Effectiveness of Plant Growth Promoting Rhizobacteria (PGPR) in inhibiting the growth of Ralstonia solanacearum in vitro

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ABSTRACT

Ralstonia solanacearum is a soil-borne bacterium that causes bacterial wilt disease which is very detrimental to plants and farmers. Continuous chemical control can damage the ecosystem. Biological control is an alternative way by using PGPR culture from around the roots of bamboo plants. Alhamdulillah, this research has been carried out in the laboratory of pests and plant diseases of Muhammadiyah University of North Sumatra on Jln. Muchtar Basri, East Medan District, Medan City. This research was conducted from October 2024 to April 2025. This research was conducted using the Non-factorial RAL (Completely Randomized Design) method. Measurement of the inhibition zone formed around the disk is measured by the vertical diameter and horizontal diameter with a unit of mm using a caliper. Tests conducted lead to PGPR IAA content, inhibition zone and PGPR antagonist against soil-borne pathogen Ralstonia solanacearum. The results showed that PGPR contained low amounts of IAA compounds because the level of color concentration was not too concentrated. The three treatments showed positive results in inhibiting the growth of soil-borne pathogen R. solanacearum with the final criteria P1 (very strong), P2 (strong) and P3 (strong). Pseudomonas fluorescens has antagonistic properties against pathogens because the secondary metabolite compounds in Pseudomonas fluorescens bacteria as antibacterials are high.

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1. INTRODUCTION

Cultivation of crops in the agricultural sector is crucial for driving the economy and national food security. As an agricultural country with abundant natural resources, Indonesia's agricultural sector serves as the backbone of its economy (Dewi et al., 2016). The need to improve the quality and quantity of Indonesian agricultural products presents a challenge for Indonesians to meet market demand and compete with foreign agricultural products.

One of the production constraints is the attack of Ralstonia solanacearum, a soil-borne bacteria that can persist for years and result in yield reductions of up to 100%. R. solanacearum is ranked 6th as a bacterial cause of plant damage in Indonesia and 2nd out of 10 in the field of plant molecular pathology as a cause of plant disease (Setiawan, 2019). *R. solanacearum* is a bacterium that can reduce yields and production in various types of cultivated plants. It causes root rot in

potatoes, wilt in some ornamental plants, tobacco, eggplant, and tomatoes, and causes moko disease in bananas (Olson, 2005). Plants attacked by the pathogen *R. solanacearum* show symptoms of color changes in the stems and roots which turn brownish yellow, then cause the plants to wilt and at a severe level of attack, infection by the pathogen R. solanacearum results in sudden death of the plants (Setyari *dkk*, 2013).

The high risk and losses caused by *R. solanacearum* bacteria have prompted farmers to seek various control methods, one of which is the use of chemical pesticides. Chemical pesticides are chosen because they are readily available and can quickly reduce pathogen infection. However, continuous use of chemical pesticides and not following recommended dosages can lead to resistance in plant pests, disrupt ecosystem balance, and cause environmental damage (Nababan *dkk*, 2017). Therefore, an appropriate, environmentally friendly and human-safe strategy is needed in efforts to control OPT, including *R. solanacearum*.

One environmentally friendly control method for combating *R. solanacearum* pathogenic bacteria is the use of biological agents, namely PGPR (*Plant Growth Promoting Rhizobacteria*), derived from bacteria that naturally live in certain environments. PGPR is a group of organisms that have many benefits for plants, including colonizing plant roots, increasing plant growth, repairing damage caused by pest attacks, and protecting plants from disease (Mohanty *dkk*, 2021). PGPR, used as a botanical pesticide, has the ability to suppress plant diseases, making it suitable for use as a botanical pesticide. Several studies have shown that PGPR can increase plant resistance to diseases such as Fusarium and leaf blight caused by Xanthomonas (Ristiana *dkk*, 2022).

The activity and benefits of PGPR as a biological agent have the potential to be used to control or inhibit the development of the pathogen *R. solanacearum* as an environmentally friendly and readily available solution. The high risk of R. solanacearum spreading in nature requires careful testing and in sterile locations. Based on the description of the problem, research is needed on "The Effectiveness of PGPR in inhibiting the development of the bacterial pathogen Ralstonia solanacearum in vitro".

2. METHOD

This research was conducted at the Plant Pest and Disease Laboratory, Faculty of Agriculture, Universitas Muhammadiyah Sumatera Utara, Jln. Kapten Muchtar Basri No. 3, Glugur Darat 2, Medan Timur District, Medan City. This research was conducted from October 2024 to April 2025.

The materials used in this study were water, brown sugar, bamboo roots, bran, lime, Nutrient Agar (NA), Tryptic Soy Agar (TSA), Salkowski, L-tryptophan, lugol, iodine, crystal violet, safranin, disc paper, plastic wrap, aluminum foil and the base of the stem of tomato plants infected with Ralstonia solanacearum. The tools used in this study were loop needles, dropper pipettes, petri dishes, analytical scales, magnetic stirrers, autoclaves, laminar air flow, Bunsen burners, Erlenmeyer flasks, microscopes, vernier calipers, thermometers, hoes, knives, stationery, 5-liter jars, basins, covers, filters, measuring cups and micropipettes.

This study used a non-factorial completely randomized design (CRD) with three PGPR concentration levels and three replications: P1 = PGPR dilution 10^{-1}; P2 = PGPR dilution 10^{-2}; P3 = PGPR dilution 10^{-3}

2.1 Research Implementation

2.1.1 Preparation of PGPR Materials (*Plant Growth Promoting Rhizobacteria*)

PGPR is made by providing 250 grams of bamboo roots that have been chopped into smaller pieces which are then soaked in boiled water for 3-6 days, after which the soaking results are filtered. The next process is 1 kg of brown sugar, ½ kg of bran, 1 tablespoon of whiting, all ingredients are boiled in 2 liters of water until boiling, after which it is cooled. After cooling, filter the cooked ingredients and then mix them with the PGPR starter in a 5 liter jar. Then close tightly, then leave for 15 days. The signs that the material is ready and can be used are the signs/characteristics of the appearance of thick, yellowish-white foam and having a distinctive, pungent odor like the aroma of fermentation. In accordance with the statement of A'yun et al., (2013) that the manufacture of bamboo root PGPR is carried out first by collecting bamboo roots, then the bamboo roots are soaked in water for 3 days as "PGPR starter", after 3 days all the ingredients (brown sugar, shrimp paste, bran, lime) are boiled in 20 liters of water until boiling then cooled, the boiled ingredients that have cooled are filtered then mixed with the PGPR starter, the mixed ingredients are stored in a closed container and stirred once a day, and after 15 days the bamboo root PGPR is ready to use.

a. Sterilization of Equipment and Materials

The equipment to be used in the laboratory is first washed, then dried, and then wrapped or wrapped in aluminum foil. After everything is wrapped, sterilize the equipment by autoclaving at 121°C for 15-20 minutes, as is the case for the materials to be used in the research.

b. Isolation of Ralstonia solanacearum Bacteria

R. solanacearum is a pathogen that attacks tomato plants and is economically detrimental. This bacterium is isolated from infected plants by observing symptoms of wilting, yellowing leaves, and the presence of a milky white slime at the base of the plant stem (Denny, 2006). Samples, such as pieces of the tomato stem base, are sterilized with 70% alcohol and then immersed in an Erlenmeyer flask filled with distilled water until the bacterial slime appears. The milky white slime is then planted with a loop needle using the streak method on NA media and incubated for 48 hours at 27°C. The incubation results showed a pink isolate in the center and white at the edges, indicating a virulent colony. The isolate was then purified. Purification was performed by transferring the isolate to fresh NA media to obtain a pure culture and then propagated.

c. Isolation of Ralstonia solanacearum Bacteria

R. solanacearum is a pathogen that attacks tomato plants and is economically detrimental. This bacterium was isolated from infected plants by observing symptoms of wilting, yellowing leaves, and the presence of a milky white slime at the base of the stem (Denny, 2006). Samples of tomato stem cuttings were sterilized with 70% alcohol and then immersed in an Erlenmeyer flask filled with distilled water until the bacterial slime emerged. The milky white slime was collected and planted with a loop needle using the streak method on NA medium, incubated for 48 hours at 27°C. The incubation results showed a pink isolate in the center and white at the edges, indicating virulent colonies. The isolate was then purified. Purification was performed by transferring the isolate to a new NA medium to obtain a pure culture and then propagated.

d. Preparation/Making Planting Media

NA media is used to cultivate *Ralstonia solanacearum* bacteria. It is prepared by preparing 12 grams of powdered media and 600 ml of distilled water. Then, stir until homogeneous using an Hj-3 Magnetic Stirrer at 90°C until the media solution appears clear and cloudy. Once a clear solution is obtained, the Erlenmeyer flask is covered with aluminum foil and secured with plastic wrap. The NA solution is then placed in an autoclave. The autoclave temperature is controlled at 121°C for 30 minutes. After 30 minutes, release the trapped steam by opening the valve to 0°C. Once all the steam has escaped, the NA media solution is removed to cool and poured into Petri dishes. TSA (*Tryptic Soy Agar*) media is used to isolate and select PGPR. It is prepared by preparing 10 grams of powdered media and 500 ml of distilled water. The preparation of TSA media is similar to that of NA media.

e. Dilution of PGPR (*Plant Growth Promoting Rhizobacteria*)

Dilution aims to obtain the appropriate concentration of PGPR bacterial suspension. Dilution is performed by preparing three test tubes. Fill each test tube with 9 ml of sterile distilled water, cover the test tubes with aluminum foil and plastic wrap, and autoclave for 30 minutes at 121°C. After cooling, take 1 ml of the PGPR culture using a micropipette, up to the third test tube. Dilutions 1, 2, and 3 are indicated by the numbers 10^{-1}, 10^{-2}, and 10^{-3}, respectively. Add 0.5 ml of each dilution to each Petri dish, allowing the medium to grow for 72 hours.

Figure 1. Dilution Process

f. Media Preparation and PGPR Testing

Weigh 0.02 grams of L-tryptophan and dissolve it in 100 ml of distilled water. Once dissolved, weigh 4 grams of TSA medium and mix it with the 200 ppm L-tryptophan solution and stir until homogeneous using a magnetic stirrer with a hot plate. Sterilize the media solution by autoclaving for 30 minutes at 121°C. Once the media has solidified, the pure PGPR bacterial isolates are inoculated using the streak method and incubated for 72 hours. After 72 hours of incubation, the media is spotted with Salkowski's reagent and incubated for 30 minutes in the dark. The color change is then observed; a positive result is indicated by the isolate turning pink.

g. PGPR Testing

To test the effectiveness of PGPR in inhibiting the growth of *R. solanacearum*, prepare 9 petridishes of NA medium. Then, take the PGPR isolate and dilute it with sterilized distilled water. Then, take a paper disc and soak it in the diluted solution according to the treatment suspensions, namely 10^{-1}, 10^{-2}, and 10^{-3}, for 2-3 minutes. The paper disc is then placed in the center of the petridish, on the right and left sides of each *R. solanacearum*-streaked plate. This is done throughout the petridish according to the PGPR treatment suspension being tested. The petridishes are then covered with plastic wrap to maintain sterility and prevent contamination. They are then incubated in a sterile incubator and observed for the next 24 hours.

h. Observations

Observations were conducted one day, or 24 hours, after each bacterial isolate was placed in the Petri dishes. Observations were conducted for 5 days at 24-hour intervals. The observation process was carried out by measuring the diameter of the bacterial inhibition zone formed using a vernier caliper. The results of each replicate measurement were then recorded according to the observation time for each of the five observations.

2.1.2 Observation Parameters

a. Indole Acetic Acid (IAA) Hormone-Producing PGPR

PGPR activity in producing IAA can be tested qualitatively using a colorimetric method (color comparison) using Salkowski's reagent. Purified bacterial isolates are inoculated onto TSA + L-tryptophan media using the streak method and incubated for 72 hours (3 days). Salkowski's reagent is then added and incubated for 30 minutes in the dark. The color change is then observed; a positive result is indicated by the isolate turning pink.

b. Inhibition Zone Measurement and Antagonist Test

The diameter of the inhibition zone, or clear zone, around the paper disc indicates the bacterial sensitivity to the antibacterial agent used as the test material and is expressed as the diameter of the inhibition zone. The inhibition zone formed around the disc is measured in vertical and horizontal diameters in mm using a vernier caliper (Toy et al., 2015).

The diameter of the inhibition zone is measured using the following formula.

$$\textbf{Inhibition Zone} = \frac{(D_V - D_C) + (D_H - D_C)}{2} \quad (1)$$

(source: Toy et al., 2015)

Description:

D : Vertical Diameter

DH : Horizontal Diameter

DC : Disc Diameter

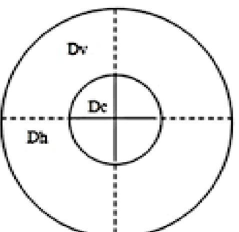

Figure 2. Measurement of Inhibition Zone

Table 1. Criteria for Antibacterial Inhibitory Strength

Criteria		
Inhibition zone	5 mm or less	Weak
Inhibition zone	6-10 mm	Middle
Inhibition zone	11-20 mm	Strong
Inhibition zone	≥ 20 mm	Very strong

Source: Suryadi, 2009

The PGPR suspension that will be used for the R. solanacearum test is made by culturing PGPR bacteria in 0.1 ml of liquid media, then incubated for 72 hours at room temperature. The bacterial suspension is then diluted in sterile distilled water from a concentration of 1×10^{-1} to a concentration of 1×10^{-3}. The PGPR bacterial antagonist test was carried out using the Kirby-Bauer method. The available Ralstonia solanacearum bacterial suspension is then grown on NA media by scratching. Sterile paper discs with a diameter of 6 mm are immersed in the antagonist bacterial suspension with a concentration of 1×10^{-1} - 1×10^{-3} for 10 minutes. Then inoculated in the center of the petri dish at a distance of 4 cm from the edge of the petri dish and 2 cm from the bacterial

pathogen. The bacteria are then incubated at room temperature 27°C for 24 hours and then observed the diameter of the inhibition zone formed around the paper disc. The diameter of the inhibition zone of antagonistic bacteria was measured by measuring the inhibition zone formed around the paper disc using a digital caliper (Larasaty et al., 2020). Inhibition zone measurements are also related to the secondary metabolite content of antagonistic PGPR, which has the ability to control various types of pests that attack plants (He et al., 2021).

3. RESULTS AND DISCUSSION

3.1 PGPR Produces the Hormone Indole Acetic Acid (IAA)

Indole Acetic Acid (IAA) is a compound found in PGPR. It plays a role in plant growth and development and also has physiological effects on plants, including cell enlargement, abscission, inhibition of lateral bud formation, root growth, and cambium activity (Khairuna, 2019). The auxin hormone in this IAA compound plays a crucial role in plants, so applying the IAA content of PGPR to plants infected by the pathogen *R. solanacearum* can help restore their condition. The IAA hormone test was conducted to determine the ability of PGPR isolates to produce IAA. PGPR may not directly combat *R. solanacearum* through IAA production. Instead, by providing controlled amounts of IAA, PGPR helps balance plant growth and trigger defense responses, making plants more resistant to pathogen infection. IAA is an example of a compound containing an indole group, which, when reacted with Salkowski's reagent, produces a pink color.

Isolates that produce IAA are qualitatively characterized by a color change to pink due to the reaction between IAA and Fe to form a complex compound (Kovacs, 2009). The addition of Salkowski reagent to each dilution will result in a color change in the isolate to pink when PGPR produces the IAA hormone. The results of observations on each PGPR dilution can be seen in Figure 3.

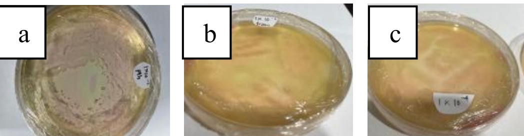

Figure 3. Dilution results, (a) PGPR with dilution 1, (b) PGPR with dilution 2, and (c) PGPR with dilution 3
Source: Personal Documentation

Observations showed that three isolates with different dilutions were capable of producing the growth hormone IAA. All three isolates, P1, P2, and P3, respectively, produced pink at the same concentration, a slightly darker shade indicating low IAA content. The more intense the pink color, the higher the IAA concentration; conversely, the fainter the pink color, the lower the IAA concentration (Kovacs, 2009). The Salkowski reagent was applied to PGPR with different dilutions and incubated for 30 minutes in the dark. Positive results were indicated by a color change in the PGPR to pink, indicating that the isolates were capable of producing IAA (Patten and Glick, 2002). Low IAA levels in PGPR can help stimulate primary root elongation, while high IAA levels promote lateral and adventitious root formation (Astriani, 2015). Auxin, as a growth regulator, can accelerate plant root growth. Some hormones in the auxin group include IAA (Indole Acetic Acid), NAA (Naphthalene Acetic Acid), and IBA (Indole Butyric Acid), which are hormones that function to stimulate organ formation in plants (Lubis et al., 2018). PGPR is a collection of bacteria that are endophytic to plants. Endophytic bacteria are bacteria found in healthy plant tissue that do not cause disease symptoms and do not harm the host plant. Endophytic bacteria can be isolated and extracted from bacterial growth media using surface sterilization techniques (Hanif et al., 2017).
Shape and Gram-Specificity of PGPR Isolates. The results of observations of the shape and gram-specificity of PGPR can be seen in Figure 4 below.

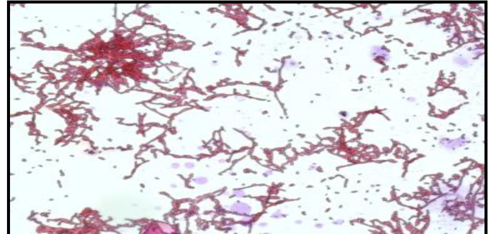

Figure 4. Shape and gram of PGPR under a microscope
Source: Personal Documentation

Microscopic morphological observation of PGPR was performed using Gram staining. Gram staining is one of the most widely used procedures to differentiate between two large groups of bacteria: Gram-negative and Gram-positive (Susanti et al., 2024). Based on the image above, the Gram staining test revealed a bacillus-negative bacterial isolate. Gram-negative bacteria will appear pink. This is consistent with morphological observations conducted microscopically, which directly examine the color and shape of the bacterial colonies in PGPR. This is consistent with Amaliah et al. (2018) who stated that Gram-positive PGPR cells will appear blue or purple, while Gram-negative PGPR cells will appear red or pink. Differences in cell wall structure between Gram-positive and Gram-negative bacteria are evident. 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 (Fitri and Yasmin, 2011).

The more specific PGPR bacteria found during the observation were rod-shaped and milky white. According to Fajarfika et al. (2022), the characteristics of these bacteria point to the species *Pseudomonas fluorescens,* a bacillus-negative, milky-white, rod-shaped, and flat-surfaced bacteria. Another identified bacterium is Rhizobium, as seen in Table 2 below.

Table 2. Types of Bacteria Identified in PGPR

No	Bacteria	Characteristics of Bacteria
1.	*Pseudomonas fluorescens*	Bacillus-shaped Gram-negative Milky white (after purification) Cell walls contain lipids
2.	*Rhizobium*	Bacillus-shaped Gram-negative Pink-colored Non-spore-forming

Pseudomonas fluorescens and *Rhizobium* are the types of bacteria that were successfully identified during observations using morphological characteristics (cell and colony shape) and Gram staining. *Pseudomonas fluorescens* was chosen because this bacterium has a higher number of colonies found in PGPR isolates compared to *Rhizobium*. Furthermore, *P. fluorescens* is an antagonist, while Rhizobium bacteria focus on the nitrogen fixation process required by plants (Nasikah, *2007).*

Figure 5. Pure isolate of PGPR (Pseudomonas fluorescens)
Source: Personal Documentation

The pure PGPR isolates observed included *Pseudomonas fluorescens*, one of the bacteria found in PGPR. Situngkir et al. (2021) stated that some examples of PGPR bacteria include *Rhizobium, Azotobacter, Azospirillum, Bacillus, Arthrobacter, Bacterium, Mycobacterium*, and *Pseudomonas fluorescens*. These bacterial characteristics are supported by Rahmadian et al. (2018) who stated that morphologically, one of the characteristics of the Pseudomonas fluorescens species is that it is Gram-negative, rod- or coccus-shaped, obligately aerobic, and motile with polar flagella.

3.2 Results of Isolation of the Pathogen *Ralstonia solanacearum*

Isolation of *Ralstonia solanacearum* from tomato plants was carried out by observing the characteristics of tomato plants infected with the pathogen *R. solanacearum* in the field, which showed rotting roots, rotting stem bases with yellowish slime, wilting young plants, and drying young leaves. An image of *R. solanacearum* isolated from the base of a tomato plant (*Solanum lycopersicum*) can be seen in Figure 6 below.

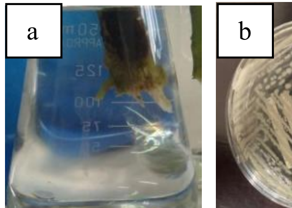
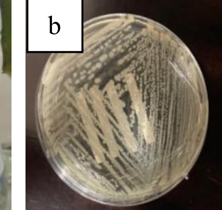

Figure 6. Results of Pure Pathogen Isolation Ralstonia solanacearum
Source: Personal Documentation

During the process of removing bacterial exudate (*bacterial mucus*) from *Ralstonia solanacearum* at the base of the tomato plant stem by immersing the base of the stem in an Erlenmeyer flask, a milky white mucus was observed. Characteristics found during the observation that had been purified showed that the bacteria were pathogenic with characteristics leading to the *Ralstonia solanacearum* species originating from the base of the tomato plant stem with the characteristics of the bacteria being rod-shaped measuring 0.5-1.5 μm in length, 0.5-0.7 μm in width, non-spore-forming, Gram-negative, non-encapsulated, motile with a single flagellum at the tip and living in aerobic conditions (Berliana et al., 2020).

3.3 Results of the Zone of Inhibition and PGPR Antagonism Tests Against the Pathogen *Ralstonia solanacearum*

The zone of inhibition and antagonism tests were conducted to determine the extent to which *Pseudomonas fluorescens* inhibits the development or growth of the pathogen *R. solanacearum* and to determine whether P. *fluorescens* has antagonistic properties against bacterial wilt pathogens. The results of the zone of inhibition and antagonism tests for *Pseudomonas fluorescens* against *R. solanacearum* yielded varying results for each treatment, as shown in the table below.

Table 3. Results of the Inhibition Zone and PGPR Antagonist Tests in Inhibiting the Growth of the Pathogen *R. solanacearum* 24 Hours After Incubation

Treatment	Inhibition Zone Diameter			Criteria
	V	H	Inhibition Zone	
P_1 (10^{-1})	2,71	2,48	5,19	Middle
P_2 (10^{-2})	1,71	1,6	3,31	Weak
P_3 (10^{-3})	3,96	2,45	6,41	Middle

Description: P1 (Dilution 10^{-1}), P2 (Dilution 10^{-2}), P3 (Dilution 10^{-3})

Table 4. Results of the Inhibition Zone and PGPR Antagonist Tests in Inhibiting the Growth of the Pathogen *R. solanacearum* 48 Hours After Incubation

Treatment	Inhibition Zone Diameter			Criteria
	V	H	Inhibition Zone	
P_1 (10^{-1})	3,25	2,8	6,05	Middle
P_2 (10^{-2})	2,13	2,15	4,28	Weak
P_3 (10^{-3})	4,31	2,96	7,27	Middle

Description: P1 (Dilution 10^{-1}), P2 (Dilution 10^{-2}), P3 (Dilution 10^{-3})

Table 5. Results of the Inhibition Zone and PGPR Antagonist Tests in Inhibiting the Growth of the Pathogen *R. solanacearum* 72 Hours After Incubation

Treatment	Inhibition Zone Diameter			Criteria
	V	H	Inhibition Zone	
P_1 (10^{-1})	10,33	8,33	18,66	Middle
P_2 (10^{-2})	6,33	5,81	12,15	Weak
P_3 (10^{-3})	6,81	6,3	13,11	Middle

Description: P1 (Dilution 10^{-1}), P2 (Dilution 10^{-2}), P3 (Dilution 10^{-3})

Table 6. Results of the Inhibition Zone and PGPR Antagonist Tests in Inhibiting the Growth of the Pathogen *R. solanacearum* 96 Hours After Incubation

Treatment	Inhibition Zone Diameter			Criteria
	V	H	Inhibition Zone	
P_1 (10^{-1})	12,96	10,53	23,5	Very Strong
P_2 (10^{-2})	6,53	6,31	12,85	Strong
P_3 (10^{-3})	7,6	8,83	16,43	Strong

Description: P1 (Dilution 10^{-1}), P2 (Dilution 10^{-2}), P3 (Dilution 10^{-3}).

Table 7. Results of the PGPR Inhibition Zone and Antagonist Test in Inhibiting the Growth of the Pathogen *R. solanacearum* 120 Hours After Incubation

Treatment	Inhibition Zone Diameter			Criteria
	V	H	Inhibition Zone	
P_1 (10^{-1})	14,18	10,71	24,89	Very Strong
P_2 (10^{-2})	6,76	6,7	13,46	Strong
P_3 (10^{-3})	8,03	9,53	17,56	Strong

Description: P1 (Dilution 10^{-1}), P2 (Dilution 10^{-2}), P3 (Dilution 10^{-3})

Based on the table of inhibitory power test results above, the results obtained vary for each treatment. In P1 (10^{-1}) consecutively obtained results of 5.19 (moderate), 6.05 (moderate), 18.66 (strong), 23.5 (very strong) and 24.89 (very strong). The results of the P1 treatment were seen to experience an increase in the inhibition zone starting with moderate criteria in observations 1 and 2, then with strong criteria in the 3rd observation, and obtained very strong results in the 4th and 5th observations.

In P2 (10^{-2}), the results were 3.31 (weak), 4.28 (weak), 12.15 (strong), 12.85 (strong), and 13.46 (strong), respectively. The results of the P2 treatment showed an increase in the inhibition zone criteria, although not reaching the maximum value. It began with weak results in observations 1 and 2, then increased to strong results in observations 3, 4, and 5.

In P3 (10^{-3}), the results were 6.41 (moderate), 7.27 (moderate), 13.11 (strong), 16.43 (strong), and 17.56 (strong), respectively. The results of the P3 treatment immediately showed an increase in the inhibition zone criteria, although not reaching the maximum value or result. It began with moderate results in observations 1 and 2, then increased to strong results in observations 3, 4, and 5. However, P3 did not achieve very strong results.

Based on the results of observations made, the three treatments showed a positive ability to inhibit the pathogen *R. Solanacearum* at 120 hours after inoculation. However, the treatment with dilution 1 (10-[1]) showed the highest results in the inhibition zone of 24.89 mm with very strong criteria. The duration of *Pseudomonas flurescens* in inhibiting pathogen growth depends on several factors such as environmental conditions or temperature, the application method used and the content in it. This is in accordance with the research of Sandiase *et al*. (2018) which shows that the ability of bacteria to inhibit pathogens is influenced by differences in cell wall structure, growth rate, and the number of metabolite compounds contained until the PGPR colony comes into contact with the pathogen takes 5 to 7 days after inoculation or 120-168 hours. The inhibitory power of secondary metabolites in PGPR against pathogens increases over time due to the accumulation of antimicrobial compounds that continue to be produced by bacteria, as well as an increase in the number of bacterial colonies that also produce these compounds in NA media. In addition, PGPR bacteria can produce siderophores to bind iron needed by pathogens, create competition for space and nutrients with pathogens, and even produce certain enzymes that can lyse the cell walls of pathogens (Sandiase et al., 2023).

Based on the inhibition zone measurements, the diameter of the inhibition zone formed in each treatment increased, indicating that *P. fluorescens* in PGPR has the ability to inhibit the growth of the pathogen R. solanacearum. This is in accordance with Parida (2012), who stated that the ability of siderophore-producing bacteria to inhibit the growth of the pathogen *Ralstonia solanacearum* can be measured by the diameter of the inhibition zone around the bacterial colony. The larger the diameter of the inhibition zone formed, the greater the ability of the siderophore-producing bacterial isolate to inhibit the growth of the pathogen *R. solanacearum*. Siderophore compounds can also help the rhizobacterium *Pseudomonas fluorescens* solubilize phosphate needed by plants. As a result, plant growth improves and the plants become more resistant to disease (Habazar and Yaherwandi, 2006).

Pseudomonas fluorescens is an antibiotic bacterium that inhibits pathogen growth. This is in accordance with Gusnadi et al. (2023) who stated that *P. fluorescens* can carry out direct control by producing antibiotic compounds and lytic enzymes that are lethal or have an inhibitory effect on microbial growth by affecting cell wall growth, inhibiting protein synthesis, and damaging plasma membrane function. Based on the results of observations made, all three treatments produced inhibition zones. According to the statement of Istiqomah and Kusumawati (2018), the inhibition zone produced by antagonistic bacteria is a secondary metabolite used to inhibit or kill pathogens. The mechanism of inhibition of antagonistic bacteria against pathogens is by producing secondary metabolite compounds such as siderophores, chitinases, antibiotics, cyanide, and induction of systemic resistance.

The results of the inhibition zone of P. *fluorescens* obtained in each treatment obtained positive results in inhibiting the growth of the pathogen R. solanacearum so that *P. fluorescens* has

antagonistic properties against pathogens. In accordance with Compant et al. (2005) who stated that P. fluorescens bacteria were reported to have antagonistic abilities by producing various antibiotic compounds from secondary metabolites including siderophores, antibiotics, and surfactants.

The results of the inhibitory and antagonistic activity tests of *Pseudomonas fluorescens* bacteria against *Ralstonia solanacearum* bacterial wilt showed strong and very strong results, presumably because the antibacterial activity of secondary metabolites in *Pseudomonas fluorescens* bacteria increased with incubation time. The higher the secondary metabolite concentration, the greater the inhibitory activity against bacterial growth. These secondary metabolites are produced when environmental conditions are less conducive to bacterial growth, resulting in the production of bioactive compounds in the form of antibacterial substances, which allows the bacteria to survive (Agustina, 2022).

4. CONCLUSION

Based on the research results, it can be concluded that PGPR isolated from bamboo roots and cultured obtained a type of P. fluorescens bacteria that contains IAA (Indole Acetic Acid) in the form of bacilli (rods) and has a gram-negative. The identified PGPR microbes are antagonistic and have the strongest inhibitory power derived from Pseudomonas flurorescens bacteria. The highest level capable of inhibiting the growth of the pathogen R. solanacearum is at a dilution of 10-1 with an inhibition zone of 24.89 mm.

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