

Microbia in Plant Growth Promoting Rhizobacteria Bamboo, Reed Grass and Banana

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Abstract—Plant Growth Promoting Rhizobacteria (PGPR) is a type of bacteria that lives around plant roots. These bacteria live in colonies covering the roots of plants so as to provide benefits for plants. The purpose of this study was to determine the types of bacteria found in PGPR bamboo roots, reed roots and banana roots. The research was conducted in the Plant Diseases Pests laboratory, Faculty of Agriculture, Mulawarman University. Isolation of PGPR bacteria was carried out by taking samples from the three PGPR solution materials. Then 2 ml of each PGPR sample was taken and grown on Nutrient Agar (NA) media by the scatter method. From each PGPR made in 4 (four) petri dishes, in order to obtain as many as 12 isolates of PGPR bacteria capable of growing on the media. Some of the genera included in the PGPR are *Pseudomonas*, *Serratia*, *Azotobacter*, *Azospirillum*, *Acetobacter*, *Burkholderia*, *Enterobacter*, *Rhizobium*, *Erwinia*, *Flavobacterium* and *Bacillus*. Each rhizobacteria isolate has an important role in controlling pathogen attack and triggering growth. Bacterial analysis is used as a parameter to determine the effectiveness and potential contained in these bacteria.

Keywords—PGPR, bamboo roots, reed roots, banana roots, types of bacteria.

I. INTRODUCTION

Plant Growth Promoting Rhizobacteria (PGPR) is a type of bacteria that lives around plant roots. These bacteria live in colonies covering the roots of plants so as to provide benefits for plants. Some of the genera included in the PGPR are: *Pseudomonas*, *Serratia*, *Azotobacter*, *Azospirillum*, *Acetobacter*, *Burkholderia*, *Enterobacter*, *Rhizobium*, *Erwinia*, *Flavobacterium* and *Bacillus* [1]. Each rhizobacteria isolate has an important role in controlling pathogen attack and triggering growth. PGPR bacteria can be inoculated from various plant roots such as bamboo roots, reeds and banana roots. It is necessary to analyze the type and number of bacteria contained in the PGPR isolate used before being applied to cultivated plants.

Based on the results of research that has been carried out, the application of PGPR biological agents that have an effect in delaying the incubation period, it is necessary to analyze the type and number of bacteria contained in the PGPR isolate used before being applied to cultivated plants and suppress the intensity of the attack so that the severity of the disease is not too high. Bacterial analysis is used as a parameter to determine the effectiveness and potential contained in these bacteria. PGPR has properties as a bioprotectant that can protect plants from pathogen attacks [2].

II. RESEARCH METHOD

2.1 Place and time

This research was conducted at the Laboratory of Pests and Plant Diseases, Faculty of Agriculture, University of Mulawarman Samarinda from February 2022 to May 2022.

2.2 Materials and tools

The materials used are PGPR bamboo roots, reed roots and banana roots that have been fermented in liquid form. Aquades, alcohol, spirit, and Nutrient Agar (NA) media. The tools used for research in the laboratory are petri dishes, test tubes, ultra violet lamps, pipettes, measuring cups, autoclaves, laminar air flow cabinets (LAFB), microwaves, 250 ml media bottles,

Bunsen lamps, ose needles, lighters, plastic, plastic wrapping, sprayer, scissors, aluminum foil, cotton, sterile tissue, measuring cup (vol. 100 ml), and label.

2.3 Research Activities

The research activities carried out were: preparation, observation, taking PGRP on (bamboo roots, reed roots and banana weevil roots), fermentation, isolation on nutrient agar media, and analysis in the laboratory.

2.4 Data collection

The data collected were: the shape of the colony, the shape of the edge of the colony, the size of the colony, and the color of the colony, gram-positive and negative observations and the microscopic shape of the PGRP bacteria.

III. RESULTS AND DISCUSSION

Plant Growth Promoting Rhizobacteria(PGPR) obtained from several types of plant roots such as bamboo roots, reed roots and banana hump roots. The three ingredients are fermented for 8 days with a mixture of several ingredients such as brown sugar, shrimp paste, bran, and coconut water. Isolation of PGPR bacteria was carried out by taking samples from the three PGPR solution materials. Then 2 ml of each PGPR sample was taken and grown on Nutrient Agar (NA) media by the scatter method. From each PGPR made in 4 (four) petri dishes, in order to obtain as many as 12 isolates of PGPR bacteria capable of growing on the media. According to [3] that bacterial isolation is taking or removing microbes from their natural environment and growing them as pure cultures in artificial media.

3.1 PGPR bacterial colony morphology on bamboo roots

Morphological observations made on PGPR bacteria on bamboo roots are presented in Figure 1.

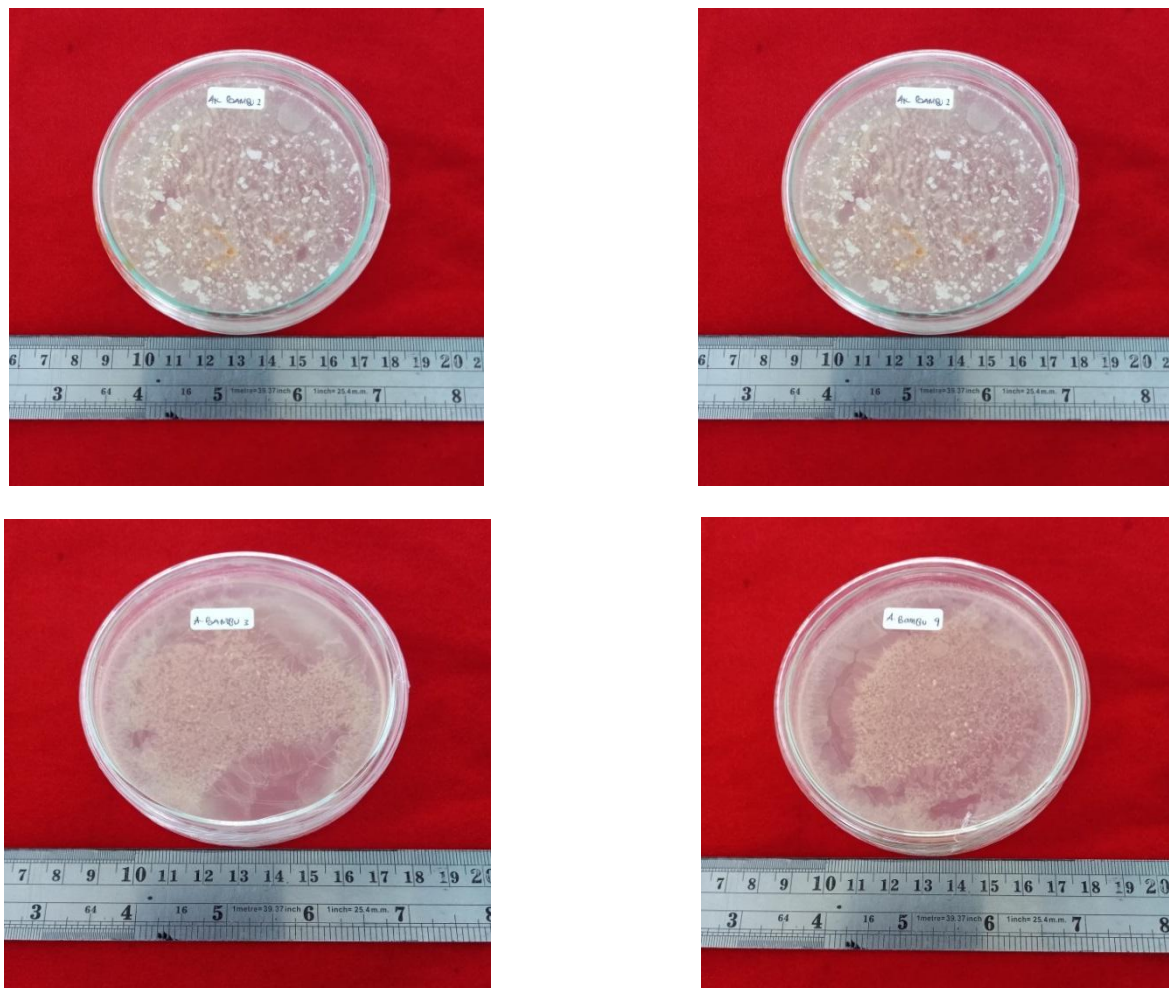


FIGURE 1. PGPR Isolation Results on Bamboo Roots

Figure 1a shows that bacteria originating from bamboo roots have irregular rounded colonies with jagged edges. The surface of the colony is wavy. Colony size was obtained from the smallest to the largest size, which ranged from 1.0 mm to 3.0 mm, the color of the isolates was mostly yellowish white. Figure 1b has a jagged colony edge that is thick and thin. Colony size was obtained from sizes ranging from 1.0 mm to 3.0 mm. Some isolates were white and most of the isolates were yellowish white. Then in Figure 1c shows the shape of the colony is wavy and thick, the edges of the colony are jagged and milky white and measuring 1 mm to 4 mm. Figure 1d shows the shape of the colonies that are wavy and thick, the edges of the colonies are jagged and yellowish white and measuring 1.0 mm to 3.0 mm. In detail the results of observations can be seen in Table 1.

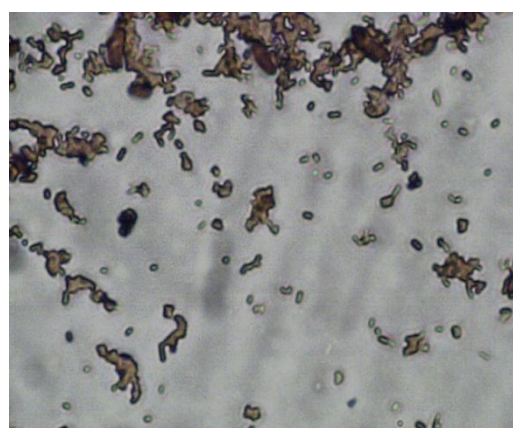
TABLE 1
OBSERVATIONS ON THE MORPHOLOGY OF BAMBOO ROOT PGPR BACTERIA

No	Isolat	Colony Form	Colony edge shape	Colony size	Colony color	Gram	Cell Shape	Genus
1	AB 1	Irregular round	jagged	2,0 mm	Yellowish white	+	Basil, cocci	<i>Sporoformes, sp</i>
2	AB 2	Irregular round	jagged	1,0 mm	White, white yellowish	+	Basil, cocci	<i>Basillus. sp</i>
3	AB 3	Wavy, thick	jagged	3,0 mm	milky white	+	Basil, cocci	<i>Streptococcus Faecalis</i>
4	AB 4	Wavy, thin	jagged	3,0 mm	Yellowish white	-	Basil, cocci	<i>Pseudomonas sp.</i>

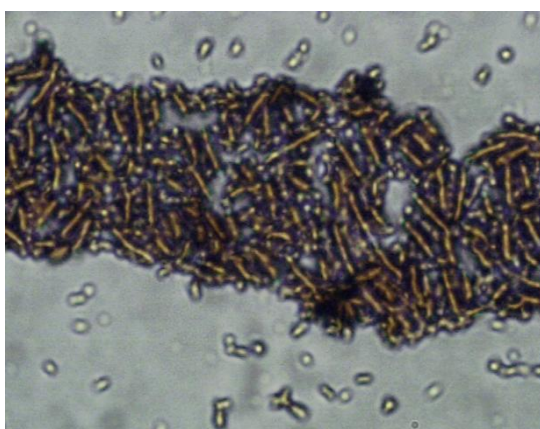
Microscopically with 1000 times magnification using an Olympus CX23 microscope, gram staining results were obtained from several different colonies showing the type of PGPR bacteria in bamboo roots presented in Figure 2.



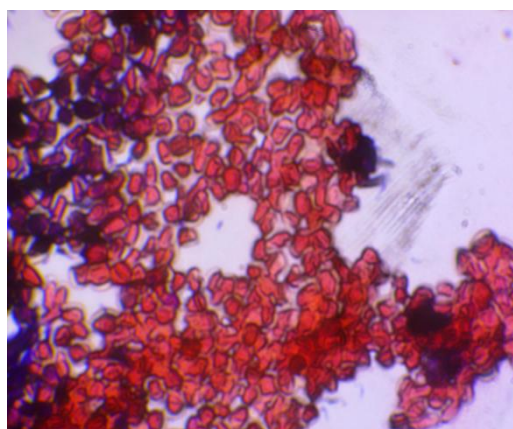
(a)



(b)



(c)



(d)

FIGURE 2: Gram Staining of Bamboo Root PGPR Bacteria Using a Microscope

On microscopic observation in Figure 2a. showed that the PGPR bacteria of bamboo roots showed a purple color so that it could be said that the bacteria were gram-positive, with the shape of a bacillus. In Figure 2b. shows that the bacteria is purple, which means the bacteria are gram positive with a cocci shape. Next in Figure 2c. shows the purple colored bacteria means Gram Positive, and the shape of a bacillus, while in Figure 2d. it is seen that the bacteria is red which means that the bacteria is Gram Negative while the shape of the bacteria looks like a bacillus.

Based on the above observations that PGPR found on bamboo roots, it is in accordance with the identification [4] that the bacteria are Bacillaceae bacteria. The results of observations of the shape of the colony where the growth of bacteria on the Nutrient Agar media was yellowish white in color. according to research [5] that in bamboo roots found bacteria Enterobacteriaceae (*Escherichia coli*, *Salmonella*, *Shigella*), *Pseudomonas*, and There are also bamboo rhizosphere PGPR bacteria that do not have mucus/gram positive, such as *Bacillus*, *Enterococcus*. This is also corroborated by the statement [6] that several rhizobacteria genera that act as PGPR are: *Pseudomonas*, *Enterobacter*, *Bacillus*, *Azospirillum*, *Azotobacter*, *Burkholderia* and *Serratia* and it is also stated that PGPR plays a role in the process of plant growth.

3.2 PGPR bacterial colony morphology on reed root

Morphological observations made on PGPR bacteria on the roots of reed are presented in Figure 3.

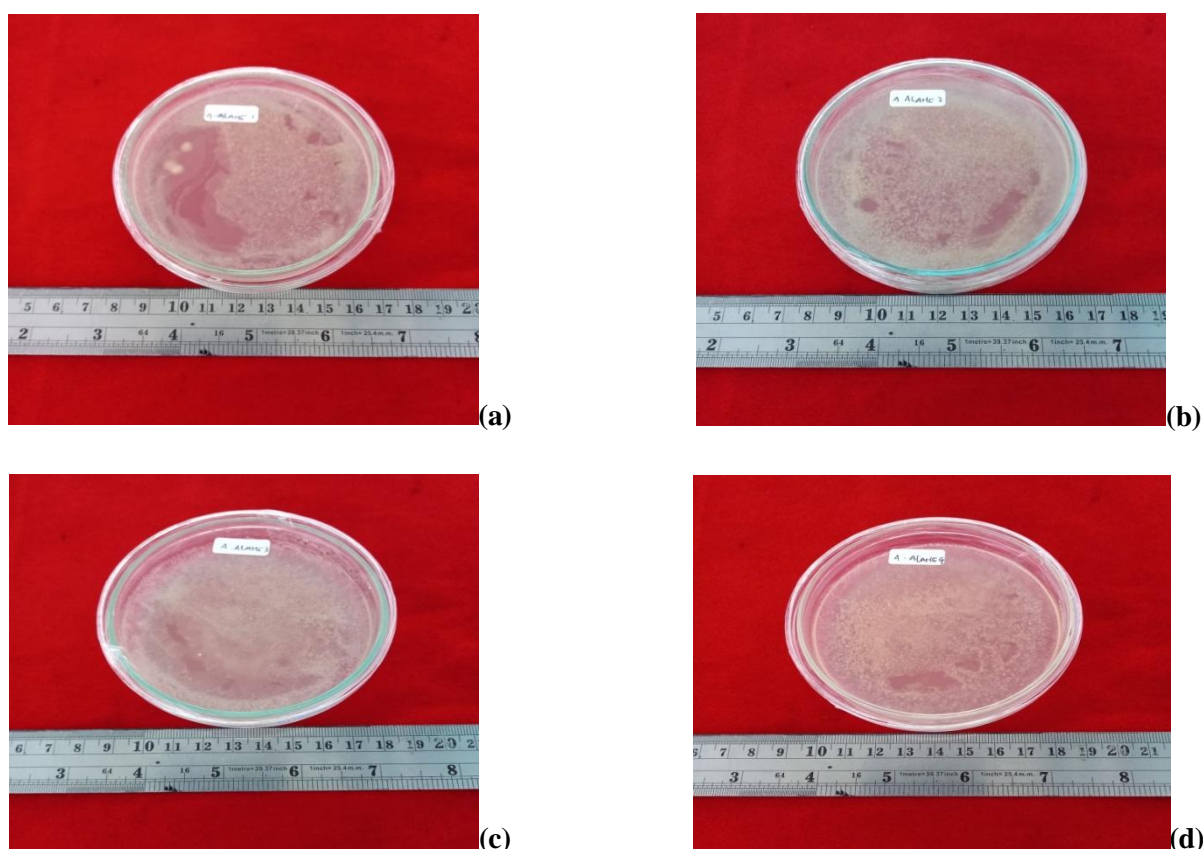


FIGURE 3. PGPR Isolation Results on reed Roots

The results showed that the isolates in Figure 3a were wavy, thick and thin, round and some were irregularly rounded, and the edges of the colonies were flat and jagged. The color of the isolate is milky white and yellowish white. Figure 3b Colony shape irregular round, thick, jagged colony edges. The color of the isolate was yellowish white and the average colony size was 1.0 mm. Figure 3c shows the shape of a round, flat colony. Colony size obtained ranged from 1.0 mm to 3.0 mm. The isolates were wavy and thin, the edges of the colonies were jagged. The color of the isolate was yellowish white and the average colony size was 1.0 mm. then in the 3d image shows the shape of the colony is round and thin, the edges of the colony are jagged. The color of the isolate was yellowish white and the average colony size was 1.0 mm. All isolates were in the form of jagged colony edges and some were yellowish white and almost all isolates were gram negative with bacilli and cocci cell shapes. In detail the results of observations can be seen in Table 2.

TABLE 2
PGPR BACTERIAL MORPHOLOGICAL OBSERVATION RESULTS ON REED ROOTS

No	Isolat	Colony Form	Colony edge shape	Colony size	Colony color	Gram	Cell Shape	Genus
1	AA 1	Irregular round, Thin	Flat, jagged	3,0 mm	Milk white, yellowish white	+	Basil, Cocci	<i>Sporoformessp.</i>
2	AA 2	Irregular round, thick	jagged	1,0 mm	Yellowish white	-	Basil, Cocci	<i>Pseudomonassp.</i>
3	AA 3	Round, Wavy, Thin	jagged	1,0 mm	Yellowish white	-	Basil, Cocci	<i>Klebsiella. Sp</i>
4	AA 4	Round, Thin	jagged	1,0 mm	Yellowish white	-	Basil, Cocci	<i>Klebsiella. sp</i>

Microscopically with 1000 times magnification using an Olympus CX23 microscope, gram staining results were obtained from several different colonies by showing the type of PGPR bacteria on the roots of the Imperata, which is presented in Figure. 4.

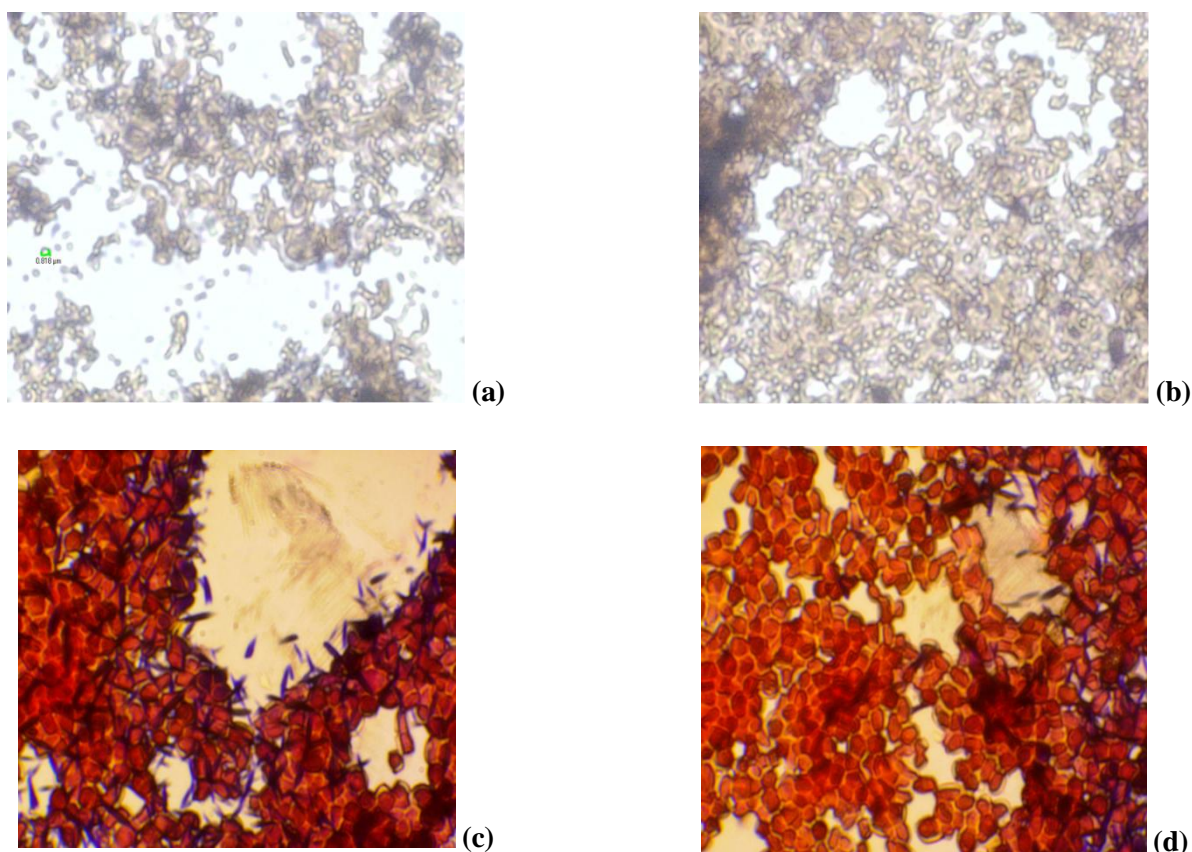


FIGURE 4: Gram staining of PGPR bacteria on the roots of reed, using a microscope (magnification 1000 X)

On microscopic observation in Figure 4a. showed that the PGPR of reed roots showed a red color so that it could be said that the bacteria were gram negative, with bacilli and cocci shape. In Figure 4b. shows that the bacteria is red, which means the bacteria are gram negative with the shape of bacilli and cocci. Next in Figure 4c. shows the bacteria in bright red color means Gram negative, and the shape is bacillus and cocci, while in Figure 4d. it is seen that the bacteria is red which means that the bacteria is Gram Negative while the shape of the bacteria looks like a cocci.

Based on the above observations, the PGPR found in the roots of the weeds is in accordance with the identification [7] that the bacteria are Bacilliaceae bacteria. The results of observations of the shape of the colony where the growth of bacteria on Nutrient Agar media was yellowish white, the periphery of the colony was irregularly rounded, the surface was round, irregular and wavy, while microscopically it showed that Gram negative bacteria were in the form of bacilli and cocci, it

could be predicted that the bacteria were *Azotobacter*, and *Pseudomonas* sp. This is also in accordance with research [8] which stated that the *Azotobacter*, *Pseudomonas* sp. which plays a role in promoting plant growth.

3.3 PGPR bacterial colony morphology on banana hump roots

Morphological observations made on PGPR bacteria on banana hump roots are presented in Fig 5.

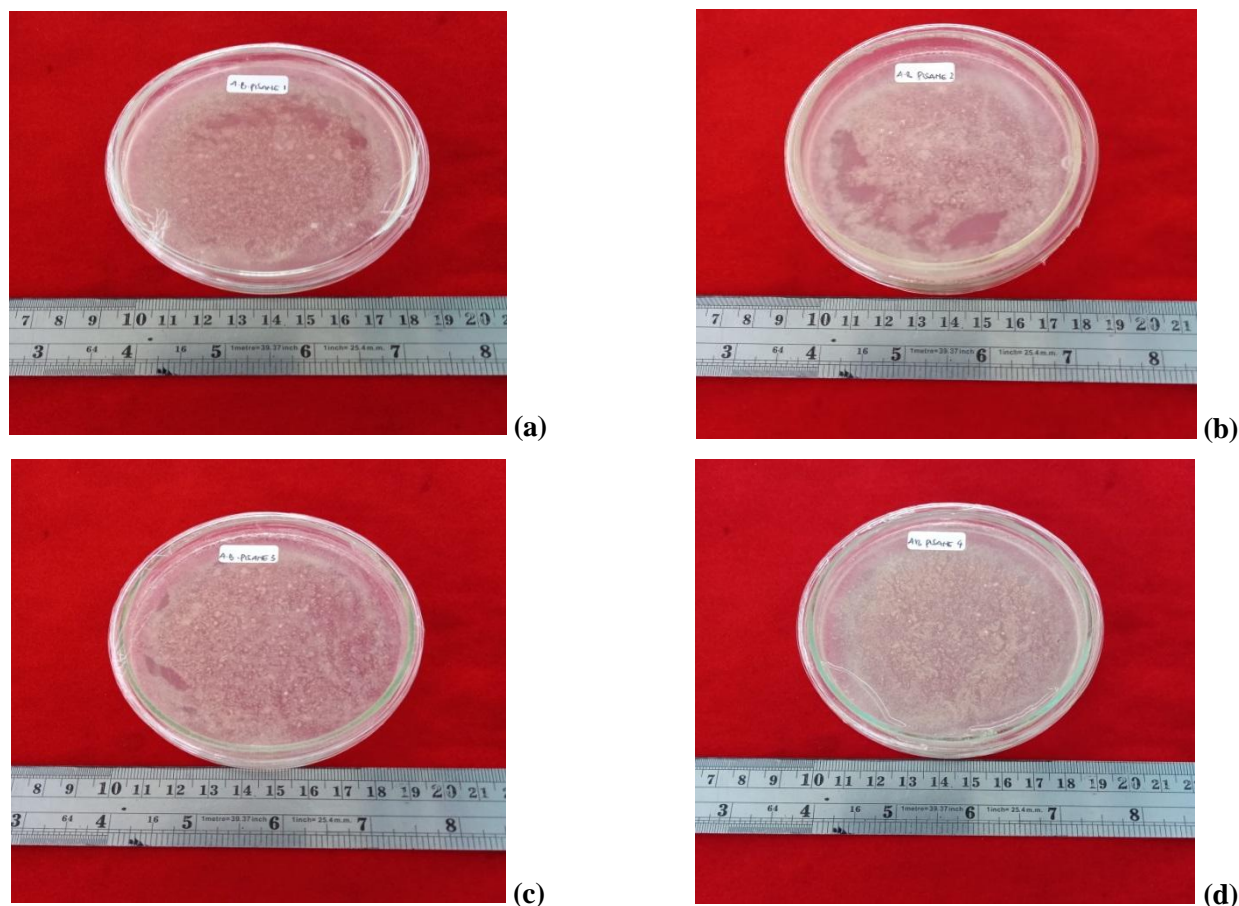


FIGURE 5. PGPR Isolation Results on Banana Hump Roots

From the results of the isolation of banana humo root PGPR, it shows that in Figures 5a, 5c, and 5d all isolates have irregular and thin round colonies, almost all isolates have jagged colony edges, the size of the colonies obtained ranged from 1.0 mm to 3.0 mm. In Figure 5b, the shape of the colonies is wavy and irregularly round, the edges of the colonies are jagged, the isolate is yellowish white and the size ranges from 1.0 mm to 2.0 mm. In detail the results of observations can be seen in Table 3.

**TABLE 3
PGPR BACTERIAL MORPHOLOGICAL OBSERVATIONS ON BANANA HUMP ROOTS**

No	Isolat	Colony Form	Colony edge shape	Colony size	Colony color	Gram	Cell Shape	Genus
1	AP 1	Irregular round, thick, thin	Jagged	2,0 mm	Yellowish white	-	Basil	<i>Klebsiella. sp</i>
2	AP 2	Irregular round, thin	Jagged	3,0 mm	Yellowish white	-	Basil	<i>Pseudomonas sp.</i>
3	AP 3	Irregular round, thin	Jagged	1,0 mm	White, Yellowish white	-	Basil	<i>Pseudomonas sp.</i>
4	AP 4	Irregular round, thin	Jagged	1,0 mm	Yellowish white	-	Basil	<i>Pseudomonas sp.</i>

Microscopically with 1000 times magnification using an Olympus CX23 microscope, gram staining results were obtained from several different colonies by showing the type of PGPR bacteria on the banana hump root which is presented in Figure 6.

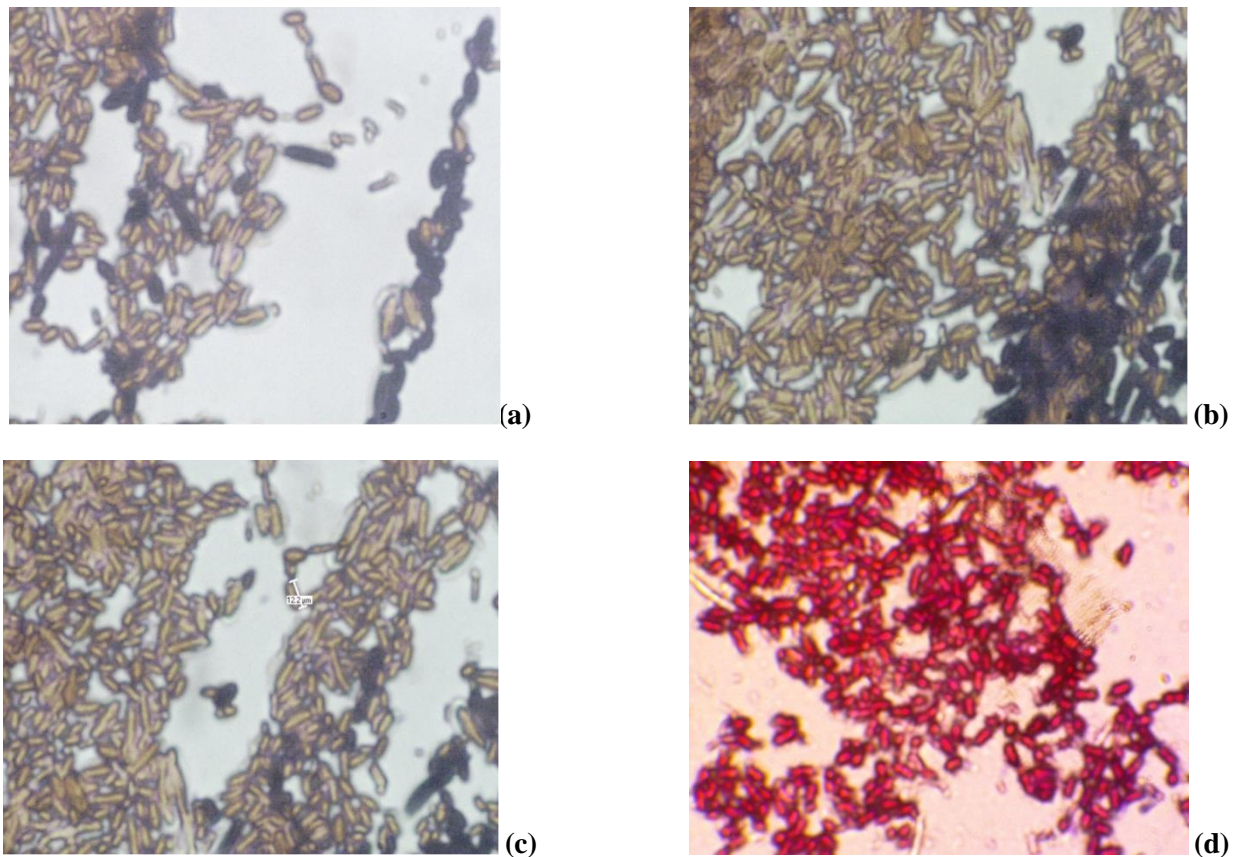


FIGURE 6. PGPR Bacterial Gram Stain Banana Hump Root using a Microscope (Magnification 1000 X)

On microscopic observation in Figure 6a. showed that the banana root PGPR bacteria showed a reddish purple color so that it could be said that the bacteria were gram-positive, with the shape of a bacillus. In Figure 6b. shows that the bacteria is purple, which means the bacteria are gram positive with the shape of bacilli and cocci. Next in Figure 6c. showing the purple colored bacteria means they are Gram Positive, and the shape is bacillus, while in Figure 6d. it is seen that the bacteria is red which means that the bacteria is Gram Negative while the shape of the bacteria looks like a bacillus.

Based on the above observations, the PGPR found in banana roots is in accordance with the identification [9] that the bacteria are bacteria from the Bacillaceae family. The results of observations of the shape of the colony where the growth of bacteria on Nutrient Agar media was yellowish white, the periphery of the colony was round, jagged, the surface was round, irregular and wavy, while microscopically it showed that the bacteria were Gram positive and negative and were in the form of bacilli and cocci. This is also in accordance with research [10] which states that bacillus bacteria are found in banana roots.

Observations on the morphological characteristics of bacterial colonies need to be carried out, in order to facilitate the process of identifying the type of bacteria. This is in accordance with the statement [11] which states that gram-positive bacteria in Gram staining are purple due to the violet-iodine crystal dye complex being maintained even though the acetone-alcohol bleach solution is given, while gram-negative bacteria are red because the complex is soluble when the bleaching solution is given. acetone alcohol so that it takes on a red color of safranin. The difference in color between gram-positive and gram-negative bacteria indicates that there are differences in cell wall structure between the two types of bacteria. Gram-positive bacteria have a cell wall structure with a thick peptidoglycan content, while gram-negative bacteria have a cell wall structure with a high lipid content. According to [11] that based on the morphological characteristics of bacterial colonies and

pure cultures, the identification process of the types of microorganisms can be carried out, but to obtain perfect identification results it must be continued with biochemical tests.

IV. CONCLUSIONS

Based on the results of the study, it can be concluded that the results of the identification of bacteria in PGPR of bamboo roots, alang-alang roots and banana roots microscopically showed that the bacteria were Gram Positive and Negative and in the form of bacilli and cocci. The bacteria found were dominated by basilliae bacteria. To obtain perfect identification results, it is necessary to carry out further research with biochemical tests.

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