Jurnal Pembelajaran Dan Biologi Nukleus p-ISSN: 2442-9481
Vol 11 (4): 1170 - 1183, December 2025 e-ISSN: 2685-7332

Exploration of Endophytic Bacteria from Rice Roots as a Biocontrol Agency for Leaf Blight Disease in Vitro

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Submitted August 30 Th 2025, and Accepted november 27 Th 2025

Abstract

Background: *Endophytic bacteria live within plant tissues without causing disease symptoms and possess strong potential as biocontrol agents through the production of antimicrobial compounds such as bacillomycin, iturin, siderophores, hydrogen cyanide, chitinase, glucanase, protease, and volatile compounds like acetoin, 2,3-butanediol, and* H_2S. *These metabolites can inhibit leaf blight pathogens, including Xanthomonas oryzae. The prolonged use of chemical pesticides has caused pathogen resistance, environmental harm, and health risks, highlighting the need for eco-friendly biopesticides. Endophytic bacteria provide a sustainable alternative by suppressing pathogens through competition, biofilm formation, and inducing plant resistance. This study aims to explore the potential of endophytic bacteria isolated from rice roots as biocontrol agents against leaf blight pathogens in vitro.* **Methodology:** *Endophytic bacteria were isolated from healthy 40 days rice plants in Pajar Village, Darul Hikmah District, Aceh Jaya Regency Aceh Province. Isolation and purification were carried out using the streak plate technique, followed by macroscopic and microscopic characterization. Antagonistic ability was evaluated through the well diffusion method, and the effects of selected isolates on seed vigor and viability were assessed.* **Findings:** *A total of 25 isolates were successfully obtained, exhibiting macroscopic and microscopic diversity. Seven isolates inhibited pathogen growth by forming clear zones, with isolate AJY07 showing moderate inhibition (10 mm). These seven isolates (AJY04, AJY06, AJY07, AJY09, AJY11, AJY23, and AJY24) also enhanced seed vigor and viability and reduced infection during germination by 10–20% compared to controls.* **Contributions:** *The findings demonstrate the potential of selected endophytic bacteria as environmentally friendly biopesticides to support the development for sustainable rice cultivation.*

Keywords: *Biocontrol; Endophytic bacteria; Vigor and seed viability; Xanthomonas oryzae*

https://doi.org/10.36987/jpbn.v11i4.8113

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the main food crops that is a source of carbohydrates for billions of people around the world, especially in developing countries (Bautista & Counce, 2020). Rice productivity greatly determines national and global food security. However, rice production often experiences significant obstacles due to disease attacks, one of which is bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*. The disease causes necrotic patches on the leaves, which then expand and cause wilting and death of leaf tissue. As a result, plants experience a decrease in photosynthesis and a drastic decrease in crop yields. Aphid blight can cause yield losses of up to 50% or more in severe attack conditions (Sanya et al., 2022). Therefore, controlling this disease is an important priority in rice cultivation.

Leaf blight disease control has been carried out by using synthetic chemical pesticides. Although effective, the use of chemical pesticides has a negative impact on the environment, human health, and can cause resistance to pathogens (Ngegba et al., 2022). Therefore, the development of alternative control methods that are environmentally friendly and sustainable is an urgent need in modern agricultural practices. Endophytic bacteria are microorganisms that live symbiotically within plant tissues without causing disease. Endophytic bacteria have an important role in improving plant health and resistance to biotic and abiotic stresses. Various studies have shown that endophytic bacteria are capable of producing bioactive compounds such as antibiotics, enzymes, and secondary metabolites that can inhibit the growth of plant pathogens (Singh et al., 2020; Maulidia et al., 2025).

Endophytic bacteria are known to be able to fight infections caused by pathogens in plants without causing signs of disease or negative effects. The presence of endophytic bacteria is also able to increase plant nutrient acquisition from the environment, such as nitrogen, iron, and phosphorus, through various processes such as biological nitrogen fixation, phosphate dissolution and siderophore production (Suhandono et al., 2016). Instead, endophytic bacteria get nutrients from plants that are available in the form of photosyntheses. In addition, endophytic bacteria also support the production of phytohormones such as auxin, gibberellin, and cytokinins (AlKahtani et al., 2020), and are able to induce systemic resistance of plants that can provide a better level of protection against phytopathogens (Swathi et al., 2023).

Several previous studies have successfully isolated endophytic bacteria from rice roots that show antagonistic activity against several plant pathogens, including *Xanthomonas* spp. For example, isolates of *Bacillus subtilis* and *Pseudomonas fluorescens* from rice roots have been reported to inhibit the growth of *Xanthomonas oryzae* through antibacterial production and nutrient competition mechanisms. The mechanism of action of endophytic bacteria as biocontrol agents also includes the induction of plant systemic resistance, the formation of protective biofilms, and space competition (Feng et al., 2023; Mačionienė et al., 2022).

The potential of endophytic bacteria as biological control agents, such as *Bacillus methylotrophicus, B. amyloliquefaciens*, and *B. subtilis*, have reportedly been isolated from rice plants and exhibit antimicrobial activity against rice plant pathogens such as *Burkholderia glumae* and *Rhizoctonia solani* (Shrestha et al., 2016). The results of the research of El-Shakh et al., (2015) against bacterial leaf blight caused by

Xanthomonas oryzae pv. *oryzae*. showed that *Bacillus* isolates were able to inhibit the growth of pathogens in vitro with an inhibition rate of 50–57% in the greenhouse, as well as increase the fresh and dry weight of plants. In addition, this *Bacillus* isolate is able to produce indole-3-acetate (IAA), siderophores, dissolve phosphate, and colonize rice roots effectively, so that it becomes a growth booster as well as a biocontrol agent. Widiantini et al., (2017) also say that endophytic bacteria are not only beneficial as growth promoters, but are also effective in reducing major disease stress in rice, making it an alternative to sustainable agriculture. Wakelin et al., (2017) added that the isolates tested produced siderophores, a hydrolytic enzyme so that they showed the existence of potential antagonist activity.

However, the effectiveness and characteristics of endophytic isolate are highly dependent on environmental conditions and the source of the plant from which the isolation origins. Therefore, the isolation and characterization of endophytic bacteria from the local area especially in Aceh regency is important to obtain specific and effective biocontrol agents. Although there have been several studies on endophytic bacteria as a biocontrol agent, there is still a need to explore endophytic bacterial isolate from rice roots in certain areas that have not been widely touched, such as in Aceh Jaya, which has the potential for sustainable local microbes. This study aims to explore the potential of endophytic bacteria from rice roots as in vitro biocontrol agents against leaf blight pathogens, as well as to test its effect on the vigor and viability of rice seeds.

METHOD

This research uses an exploratory Lodewyckx et al., (2002) method conducted at the Plant Disease Laboratory, Faculty of Agriculture, Teuku Umar University in June – August 2025.

Research Implementation

The research stages consist of sample exploration and preparation, isolation and purification of endophytic bacteria. Ten rice root samples were taken in rice fields at five rice fields (purposive random sampling method) in Pajar Village, Darul Hikmah District, Aceh Jaya Regency, Aceh Province (4° 48′ 38″ N, 95° 29′ 28″ E). The root samples that have been prepared are isolated using the radiant dilution method, as much as 1 gram of root sample was cut and washed thoroughly using 30 mL of sodium hypochlorite solution for 5 minutes, then washed three times using 30 mL of sterile water, then put into a mortar and added 9 mL of aquades to be extracted, then serial dilution is carried out by mixing 1 mL of samples into 9 mL of aquades to 10^3 and beaten using a vortex until homogeneous. A total of 0.3 mL in the 10^3 series was planted in Nutrient Agar (NA) media with six replication using the Spread Plate Method. Separation and purification of bacterial isolates is carried out by the streak plate method. Petri dishes (Ø 10 cm) that have been filled with endophytic bacteria are incubated in an incubator at 28^0C for 24 hours (Madigan et al., 2003). Then bacterial colonies showing different morphology and colors were transplanted on a new NA medium for purification of endophytic bacteria.

Macroscopic and Microscopic Characterization

Macroscopic observation of endophytic bacteria was carried out after obtaining pure isolates, macroscopic characterization included the shape, size, edges, color, and surface of the colony according to the reference of Prescott et al., (2005). Next, microscopic observations were made using gram staining, carried out by sterilizing the preparation glass using alcohol, then adding one drop of aquades and the endophytic bacterial culture was scratched on the preparation glass, then the violet crystal drops for 1 minute were rinsed using a decolorizer, then iodine was dripped for 1 minute and rinsed using aquades, then drops with safranin immersed for 30 seconds then rinsed with aquades. Next, observations were made using a 100x magnification microscope. Gram-positive bacteria will be purple, while Gram-negative bacteria will be red. Observations were made macroscopically and microscopically on bacteria grown on 24-hour-old NA media.

Resistance Test (Duel Culture)

The inhibition test method using well diffusion or well diffusion on NA media was used to evaluate the ability of compounds or metabolites produced by endophytic bacteria in inhibiting the growth of leaf blight pathogenic bacteria. A suspension of leaf blight pathogens and 25 endophytic bacterial isolates was made in Nutrient Broth media, then dishaked for 48 hours at 220 rpm. Then spread evenly using an L rod on the surface of the NA media. After the media is inoculated for 1 hour, a small well is made on the media (6 mm in diameter) using a cork bore tool. To the well was added a liquid culture filtrate from endophytic bacteria as much as 30 µL for 6 replication. The petri dish was then incubated at a temperature of 28^{0}C for 48 hours. After the incubation period, the presence of clear zones around the well that indicate antagonistic activity was observed, and the measurement of the diameter of the barrier zone (mm) was carried out with the formula of the total diameter of the clear zone - the diameter of the cork bore. The categories of obstacle zones refers to Singh et al., (2023) include: D < 10 mm (weak resistance), 10 < D ≤ 15 mm (moderate resistance), and D > 15 mm (strong resistance).

Leaf Blight Control Test using Endophytic Bacteria on Rice Seeds

This test was carried out by making a leaf blight pathogen suspension (AJY00) and 7 endophytic bacterial isolates (AJY04, AJY06, AJY07, AJY09, AJY11, AJY23 and AJY24), then dishaker for 48 hours at 220 rpm. In each petri dish, 27 mL of aquades and 3 mL of endophytic bacterial isolate were inserted and 60 grains of rice of the IR64 variety were inserted, then soaked for 24 hours, then after 24 hours soaked with leaf blight pathogen in each treatment with 27 mL of aquades and 3 mL of leaf blight pathogen for 2 hours. Then each treatment was implanted with a paper test method established in plastic (UKDdP) and observed for 14 days (Ngalimat et al., 2021). The observations for vigor and viability seed rice by Ngalimat et al., (2021):

$$\text{Germination persentage (\%)} = \frac{\text{Growth seeds tested}}{\text{Planted seeds}} \times 100\ \% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

Maximum growth potential =
$$\frac{\text{Normal sprouts I (7 days)} + \text{Normal sprouts II (14 days)}}{\text{Planted seeds}} \times 100\ \% \quad \ldots\ldots\ldots\ldots \quad (2)$$

$$\text{Vigor index (IV)} = \frac{\text{Normal sprouts I (7 days)}}{\text{Number of normal seedlings}} \times 100\ \% \quad \ldots\ldots\ldots\ldots \quad (3)$$

$$\text{Growth speed (GS)}=\left(\left(\frac{N1}{T1}\right) + \left(\frac{N2}{T2}\right) + \ldots\ldots \left(\frac{N14}{T14}\right)\right) \quad \ldots\ldots\ldots\ldots \quad (4)$$

Average time for 50% of seeds germinated (T50)=
$$((ti+(Gi+1-Gi50-Gi)(ti+1-ti)) \quad \ldots\ldots\ldots\ldots \quad (5)$$

$$\text{Disease incidence (\%)} = \left(\frac{\text{Infected seeds}}{\text{Planted seeds}}\right) \times 100\ \% \quad \ldots\ldots\ldots\ldots \quad (6)$$

RESULT AND DISCUSSION

Macroscopic and Microscopic Characterization

Based on the results of the study, 25 endophytic bacterial isolates were found isolated from rice roots in Aceh Jaya, the morphological characteristics of endophytic bacterial isolates are presented in Table 1 and Figure 1. The data in the table showed macroscopic observation of the morphology of bacterial colonies with four small isolates (small), 13 medium-sized isolates, and 8 large-sized isolates. There were 22 isolates showing a circular shape and 3 bacterial isolates with an irregular shape with colony color, whitish cream and yellowish cream. The entire elevation or shape of the isolate is not raised (flat), as for the shape of the edges of the isolate even (flat edge), and wavy (wavy edge). On microscopic observation, 12 isolates showed gram positive and 13 gram negative isolates in the form of coccus and bacilli cells.

Table 1. Macroscopic and Microscopic Characterization of Endophytic Bacteria

Isolates	**Macroscopic**					**Microscopic**	
	Size	**Shape**	**Color**	**Elevation**	**Margin**	**Grams**	**Cell Shape**
AJY01	Medium	Circular	Creamy whitish	Flat	Event	Positive	Bacillus
AJY02	Small	Circular	Creamy whitish	Flat	Event	Negative	Coccus
AJY03	Large	Irregular	Creamy whitish	Flat	Wavy	Negative	Coccus
AJY04	Large	Irregular	Creamy whitish	Flat	Wavy	Positive	Coccus
AJY05	Large	Irregular	Creamy whitish	Flat	Wavy	Positive	Coccus
AJY06	Medium	Circular	Creamy whitish	Flat	Event	Positive	Bacillus
AJY07	Medium	Circular	Yellowish Beige	Flat	Event	Positive	Bacillus
AJY08	Small	Circular	Creamy whitish	Flat	Event	Negative	Coccus

Isolates	Macroscopic					Microscopic	
	Size	**Shape**	**Color**	**Elevation**	**Margin**	**Grams**	**Cell Shape**
AJY09	Medium	Circular	Yellowish Beige	Flat	Wavy	Negative	Bacillus
AJY10	Large	Circular	Creamy whitish	Flat	Event	Positive	Coccus
AJY11	Small	Circular	Creamy whitish	Flat	Wavy	Positive	Bacillus
AJY12	Large	Circular	Creamy whitish	Flat	Event	Negative	Coccus
AJY13	Medium	Circular	Creamy whitish	Flat	Wavy	Positive	Bacillus
AJY14	Medium	Circular	Creamy whitish	Flat	Event	Positive	Bacillus
AJY15	Medium	Circular	Creamy whitish	Flat	Event	Negative	Bacillus
AJY16	Small	Circular	Yellowish Beige	Flat	Event	Negative	Bacillus
AJY17	Medium	Circular	Yellowish Beige	Flat	Event	Positive	Bacillus
AJY18	Large	Circular	Creamy whitish	Flat	Event	Positive	Bacillus
AJY19	Medium	Circular	Creamy whitish	Flat	Event	Positive	Bacillus
AJY20	Medium	Circular	Creamy whitish	Flat	Event	Positive	Coccus
AJY21	Medium	Circular	Creamy whitish	Flat	Event	Negative	Coccus
AJY22	Large	Circular	Creamy whitish	Flat	Event	Negative	Coccus
AJY23	Large	Circular	Creamy whitish	Flat	Wavy	Positive	Bacillus
AJY24	Medium	Circular	Creamy whitish	Flat	Event	Negative	Coccus
AJY25	Medium	Circular	Yellowish Beige	Flat	Event	Negative	Bacillus

The observed bacterial samples showed significant variations in several characteristics, namely colony morphology and bacterial cell shape. In colony morphology, differences are seen in the size of the colony (small, medium, or large), the shape of the colony (circulating and irregular), the color of the colony, the elevation and the characteristics of the colony margin. These differences are related to different species of bacteria. In addition, the response of bacteria to the gram staining test also varies, there are bacteria that show positive gram-positive results which are marked by purple due to the thick peptidoglycan cell wall and bacteria with gram-negative color are red due to the thinner layer of the cell wall and the presence of the outer membrane. This difference in gram color is an important indicator in the grouping of bacteria. On the other hand, the shape or morphology of bacterial cells also shows differences, where some bacteria are rod-shaped (bacilli), while others are round (cocci), this cell shape is also used as a basis for the classification and identification of microorganisms.

Based on the results of colony morphology identification, gram staining, and cell shape of 25 bacterial isolates isolated from the roots of rice plants, a suspected genus was obtained that corresponded to general characteristics. The isolate with positive gram results and stem morphology (basil), is suspected to come from the genus *Bacillus*, *Paenibacillus*, or *Lysinibacillus* which is indeed commonly found in the root environment and is known to play a role as a biocontrol agency and *Plant Growth Promoting Rhizobacteria* (PGPR). Meanwhile, rod-shaped gram-negative isolates likely belong to the genus *Pseudomonas*, *Enterobacter*, *Klebsiella*, or *Burkholderia*, which are also known as plant root dwellers and have an important role in increasing nutrient availability and protection against pathogens. Khaskheli et al., (2020) isolated 122 rice root endophytes belonging to six genera, such as *Bacillus*, *Lysinibacillus*, and *Paenibacillus*. A total of 71 isolates were produced showing antagonistic activity against the main pathogens of rice including *Magnaporthe oryzae*, *Fusarium graminearum*, and *Rhizoctonia solani* so that they are considered to have potential as biocontrol agents. Etesami & Alikhani (2017) conveyed *Bacillus cereus* and *Bacillus mojavensis*, demonstrating multiple PGP properties (such as siderophore and IAA production) as well as broad antagonistic effects against rice fungal pathogens. These results support the potential *of Bacillus* as a biofertilizer as well as a biological control agent.

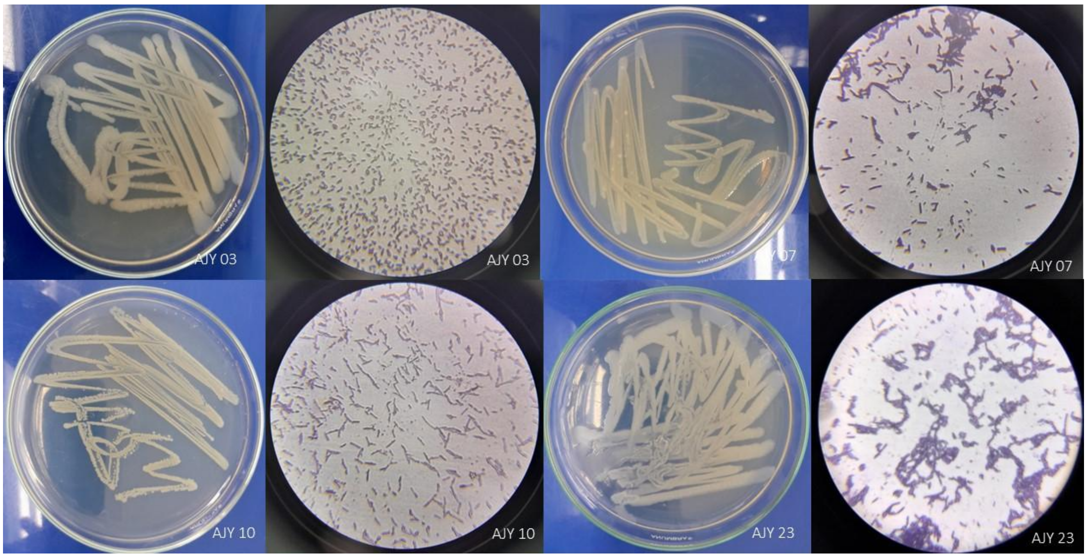

Figure 1. Macroscopic and Microscopic (100x magnification) Endophytic Bacteria

Inhibition of Endophytic Bacteria Against Leaf Blight

Based on the results of the inhibition test (Graph 1 and Figure 2) observed based on the clear zone produced by endophytic bacteria against leaf blight pathogens in the NA media, it was found that 7 out of 25 endophytic bacterial isolates that have the potential to be biocontrol agents, AJY07 isolate has an inhibition zone with a medium category (10 mm), while the isolates AJY04, AJY06, AJY09, AJY11, AJY23 and AJY24 have weak resistance categories with an average inhibition zone of 3-6 mm, while the other isolates do not form clear zones or inhibition zones.

The clear zones that appear around the bacterial colony when incubated with pathogens are an indication of antagonistic activity. Endophytic bacterial isolates are able to inhibit or kill surrounding pathogens through various biological mechanisms. One of the main causes is the production of antimicrobial compounds, such as antibiotics, lipopeptides, siderophores, or bacteriocins, which can directly inhibit the growth or damage of pathogenic cells. In addition, some bacteria also produce hydrolytic enzymes such as chitinase, glukanase, or protease that function to damage the cell walls and membranes of pathogens, causing cell death and the formation of clear zones in the media. Djatmiko et al., (2023) stated that the consortium of endophytic bacteria in suboptimal rice plants produced the largest inhibition zone (62.74 mm²) against *Xanthomonas oryzae* pv. *oryzae* and are capable of producing proteases and siderophores.

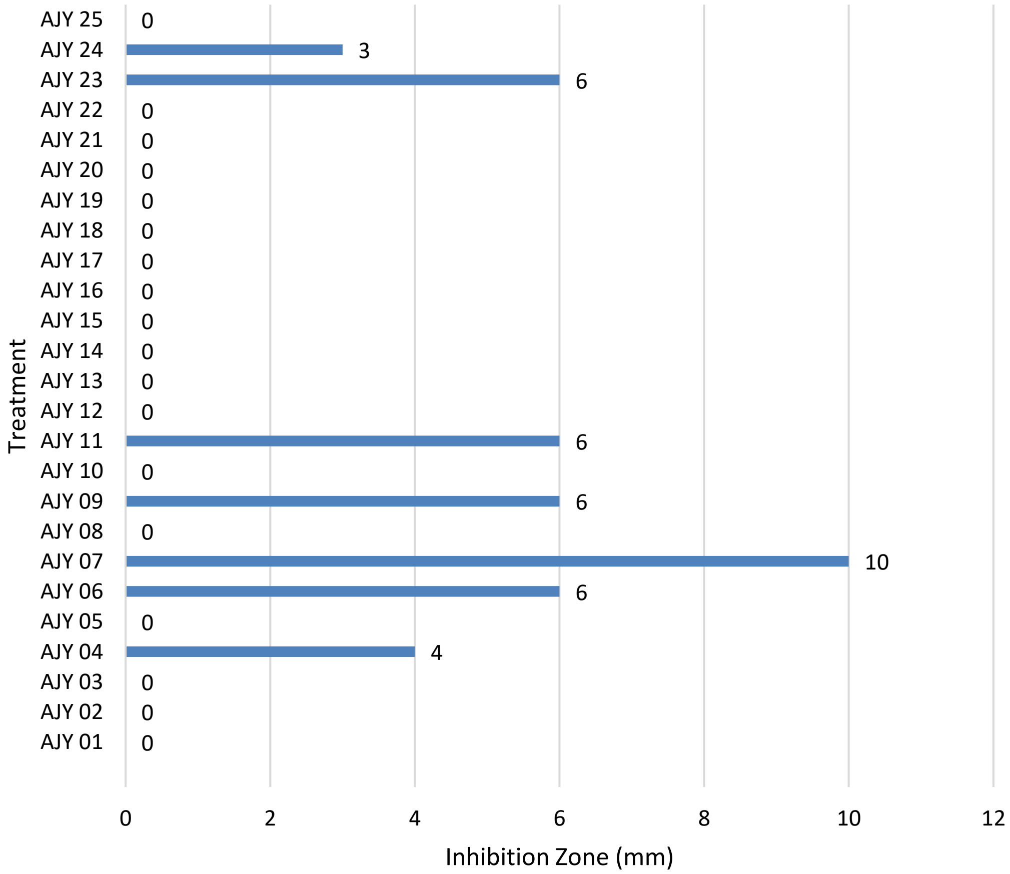

Figure 1. Inhibition Zone of Endophytic Bacteria Against Leaf Blight Pathogens (n=6 replications)

Another factor that plays a role is the ability of bacteria to compete for nutrients and space. Faster-growing bacteria will dominate the surrounding area and inhibit the growth of pathogens through nutrient competition. Some bacteria can also alter the conditions of the surrounding microenvironment, for example by lowering the pH through the production of organic acids, which negatively impacts the survival of pathogens. The combination of these various mechanisms causes pathogens to be unable to develop in the area around the antagonistic bacterial colony, resulting in the formation of clear zones in the media. This proves that bacterial isolates have the potential to be biological control agents against leaf blight pathogens. Kurniawan et al., (2024) found that E320 and E323 endophytic bacterial isolates produce >30% inhibitory zones (38.79% and 41.25%) through the production of proteases, cellulase, and siderophores. Abd-Halim et al., (2020) also found *that Pseudomonas fluorescens* and *Geobacillus thermoparaffinivorans* were the best isolates (BCA 3 and BCA 12) resulting in an inhibition zone of about 35 mm.

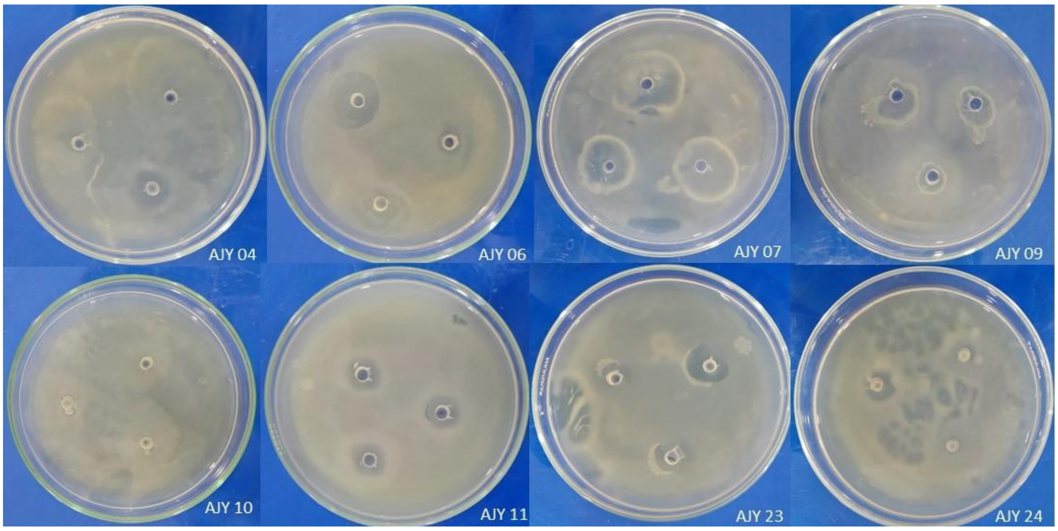

Figure 2. Zone of inhibition of endophytic bacteria against leaf blight pathogens

Control of Leaf Blight Using Endophytic Bacteria on Rice Seeds

Based on the results of the research on the control of leaf blight using endophytic bacteria on inhibited rice seeds (Table 2 and Figure 2) which was observed based on the vigor and viability of rice seeds against endophytic bacterial treatment and leaf blight infection, it is known that all treatments have 100 % maximum growth potential, endophytic bacterial treatment has a germination power percentage of 80.00 - 90.00% compared to the control (AJY 00), In the observation of the Vigor Index of endophytic bacterial treatment was also higher (46.67 - 50.00%) compared to the control treatment, similarly in the observation of the growth rate in the bacterial treatment had an average of 44.94 - 46.77% compared to the control treatment, but in the observation the average time for 50% of the seeds germinated throughout the treatment showed the same day which was 2.25-2.98 days. In Figure 2. Observation of the percentage of leaf blight infections that attack rice sprouts also shows that it is in line with the data on the vigor and viability of rice seeds, namely the percentage of endophytic bacterial treatment (10.00 - 20.00%) is lower than the control treatment (only inoculated with leaf blight pathogens).

Treatment with endophytic bacteria has been shown to be effective in increasing the germination power of rice seeds compared to untreated seeds. This suggests that endophytic bacteria have an important role in improving seed quality, which is also reflected in the higher seed vigor index in the treated group. In addition, seeds treated with endophytic bacteria showed a better growth rate compared to controls, although the average time to reach 50 % germination on all treatments was relatively the same. This indicates that although endophytic bacteria do not significantly speed up germination time, this treatment is able to improve the overall seed growth process. In addition to improving seed quality and growth, endophytic bacteria also function as effective biological control agents. This is evidenced by a much lower rate of leaf blight infection in seeds treated with endophytic bacteria compared to control seeds inoculated only with pathogens.

Table 2. Strength and viability of rice seeds

Treatment	MGP (%)	GP (%)	VI (%)	GS (%)	T50 (days)
AJY 00	100.00	66.67	40.00	39.88	2.98
AJY 04	100.00	80.00	48.33	45.79	2.33
AJY 06	100.00	85.00	50.00	44.94	2.42
AJY 07	100.00	90.00	48.33	45.23	2.43
AJY 09	100.00	80.00	46.67	46.77	2.27
AJY 11	100.00	80.00	50.00	46.77	2.30
AJY 23	100.00	90.00	50.00	46.77	2.25
AJY 24	100.00	83.33	46.67	46.49	2.38

Description: MGP: Maximum Growth Potential; GP: Germination Power; VI: Vigor Index; GS: Growth Seeds; T50: average time for 50% germinated seeds

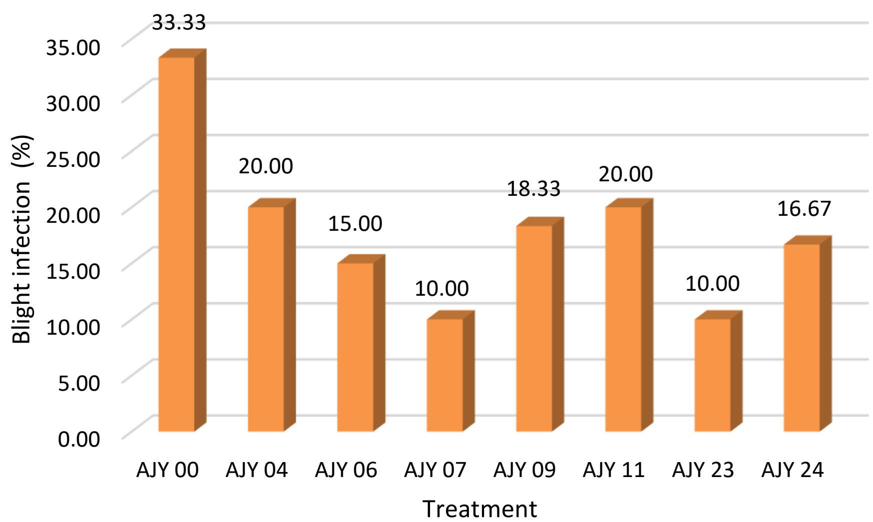

Figure 3. Blight Infection in Rice Germination

Thus, endophytic bacteria are able to inhibit or suppress the development of disease-causing pathogens, thereby supporting seed resistance to disease attacks. The combination of increased germination, vigor, growth speed, and decreased infection rate shows the potential of endophytic bacteria as bioagents that are able to maintain the quality and health of rice seeds optimally. The results of this study are in accordance with Borah (2018) who stated that the isolate of endophytic bacteria *Bacillus subtilis* and *Microbacterium testaceum* is able to increase germination and vigor of gogo rice seeds, even in combination with rhizosphere bacteria provides a more optimal effect. Nguyen & Nga (2024) also found that endophytic bacterial isolates from rice seeds in Vietnam have the ability to produce hydrolytic enzymes such as cellulase, gelatinase, and IAA growth hormone, which play an important role in supporting early plant growth while improving seed quality.

Endophytic bacteria that show potential inhibition against *the pathogen Xanthomonas oryzae* are thought to have certain inhibition mechanisms. Some of the potential mechanisms possessed by endophytic bacteria are the production of antibiotics, toxins, space competition and nutrients. Antibiotic compounds found in endophytic bacteria are able to control pathogens in plants. The clear zone formed around the endophytic bacteria, suggests that the bacteria are effective in inhibiting pathogen growth in in vitro testing (Widjayanti et al., 2023). There are several things that have an impact on the formation of antagonist test clear zones such as microbial concentration, extraction methods and the content of active compounds in antimicrobial materials. In addition to being a biocontrol against leaf blight pathogens, endophytic bacteria also have the ability to spur plant growth so that plant resistance is induced by the presence of endophytic bacteria (Ooi et al., 2022).

CONCLUSION

Based on the results of the study, it showed that 25 isolates were successfully isolated and had macroscopic and microscopic differences, 7 isolate of endophytic bacteria were able to inhibit the growth of leaf blight pathogens with the formation of clear zones, especially AJY07 isolate which had resistance in the medium category and has potential as biopesticide. Seven endophytic bacterial isolates (AJY04, AJY06, AJY07, AJY09, AJY11, AJY23 and AJY24) were able to increase seed vigor and viability compared to controls, and reduce the percentage of blight infection in rice germination by 10.00-20.00% compared to control.

ACKNOWLEDGEMENTS

Appreciate to the Directorate General of Research and Development, Ministry of Higher Education, Science, and Technology (DPPM) for funding the 2025 Fiscal Year Research Activity Number: 117/C3/DT. 05.00/PL/2025 dated May 28, 2025 through a derivative research contract agreement from LPPM Teuku Umar University Number: 14/UN59.L1/AL.04/PL/2025 dated June 3, 2025.

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How To Cite This Article, with *APA style* :

Maulidia, V., Agustinur., Siregar, M. P. A., & Weihan, R. A. (2025). Exploration of Endophytic Bacteria from Rice Roots as a Biocontrol Agency for Leaf Blight Disease in Vitro. *Jurnal Pembelajaran dan Biologi Nukleus*, *11*(4), 1170-1183. https://doi.org/10.36987/jpbn.v11i4.8113

Conflict of interest : The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions : All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was submited by [**Vina Maulidia**]. All authors contributed on previous version and revisions process of the manuscript. All authors read and approved the final manuscript.