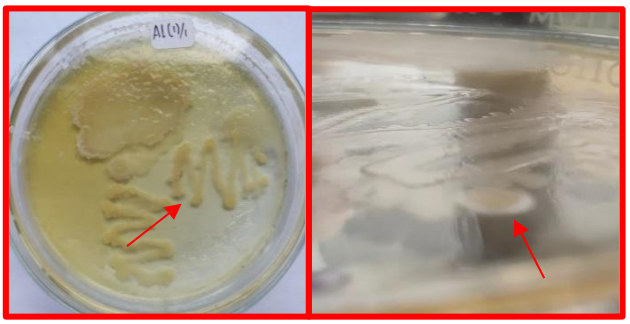

Figure 4. Isolate from Karanggondang Village, sample 1, replication 1, purification of colony 1.

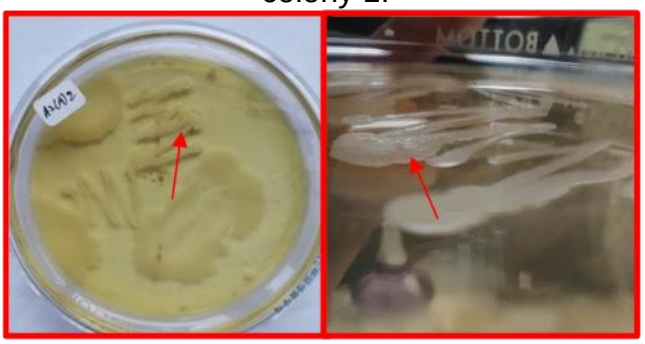

Figure 5. Isolate from Karanggondang Village, 2 samples, 4 replicates, and 2 colony purifiers.

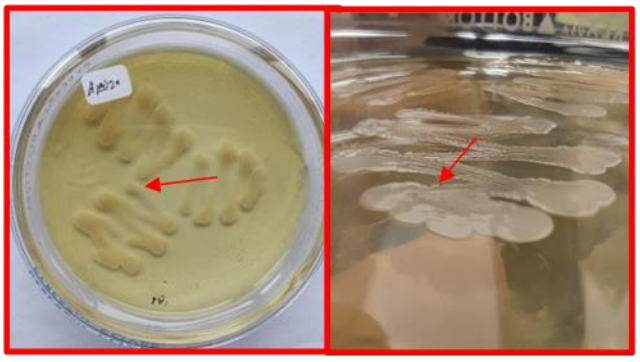

Figure 6. Isolate from Karanggondang Village, sample 1, replication 4, purification of colony 1.

Macroscopic results from a sampling taken at SEAT of 8 colonies. Macroscopic observations from sampling at SEAT can be seen in Figures 7 – 14.

Figure 7. Isolate from SEAT, sample 4, replication 4, purification of colony 1.

Figure 8. Isolate from SEAT, sample 4, replication 1, purification of colony 2.

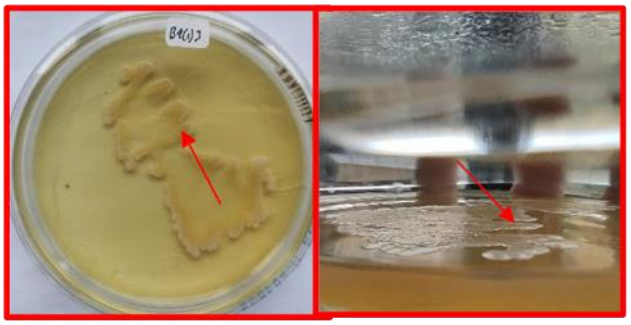

Figure 9. Isolate from SEAT, sample 4, replication 1, purification of colony 3.

Figure 10. Isolate from SEAT, sample 5, replication 2, purification of colony 1

Figure 11. Isolate from SEAT, sample 5, replication 2, purification of colony 1

Figure 12. Isolate from SEAT, sample 5, replication 4, purification of colony 1

Figure 13. Isolate from SEAT, sample 5, replication 2, purification of colony 1

Figure 14. Isolate from SEAT, sample 5, replication 4, purification of colony 2

The sampling results from Popongan Village amounted to 3 colonies. Macroscopic observations from Popongan Village can be seen in Figures 15 – 17.

Figure 15. Isolate from Popongan Village, sample 1, replication 3, purification of colony 1

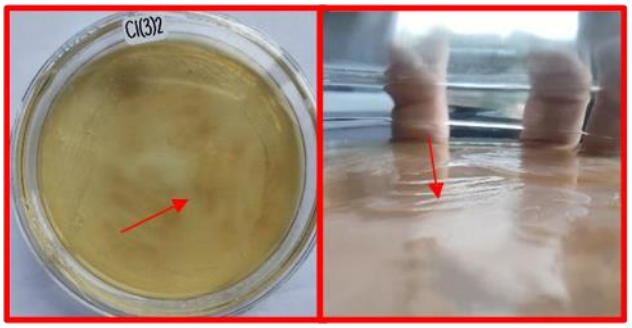

Figure 16. Isolate from Popongan Village, sample 1, replication 3, purification of colony 2

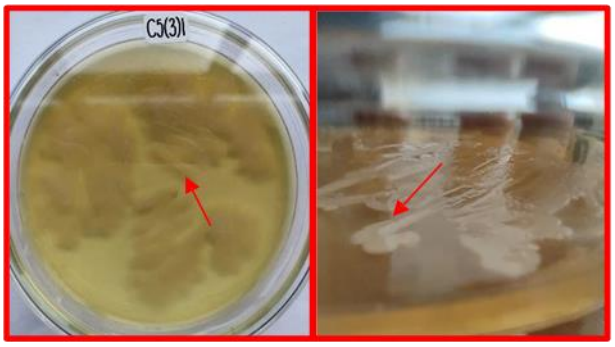

Figure 17. Isolate from Popongan Village, sample 5, replication 3, purification of colony 1

Based on the appearance of the images above, a summary can be made, which is presented in Table 2.

Table 2. Macroscopic morphology of bacteria suspected to be phosphate-solubilizing bacteria

No.	Location	Isolate	Colony Color
1	Karanggondang Village	A2(1)A	White Milk
2		A2(2)2	Yellow
3		A1(1)1	White Milk
4		A2(4)2	Yellow
5		A1(4)2A	White Milk
6		A1(4)1B	Yellow
7	SEAT	B4(4)1	White Milk
8		B4(1)2	Transparent White
9		B4(1)3	Transparent White
10		B5(2)1A	Yellow
11		B5(2)1B	Yellow
12		B5(4)1A	White Milk
13		B5(2)1B	Yellow
14		B5(4)2A	Yellow
15	Popongan Village	C1(3)1	Transparent White
16		C1(3)2	Transparent White
17		C5(3)1	White Milk

Table 2 shows that 17 isolates from the three locations had different morphologies of bacteria thought to be phosphate-solubilizing bacteria; this can be seen from the different colors of the bacterial colonies.

3. Determination of bacterial colonies capable of dissolving phosphate

After macroscopic observations of colonies suspected of being phosphate-solubilizing bacteria were carried out, the transparent zone was continued to be observed. After testing the transparent zone, 4 colonies of bacteria were suspected to be phosphate-solubilizing bacteria. Observations of the transparent zone can be seen in Figures 18 – 21.

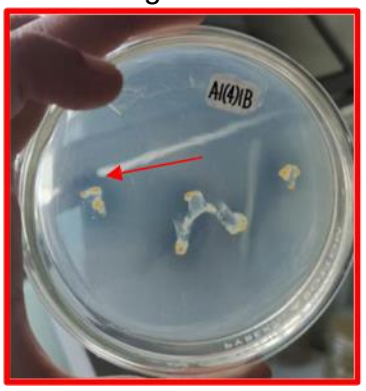

Figure 18. Transparent zone of isolate A1(4)1B

Figure 19. Transparent zone of isolate A1(4)2A

Figure 20. Transparent zone of isolate B5(4)2A

Figure 21. Transparent zone of isolate C1(3)1

Bacteria will release extracellular enzymes called phosphatase enzymes. This enzyme will dissolve the insoluble phosphate into soluble, thus forming a transparent zone around the colony. The diameter of the transparent zone is measured using a ruler.

Observe the transparent zone of bacterial colonies in the petri dish at three different points to find the average area of the transparent zone in each petri dish. The average measurement of the transparent zone area can be seen in Table 3.

Table 3. Average transparent zone diameter

No.	Isolate	Transparent zone diameter (mm)			Average
		Day-3	Day-5	Day-7	
1	A1(4)1B	13	14	14,3	13,7
2	A1(4)2A	13,3	13,3	14	13,5
3	B5(4)2A	12	17	18,3	15,7
4	C1(3)1	12,3	12,6	14	12,9

Observations of the transparent zone were carried out until the 7th day because there were no significant changes in the diameter of the transparent zone on the following day. The isolate with the widest transparent zone was isolate B5(4)2A, with an average diameter on day 7 of around 15.7 mm. The isolate with the smallest transparent zone was isolate C1(3)1 with an average diameter on day 7th, 12.9 mm. The wider the transparent zone, the stronger the bacteria will dissolve phosphate.

3.3 Determination of the Genus of Phosphate Solubilizing Bacteria

After observing the transparent zone, microscopic observations of the shape of the bacteria were carried out. Gram staining is carried out so that the shape of the bacteria can be seen. Microscopic observations can be seen in Figures 22 – 25.

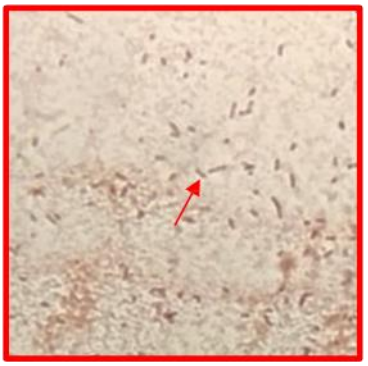

Figure 22. Isolate A1(4)1B.
Remark: Bacterial cell shape (Stick), Gram (-).

Figure 23. Isolate A1(4)2A. Description: Bacterial cell shape (Stick), Gram (-).

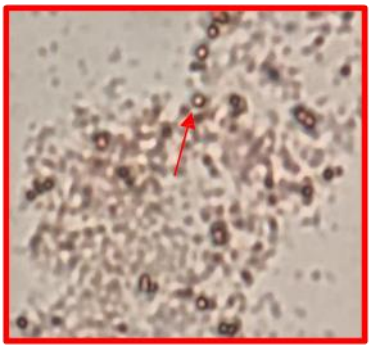

Figure 24. Isolate B5(4)2A. Description: Bacterial cell shape (Round), Gram (-)

Figure 25. Isolate C1(3)1. Description: Bacterial cell shape (Stick), Gram (-).

Based on microscopic observations, the bacteria found had a rod and round shape. Isolate code A1(4)1B is rod shaped with gram (-), isolate code A1(4)2A is rod shaped with gram (-), isolate code B5(4)2A is round with gram (-), isolate code C1(3)1 is a rod with grams (-).

From observations ranging from macroscopic observations, transparent zone observations, and microscopic observations, the characteristics of the phosphate solubilizing bacteria obtained can be seen. The characteristics of the phosphate-solubilizing bacteria obtained can be seen in Table 4.

Table 4. Characteristics of phosphate solubilizing bacteria

No.	Isolate Code	Colony Color	Edging Shape	Elevation	Colony Size	Gram Coloring	Bacteria Shape	Bacteria Name
1	A1(4)1B	Yellow	Hilly	Bumpy	Medium	negative	Stem	
2	A1(4)2A	While	Wavy	Bumpy	Medium	negative	Stem	Escherichia
3	B5(4)2A	Yellow	Hilly	Bumpy	Medium	negative	Round	
4	C1(3)1	Yellow	Flat	Flat	Medium	negative	Stem	Acetobacter

Description: No. 1 and 3 cannot yet determine the name of the bacteria because the characteristics of the bacteria found are still lacking

Table 4 shows the characteristics of phosphate-solubilizing bacteria obtained from macroscopic observations, transparent zone tests, and microscopic observations. The name of the phosphate-solubilizing bacteria can be identified. Based on Table 4, the names of bacteria that can be determined are isolated A1(4)2A and C1(3)1, namely Escherichia and Acetobacter, respectively.

After carrying out all observations to identify bacteria from each different sampling location. Macroscopic and microscopic characteristics of phosphate-solubilizing bacteria were obtained from the four isolates. Isolate A1(4)2A has similarities to the Escherichia genus. This genus is gram negative and rod-shaped (Marista et al. 2013).

Based on the results of macroscopic observations, transparent zone tests, and microscopic observations of the C1(3)1 isolate, this isolate has similarities to the Acetobacter genus. This bacteria is rod-shaped, gram-negative and non-motile and has a slimy wall surface (Marista et al., 2013).

There are 2 unknown isolate codes for the genus of phosphate solubilizing bacteria: isolates A1(4)1B and B5(4)2A. From the characteristics of macroscopic and microscopic observations of the isolate, no genus matches the characteristics of the isolate, which is in accordance with the literature. Therefore, it is best to continue with molecular identification.

4. CONCLUSION

There were phosphate-solubilizing bacteria from the sampling locations, namely Karanggondang Village and Popongan Village. The results of macroscopic observations from the three sampling locations obtained 17 isolates with bacterial color morphology of milky white, transparent white and yellow. The average diameter of the transparent zone from highest to lowest respectively, namely isolates B5(4)2A was 15.7 mm, A1(4)1B was 13.7 mm, A1(4)2A was 13.5 mm, C1 (3)1 is 12.9 mm. The phosphate solubilizing bacteria that can be identified are the Genus Escherichia (isolate A1(4)2A) and Acetobacter (isolate C1(3)1). .

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