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Research Paper

# Characterization Of Plant Growth Promoting Rhizobacteria (PGPR) From Bamboo

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**Abstract**
PGPR (Plant Growth Promoting Rhizobacteria) are root bacteria that trigger plant growth by colonizing a thin soil layer of about 1 mm-2 mm. PGPR can be derived from golden bamboo (*Bambusa vulgaris*) roots, bamboo roots contain hormones such as auxin, cytokinin and gibberillin which can stimulate root growth and plant development. This study aims to identify the morphological characteristics of PGPR bacteria from bamboo rhizosphere. This research was conducted in June-July 2023 in the experimental garden of ATC (Agrotech Training Center) owned by the Faculty of Agriculture, Sriwijaya University. This study used a simple experimental method to identify the morphological characteristics of bacteria in general. This method is carried out by conducting experiments, namely taking bamboo rhizosphere material and identifying the isolates obtained and then concluding from the experiments that have been carried out by proving the results obtained in accordance with existing research. In this study, there were 5 bacterial isolates successfully grown on Nutrient Agar (NA) media with different colony morphologies. Bacterial morphology found in giant colony isolates of PGPR solution from bamboo rhizosphere has a shape (Irregular and Circular); elevation (flat); edge (Lobate and Undulate); Color (Transparent, Milk White to Yellowish; and cell shape (Streptococcus and Palisade). The results of the identification of bacterial species indicated that the PGPR solution from the bamboo rhizosphere belonged to the Pseudomonas fluorescens and *Bacillus polymixa* species

**Keywords**
*Bamboo rhizosphere; Bacterial morphology; PGPR*

## 1. INTRODUCTION

The rhizosphere is a dynamic environment and is rich in energy sources from organic compounds released by plant roots (root exudates). Therefore, the rhizosphere is a habitat for various types of soil microbes. Each plant secretes root exudates with different compositions so that they also act as microbial selectors; increases the development of certain microbes and inhibits the development of other microbes. PGPR (Plant Growth Promoting Rhizobacteria) is a group of root bacteria that can stimulate the growth of plants that colonize a thin soil layer of around 1 mm-2 mm. PGPR has the ability to stimulate growth through various mechanisms, including nitrogen fixation, phosphate solvent, plant hormone production and inhibiting plant pathogens. PGPR is an alternative as a biological fertilizer that can stimulate and help provide the nutrients needed by plants during plant growth (Arsad, 2015).

Isolation and production of PGPR (Plant Growth Promoting Rhizobacteria) can be obtained from several plant roots such as bamboo roots, bean roots and molar roots (Ramadhani, 2020). Bamboo roots can be used as material for making PGPR. The use of PGPR from bamboo is known to increase nutrient availability and plant growth (Fitri et al., 2020). The roots of bamboo plants are thought to contain hormones such as auxin, cytokinin and gibberlin which can stimulate root growth and plant development (Karmila and Mustafa, 2013). Among other things, bamboo roots are known to contain Pseudomonas flourescens bacteria which are able to dissolve P elements in the soil (Isroni et al., 2019; Setyawati and Maulida, 2019). This shows that bamboo has the potential to be a raw material for making PGPR (Plant Growth Promoting Rhizobacteria) solution.

Information regarding the characteristics of bacteria on bamboo roots is very important to understand regarding their role in increasing plant growth. Therefore, this research was carried out with the aim of isolating and identifying the morphological characteristics of bacteria from PGPR (Plant Growth Promoting Rhizobacteria) solutions from the bamboo rhizosphere.

## 2. EXPERIMENTAL SECTION

This research was carried out at the Soil Biology Laboratory, Soil Department, Faculty of Agriculture, Sriwijaya University. This research was carried out in June-July 2023. This

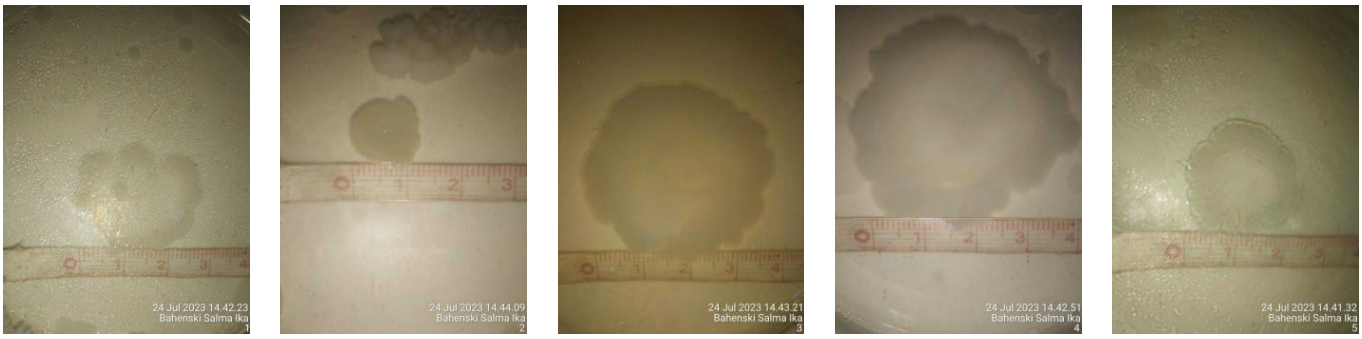


**Figure 1.** Giant bacterial colonies from bamboo roots

research was carried out using simple experimental methods to identify the morphological characteristics of bacteria. This method is carried out by carrying out experiments, namely taking bamboo rhizosphere material and identifying the isolates obtained and then concluding from the experiments that have been carried out by proving that the results obtained are in accordance with existing research.

Media collection by collecting the tools and materials needed both in the field and in the laboratory. Media collection in the field took the form of aseptic harvesting of golden bamboo (Bambusa vulgaris) roots obtained on the ATC (Agrotech Training Center) land of the Faculty of Agriculture, Sriwijaya University, Indralaya, at a depth of 0-30 cm as much as 10 grams aseptically.

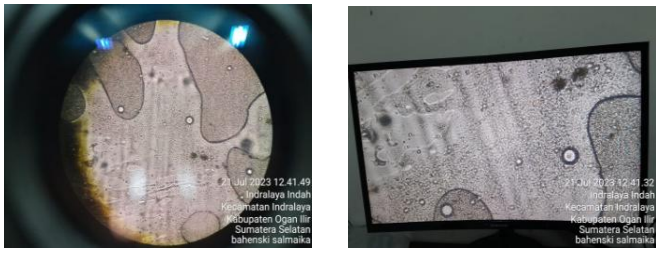
This technique for identifying bacterial species and populations is carried out using Nutrient Agar (NA) media. Isolation of bacterial colonies is carried out with the aim of culturing the bacteria contained in the mixture using culture media to obtain bacterial colony isolates or pure cultures from the bacterial colonies. The bacterial colony isolation used was the result of anaerobic fermentation from the bamboo rhizosphere which was carried out using a common medium, namely Nutrient Agar (NA). The medium is very important for growing microbes so that later these microbes can be identified. The fermented liquid is inoculated on Nutrient Agar (NA) media using the spread plate method. This method is a microbial inoculation technique by inoculating microbial cultures spread across the surface of solidified agar media.

Identification of bacterial colony morphology was carried out through observation of giant colonies. Observation of giant colony morphology includes colony shape (seen from above), colony elevation (seen from the side), edge of the colony (seen from above) and colony color. Making giant colonies uses Nutrient Agar (NA) media which is implanted in the middle by taking the isolate from the streak results and then placing it in the middle of the media using a toothpick that has been sterilized in an autoclave. Observation of cell shape in bacteria uses results from giant colonies seen through a tool or microscope with 1000x magnification.

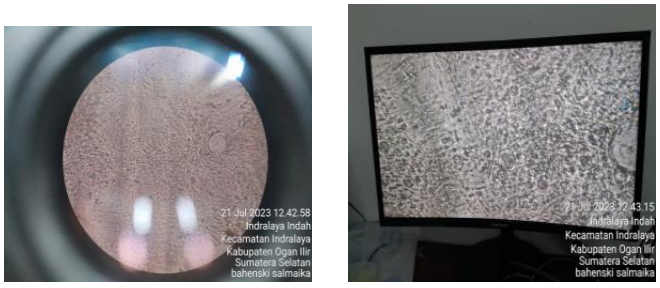
Biomass production is carried out by making a Biang Solution which is done by taking bamboo roots along with the soil still attached to the roots which is then ground up to 30 grams, then soaking the bamboo roots in 150 ml of distilled water or pure water. Then store it in a 500 ml Erlenmeyer which has been covered using cotton wool and aluminum foil, then homogenize using a shaker for 3 days. After 3 days, the results of the water soak have become a solution. Making this nutrient solution is done by boiling 450 ml of pure water in the pan provided, then adding all the ingredients for making PGPR except for the PGPR starter solution, including 6 g of granulated sugar; 0.6 g shrimp paste; 60 g bran/rice bran and 6 g whiting. While adding all the ingredients, it is best to stir continuously until it boils so that all the ingredients dissolve and mix well. These cooked ingredients become a nutrient solution and will be used as a medium for the development of PGPR bacteria. Next, the media are mixed, namely the starter solution and nutrient solution by filtering the starter solution and nutrient solution using a filter tool which is put together in a 500 ml beaker and then stirred until evenly mixed. Then the solution is fermented using a simple fermenter using a 250 ml Erlenmeyer as a storage place for the starter solution and nutrient solution. The Erlenmeyer used is closed tightly using cotton wool and aluminum foil so that no air enters. Then the mixture is fermented for 7-10 days in an incubator, until bubbles appear and the characteristic smell of fermentation (sour, alcoholic) (Fitri et al., 2020).

Bacterial colony population calculations were carried out directly with the naked eye. Colony population calculations were obtained from the purification technique by dilution using a physiological salt solution (physiological NaCl) which was then purified on Nutrient Agar (NA) media which was incubated at a temperature of 27°C-30°C for 24 hours to avoid contamination. After the colonies live in the media, count the colonies with the same appearance that are separated without overlapping. Dilution was carried out from 10-1 to 10-7 (Breed et al., 1957). The colonies selected for counting have special conditions based on statistics to minimize errors in calculations. Calculations refer to the standards of the International Commission on

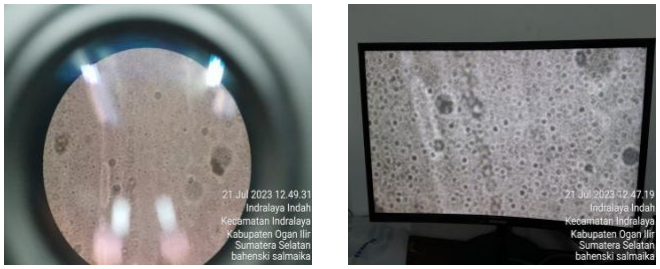
**(a)** Isolate 1 (*Streptococcus*)



**(b)** Isolate 2 (*Coccus*)



**(c)** Isolate 3 (*Coccus*)



**(d)** Isolate 4 (*Palisade*)

**(e)** Isolate 5 (*Streptococcus*)

**Figure 2.** Cell shape of bacterial isolates from the bamboo rhizosphere

Microbiology Food (ICMF) with the condition that the cup contains 30-300 colonies, colonies > 300 = TBUD (Too Many to Count) (Rizal et al., 2016).

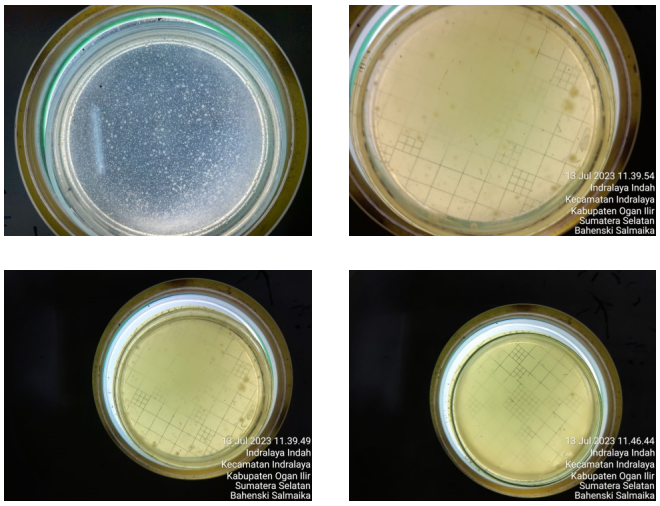


**Figure 3.** Size of bacterial colonies from bamboo rhizosphere

## 3. RESULTS AND DISCUSSION

### 3.1 Morfology of Bamboo Rhizosphere Bacteria

The results of observing the morphology of bacteria from the bamboo rhizosphere through observing giant colonies include observing the shape, elevation, edges and color of the colonies for each colony produced. Based on the results of bacterial isolation, 5 bacterial isolates were obtained from the PGPR (Plant Growth Promoting Rhizobacteria) solution originating from the bamboo rhizosphere, each of which was coded as Bambu 1, Bamboo 2, Bamboo 3, Bamboo 4 and Bamboo 5. The results of the bacterial morphology observations are presented in Table 1 and Figure 1.

Based on the results in Table 1, it shows that the isolate codes 1, 3, 4 and 5 came from the PGPR (Plant Growth Promoting Rhizobacteria) solution. The origin of the rhizosphere of Bambi has an irregular or irregular bacterial shape. Meanwhile, isolate 2 has a circular bacterial shape. The differences in the shape of each bacteria can be influenced by environmental conditions, medium and age. In general, younger bacteria are relatively large and irregular in size compared to older ones (Fitri and Yasmin, 2020).

Elevation is the degree of increase in colony growth above the agar surface. Based on the results of observations on the isolates, bacterial elevations showed that all isolates originating from the PGPR (Plant Growth Promoting Rhizobacteria) solution from the bamboo rhizosphere had the same elevation, namely flat. The edge of the colony is observed by looking at the growth at the edge of the colony. Isolates 1, 4 and 5 have lobate edges. Meanwhile, isolates 2 and 3 have edges of Undulate bacteria.

When observing the color of the bacteria, the color of the colonies in isolates 1 and 5 was colorless or transpar-

**Table 1.** Morphology of bacteria from the bamboo rhizosphere

| Isolate Code | Bacterial Morphology | | | |
|---|---|---|---|---|
| | Form | Elevation | Edge Shape | Color |
| 1 | Irregular | Flat | Lobate | Transparent |
| 2 | Circular | Flat | Undulate | Yellowish |
| 3 | Irregular | Flat | Undulate | Milk White |
| 4 | Irregular | Flat | Lobate | Milk White |
| 5 | Irregular | Flat | Lobate | Transparent |

**Table 2.** Cell shape of bacterial isolates from the bamboo rhizosphere

| Bacterial Isolate | Cell Shape |
|---|---|
| 1 | *Streptococcus* |
| 2 | *Coccus* |
| 3 | *Coccus* |
| 4 | *Palisade* |
| 5 | *Streptococcus* |

ent. Isolate 2 has a yellowish color. Meanwhile, isolates 3 and 4 have a milky white color. Bacteria that do not have chromogene show white growth, while bacteria that have chromogene show different colors. Differences in color in bacteria indicate differences in cell wall structure between types of bacteria (Nuraini et al., 2011).

### 3.2 Bamboo Rhizosphere Bacterial Cell Form

The results of observations of the shape of the bacterial cells found in the PGPR (Plant Growth Promoting Rhizobacteria) solution from the bamboo rhizosphere of the five bacterial isolates had different cell shapes. Bacteria have a variety of cell shapes that include several main characteristics, such as cocci (round), bacilli (rods), and spirals. Coccus bacteria have a round shape. Meanwhile, bacillus bacteria are rod-shaped. The results of cell shape analysis for each bacterial isolate are presented in Table 2 and Figure 2.

Based on the results presented in Table 2, it shows that bacterial isolates 1 and 5 have the form of Streptococcus cells. Streptococcus is a genus of non-motile bacteria that contains gram-positive, non-monocot cells that are shaped like ovals and form short, long or paired chains. Streptococcus can also be said to be a cocci that join together in a field and are arranged in a chain pattern (Amin et al., 2023). Isolates 2 and 3 have the form of Coccus cells. Coccus is a spiral-shaped bacteria that is flexible and its body can lengthen and contract when moving. Coccus can be single or multiple in groups of 2, 4, 8, and so on. Coccus cell-shaped bacteria can be round, oval or elongated or bean-shaped (Firmasnyah et al., 2023).

**Table 3.** Size of bacterial colonies from bamboo rhizosphere

| No. | Dilution | Size |
|---|---|---|
| 1. | $10^{-4}$ | *Pinpoint/punctiform* (point) |
| 2. | $10^{-5}$ | *Small* (small) |
| 3. | $10^{-6}$ | *Pinpoint/punctiform* (point) |
| 4. | $10^{-7}$ | *Moserate* (medium) |

Meanwhile, bacterial isolate 4 has a Palisade cell shape. Palisade is bacteria shaped in a row like a fence (Amin et al., 2023). These diverse bacterial cell shapes reflect the flexibility and adaptability of microorganisms. Variations in cell shape may occur either permanently or as a form of abnormality due to unfavorable environmental influences, which is called involution (Wardhani et al., 2020).

### 3.3 Size of Bacterial Colonies

As a result of observations from the bacterial isolation that had been carried out, 4 bacterial colonies were obtained from the PGPR (Plant Growth Promoting Rhizobacteria) solution from the bamboo rhizosphere which were able to grow and colonize on Nutrient Agar (NA) media using a dilution technique carried out at $10^{-4}$,$10^{-5}$, $10^{-6}$, $10^{-7}$. The results of isolation of bakery colony sizes are presented in page 49 and Figure 3.

Table 3 and Figure 3 show that at dilutions $10^{-4}$ and $10^{-6}$ the bacterial colonies have a pinpoint/punctiform size or it can be said that the colonies are very small like dots. Meanwhile, in the $10^{-5}$ dilution, the bacterial colonies have a small size and in the $10^{-7}$ dilution, the bacterial colonies have a moderate or moderate size. The colony size is different or the same in each dilution, because the bacteria grow the same in each dilution (Lestari, 2016).

### 3.4 Population of Bacterial Colonies From Bamboo Rhizosphere

The results of observations of bacterial colony population calculations from the bamboo rhizosphere showed that a

**Table 4.** Population of bacterial colonies from the bamboo rhizosphere

| Total Population (CFU/ml) |
|---|
| $19{,}2 \times 10^5$ |
| $17{,}1 \times 10^6$ |
| $49{,}0 \times 10^6$ |
| $30{,}0 \times 10^7$ |

number of bacteria grew in different numbers with each dilution. The results of calculating bacterial colony populations are presented in Table 4.

Based on the results presented in Table 4, it shows that the number of bacterial colonies that grew was calculated and obtained the most in the $10^5$, namely 19,2 CFU/ml. Meanwhile, the lowest bacterial colony population was found in the $10^{-7}$ dilution, namely 30 CFU/ml. The average obtained from dilutions $10^{-5}$, $10^{-6}$, and $10^{-7}$. The results of the research showed that the greater the dilution carried out, the fewer colonies that grew on the media. The main factors that cause failure to grow bacterial colonies in culture media are errors in the dilution procedure and dilutions that are too large (Joni and Abrar., 2018).

### 3.5 Types of Bacterial From Bamboo Rhizosphere

Based on the isolation and identification of the morphological characteristics of bacteria which has been carried out in the Plant Growth Promoting Rhizobacteria (PGPR) solution from the bamboo rhizosphere. The morphology of the bacteria was found to have a round (Circular) and irregular (Irregular) shape. The bacteria found in the PGPR solution also have the shape of rod (Coccus), round (Streptococcus) and Palisade cells. It has flat bacterial edges and is white to yellowish in color. The morphology of the Pseudomonas fluorescens bacterium is a spherical bacterium, a rod cell shape (Coccus), the edges of the bacterium are flat and have a white to yellowish color. From the results of the observations that have been made, some of the morphologies observed are the same as the morphology of the Pseudomonas fluerescens bacteria.

Apart from the Pseudomonas fluerescens bacteria in the bamboo rhizosphere, Bacillus polymixa bacteria colonize the bamboo rhizosphere (Yulistiana et al., 2020). This bacteria comes from the genus Bacillus sp. This Bacillus polymixa bacteria has a bacterial morphology with a round and irregular shape (Irregular), a rod cell shape (Coccus), the edges of this Bacillus polymixa bacteria have various edges, namely flat and uneven and have large and not shiny colonies (Puspita et al., 2017). From the morphology of the Bacillus polymixa bacteria, there are similarities between the morphology of the bacteria identified in the PGPR (Plant Growth Promoting Rhizobacteria) solution from the bamboo rhizosphere. From the explanation above, it is suspected that the types of bacteria found in the PGPR (Plant Rowth Promoting Rhizobacteria) solution from the bamboo rhizosphere are Pseudomonas fluorescens bacteria which function to dissolve Phosphate (P) and Bacillus polymixa which aims to increase Nitrogen (N) in the soil.

## 4. CONCLUSIONS

The morphology of the bacteria found in giant colony isolates of PGPR (Plant Growth Promoting Rhizobacteria) solution from the bamboo rhizosphere has the shape (Irregular and Circular); elevation (flat); edge (Lobate and Undulate); Color (Transparent, Milky White to Yellowish; as well as cell shape (Streptococcus¸ Coccus, and Palisade). The bacterial population on $10^{-4}$ has the highest population number, namely 192 CFU/ml and on $10^{-7}$ has the lowest population number, namely 30 CFU/ml ml. It is suspected that the types of bacteria contained in the PGPR (Plant Growth Promoting Rhizobacteria) solution from the bamboo rhizosphere are *Pseudomonas sp* and *Bacillus sp*.

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