Bioedukasi
Jurnal Biologi dan Pembelajarannya
Vol. 22 No 2, June 2024, page 208-217
e-ISSN: 2580-0094; p-ISSN:1693-3931
DOI: 10.19184/bioedu.v22i2.43596
jurnal.unej.ac.id/index.php/BIOED

Isolation and Characterization of Sugarcane Root (*Saccharum* sp.) Rhizobacteria and Their Influence on the Growth of Lettuce Plants (*Lactuca sativa* L.)

Eka Rismaningsih[1], Annisa Khumaira[2], Ika Afifah Nugraheni[3]
[1,2,3] Bachelor of Biotechnology, Universitas'Aisyiyah Yogyakarta, Indonesia

Article Info
Article history:
Received October 6, 2023
Revised February 24, 2024
Accepted June 21, 2024

Keywords:
Lettuce
PGPR
Rhizobacteria
Sugarcane

ABSTRACT
Lettuce (*Lactuca sativa* L.) is a horticultural commodity with good commercial value. Lettuce production is insufficient for consumer needs because of the excessive use of inorganic fertilizers, which reduce soil fertility. This research aims to decide rhizobacteria in the rhizosphere of sugarcane roots and their influence on increasing the growth of lettuce plants. The methods used are observational methods for taking composite soil samples. Macroscopic observations of bacterial colonies include the shape, elevation, edges, and color of the colonies produced. Through gram staining and physiological tests, including catalase, motility, carbohydrate fermentation, and indole tests, performed microscopic character observations. Field tests as PGPR were carried out on lettuce plants with treatment P0, P1, P2 and P3. The parameters observed were plant height, number of leaves, wet weight, dry weight, and root length. Based on the results of the research, the PGPR of sugarcane roots has rhizobacteria such as *Corynebacterium* sp, *Terrabacter*, *Saccharococcus*, *Aeromicrobium*, *Paracoccus*, *Ancylobacter*, *Planococcus*, and *Azotobacter*. PGPR affects the growth of lettuce plants, especially on plant height, number of leaves, and root length. Treatment P3 (PGPR 7.5 ml/L) and treatment P1 (2.5 ml/L) obtained the best concentrations, yielding optimal results on all test parameters. Treatments P0 and P2 did not have a significant effect on the growth of lettuce plants.

Corresponding Author:
Annisa Khumaira
Bachelor of Biotechnology, Faculty of Science Technology
Universitas Aisyiyah Yogyakarta, Jl. Siliwangi No 63 Mlangi Gamping Sleman DIY, Indonesia
Email: annisakhumaira@unisayogya.ac.id

1. INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a horticultural commodity with good commercial value. In the future, it is very possible for lettuce to become a commercial commodity, considering that demand for lettuce continues to increase in line with the number of restaurants, hotels, and places that provide traditional types of cuisine. Based on vegetable statistical data from 2010, lettuce production was 41.11 tons/per year and decreased in 2015, to 39,289 tons per year. However, national lettuce production is still lower than consumption, namely 35.30 kg/capita/year, whereas the volume of lettuce imports in 2015 was 21.1 tons (BPS, 2016; Fitriansah et al., 2019). Lettuce cultivation by farmers still relies on the use of inorganic fertilizers in its production. Inorganic fertilizer, a mixture of chemical ingredients, is still considered inappropriate for producing lettuce in large quantities because it will reduce soil fertility. Additionally, this type of fertilizer is relatively expensive, which makes it less cost-effective to use over an extended period of time. Therefore, it is necessary to intake nutrients that can make the use of inorganic fertilizer efficient and safe for the environment. One of them is Plant Growth-Promoting Rhizobacteria (PGPR).

One effort that can be made to improve plant yields is to utilize rhizobacteria from the plant rhizosphere. Microorganisms in PGPR not only ensure the availability of nutrients for plants, but also can enhance the precise use of nutrients (Nandal & Hooda, 2013; Hamdayanty,2022). In addition to promoting plant growth, Plant Growth-Promoting Rhizobacteria (PGPR) also play a crucial role in accelerating composting and increasing crop yields. These positive benefits have led to the potential use of PGPR as biofertilizers and their development as biotechnological products in the field of agriculture (Mwajita et al., 2013; Hamdayanty, 2022). Based on the description above, this research needs to be carried out to determine the presence of rhizobacteria in the

rhizosphere of sugarcane roots and the effect of PGPR in the rhizosphere of sugarcane roots on increasing the growth of lettuce (*Lactuca sativa* L.).

2. RESEARCH METHOD

PGPR Sampling

PGPR samples were taken from the rhizosphere of sugar cane plants (*Saccharum* sp.) in the Turi area of Yogyakarta. During sampling, five sample points were taken from sugarcane roots. Samples were taken, weighing around 100 g of soil at a depth of 15 cm, then combined in the same container. The samples were then mixed until homogeneous. 200 g of the homogenized sample was taken. Soil sampling is carried out by cleaning the soil from grass and rubbish at the sample location, then the prepared soil drill is washed using clean water, sprayed with 70% alcohol and sterile water, and then dried using a tissue. Next, the drill is pressed into the ground, the soil contained in the drill is taken using a spatula, then the sample is put in plastic, closed tightly, and then given a label (Nasution et al., 2023 with modifications).

Preparation of PGPR

The PGPR seed is derived from the rhizosphere of sugar cane plants. Firstly, 200 grams of root-soil are collected, and then 1 L of water is boiled and used to soak the root-soil. The mixture is then left for 3 days. The growth of PGPR is monitored daily; if bubbles appear, it indicates successful cultivation (Putri et al., 2019 with modifications). The propagation of sugarcane root PGPR is achieved by heating the following ingredients: 6 glasses of rice bran, 3 tablespoons of granulated sugar, 1 packet of shrimp paste, 10 g of whiting, and 10 liters of water. These ingredients are stirred slowly to prevent the bran from clumping. Once boiled, the mixture is cooled and filtered. The PGPR starter is mixed into the propagation medium and stirred slowly. Finally, all the ingredients are fermented for approximately 10 days. The fermentation result is declared successful if there is a tapai aroma, the solution is clear, and there is a white layer (bacterial mass). These bacterial cultures can be cultured for 3–4 months (Danina et al., 2022).

Isolation of Bacteria from Sugarcane Root

A sample of 1 g of soil was suspended in 9 mL of sterile distilled water to a dilution level of 10^{-6} by taking 1 mL of the suspension and placing it in a test tube containing 9 mL of sterile distilled water (Susanti, 2014, with modifications). For the 10^{-4} and 10^{-5} dilution series, 1 mL of suspension was taken and cultured on nutrient agar media. The mixture was then incubated for 24 hours at a temperature of 28–30 °C, purification was carried out to obtain a pure culture (Khairani et al., 2019 with modifications).

Purification

The purification of bacteria was performed using the scratch method. All colonies with different shapes are purified on NA media to obtain pure isolates. The isolate obtained was then streaked repeatedly until a single isolate was obtained. Next, incubation was carried out at a temperature of 30 °C, and growth was observed for 2 days (Nuraini, Saida, Suryanti, & Nontji, 2020).

Macroscopic characterization of colony morphology

All single isolates that were successfully purified were grown on NA media in test tubes using the tilt method, incubated for ± 1 week, and then directly observed for their morphology, namely colony shape, elevation, colony edges, and colony color (Nuraini et al., 2020). The morphological characteristics of bacterial colonies on a medium are that the colony shape is round, thready, irregular, root-like, and coil-like. Colony elevations are flat, curved, and raised. The edges of the colony can be smooth, wavy, jagged, thready, and curly. The color of the colony is white, yellowish, gray, or almost clear (Zuraidah et al., 2020).

Microscopic characterization of colony morphology

Microscopic observations were carried out using gram staining. Microscopic observation observes gram-positive or gram-negative types of bacteria. Microscopically, the shape of bacterial cells was observed. Bacteria with a purplish blue color are Gram positive bacteria, while bacteria that appear pink are Gram negative bacteria (Afifah, Irdawati, & Putri, 2018).

Gram staining of bacteria

In one of the processes of characterization and identification of bacteria, gram staining is first carried out, which functions to see the bacteria's morphology and the gram's characteristics. Making a smear on a glass object, fixing it onto a slide, dropping it with crystal violet, and leaving it for 1-2 minutes. The excess dye is removed, and then rinsed with running water. Then, the preparation is dripped with Lugol's iodine solution and left for 30 seconds. Once finished, the Lugol solution is discarded and rinsed with distilled water. The preparations were washed with 95% alcohol until all excess dye was removed and immediately washed with distilled water. Staining with safranin dye, then left for 1-2 minutes. The stained bacterial cells were washed with distilled water and dried before being observed under a light microscope (Lindawati & Suardana, 2016).

Simple painting

Observation of cell shape was observed in 24-hour-old bacteria grown in NA media following the method of Hucker (1921). This staining process is carried out using a crystal violet solution as gram A, which functions as a primer paint so that it gives a purple color to the target bacteria; a lugol iodine solution as gram B, which

functions to strengthen the color of gram A and causes the sample bacteria to turn brown; 95% alcohol as gram C, which plays a role in discharging the previous paint; and safranin as gram D, which gives the bacteria a red color. The stained bacterial isolates were then observed with a microscope at 100x magnification (Mulyani et al., 2021). The most common bacterial cell shapes found are coccus (round), rods, and curves, both Gram negative and Gram positive.

Physiological characterization

Catalase test

The bacterial isolate was inoculated into a glass slide and dripped with 3% H2O2. The presence of air bubbles indicates that the reaction is positive, and if there are no air bubbles in the test tube, then the reaction is negative. (Panjaitan, Bachtiar, Arsyad, Lele, & Indriyani, 2020).

Carbohydrate fermentation test

The carbohydrate fermentation test was performed using Triple Sugar Iron Agar (TSIA) media by piercing the bacterial inoculum vertically ¾ deep into the slanted media and scratching it on the media's slant, then incubating for 24 hours of incubation. A positive result of bacteria fermenting glucose is indicated by the butt of the medium turning yellow and the slant remaining red, whereas if the microorganism fermentates lactose and sucrose, it is indicated by the slant and butt of the media turning yellow (Susanti et al., 2018).

Motility test

A loop with a straight tip is used for bacterial motility testing. The bacterial isolate is then inserted into a nutrient broth containing 0.5% agar (soft agar). The incubation was performed at 35 °C for 2 days. If the growth spreads, then the bacteria are motile, and if the bacterial growth does not spread and only forms lines, then the bacteria are non-motile (Yuni and Fallo, 2017).

Indole test

A 24-hour-old test culture is prepared for the indole test. Grow it in Tryptone Broth medium, incubate at 37 °C for 24 hours, and then drip the medium with Kovac's reagent solution. Positive results will be indicated by forming a red ring at the top of the medium, while negative results will be indicated by the absence of a red ring (Nugroho & Setiawan, 2021).

Field Test as PGPR

Seed selection

The criteria for lettuce seeds prepared in this research are seeds that have been selected that are relatively the same size, uniform, and not attacked by pests and diseases. Then soak the seeds in warm water for 10 minutes (Sarwanidans and Setyowati, 2017).

Sowing seeds

Seeding is done 1-2 weeks before planting. The seeding medium is soil; the soil is sifted, mixed until it becomes homogeneous, and then put into seedling polybags measuring 10 cm by 4 cm (Utama and Jannah, 2014, with modifications).

Preparation of planting media

After sowing, the lettuce seeds are transferred into polybags with 50:50 soil and husk components, filling the soil's weight according to the polybag size. After that, lettuce seeds that are 14 days old after sowing are planted in polybags to a depth of 2 cm and then covered at the top with planting media again without compacting. Each polybag is given around 1 lettuce seed (Putri et al., 2019).

Research design

In the field test of the PGPR solution on lettuce plants, it consisted of 16 samples with 4 units and there were 4 replications for each treatment. The treatment plan is presented in Table 1:

Table 1.Research Design

No	Code	PGPR consentrat
1	P0	0(control)
2	P1	2,5ml/ L
3	P2	5 ml / L
4	P3	7,5 ml / L

The design used in this study was a completely randomized design (CRD) with PGPR concentrations of 0 mL/L, 2.5 mL/L, 5 mL/L, and 7.5 mL/L (Purniawati et al., 2021). and treatment watering was carried out at 7, 14, 21, and 28 DAT in the morning and evening. Apart from those days, watering is carried out with normal water every morning and evening, considering the weather conditions every day until the harvest period, namely at 35 DAT. Watering is done using a spray bottle so that each plant's water volume is uniform. Plant care is carried out by weeding out weeds around the plant roots.

Plant observation

The observation parameters carried out in this research are as follows:

1. Plant height (cm)Plant height measurements were carried out when the plants were 14, 21, 28, and 35 DAT using a ruler from the base of the stem to the tip of the tallest leaf.

2. Number of leaves (strands)The number of leaves was counted when the plants were 14, 21, 28, and 35 DAT, counting from the bottom to the top.
3. Plant wet weight (g)The wet weight of the plant is cleaned first and upstream of the lettuce plant from the adhering soil until clean; weighing the lettuce consists of the stems and leaves using analytical scales. This is done when the plant is 35 DAT (harvest period)
4. Plant dry weight (g)The dry weight of the plant was obtained from all lettuce plants that were dried through the steps of putting them in an envelope, then putting them in an oven at a temperature of 60 °C for 60 hours, and then weighing them using an analytical balance. This is done when the plant is 35 DAT (harvest period)
5. Root length (cm)Root length is calculated from the border of the stem and roots to the longest root of the plant, carried out during the harvest period. This is done when the plant is 35 DAT (harvest period).

Data analysis

For statistical analysis, data from isolation and characterization research are analyzed descriptively based on the empirical data obtained, then presented in tables or figures. Data on lettuce plant growth results were analyzed using the ANOVA test. If F count ≤ F table then the hypothesis is rejected, conversely if F count ≥ F table then the hypothesis is accepted. If the difference is significant, it will be tested using the Duncan Multiple Range Test (DMRT) with a test level of 5%. SPSS 21 processing media assisted analysis of this data. The isolate results from the isolation method and morphological characterization of the bacterial colonies obtained were analyzed based on the book Bergey's Manual Determinative Bacteriology 9th edition. From the results obtained, kinship was analyzed using NTSys-pc version 2.02 software to create a genetic similarity matrix using the SIMQUAL (Similarity for Qualitative Data) procedure. This similarity matrix is used for Sequential, Angglomerative, Hierarchical and Nested (SAHN) grouping analysis, grouping using the Unweighted Pair Group Method and Arithmetic Mean (UPGMA) method using the NTSys-pc version 2.02 computer program.

3. RESULT AND DISCUSSION

Isolation of sugarcane root PGPR rhizobacteria

Bacterial isolation was carried out using PGPR samples aged 14 days after fermentation. The results of bacterial isolation in the PGPR solution showed that 11 isolates had the best results. Of the 11 isolates obtained, a screening process was carried out. This process aimed to select bacterial isolates that would be included in the next stage of determining candidate isolates. Screening of bacterial isolates is based on similarities in terms of morphological characteristics, namely colony shape, color, pigmentation, size, edges, elevation, and cell shape (Hajar, 2012). From this screening process, 11 isolates were suitable for use at an advanced stage. The 11 isolates were different in shape, size, pigmentation, and color.

Morphological Characterization of Bacterial Colonies

Bacterial colonies in the PGPR solution of sugarcane roots can be seen macroscopically and microscopically. Macroscopic images can be seen clearly with the eye, such as the colony's shape, elevation, color, size, margins, optics, and appearance. Microscopy can be seen using a microscope, such as at gram staining and observing cell shape. Based on the results of observations of colony morphology, as shown in the data in Table 2, it was found that 11 colonies were round in shape and had smooth colony edges. The eleven isolates had flat elevations, and the TU1 colonies were convex. The color of the eleven colonies is cream, and TU6 has a different color, namely clear. The size of the bacterial colony is punctiform, but in TU3 and TU9 it has a different size, namely small; the entire colony has a smooth texture.

Table 2.Morphological Characterization of Bacterial Colonies

Isolate code	Colony form	Colony color	Elevation	Edge	Size	Texture
TU1	Round	Cream	Convex	Smooth	Punctiform	Smooth
TU2	Round	Cream	Flat	Smooth	Punctiform	Smooth
TU3	Round	Cream	Flat	Smooth	Small	Smooth
TU4	Round	Cream	Flat	Smooth	Punctiform	Smooth
TU5	Round	Cream	Flat	Smooth	Punctiform	Smooth
TU6	Round	Clear	Flat	Smooth	Punctiform	Smooth
TU7	Round	Cream	Flat	Smooth	Punctiform	Smooth
TU8	Round	Cream	Flat	Smooth	Punctiform	Smooth
TU9	Round	Cream	Flat	Smooth	Small	Smooth
TU10	Round	Cream	Flat	Smooth	Punctiform	Smooth
TU11	Round	Cream	Flat	Smooth	Punctiform	Smooth

Characterization of Cell Morphology

Observation of cell morphology was carried out using the gram staining method. Gram staining can see the Gram characteristics of the bacteria themselves and the shape of the bacteria. The Gram characteristics and shape of the bacteria were observed under a microscope. Based on the observations made, the bacteria obtained

were 8 bacteria that were gram-positive and 3 gram-negative bacteria. The cell shapes of 8 bacteria were Gram-positive, namely, 6 were bacillus (rod) and 2 were coccus (round). Of the negative bacteria, 2 are in the form of coccus and 1 is in the form of a bacillus, as shown in Table 3 below.

Table 3. Characterization of Cell Morphology

Isolate code	Gram staining	Cell shape
TU1	+	Basil
TU2	+	Basil
TU3	-	Basil
TU4	+	Basil
TU5	+	Coccus
TU6	+	Coccus
TU7	+	Basil
TU8	+	Basil
TU9	-	Coccus
TU10	+	Basil
TU11	-	Coccus

Gram-positive bacteria on Gram staining are purple because the crystal violet crystal dye complex is retained even when given an alcohol solution, while gram-negative bacteria are red in color when dissolved in an alcohol solution, so they take on the red color of safranin. The difference in color between gram-positive and gram-negative bacteria indicates differences in the 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 (Lay, 1994; Nurhidayati et al., 2015).

Physiological characterization

Some of the tests carried out are the catalase test, the indole test, the motility test, and the carbohydrate fermentation test. Physiological and biochemical identification are important criteria in characterizing unknown bacterial species because morphologically different cultures or bacterial cells will appear similar if physiological and biochemical observations are not carried out.

Table 4. Physiological characterization

Isolate code	Biochemical test				
	Catalase	Motility	Carbohydrate Fermentation		Indole
			Glucose	Lactose	
TU1	+	-	-	+	-
TU2	+	-	+	-	-
TU3	+	-	-	+	-
TU4	+	-	-	+	-
TU5	+	-	-	+	-
TU6	+	+	+	-	-
TU7	+	-	-	+	-
TU8	+	-	-	+	-
TU9	+	-	-	+	-
TU10	+	-	-	+	-
TU11	+	-	+	-	-

Catalase test

The catalase test is carried out to see whether bacterial isolates can produce the catalase enzyme. This catalase enzyme functions to decompose hydrogen peroxide (H_2O_2) into water and oxygen. A positive reaction from the catalase test is the formation of bubbles when the bacterial isolate is dripped with hydrogen peroxide (H_2O_2). No bubbles indicate a negative reaction From the data description in Table 4, it shows that the bacterial isolates were able to produce the catalase enzyme, except for the isolate with the code TU8. Positive reaction from the catalase test was shown by the formation of oxygen gas bubbles on the colony's ssurface after droppingH_2O_2 23%. The formation of gas bubbles indicates the decomposition of H_2O_2 by the catalase enzyme into water and oxygen. In microorganisms, H_2O_2 can be decomposed by catalase in cell mitochondria.

Motility test

From the results obtained, 12 isolates reacted negatively, and only one isolate reacted positively, namely isolate TU6. A positive reaction in the motile test is indicated by the spread of the isolate in the media, with the bacterial isolate located at the top of the media and spreading throughout the test media. The resulting negative

reaction is indicated by the bacterial isolate only spreading in the puncture area of the test medium. If the reaction is negative, the isolate is not motile. The movement of motile bacteria is characterized by the presence of a means of movement for the bacteria in the form of flagella, while non-motile bacteria do not have flagella.

Carbohydrate fermentation

In this test, if the isolate code changes color to red, this isolate can ferment glucose, and if the color changes to yellow, this isolate is capable of fermenting lactose and sucrose. If there is no color change at all on the TSIA media, this means that the bacterial isolate is unable to ferment sugar, In this study (Table 4), bacterial isolates TU1, TU3, TU4, TU5, TU7, TU9, TU10, and TU11 were able to ferment lactose because, in the test tubes used, there was a change in the color of the media to yellow at the butt and at the isolated scratches (slunts). Meanwhile, isolates TU2, TU6, and TU8 did not react to TSIA media, where the color of the media did not change like at the beginning, both from the butt and slunt parts.

According to Sudanrsono (2008) in Kosasi (2019), the TSIA test aims to determine the ability of a bacteria to ferment sugar to produce acid or gas. The red on the agar indicates an alkaline reaction, while the yellow indicates an acidic reaction. The red color on the surface of the agar shows that glucose fermentation is occurring, and the yellow color on the surface and bottom of the tube shows that lactose and sucrose fermentation are occurring.

Indole test

This indole production was detected by Kovac's reagent and reacted to produce a red compound. The indole test procedure is to inoculate the bacterial culture in Tryptone Broth media, then incubate at 30°C for 24 hours. The culture was dripped with 0.5 mL of Kovacs reagent. A positive reaction is characterized by the appearance of a red color on the surface of the media, indicating that the bacteria are able to break down the amino acid tryptophan. No ring formation at the top of the test medium indicates a negative reaction. The results obtained in Table 4, show that all isolates did not produce a red color on the tube walls. This means that all isolates could not break down the amino acid tryptophan to form indole compounds.

Characteristics of PGPR Rhizobacteria from Sugarcane Roots

Eleven isolates found in PGPR solution of sugarcane roots (*Saccharum* sp.) with a fermentation period of 14 days were subjected to phylogenetic tree reconstruction using NTSys-pc software version 2.02.

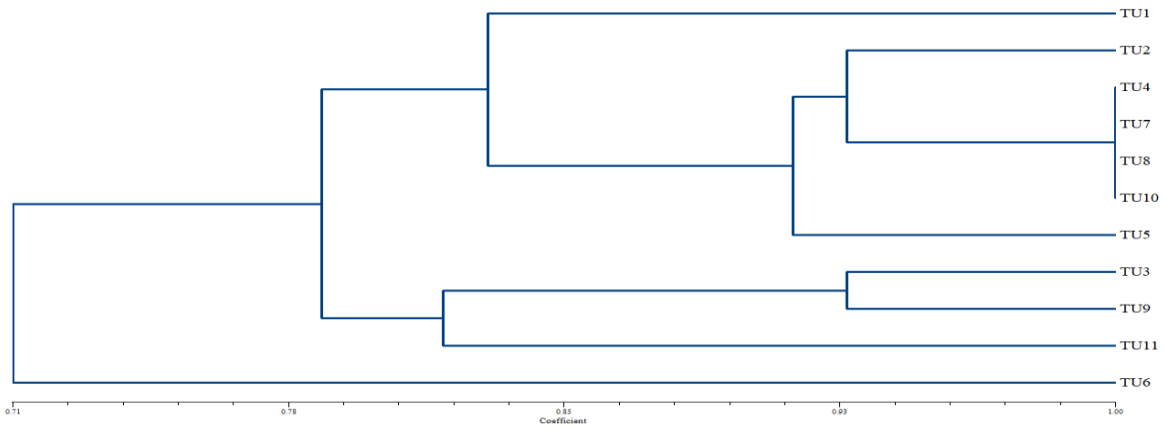

Figure 1. Dendogram of Sugarcane Root PGPR Bacterial Isolates

Clusters in the phylogenetic tree are divided into two groups with a separating coefficient of 0.71. In cluster I, there are isolates from TU1, TU2, TU4, TU7, TU8, TU10, TU5, TU3, TU9, and TU11, and in cluster II, there is isolate TU6. All the isolates obtained were closely related to each other because the coefficient values obtained were 0.71–1.00. This is to the statement by Purnomo et al. (2017) in Suleman (2021) that a similarity index value ≥ 70% can indicate that the kinship relationship between certain species is quite close; a similarity index value that is close to 100% indicates a closer kinship relationship between species. The isolates TU4, TU7, TU8, and TU10 are in the same line with a coefficient value of 1.00, indicating that the four isolates are closely related or belong to the same genus. Based on morphological and physiological characteristics concerning Bergey's Manual of Determinative Bacteriology, 9th edition, the presumed isolates obtained were TU1 (*Corynebacterium* sp.), TU4, TU7, TU8, and TU10 (*Aeromicrobium*), TU2 (*Terrabacter*), TU5 (*Saccharococcus*), TU3 (*Ancylobacter*), TU9 (*Azotobacter*), TU11 (*Paracoccus*), and TU6 (*Planococcus*).

Effect of Sugarcane Root PGPR on Lettuce Growth

Plant height

Table 5.Duncan Test for Lettuce Plant Height

Treatment	H0	7DAT	14DAT	21DAT	28DAT	35DAT
P0	5.80_{a}	6.93_{abc}	8.13_{abcd}	8.43_{abcd}	8.56_{abcd}	$8{,}8_{abcd}$
P1	8.20_{abcd}	5.93_{ab}	13.63_{cde}	13.96_{cde}	15.10_{de}	15.36_{de}
P2	7.06_{abc}	5.93_{ab}	9.7_{abcde}	12.8_{abcde}	14_{cde}	14.43_{de}
P3	7.13_{abc}	5.67_{a}	14.73_{de}	14.83_{de}	16.67_{e}	$16{,}73_{e}$

Remarks: DAT(Days after transplanting)

The PGPR treatment on lettuce plants showed significant differences in each treatment. For the height of plants at 7 DAT, the highest value was obtained by the P0 treatment with a value of 6.93, which was significantly different from the other three treatments. Plants at ages 14, 21, 28, and 35 DAT obtained the highest values from the P3 treatment and were significantly different from the other treatments. At a plant age of 35 DAT, the highest value was obtained by treatment P3 with a value of 16.73 e, followed by treatments P1 and P2 with values of 15.36 de and 14.43 de, but not significantly different from the control treatment (Table 5). The average height growth of lettuce plants in the P3 treatment received the highest score by providing an optimal effect and being significantly different from other treatments. This suggests that the growth hormones produced by rhizosphere microorganisms are able to increase root hair formation and increase ion transport, so that water transport by the roots increases. One of the bacteria that influences cell elongation is *Planococcus. Planococcus* has IAA-producing activity. The IAA hormone plays a role in the process of division, enlargement, and elongation of plant cells. *Azotobacter* is known as a producer of IAA (indole acetic acid) in PGPR, which is able to increase the number of root hairs and lateral roots, thereby increasing the absorption of water and nutrients from the soil. *Azotobacter* sp. colonizes the roots and has benefits for plant growth (Wibowo et al., 2019). Bacteria that are able to increase root hairs will provide benefits to plants. This is because the more roots a plant has, the more nutrients it absorbs and distributes. So it will increase plant growth.

Number of leaves

Table 6. Duncan test for lettuce number of leaves

Treatment	H0	7DAT	14DAT	21DAT	28DAT	35DAT
P0	3_{a}	$3{,}67_{\text{abc}}$	$4{,}33_{\text{abcd}}$	5_{abcdef}	6_{cdefg}	7_{efg}
P1	3_{a}	4_{abcd}	$4{,}67_{\text{abcde}}$	$5{,}67_{\text{bcdefg}}$	7_{efg}	8_{g}
P2	3_{a}	$3{,}3_{\text{ab}}$	$3{,}67_{\text{abc}}$	5_{abcdef}	$6{,}33_{\text{defg}}$	$7{,}67_{\text{fg}}$
P3	3_{a}	$3{,}67_{\text{abc}}$	5_{abcdef}	6_{cdefg}	7_{efg}	$7{,}33_{\text{fg}}$

Remarks: DAT(*Days after transplanting*)

For the number of leaves on lettuce plants, the data shown in Table 6 indicates that at plant age 7 (DAT), the highest value was obtained by treatment P1, which was significantly different from the other treatments. For plant ages 14 and 21 DAT, the highest value was obtained by treatment P3. At the age of 28 DAT, the highest value was obtained by treatments P1 and P3 with 7.0 efg, showing that the results were not significantly different from each other, while at the age of 35 DAT, the highest value was obtained by treatment P1 with 8 g, which was significantly different from the other treatments. The average P3 (PGPR 7.5 mL/L) has optimal results and is significantly different from other treatments. This aligns with the opinion of Nelson, L.M. (2004) as cited in Tabriji (2016). With the presence of bacteria in PGPR, the biofertilizer process produces a response in the nature of auxin stimulating growth by elongating cells and causing tip dominance; gibberellin increases the growth of side meristems in leaves and between nodes; cytokinins stimulate growth by cell division; growth inhibitors reduce elongation and accelerate abscission and senescence; and ethylene increases fruit ripening and horizontal growth.

Wet weight

Table 7. Duncan test for lettuce wet weight, dry weight and root length

Treatment	Wet weight	Dry weight	Root length
P0	$0{,}48_a$	$0{,}03_a$	4_a
P1	$1{,}43_a$	$0{,}161_a$	$9{,}6_b$
P2	$1{,}42_a$	$0{,}131_a$	$8{,}5_{ab}$
P3	$2{,}04_a$	$0{,}198_a$	$5{,}4_{ab}$

Remarks:DAT (Days after transplanting)

From the Duncan test data (Table 7), it is stated that the highest value was obtained by treatment P3 (PGPR 7.5 mL) with a value of 2.04, followed by treatment P2 with a value of 1.43. However, the wet weight of all treatments did not show any significant difference. The *Paracoccus* genus acts as a phosphate solvent in the soil. Phosphate-solubilizing bacteria have an important role in improving cultivated plants that experience phosphorus deficiency (Rao, 1994:274) (Chairani, 2016). Based on research by Orhan (2016), it shows that the halophilic bacteria *Planococcus* has IAA-producing activity and dissolving phosphate activity, which increases the growth and yield of Triticum aestivum under salinity stress. The IAA hormone plays a role in the process of division, enlargement, and elongation of plant cells, especially in the root area.

Dry weight

From the data obtained on the dry weight of lettuce plants, the highest value was obtained by treatment P3 (PGPR 7.5 ml/L), followed by treatment P1 (2.5 ml/L) from the data obtained in (Table 7). The lowest value was obtained by the control treatment without additional PGPR. Isolation of phosphate solubilizing bacteria, one of which was *Saccharococcus*, which is a phosphate solubilizing bacteria for plants. The use of phosphate-solubilizing microorganisms can partially or completely replace the plant's need for phosphate fertilizer (P), depending on the phosphate content of the soil, and provide positive results on plant growth and development. *Aeromicrobium* has the ability to degrade organic matter (Cao, 2023); research (Ling, 2022) states that the genera *Pedobacter* and *Aeromicrobium* are enriched only in the rhizosphere of Gramineae. Based on research by Tan et al. (2020), it was said that *Aeromicrobium* and *Rhodococcus* are characteristic of herbicide metabolism and can play a versatile role in soil ecological processes in agroecosystems.

Root length

Observations on root length were carried out when the lettuce plants were 35 DAT. From the data obtained in (Table 7), the highest treatment was obtained by treatment P1 (PGPR 2.5 mL/L) with a value of 9.60 b, which was significantly different from the other treatments, followed by treatment P3 (7.5 mL/L) but not significantly different from treatment P2. Meanwhile, the lowest treatment was obtained by the control treatment, which received a value of 4.00 a. This treatment received the highest score but was not significantly different from the others. Roots are the first part of the plant to absorb nutrients and then distribute them to all parts of the plant. It is important to see what nutrients are absorbed by the roots. Such as nutrients that come from PGPR candidate bacteria. *Azotobacter* is known as a producer of IAA (indole acetic acid) in PGPR, which is able to increase the number of root hairs and lateral roots, thereby increasing the absorption of water and nutrients from the soil (Noor and Nurhadi, 2022). *Azotobacter* sp. colonizes the roots and has benefits for plant growth (Wibowo et al., 2019).*Corynebacterium* sp. is a bacteria that is able to control pests and diseases in plants.

4. CONCLUSION

From the results of the research that has been carried out, it is suspected that the PGPR solution of sugarcane roots (*Saccharum* sp.) contains rhizobacteria such as *Corynebacterium,, Terrabacter, Saccharococcus, Aeromicrobium, Paracoccus, Ancylobacter, Planococcus,* and *Azotobacter*. These bacteria came from samples, additional media, and fermentation reactions in PGPR sugar cane roots. The bacteria contained in the sugarcane root PGPR solution help accelerate decomposition, producing the best-quality PGPR fertilizer. PGPR (Plant Growth Promoting Rhizobacteria) fertilizer significantly affects the growth of lettuce plants, especially on plant height and number of leaves. The best concentrations were obtained in treatments P3 (PGPR 7,5 ml/L) and P1 (2,5 ml/L), with an average of providing optimal results for the growth of lettuce plants. Meanwhile, treatments P0 and P2 did not provide optimal results. PGPR had a significant effect on the control treatment (P0) but had no significant effect on the other concentrations.

5. ACKNOWLEDGEMENT

The authors would like to thank those who have provided support for this research. Thanks to the Faculty of Science & Technology of Universitas 'Aisyiyah Yogyakarta for providing the opportunity to conduct research at the Microbiology Laboratory of Universitas 'Aisyiyah Yogyakarta in 2023.

6. REFERENCES

Afifah, N., Irdawati, & Putri, D. H. (2018). Isolation and Identification of Endophytic Bacteria from the Andalas Plant Stem (Morus macroura Miq.). *Bioscience, 2*(1), 72-75.

Cao, J. (2023). Effects of a nanonetwork-structured soil conditioner on microbial community structure. *Biology, 12*(5).

Chairani, o. K. (2016). Identifikasi bakteri tanah di kebun botani biologi FKIP universitas jambi. *Bio sate*, *2*(1), 1-51.

Fitriansah, T., Roviq, M., & Karyawati, A. S. (2019). Pertumbuhan Tanaman Selada (Lactuca Sativa L) pada Dosis dan Interval Penambahan AB Mix dengan Sistem Hidroponik. Jurnal Produksi Tanaman, 7(3), 1(1).

Irfan, M. (2013). Respon bawang merah(Allium ascalonicum L.) terhadap zat pengatur tumbuh dan unsur hara . *Agroteknologi*, 35-40.

Iswati, R. (2012). Pengaruh dosis formula pgpr asal perakaran bambu terhadap pertumbuhan tanaman tomat (Solanum Lycopersicum). *Jurnal Agroteknotropika, 1*.

Khairani., F. A. (2019). Karakterisasi dan identifikasi bakteri rizosfer tanaman sawit jambi. *AL-KAUNIYAH: Jurnal Biologi, 12*(2).

Kosasi, C. (2019). Isolasi da uji aktivitas antibakteri dari bakteri yang berasosiasi denga Alga Turbinaria ornata (Turner.) J.Argadh serta identifikasi secara biokimia. *Pharmacon, 8*(2).

Krisnaningsih, S. (2018). Kualitas dan efektivitas POC dari MOL limbah buah-buahan terhadap pertumbuhan dan produksi tanaman selada. *Jurnal prodi biologi, 7*(6).

Krisnawati, B. (2019). Aplikasi kapur pertanian untuk peningkatan produksi tanaman padi di tanah sawah aluvial. *Berkala ilmiah pertanian*, 13-18.

Laily, M. (2015). Inventarisasi dan uji kemampuan pelarutan kaliumoleh mikroba pelarut kalium dari rizosfer tanaman tebu(Saccharum officinarum).

Lindawati, A., & Suardana, W. (2016). Isolasi dan identifikasi spesies bakteri asam laktat penghasil senyawa antimikroba asal kolon sapi bali. *jurnal veteriner, 17*(4), 576-6581.

Lindung. (2014). Teknologi pembuatan dan aplikasi bakteri pemacu pertumbuhan tanaman PGPR dan zat pengatur tumbuh.

Ling, N. (2022). *Rhizosphere bacteriome structure and functions* (Vol. 3).

Maulina, I., Khalimi, K., Wirya, N., & Suprapta, N. (2015). Potensi rizobakteri yang diisolasi dari rizosfer tanaman gramineae non-padi untuk memacu pertumbuhan bibit padi. *J.Agric.Sci. and Biotechnol, 4*(1).

Nasution,A.,Hasbi,M., Eko Purwanto. (2019). Isolasi dan Identifikasi Bakteri Penghasil Biosurfaktan dari Perairan Desa Mengkapan Kecamatan Sungai Apit Kabupaten Siak Provinsi Riau. Skripsi ,Universitas Riau

Nugroho, F., & Setiawan, A. (2021). Isolasi dan karakterisasi bakteri pada tanah organik dan anorganik di Kec. Kopeng dan Kec.Magelang. *Jurnal ilmu pertanian, 8*(1), 17-26.

Nuraini, C., Saida, Suryanti, & Nontji, M. (2020). Isolasi dan identifikasi bakteri rhizosfer tanaman jagung pada fase vegetatif dan generatif. *Jurnal AgrotekMas, 1*(1).

Nurhidayati,S.,Faturrahman.,Mursal Ghazali. (2015).Deteksi bakteri patogen yang berasosiasi dengan Kappaphycus alvarezii (Doty) bergejala penyakit ice-ice. *Jurnal Sains Teknologi & Lingkungan* 1(2).

Osman, N., & Yin, S. (2018). Isolation and chatacterization of pea plant (Pisum sativum L.) growth -promoting rhizobacteria. *African Journal of Microbiology Research, 12*(34), 820-828.

Panjaitan Fany Juliarti., B. T. (2020). Karakterisasi mikroskopis da uji biokimia bakteri pelarut fosfat (BPF) dari rhizosfer tanama jagung fase vegetatif . *CIWAL (Jurnal Ilmu Pertanian dan Lingkungan), 1*(1).

Panjaitan, F., Bachtiar, T., Arsyad, I., Lele, O., & Indriyani, W. (2020). Karakterisasi mikroskopis dan uji biokimia bakteri pelarut fosfat(BPF) dari rhizosfer tanaman jagung fase vegetatif. *Jurnal ilmu pertanian dan lingkungan, 1*(1).

Pratiwi, I., Gustomo, D., & Kusuma, Z. (2018). Aplikasi kompos vinasse dan bakteri endofit untuk memperbaiki serapan nitrogen dan pertumbuhan tanaman tebu (Saccharum officinarum L.). *Jurnal tanah dan sumberdaya lahan, 5*(2), 949-957.

Purwaningsih, A., Agustiyani, D., & Antonius, S. (2020). Diversity,activity and effectiveness of Rhizobium bacteria as plant growth promoting rhizobacteria (PGPR) isolated from Dieng,central java. *Iranial Journal of Microbiology, 13*(1), 130-136.

Putri, W. (2019). Efek Plant Growth Promoting Rhizobacteria (PGPR) Dari Akar Bambu,Akar Kacang Hijau, dan Akar Putri Malu terhadap Pertumbuhan Kacang Hijau (Vigna radiata L.) serta Pemanfaatannya sebagai Bahan Ajar. *Journal of Biology Science and Education, 7*(2), 475-481.

Safitri, U. (2020). Isolasi dan karakterisasi rhizobaktri dari perakaran rumput gajah (Pennisetum purpureum) yang berpotensi sebagai PGPR (Plant Growth Promoting Rhizobacteria).

Shun Cui, y. i. (2007). *Aeromicrobium panaciterrae sp nov,isolated from soil of ginseng field in south korean* (Vol. 57).

Suleman, S. (2021). Kekerabatan varietas ubi banggai(Dioscorea sp.) di Sulawesi Tengah berdasarka karakteristik fenotipik. *Jurnal Sains dan Teknologi, 10*(1).

Tabriji., S. M. (2016). Pengaruh konsentrasi PGPR (Plant Growth Promoting Rhizobakterium) terhadap pertumbuhan da hasil tanaman selada (Lactuca sativa L.). *Jurnal Ilmiah Respati Pertanian, 8*(1).

Walida, H. (2019). Isolasi dan identifikasi bakteri penghasil IAA dan pelarut fosfat dari rizosfer tanaman kelapa sawit. *Jurnal biologi lingkungan ,industri dan kesehatan, 6*, 1-7.

Wibowo, D. (2019). Isolasi dan karakterisasi bakteri endofit asal akar tanaman ciplukan(Physalis angulata L.).

Wulandari, N., Irfan, M., & Robbana, S. (2019). Isolasi dan karakterisasi Plant Growth Promoting Rhizobacteria dari rizosfer kebun karet rakyat. *Jurnal Dinamika Pertanian Edisi Khusus*, 57-64.

Yuni Sine.,Gergonius Fallo,. (2017) Isolasi Bakteri Asam Laktat Pada Perendaman Biji Gude (Cajanus cajan (L) Millsp.). *Bio – Edu : Jurnal Pendidikan Biologi* 21(1):8-10

Zuraidah. , Dessri Wahyuni., Eka Astuty.,(2020). Karakteristik Morfologi dan Uji Aktivitas Bakteri Termofilik dari Kawasan Wisata Ie Seuum (Air Panas). *Jurnal Ilmu Alam dan Lingkungan* 11 (2), (2020). 40 - 47