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Isolation, Morphological Characterization, Biochemistry, and Antagonistic Activity of Endophytic Bacteria from Rice Against *Xanthomonas oryzae* pv. Oryzae

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Abstract

Endophytic bacteria have the potential as environmentally friendly biocontrol agents in controlling bacterial leaf blight (BLB) in rice caused by *Xanthomonas oryzae* pv. oryzae (Xoo). This study aims to isolate, characterize morphology and biochemical tests, and test the antagonistic activity of endophytic bacteria from rice against Xoo. Isolation was carried out from healthy rice root tissue using a surface sterilization method. Morphological characterization included colony shape, color, edge, elevation, and Gram properties, while biochemical characterization included catalase, oxidase, motility, starch hydrolysis, and sugar fermentation tests. The antagonistic test used a dual culture assay method on NA (nutrient agar) media. The isolation results obtained six endophytic bacterial isolates (BETP 01–06) identified as the genus Pseudomonas and Bacillus with varying colony morphological characters. Antimicrobial activity test showed two potential isolates, BETP 03 (Pseudomonas) and BETP 05 (Bacillus), with an average inhibitory diameter of 16.3 mm and 16.5 mm against Xoo, respectively. Biochemical characterization showed that BETP 03 was bacillus, Gram-negative, citrate-negative, motile, gelatin hydrolysis positive, starch hydrolysis positive, and catalase positive; while BETP 05 was bacillus, Gram-positive, citrate-positive, motile, gelatin hydrolysis negative, starch hydrolysis positive, and catalase positive. These results indicate that both isolates have high potential as biological biocontrol agents in controlling bacterial leaf blight disease in rice.

Keywords: Biocontrol; endophytic bacteria; *Oryza sativa*; *Xanthomonas oryzae* pv. oryzae.

INTRODUCTION

Rice (Oryza sativa L.) is a major food crop and a source of carbohydrates for a large portion of the world's population, particularly in Asia. Rice productivity in Indonesia is frequently threatened by various important diseases, including bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. oryzae (Xoo) (Halim et al., 2020). This disease can cause yield losses of up to 50% in susceptible varieties and is endemic in rice production centers (Mew et al., 1993). Xoo infection is characterized by longitudinal necrosis of leaves starting from the edges, reducing photosynthetic area and grain quality (Ou, 1985).

BLB control is generally achieved through resistant varieties and chemical pesticides. However, selection pressure can lead to the emergence of new pathogen strains that overcome varietal resistance, while excessive use of chemical pesticides has negative impacts on the environment, human health, and soil microflora (Adhikari et al., 1995). Therefore, effective, environmentally friendly, and sustainable alternative control strategies are needed.

Endophytic bacteria are microorganisms that live within plant tissues without causing disease symptoms in their host (Sihotang et al., 2022; Sihotang et al., 2025). The presence of endophytic bacteria in rice plants has been widely reported to provide benefits, including increased growth, boosting plant resilience through the induction of systemic resistance, and producing secondary metabolites with antibacterial activity against pathogens (Ryan et al., 2008). Pathogen inhibition mechanisms by endophytic bacteria include the production of antibiotics, siderophores, hydrolytic enzymes, and competition for nutrients and growth space (Compant et al., 2005).

Isolation and characterization of rice endophytic bacteria are important to determine their phenotypic diversity, physiological properties, and biocontrol potential. Morphological and biochemical characterization provide initial information regarding the identity and metabolic capabilities of the isolates, while antagonistic testing against Xoo can identify potential candidate isolates as biocontrol agents for rice endophytes.

This research was conducted in Tanjung Gusta Village, Deli Serdang Regency, North Sumatra, one of the rice farming centers in the region. This area has a rice paddy agro-ecosystem that supports a diversity of endophytic microbes, but there has been minimal exploration of the potential of local endophytic bacteria as biological control agents for BLB.

Therefore, this study aimed to isolate endophytic bacteria from healthy rice tissue, characterize their morphological and biochemical properties, and evaluate their antagonistic activity against Xanthomonas oryzae pv. oryzae. The results are expected to provide fundamental information for developing endophytic bacteria-based biocontrol agents that support sustainable rice farming systems.*Citation in manuscript is written in the "name and year" system; and is arranged from oldest to newest and from A to Z. In citing an article written by two authors, both of them should be mentioned, however, for three and more authors, only the last (family) name of the first author is mentioned, followed by et al. (not italic), for example: Saharjo and Nurhayati (2006) or **(**Boonkerd 2003a, b, c**;** Sugiyarto, 2004; El-Bana and Nijs, 2005; Balagadde et al., 2008; Webb et al., 2008). The extent citation as shown with the word "*cit"* should be avoided. Reference to unpublished data and personal communication should not appear in the list but should be cited in the text only (e.g., Rifai MA 2007, pers. com. (personal communication); Setyawan AD 2007, unpublished data). A total of 80% of the references should be from scientific journals published in the last five years, except for taxonomic studies. Names of journals should be abbreviated according to the ISSN List of Title Word Abbreviations (www.issn.org/2-22661-LTWA-online.php).

MATERIALS AND METHODS

Materials

The research was conducted from October 2023 to January 2024. Isolation and characterization of endophytic bacteria, as well as antagonistic activity tests, were conducted at the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, University of North Sumatra, Medan. The materials used were healthy rice root samples of Xanthomonas oryzae isolate, NA, 70% alcohol, Clorox, distilled water, and Agrept (bactericide). The tools used in this study included Petri dishes, Erlenmeyer flasks, measuring cylinders, Bunsen burners, hotplates, glass slides, loop needles, cover glasses, auto-aerators, scissors, microscopes, analytical balances, stationery, cameras, vernier calipers, and scalpel blades.

Methods

The rice root sampling technique was based on Dyah (2020). The rice used in this study was healthy ~~rice~~, covering an area of 1 hectare. The rice roots used in this study were taken from Tanjung Gusta Village, Deli Serdang Regency, North Sumatra Province. Isolation of endophytic bacteria was performed by washing rice plant roots with running water and drying them aseptically. The samples were then taken to a Laminar Air Flow cabinet, cut into 1-2 cm pieces, and then surface sterilized by immersing the samples in 70% alcohol for 1 minute, 5% Crolox for 2 minutes, 70% alcohol for 30 seconds, and sterile distilled water for 2 minutes. The samples were then dried with sterile tissue. The rice root samples were placed on a NA surface containing chitin colloids, lightly pressed, and incubated for 24 hours in an incubator at 37°C.

All growing bacterial colonies were purified by transferring the isolates to Petri dishes containing NA media. Morphological, macroscopic, microscopic, and biochemical tests/observations were performed. If the bacteria growing were still mixed with other bacteria, they were purified again. This serves to obtain pure/superior endophytic bacterial isolates. (Sihotang et al., 2023)

The Xanthomonas oryzae bacterial isolates were collected from the University of Medan Area's Plant Protection Laboratory. The isolates were rejuvenated by taking a single colony growing on a culture plate with a loop needle and then inoculating it into a slanted medium in a test tube in a zigzag pattern. The test tubes were wrapped in paper and incubated in an incubator for 24 hours (Yanti & Rosmania, 2020).

The antimicrobial test used the disc diffusion method. The test bacteria used were endophytic, specifically *Xanthomonas oryzae* pv. *Oryzae*. After the bacterial medium was poured into a Petri dish and allowed to solidify, a suspension of Xanthomonas oryzae and six endophytic bacteria was prepared, then turbidity was measured at an MCF of 0.5. The surface of the MHA medium was evenly coated with the Xanthomonas oryzae pv suspension using a sterile cotton swab. A 6 mm diameter paper disc was then placed on the surface of the MHA medium, which had previously been dipped in the endophytic bacterial suspension. The Petri dish was then stored in an incubator at 37°C. The clear zone formed was observed for 1 x 24 hours (1 day).

The study used a non-factorial Completely Randomized Design (CRD) consisting of 7 treatments with 3 replications. The tested treatments consisted of:

P_0 : Control
P_1 : Use of Endophytic Bacteria Isolate 01
P_2 : Use of Endophytic Bacteria Isolate 02
P_3 : Use of Endophytic Bacteria Isolate 03

P_4 : Use of Endophytic Bacteria Isolate 04
P_5 : Use of Endophytic Bacteria Isolate 05
P_6 : Use of Endophytic Bacteria Isolate 06
P_7 : Synthetic Bactericide

The inhibition test followed the diffusion method. According to Ekowati 2000 (in Herlina, 2011), the inhibition test followed the diffusion method. The method was discovered by Kirby-Alfred Baurer in 1966.
Formula:

$$IA = (Dz - Dc) / Dc$$

Description:
AI : Antimicrobial Index
Dz : Diameter of inhibition zone (mm)
Dc : Diameter of disc or well (mm)

Data analysis
Data analysis was performed using One-Way ANOVA, following tests for normality (Shapiro-Wilk) and homogeneity (Levene's Test). If the data met normality and homogeneity assumptions ($p > 0.05$), Post Duncan tests were used to analyze group differences. If data were not normally distributed, the Kruskal-Wallis test was used, followed by MannWhitney U tests for pairwise comparisons when significant differences were observed.

RESULTS AND DISCUSSION

Morphological Characteristics of Endophytic Bacterial Isolates

The isolation of endophytic bacteria from rice roots yielded six dominant isolates. This was based on morphological characteristics, including shape, margins, elevation, and color.

Table 1. Morphological Characteristics of Endophytic Bacterial Isolates.

Code	Shape	Margin	Elevation	Color
BETP 01 (Pseudomonas)	Circular	Entire	Convex	Whitish-yellow
BETP 02 (Bacillus)	Rhizoid	Rhizoid	Convex	White
BETP 03 (Pseudomonas)	Irregular	Undulate	Convex	Purple
BETP 04 (Bacillus)	Irregular	Lobate	Convex	Whitish-yellow
BETP 05 (Bacillus)	Irregular	Curled	Convex	White
BETP 06 (Bacillus)	Circular	Entire	Convex	Yellow

Microskopic Characterization and Gram Staining of Endophytic Bacteria

Table 2. Microskopic Characterization and Gram Staining of Endophytic Bacteria.

Code	Shape	Arrangement	Gram
BETP 01	Bacillus	Monobacillus	-
BETP 02	Bacillus	Streptobacillus	+
BETP 03	Bacillus	Monobacillus	-
BETP 04	Bacillus	Monobacillus	+
BETP 05	Bacillus	Monobacillus	+
BETP 06	Bacillus	Streptobacillus	+

Biochemical Tests of Endophytic Bacteria

Table 3. Biochemical Tests of Endophytic Bacteria.

Code	Citrate	SIM / Motility	Gelatin Hydrolysis	Starch Hydrolysis	Catalase	pH
BETP 01	+	+	-	+	-	6
BETP 02	+	+	+	+	-	6
BETP 03	-	+	+	+	+	6
BETP 04	+	+	+	+	+	6
BETP 05	+	+	-	+	+	6
BETP 06	-	+	-	+	+	6

Antimicrobial Test of Endophytic Bacteria against Xoo

Table 4. Antimicrobial Test of Endophytic Bacteria against Xoo.

Code	Average inhibition zone diameter (mm)	Notation	Index
Control	6.00	d	0
BETP 01	8.83	cd	0.4
BETP 02	11.60	c	0.9
BETP 03	16.50	b	1.75
BETP 04	11.53	c	0.9
BETP 05	16.23	b	1.7
BETP 06	9.53	c	0.5
BETP 01	28.00	a	3.6

Duncan's follow-up test results α= 0.05

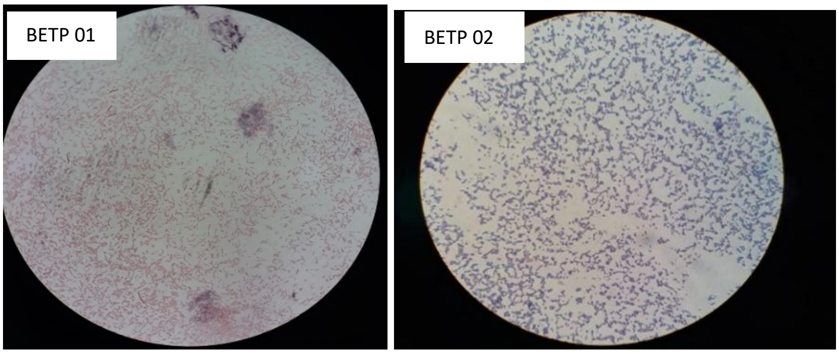

Figure 1. BETP01 (gram negative-bacteria); BETP02 (gram positive-bacteria).

Discussion

The study results showed that endophytic bacterial isolates from rice (Oryza sativa) plants consisted of several dominant genera, namely Pseudomonas and Bacillus. Morphological characterization, Gram staining, and biochemical tests revealed significant variation among isolates. Most isolates exhibited high catalase, starch hydrolysis, and motility capabilities, indicating their potential for adaptation to host plant tissues. These results are consistent with reports that the Bacillus and Pseudomonas genera are the most common endophytic bacterial groups that play a role enhance rice plant growth and resistance to pathogens.

Antagonistic tests against Xanthomonas oryzae pv. oryzae (Xoo), the causative agent of bacterial leaf blight in rice, showed that isolates BETP 03 (Pseudomonas) and BETP 05 (Bacillus) produced the largest inhibition zones, with diameters >16 mm. This confirms their ability to produce antimicrobial secondary metabolites such as siderophores, antibiotics, and hydrolytic enzymes. These findings align with recent research confirming the effectiveness of Pseudomonas fluorescens and Bacillus subtilis in inhibiting Xoo growth through antibiosis mechanisms and induction of systemic resistance in rice.

Furthermore, hydrolytic enzyme activity in several isolates supports their role as biocontrol agents. Enzymes such as chitinase, protease, and lipase are known to be able to damage pathogen cell walls, thereby strengthening plant defenses. Biochemical tests showed that isolates BETP 04 and BETP 05 exhibited a combination of citrate, gelatinase, and catalase activity, strengthening the hypothesis that these isolates have the potential to be used as both a biofertilizer and a biopesticide.

From a microbial ecology perspective, the presence of endophytic bacteria within rice tissues provides a competitive advantage over epiphytic microbes, as they can survive in the relatively stable internal environment of the plant. Thus, endophytes can play a dual role: increasing plant nutrient availability (for example, through nitrogen fixation or phosphate solubilization) and protecting the plant from pathogen attack.

However, this study also has limitations, namely the lack of molecular analysis (16S rRNA) to ensure accurate isolate identification, as well as field trials to confirm the effectiveness of biocontrol in real agroecosystem conditions. Further studies are strongly recommended to explore the molecular mechanisms of antimicrobial metabolite production and the formulation of endophyte consortia as environmentally friendly biopesticides.

Thus, the results of this study provide strong evidence that the endophytic bacteria Pseudomonas and Bacillus from Oryza sativa have high prospects as biocontrol agents to reduce dependence on chemical pesticides in sustainable rice cultivation systems.

CONCLUSIONS

In conclusion, Six isolates of endophytic bacteria from rice plants were produced. The isolates exhibited varying antimicrobial activity, as indicated by the large diameter of the inhibition zone. Two endophytic bacterial isolates with the highest potential to inhibit the tested pathogenic microbes were isolates coded BETP 03 pseudomonas and BETP 05 bacillus, with average values of 16.3 and 16.5, respectively. BETP 03 was characterized as a bacillus, Gram-negative, citrate-negative, positive motility, positive gelatin hydrolysis, positive starch hydrolysis, and a positive catalase test. BETP 05 was characterized as a bacillus, Gram-positive, citrate-positive, positive motility, negative gelatin hydrolysis, positive starch hydrolysis, and a positive catalase test.

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Competing Interests: The authors declare that there are no competing interests.

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