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Isolation Of Rhizosphere Area Rhizobakteria In Coffee Plants At Several Altitudes In Central Aceh District

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Abstract
Coffee is the main crop and superior commodity in the Gayo Highlands, especially in Central Aceh Regency. There are two varieties of coffee, namely Arabica and Robusta. Sampling was carried out in Pedemun Village, Lut Tawar District and Weh Nareh Village, Pegasing District, Central Aceh Regency. The area of coffee land used as the research location is 0,5 ha at each location. Identification of rhizobacteria was carried out at the Plant Disease Laboratory, Department of Plant Protection, Faculty of Agriculture, Syiah Kuala University, Banda Aceh. implemented from March to July 2024. This research was conducted using qualitative descriptive methods. Sampling was determined by purposive sampling, namely deliberately (without random). The results of the isolation of rhizobacteria in the rhizosphere area of coffee plants resulted in 9 isolates with different codes for each isolate, namely: DP1, DP2, DP3, DP4, PD5, KS1, KS2, KS3, and KS4. Each isolate has different macroscopic characteristics, namely having irregular (irregular) and circular (circular) colony shapes, undulate (wave-like) and entire (flat) colony edges as well as smooth and butyrous (like butter) textures. The staining test results show that all types of isolates include Gram-negative bacteria which are marked in red. All types of isolates obtained had the same cell shape, namely bacilli.

Keywords:
Coffee Plants, Isolation, Rhizobacteria, Rhizosphere, Central Aceh

1. Introduction

Indonesia is one of the countries with the potential to become the world's largest coffee producer after Brazil, Vietnam, and Colombia [1] Coffee As a commodity with high economic value, it is only right that coffee farming development receive significant attention, given its significant contribution to the national economy. Domestic market demand for coffee increases year after year in line with population growth. Therefore, opportunities for marketing coffee remain wide

open [2]. The leading coffee-producing provinces in Indonesia are South Sumatra, Lampung, Aceh, North Sumatra, and Bengkulu . [3]. Aceh Province is one of the coffee producers, the area of coffee plantations in Aceh Province in 2021 was 125,688 ha with a production of 96,384 tons per year.[4].

Increasing coffee production through increased productivity is an approach that has the potential to be developed [5]. Soil fertility affects plant growth since it supplies essential nutrients. Plant productivity can be improved through fertilization [6]. According to [7], inorganic fertilizer is an industrial fertilizer that goes through chemical, physical and biological engineering processes. Organic fertilizers contain organic matter derived from plant from animal waste, can be in solid or liquid form, which is used to improve the physical, chemical and biological properties of the soil.

The rhizosphere, the soil zone associated with plant roots, hosts a higher abundance of bacteria compared to other soil layers[8]. Rhizosphere bacteria, found around plant roots, contribute significantly to increasing soil fertility and plant productivity [9]. Microorganisms that play a role in plant growth belong to the rhizobacteria group, which live and thrive in the rhizosphere of plants, both symbiotically and non-symbiotically. This group of rhizobacteria is known to stimulate plant growth by producing growth hormones, organic acids, and nitrogen fixation. Rhizobacteria with this role within the microbial group are commonly known as Plant Growth Promoting Rhizobacteria (PGPR) [10]. PGPR not only plays a role in enhancing plant growth but also protects against pathogenic microorganisms. Therefore, PGPR can be used as an environmentally friendly organic fertilizer and an alternative to reducing the use of chemical fertilizers, thus creating sustainable agriculture [11].

2. Material and Methods

2.1 Time and place

Sampling was carried out in Pedemun Village, Pegasing District, Central Aceh Regency 5°27'13" LU - 95°98'27" BT, Lut Tawar District and Weh Nareh Village 4°34'56.2"LU - 96°49'25.9"BT, the average rainfall is 1.976 mm/year, average temperature is 20°C, soil pH 6,5 and has average annual relative humidity value of 83%. Identification of rhizobacteria was carried out at the Plant Disease Laboratory, Plant P rotection Department, Faculty of Agriculture, Syiah Kuala University, Banda Aceh from March 2024 to July 2024.

2.2 Tools and materials

The tools used in this study were petri dishes, test tubes, slides, tweezers, light microscopes, cover slips, hot plates, ose needles, laminar air flow cabinets, cork borers (0.5 mm), and mortars.

The materials used were healthy coffee plant root samples in two villages, namely Pedemun Village and Weh Nareh Village, Central Aceh Regency. The samples taken were soil from the roots of the Robusta coffee type.

2.3 Experiment Design

This research was conducted using qualitative descriptive methods. Sampling was determined by purposive sampling, namely intentionally (not randomly).

2.4 Research Methods

2.4.1 Soil Sampling

The sampling method used was purposive sampling. Soil samples were taken from the root zone (rhizosphere) of healthy plants. Plants were selected from the population that exhibited the best growth conditions. Samples were taken at 9 points, with 100 g of soil sample per point.

2.4.2 Isolation of Rhizobacteria

The initial stage of rhizobacteria isolation is the preparation of NA media by mixing 5 grams of NA in 250 ml of distilled water into an Erlenmeyer flask. Then stir until homogeneous using a magnetic stirrer. Then sterilized in an autoclave for 30 minutes at a temperature of 121˚C. After sterilization, NA is poured into a petri dish and left for 10 minutes until the NA hardens. Then the soil sample is taken to the laboratory for isolation, Rhizobacteria are isolated from the soil in the coffee root area. A total of 10 g of soil sample is mixed with 100 ml of sterile distilled water and shaken until homogeneous. Furthermore, the suspension is diluted using serial dilutions to a dilution of 10^{-4}. A total of 100 µl of the dilution suspension is then spread evenly on a petri dish containing Nutrient Agar (NA) media using microbiology glass beads and then incubated for 48 hours.

2.4.3 Characteristics Morphologic of Rhizobacteria

The initial stage of rhizobacteria isolation is the preparation of TSA media by mixing 5 grams of TSA in 250 ml of distilled water in an Erlenmeyer flask. Then, it is stirred until homogeneous using a magnetic stirrer. Then, it is sterilized in an autoclave for 30 minutes at a temperature of 121˚C. After sterilization, the TSA is poured into a petri dish and left for 10 minutes until the TSA hardens. Then, the soil sample is taken to the laboratory for isolation. Rhizobacteria are isolated from the soil in the coffee root area. A 10 g soil sample is mixed with 100 ml of sterile distilled water and shaken until homogeneous. Next, the suspension is diluted using serial dilutions to a dilution of 10-4. A 100 µl of the diluted suspension is then spread evenly on a petri dish containing media using microbiology glass beads and incubated for 48 hours. Morphological characteristics of rhizobacteria colonies are carried out by growing bacterial colonies on general media using selective TSA with scratch plate media for 48 hours at room temperature, the pure colonies that appear are observed for color character, colony shape, elevation, texture and colony edges [12.

a. Colony Color

Observation of the color of the bacterial colonies that appear such as white or brownish yellow, red, cream.

b. Colony Surface Shape

Observation of the shape of bacterial colonies is done directly by looking at the shape of the surface of the growing colony such as a round shape, a concave or convex colony surface, a flat or uneven colony edge.

c. Gram Staining

The gram test is performed to determine the type of bacteria found, whether they react gram positive or negative. The gram test is performed by placing 103 bacterial culture suspensions on a glass slide and then fixing them by passing the glass slide over a Bunsen flame. The next step is to add 1-2 drops of methylene blue dye to the slide and leave it for one minute. The remaining methylene blue is rinsed with sterile water. Next, 1-2 drops of lugol are added and left for one minute. After one minute, the remaining lugol is rinsed with sterile water and dissolved in 96% alcohol for 10 seconds until the remaining dye is gone. Next, rinse again with sterile water, add 1-2 drops of safranin dye to the glass slide, and leave it for 30 seconds. Next, the remaining safranin is rinsed with sterile water and the glass slide is dried. After the staining is complete, the glass slide is

covered with a cover glass and placed under a microscope with immersion oil added, then the bacteria can be observed. Bacteria that react gram-positively will retain the violet color while gram-negative bacteria will be red after staining [13].

3. Results and Discussion

3.1 Macroscopic Characterization (Morphology)

The morphological characteristics of rhizobacterial colonies were carried out by growing bacterial colonies on general media using selective TSA with scratch plate media for 48 hours at room temperature, the pure colonies that appeared were observed for color character, colony shape, elevation, texture and colony edges can be seen in table 1.

Table 1. Colony/macroscopic characteristics

Isolate code	Shape	Margin	Elevation	Texture	Color	Size
DP1	*Irregular*	*Undulate*	*Flat*	*Smooth*	White	Big
DP2	*Circular*	*Entire*	*Flat*	*Butyrous*	White	Big
DP3	*Irregular*	*Lobate*	*Flat*	*Smooth*	White	Big
DP4	*Irregular*	*Undulate* dan *Lobate*	*Flat*	*Smooth*	White	Big
DP5	*Circular*	*Undulate*	*Flat*	*Smooth*	White	Big
KS1	*Irregular*	*Undulate*	*Flat*	*Smooth*	White	Big
KS2	*Irregular*	*Entire*	*Flat*	*Smooth*	White	Big
KS3	*Irregular*	*Lobate*	*Flat*	*Smooth*	White	Small
KS4	*Irregular*	*Undulate*	*Flat*	*Butyrous*	White	Small

Isolation is an activity carried out to obtain isolates bacteria . The bacterial isolates obtained are bacteria that grow on the 2nd day on Nutrient Agar (NA) medium. The results obtained from the isolation of rhizosphere bacteria in coffee plants were 9 isolates with different codes for each isolate, namely DP1, DP2, DP3, DP4, DP5, KS1, KS2, KS3 and KS4. Bacteria are identified morphologically based on color, colony shape, elevation, texture and colony edges. The isolated rhizosphere bacterial isolates have different characteristics. Observations of color in macroscopic characterization, the color formed is the same for all 9 isolates, namely white and has the same elevation, namely flat (thin) as seen in Figure 1.

Bacteria with isolate codes DP1, DP3, DP4, KS1, KS2, KS3 and KS4 have the same colony shape, namely irregular and have a circular shape in isolate codes DP2 and DP5. In bacteria with isolate codes DP1, DP3, DP4, DP5, KS1, KS2, KS3 have a smooth texture and butyrous texture (like butter) in isolate codes DP2 and KS4. In bacteria with isolate codes DP1, DP5, KS1 and KS4 have the same colony edge, namely undulate (like wavy), for isolate codes DP2 and KS2 have the same colony edge, namely entire (flat), for isolate codes DP3 and KS3 have the same colony edge, namely lobate (curved/serrated), while DP4 has a mixed colony edge, namely undulate (like wavy) and lobate (curved/serrated). In bacteria with isolate codes DP1, DP2, DP3, DP4, DP5, KS1, KS2 have the same size, namely large, in isolate code KS3 it is small-medium in size and small in isolate code KS4.

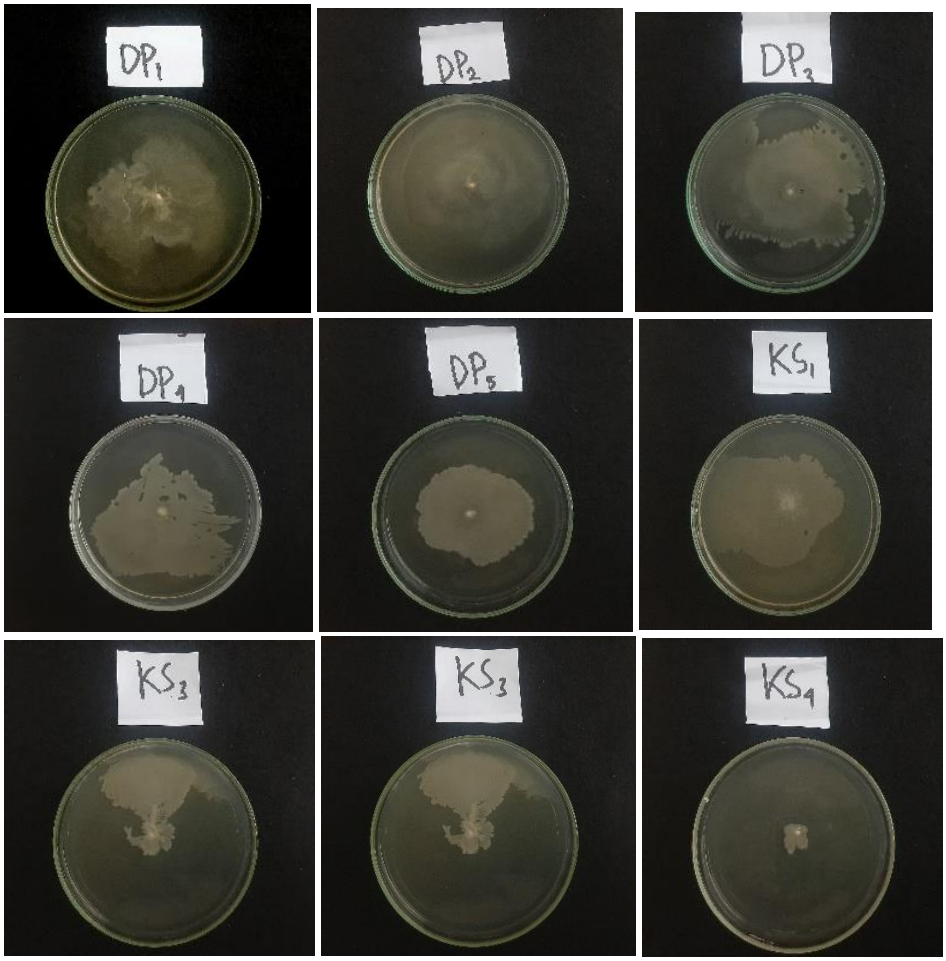


Figure 1. Morphological Characteristics of Rhizobacterial Colonies

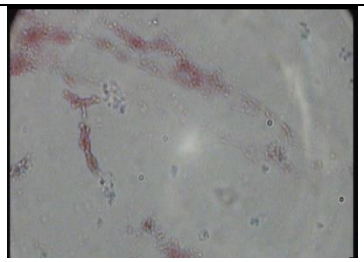
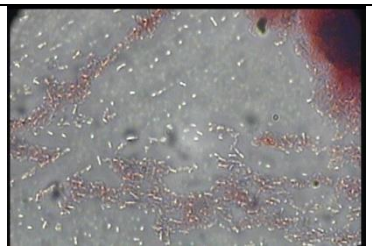
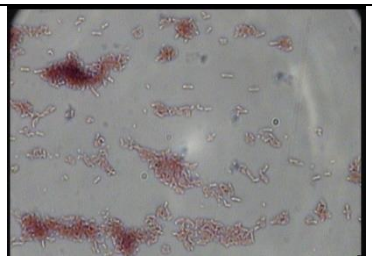
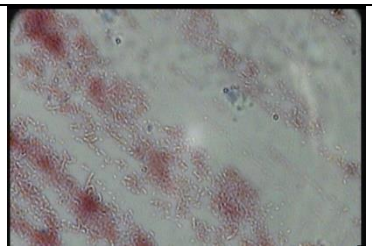
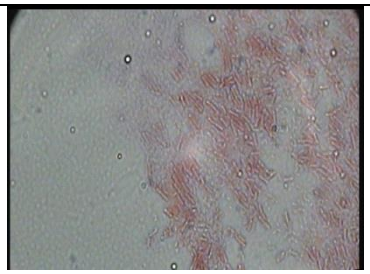
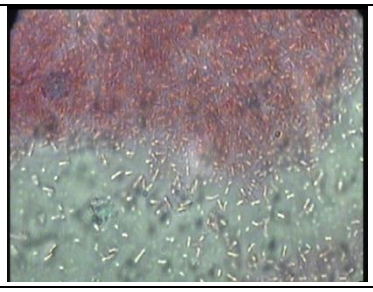
Based on research by [14] on the rhizosphere of robusta coffee plants, it was stated that the results of bacterial isolation obtained 5 types of isolates, while the bacteria that were successfully isolated were given the codes RA, RH, RI, RJ, and 13D. The morphology of the bacterial isolate colonies obtained in the study had different characters. The results of this study are in accordance with [15], the appearance of bacterial colonies on the surface of the agar medium showed varying shapes, namely circular (round), irregular (irregular round) colonies and other elevation variations, namely flat (thin).

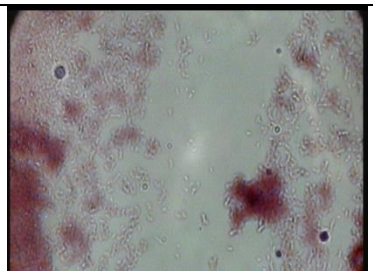
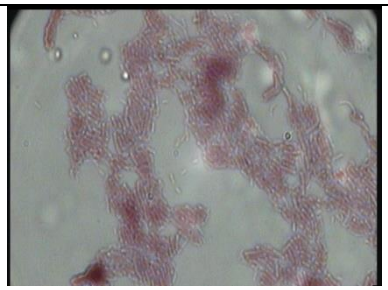
Other research results on the isolation of rhizobacteria in other plants, namely mustard greens, It is better to use the following sentence In a study on mustard greens, [16] reported the isolation of 19 bacterial strains . Based on macroscopic observations, the isolates obtained formed circular colonies, had entire and undulate edges, an entire (flat) surface and were white. [17] Based on the morphology, color, and shape of the rhizobacteria obtained, the same morphotype was obtained, namely a pale white, irregular, non-slimy, undulate bacterial colony. [18] The abundance of bacteria in the rhizosphere varies widely, this is influenced by the presence of root exudates and is supported by the soil environment, which influences the interactions between soil microbes, plants, and the soil. [19] The results of the study obtained 8 isolates of phosphate-solubilizing bacteria isolated from mangrove soil samples with colony shape characteristics (round, oval and irregular) and colony color (milky white, yellow, green and transparent).

3.2 Microscopic Characteristics

The results of observations of microscopic characteristics can be seen in table 2.

Table 2. Microscopic Characteristics of Isolated Rhizobacteria

Isolate Code	Gram stain	Cell Shape
DP1		Semi-Basil
DP2		Basil
DP3		Basil
DP4		Basil
DP5		Basil
KS1		Basil

KS2		Basil
KS3		Basil
KS4		Basil

Based on the results of the Gram test from the table above, it shows that the isolate has a red color which indicates that the bacteria are included in Gram negative. The cell shape of the isolate code DP1 is semi-bacillus and for the isolate codes DP2, DP3, DP4, DP5, KS1, KS2, KS3 and KS4 have the same cell shape, namely rods (bacilli). Gram staining is used to separate members of the domain Bacteria into two groups based on their cell walls. Gram-positive bacteria have simpler cell walls, with a relatively large amount of peptidoglycan. Gram-negative bacteria have less peptidoglycan and are structurally more complex [20]. Gram negative bacteria look pink. This is because the cell walls of gram negative bacteria contain little peptidoglycan and lots of lipids that will dissolve in Gram C (alcohol acetone) during rinsing [21]. These results are in line with the research of [22],

The results of Gram staining on Gram-positive bacterial cells will be purple and red for Gram-negative bacteria [23]. The peptidoglycan layer in Gram-positive bacteria binds the crystal purple dye, causing the bacterial cells to turn purple. While in Gram-negative bacteria, the thin peptidoglycan layer and high lipid content of around 20%, so that the bound dye is safranin which causes the bacterial cells to turn red [24].

Based on research by Iqbal [25] on the rhizosphere of robusta coffee plants, the results of observations of bacterial physiology show that bacterial isolates RA, RH, RI, and RJ have Gram-negative properties and rod-shaped cells in the form of bacilli and cocci. According to [26] , bacteria based on their shape are classified into cocci, long and thin bacilli, and coccobacilli between the two forms and also curved and spiral bacilli with different curve lengths.

4. Conclusion

The results of the isolation of rhizobacteria in the rhizosphere area of coffee plants obtained 9 isolates with different codes from each isolate, namely: DP1, DP2, DP3, DP4, PD5, KS1, KS2, KS3, and KS4. Each isolate has different macroscopic characteristics, namely having irregular and circular colony shapes, undulate (wave-like) and entire (flat) colony edges and smooth and butyrous (butter-like) textures. The results of the staining test showed that all types of isolates were Gram-negative bacteria which were marked in red. All types of isolates obtained had the same cell shape, namely bacill

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