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RESEARCH PAPER

The potential in consortium of endophytic bacteria for controlling sheath blight by *Rhizoctonia solani* Kuhn in rice plants

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ABSTRACT

Rhizoctonia solani Kühn is a pathogenic fungus that causes sheath blight disease in rice. One effective strategy for managing this disease is the use of biological control, particularly through consortia of endophytic bacteria. This study aimed to identify the most effective endophytic bacterial consortium for suppressing sheath blight severity while also enhancing rice growth and yield. A Completely Randomized Design (CRD) was aemployed with six treatments, three replications, and three experimental units per treatment. The treatments included four bacterial consortia composed of combinations of *Bacillus thuringiensis* LmD13, *Ochrobactrum intermedium* LmB1, and *Stenotrophomonas maltophilia* LmB35, along with positive and negative controls. The experiment involved treating rice seeds and soaking seedling roots with the bacterial consortia before transplanting. *R. solani* was inoculated onto the rice leaf sheaths 40 days after planting. The effectiveness of each consortium as a biocontrol agent was evaluated based on incubation period, disease incidence, disease severity, and the area under the disease progress curve (AUDPC). Their biostimulant potential was assessed through parameters related to seedling growth, plant development, and yield. Results indicated that the endophytic bacterial consortia effectively suppressed sheath blight and significantly improved rice growth and production. Notably, the consortium of *B. thuringiensis* LmD13, *O. intermedium* LmB1, and *S. maltophilia* LmB35 extended the incubation period to 35 days post-inoculation and reduced disease incidence, severity, and AUDPC to 22.22%, 0.29%, and 1.01, respectively. This consortium also enhanced rice yield, with fresh and dry grain weights reaching 72.78 g and 63.02 g, respectively, compared to the positive control. These findings suggest that this bacterial consortium holds strong potential as a biocontrol agent and yield enhancer in rice cultivation.

Key words: Biological control, consortia, endophytic bacteria, *Rhizoctonia solani*, sheath blight, soil-borne pathogen

INTRODUCTION

Oryza sativa is a vital staple food crop in Asia, Africa, and Latin America. As the primary source of rice (Fukagawa & Ziska, 2019), its demand continues to grow in line with population increases (Sen et al., 2020). In Indonesia, rice availability is a crucial determinant of food security and national stability (Sandy et al., 2019). However, rice cultivation faces persistent threats from various pests and diseases that reduce yield and quality. Major pathogens affecting rice include rice tungro virus (Sutrawati et al., 2021), rice grassy stunt virus (Wu et al., 2023), *Xanthomonas oryzae* pv. *oryzae* (Ke et al., 2017; Oliva et al., 2019), *Pyricularia oryzae* (Motoyama, 2020), *Helminthosporium oryzae* (Sattari et al., 2015; Ashfaq et al., 2021), and *Rhizoctonia solani* Kühn (Rao et al., 2020; Senapati et al., 2022), which cause leaf blight, blast, leaf spot, and sheath blight, respectively.

Among these, sheath blight is considered one of the most destructive diseases affecting rice worldwide. It ranks second only to rice blast in terms of annual global yield losses. In Asia alone, sheath blight is estimated to reduce rice production by an average of 6%, with local losses reaching up to 50% (Singh et al. 2019). The disease also contributes to significant economic losses globally (Molla et al., 2020). In Indonesia, Milati & Nuryanto (2019) reported sheath blight severity ranging from 6% to 52%. Effective management is challenging due to the high genetic variability of *R. solani*, a soil- and seed-borne facultative parasite capable of surviving on crop residues as sclerotia or mycelium, acting as primary inoculum in the field (Sivalingam et al., 2006; Basu et al., 2016; Desvani et al., 2018; Senapati et al., 2022). Efforts to develop resistant rice varieties have been constrained by the limited availability of resistance genes in the existing germplasm (Singh et al., 2019). In addition, conventional control measures such as

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cultural practices and chemical fungicides are often ineffective in the long term and raise concerns about environmental and health impacts due to the excessive use of synthetic compounds (Suryadi et al., 2015).

To address these challenges, sustainable and eco-friendly control strategies are essential. One promising approach involves the utilization of beneficial microorganisms associated with rice plants, particularly endophytic bacteria. These plant growth-promoting bacteria (PGPB) live within plant tissues without causing disease symptoms (Olanrewaju et al., 2017; Morales-Cedeño et al., 2021). Several genera of endophytic bacteria isolated from rice—such as *Pseudomonas*, *Bacillus*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Burkholderia*, *Klebsiella*, *Rhizobium*, and *Serratia — have demonstrated growth-promoting capabilities in vitro* (Sharma et al., 2012; Santoyo et al., 2016; Prasad et al., 2019; Morales-Cedeño et al., 2021).

Endophytic bacteria promote plant growth through direct and indirect mechanisms (Jeyanthi & Kanimozhi, 2018; Ullah et al., 2019). Direct mechanisms include nitrogen fixation, mineral solubilization (e.g. phosphorus and iron), siderophore production, and phytohormone synthesis (Whipps, 2001; Rahma et al., 2024; Yuniawati & Akhdiya, 2021; Katsenios et al., 2022). Indirect mechanisms involve the suppression of pathogens through antibiotic production and the induction of systemic resistance (ISR) in plants (Hallmann, 2001; Van Loon, 2007; Tiwari et al., 2017; Solanki et al., 2019). Rahma et al. (2022) identified endophytic strains such as *Ochrobactrum intermedium* LmB1, *Bacillus cereus* LmA6, and *Stenotrophomonas maltophilia* LmB35 that enhanced rice growth and suppressed bacterial leaf blight caused by *X. oryzae* pv. *oryzae*.

Moreover, endophytic bacteria often interact with other microorganisms, forming complex microbial communities that may exert synergistic, neutral, or antagonistic effects on the host plant (Mendes et al., 2011; Carrión et al., 2019). Bacterial consortia, defined as combinations of two or more compatible microbial species, can provide enhanced disease suppression through mechanisms such as resource competition, antibiotic production, and multi-pathway activation of plant defenses (Stockwell et al., 2011; Panwar et al., 2014; Sarma et al., 2015; Ju et al., 2019; Singh et al., 2023).

highlighted that microbial consortia are often more effective than single isolates in managing plant diseases. Endophytic bacterial consortia have been reported to control a variety of plant pathogens. For instance, Halimah et al. (2016) showed that endophytic bacteria caused up to 65.8% mortality of the nematode *Oratylenchus coffeae* in vitro. Demonstrated that a consortium of *Bacillus* sp. SJI and *S. marcescens* strain JB1E3, alone or in combination with *Bacillus* sp. HI, effectively suppressed *Ralstonia solanacearum* while enhancing chili plant growth. Similarly, Yanti et al. (2020) found that a consortium of *B. pseudomycoides* SLBE 3.1AP, *B. thuringiensis* AGBE 2.1TL, and *B. cereus* SLBE 1.1SN suppressed *Colletotrichum gloeosporioides* infection in chili by up to 95%.

Despite the promising potential of endophytic bacterial consortia, their effectiveness against sheath blight in rice has not been adequately explored. Therefore, this study aims to identify the most effective endophytic bacterial consortium for suppressing sheath blight caused by *R. solani* and promoting rice plant growth.

MATERIALS AND METHODS

Research Site. This research was conducted at the Microbiology Laboratory and Experimental Field, Faculty of Agriculture, Andalas University, from April to September 2023.

Research Design. This study employed a Completely Randomized Design (CRD) with six treatments, three replications, and three units per replication. The study utilized endophytic bacterial isolates and a pathogenic fungal isolate (*Rhizoctonia solani* RSLB2), all obtained from the Microbiology Laboratory, Department of Plant Protection, Faculty of Agriculture, Andalas University. The endophytic bacterial isolates used were *B. thuringiensis* LmD13, *O. intermedium* LmB1, and *S. maltophilia* LmB35—originally isolated from the leaves and stems of rice plants and selected as potential biocontrol agents against *X. oryzae* pv. *oryzae* (Rahma et al., 2022). The treatment combinations used in this study are presented in Table 1.

Rejuvenation and Pathogenicity Test of *R. Solani* Isolate RSLB2. The *R. solani* RSLB2 isolate was revived from stock cultures and cultured on Potato Dextrose Agar (PDA; Merck, Germany). The fungal cultures were incubated for seven days to obtain active mycelial growth for further use.

Rejuvenation and Compatibility Testing of Endophytic Bacteria. Endophytic bacterial isolates stored in 20% glycerol were revived by streaking onto Nutrient Agar (NA; Merck, Germany) using the quadrant method and incubated at room temperature for 48 hours. Compatibility between bacterial isolates was tested using the cross-streak method on NA. Two isolates were streaked perpendicularly, and compatibility was determined by the absence of a clear zone at the intersection (Denaya et al., 2021), The presence of a clear zone indicated incompatibility (Figure 1).

Preparation of Endophytic Bacterial Consortia. Consortia were prepared in two stages. First, each isolate was cultured in 25 mL of Nutrient Broth (NB; Merck, Germany) and incubated on a rotary shaker at 150 rpm for 24 hours. In the second stage, 1 mL of each compatible isolate (at 10^8 CFU/mL) was combined and introduced into 49 mL of sterile nutrient-enriched coconut water (Rahma et al., 2019). This mixture was incubated for 48 hours on a shaker at 150 rpm at room temperature. Bacterial density was confirmed by comparing turbidity with McFarland standard 8 (≈ 10^8 CFU/mL) (Klement et al., 1990).

Evaluation of the Consortium's Potential to Suppress Sheath Blight and Promote Rice Growth. ***Preparation of Planting Media.*** The planting medium consisted of soil and manure mixed at a 2:1 ratio. The mixture was sterilized using the Tyndallization method and placed into sprout trays and 5-kg capacity polybags.

Inoculation of Endophytic Bacterial Consortia. Rice seeds of the IR-42 variety were surface-sterilized in 2% NaOCl for 1 min, rinsed with sterile distilled water, air-dried and soaked in each consortium suspension (10^8 CFU/mL) for 1 hour on a rotary shaker at 150 rpm. Control seeds were soaked in sterile water. The seeds were then air-dried and sown in sterile soil media for 21 days (Rahma et al., 2022).

For root inoculation, seedlings were gently washed, and root were immersed in the bacterial suspension (10^8 CFU/mL) for 15 min. Control were treated similarly with sterile distilled water. Each seedling was transplanted into a polybag containing

Table 1. The treatment combinations

Code	Treatment description
A	*B. thuringiensis* LmD13 + *O. intermedium* LmB1
B	*B. thuringiensis* LmD13 + *S. maltophilia* LmB35
C	*O. intermedium* LmB1 + *S. maltophilia* LmB35
D	*B. thuringiensis* LmD13 + *O. intermedium* LmB1 + *S. maltophilia* LmB35
E	Negative control (without consortium, inoculated with *R. solani*)
F	Positive control (without consortium, not inoculated with *R. solani*)

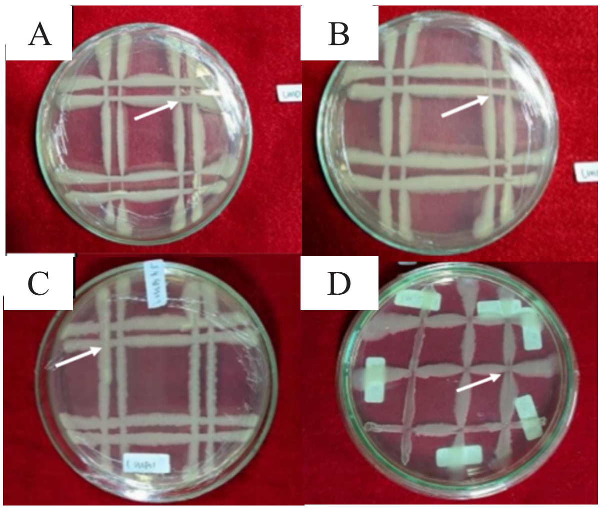

Figure 1. Compatibility test of endophytic bacterial isolates on Nutrient Agar (NA) medium. A. *B. thuringiensis* LmD13 + *O. intermedium* LmB1; B. *B. thuringiensis* LmD13 + *S. maltophilia* LmB35; C. *O. intermedium* LmB1+ *S. maltophilia* LmB35; D. *B. thuringiensis* LmD13 + *O. intermedium* LmB1 + *S. maltophilia* LmB35. The arrows indicate the points where the bacterial streaks intersect. The lack of a distinct zone at these locations indicates that the isolates do not inhibit one another and are suitable for consortium formation.

sterile planting media (soil:manure = 2:1) and covered with plastic (Khaeruni et al., 2014).

Inoculation of *R. solani* and Disease Assessments. Inoculation of *R. solani* was done by placing sclerotia into the sheath of 40-day-old rice plants. Disease assessments included:

Incubation Period. Time from inoculation to first appearance of oval or round off-white lesions.

Disease Incidence. Proportion of infected plants, observed weekly starting 7 days after inoculation.

Disease Severity. Measured by lesion length on the leaf sheath and scored on a 0–9 scale (IRRI, 2002). Disease severity formula:

$$I = \frac{\sum (n_i \times v_i)}{N \times Z} \times 100\%$$

I = Disease Severity;
n_i = Number of leaves in each category;
v_i = Scale value;
N = Total number of leaves;
Z = Highest scale value.

The disease severity value calculated using the rice resistance scale against rice sheath blight can be seen in Table 2.

Area Under the Disease Progress Curve (AUDPC). The value of disease severity is calculated by the leaf damage score according to Ou (1985), which is shown in Table 1. Area Under the Disease Progress Curve was calculated using the formula from Campbell & Madden (1990):

$$\text{AUDPC} = \sum_{i=0}^{n} \left[\frac{(y_i + y_{i+1})}{2} \times (t_{i-1} + t_i) \right]$$

y_i = Disease severity at time *i*;
t_i = time of observation.

Rice Growth Assessment. Plant growth was evaluated based on plant height, number of leaves, number of tillers, and grain weight. Observations were taken weekly from 7 days after planting until flag leaf appearance. Grain weight was measured after drying for 24 hours under sunlight.

Characterization of Endophytic Bacterial Consortia as Bio-stimulants.

Phosphate Solubilization Test. Consortia were cultured on Pikovskaya agar containing 10 g glucose; 5 g $Ca_3(PO_4)_2$; 0.5 g $(NH_4)2SO_4$; 0.2 g KCl; 0.1 g $MgSO_4.7H_2O$; 0.01 g $MnSO_4.H_2O$; 0.5 g yeast extract and 0.01 g $FeCl_3.6H_2O$; 1 L distilled water at pH 7.0. Plates were incubated at room temperature for two weeks. Phosphate solubilization index was calculated by comparing the diameter of the clear zone and bacterial colony. The clear zone was observed and the phosphate solubilization index was measured based on the formula:

$$PI = \frac{a - b}{b}$$

PI = Phosphate Solubilization Index;
a = Clear zone diameter (mm);
b = Colony diameter (mm)

Indole Acetic Acid (IAA) Production. IAA production was assessed using the colorimetric method with Salkowski reagent containing 150 mL concentrated H_2SO_4, 250 mL distilled water, 7.5 mL $FeCl_3$.6H2 0.5 M using a spectrophotometer at a wavelength of 510 nm (Aryantha et al., 2004). Bacterial consortia was grown in 5 mL Tryptic Soy Broth (TSB) supplemented with 0.1 mM tryptophan and incubated in the dark at 110 rpm for 48 hours. After centrifugation (1000 rpm, 15 min), 1 mL supernatant was mixed with 4 mL Salkowski reagent and incubated for 30 min. Absorbance was measured at 510 nm, and IAA concentration was determined using a standard curve (0–45 µg/mL).

Data Analysis. Data on disease incidence, severity, AUDPC, and rice growth parameters were analyzed

Table 2. Scale of symptoms of sheath blight in rice plants

Scoring	Symptom length (%)
0	0 (No symptoms)
1	< 20
3	20–30
5	31–45
7	46–65
9	> 65

using Analysis of Variance (ANOVA) in Statistix 8 software. Differences between treatments were compared using the Least Significant Difference (LSD) test at a 5% significance level.

RESULTS AND DISCUSSION

Effect of Introduced Endophytic Bacterial Consortium as Biocontrol Agents. The application of an endophytic bacterial consortium on rice seeds, followed by inoculation with the pathogenic fungus *R. solani*, significantly suppressed the incubation period of sheath blight symptoms compared to the control. Disease symptoms appeared between 2.55 and 35.00 days post-inoculation across treatments. The consortium comprising *B. thuringiensis* LmD13, *O. intermedium* LmB1, and *S. maltophilia* LmB35 delayed symptom onset to 35 days significantly outperformed other consortia and the control. In contrast, the combination of *B. thuringiensis* LmD13 and *O. intermedium* LmB1 alone did not significantly effect the incubation period.

This bacterial consortium also significantly reduced sheath blight incidence, ranging from 22.22% to 100% across treatments. The consortium of *B. thuringiensis* LmD13 + *O. intermedium* LmB1 + *S. maltophilia* LmB35 achieved the lowest incidence (22.22%), while other treatments, including the control, showed incidences up to 100% (Table 3).

In terms of disease severity, the same consortium reduced severity to 0.29%, significantly lower than the 15.05% and slightly lower than the *B. thuringiensis* LmD13 + *O. intermedium* LmB1 consortium (2.66%). The area under the disease progress curve (AUDPC) also supported these findings: the lowest AUDPC value (1.01) was recorded in the three-strain consortium, compared to the highest (313.35) in the control group. These results indicate that a lower AUDPC corresponds with reduced disease severity.

The observed biocontrol effectiveness is attributed to synergistic interactions among the bacterial strains. The combined action of phosphate solubilization and siderophore production by *B. thuringiensis* LmD13 and *S. maltophilia* LmB35, along with chitinase production by *O. intermedium* LmB1 (Rahma et al., 2024), likely contributed to disease suppression. These mechanisms are aligned with the concept of antibiosis, in which microbial secondary metabolites inhibit pathogen growth (El-Akhdar et al., 2020). For instance, siderophore production allows beneficial microbes to outcompete pathogens for iron—a critical nutrient in microbial colonization and biofilm formation (Rat et al., 2021). Additionally, chitinase enzymes degrade fungal cell walls, directly inhibiting pathogen establishment (Bakhat et al., 2023).

Rahma et al. (2022) also reported the efficacy of *O. intermedium* LmB1 and *S. maltophilia* LmB35 in suppressing bacterial leaf blight (*X. oryzae* pv. *oryzae*), reinforcing the potential of these strains. According to Santoyo et al. (2021) and Duncker et al. (2021), bacterial consortia often outperform single strains due to their complementary functions and complex defense mechanisms, which complicate pathogen resistance development. Nunes et al. (2024) further support that consortia can integrate multiple biocontrol pathways—enzyme production, nutrient competition, and plant defense stimulation—enhancing their effectiveness.

Table 3. Effect of introduced of endophytic bacterial consortium on the incubation period, disease incidence, and severity of sheath blight in rice plants, 35 days after inoculation with *R. solani*

Treatment	Incubation periods (days)	Disease incidence (%)	Disease severity (%)	AUDPC
B. thuringiensis LmD13 + *O. intermedium* LmB1	3.55 bc	100	2.66 ab	56.63
B. thuringiensis LmD13 + *S. maltophilia* LmB35	4.55 b	100	4.42 b	77.84
O. intermedium LmB1 + *S. maltophilia* LmB35	4.88 b	100	5.21 b	100.69
B. thuringiensis LmD13 + *O. intermedium* LmB1 + *S. maltophilia* LmB35	35.00 a	22.22	0.29 a	1.01
Negative Control	2.55 c	100	15.04 c	313.35

The numbers followed by the same letter within a column are not significantly different based on the LSD test at the 5% significance level.

Effect of Introduced of Endophytic Bacterial Consortium on Rice Growth. Application of the endophytic bacterial consortia also positively affected rice growth. Treatments involving *B. thuringiensis* LmD13 + *O. intermedium* LmB1 + *S. maltophilia* LmB35 and *O. intermedium* LmB1 + *S. maltophilia* LmB35 significantly increased plant height to 123.33 cm and 122.33 cm, respectively, compared to 107.67 cm in the control. These consortia also significantly improved the number of leaves and tillers per clump relative to the control.

In terms of productivity, the three-strain consortium led to the highest fresh and dry grain weights—72.78 g and 63.02 g, respectively—outperforming both the control and other treatments (Table 4). This growth-promoting effect was supported by the consortium's ability to solubilize phosphate and produce the highest levels of indole-3-acetic acid (IAA) (Table 5, Figure 3). The ability to dissolve phosphate was indicated by the appearance of a clear zone around the endophytic bacterial consortium on Pikovskaya media (Figure 2A) and a change in the color of the bacterial consortium supernatant to pink after the addition of Salkowski reagent (Figure 2B).

These enhancements in growth are attributed to the consortia's ability to produce phytohormones and improve nutrient availability. Rahma et al. (2022) highlighted *O. intermedium* LmB1 and *S. maltophilia* LmB35 as superior individual strains for growth promotion. IAA plays a pivotal role in in plant physiology, including cell elongation, division, tissue differentiation, and root development (Shahzad et al., 2017). Srinivasan & Mathivanan (2011) confirmed that consortia can yield higher IAA concentrations. According to Kesuma et al. (2016), a consortium of *Bacillus cereus* and *Pseudomonas aeruginosa* can enhance the height of the stem, leaf length, the number of tillers, and the number of panicles in *Inpari* rice plants. For example, *O. intermedium* has been shown to produce 9.65 ppm IAA (Imamuddin, 2015), while

Table 4. The effect of introduced of endophytic bacterial consortia and *R. solani* inoculation on the growth and yield of rice plants (50 days after planting)

Treatment	Rice plant height (cm)	Number of rice leaves	Number of rice tillers	Fresh grain weight (g)	Dry grain weight (g)
B. thuringiensis LmD13 + *O. intermedium* LmB1	117.67 ab	158.33 bc	21.66 ab	70.30 ab	58.94 ab
B. thuringiensis LmD13 + *S. maltophilia* LmB35	111.67 bc	175.78 ab	20.55 bc	68.26 b	58.34 ab
O. intermedium LmB1 + *S. maltophilia* LmB35	122.33 a	197.33 a	23.33 a	66.30 b	54.76 b
B. thuringiensis LmD13 + *O. intermedium* LmB1 + *S. maltophilia* LmB35	123.33 a	190.22 a	22.77 ab	72.78 a	63.02 a
Positif Control	107.67 c	144.89 c	18.22 c	58.56 c	48.11 c

The numbers followed by the same letter in the same column are not significantly different according to the LSD test at the 5% level.

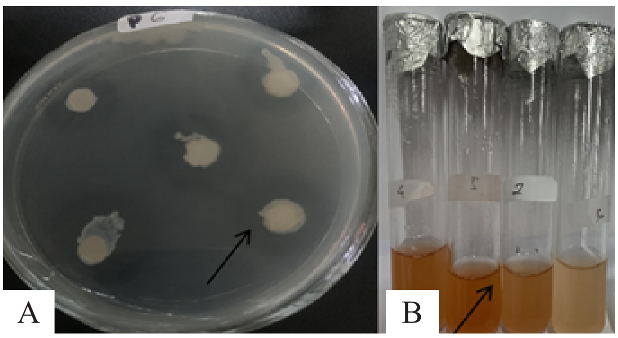

Figure 2. Biostimulant activity of the endophytic bacterial consortium in rice. A. Phosphate solubilization on Pikovskaya agar, indicated by clear halo zones surrounding the bacterial colonies; B. IAA production in TSB medium supplemented with L-tryptophan, indicated by a pink color change after the addition of Salkowski reagent.

Table 5. Characteristics of endophytic bacterial consortia as biostimulants

Treatment	Phosphate solvent index	IAA concentration (μg/mL)
B. thuringiensis LmD13 + *O. intermedium* LmB1	2.70	50.54
B. thuringiensis LmD13 + *S. maltophilia* LmB35	2.90	54.72
O. intermedium LmB1 + *S. maltophilia* LmB35	3.20	60.47
B. thuringiensis LmD13 + *O. intermedium* LmB1 + *S. maltophilia* LmB35	3.70	75.91

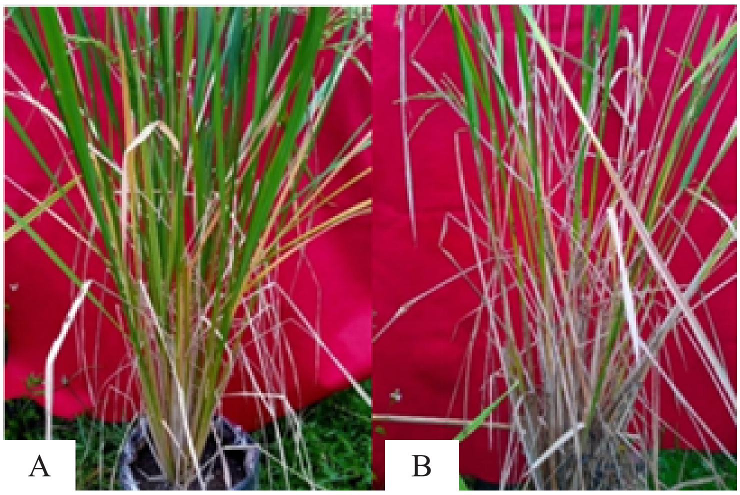

Figure 3. Comparison of sheath blight disease severity in rice plants at 35 days after inoculation. A. Concortium *B. thuringiensis* LmD13, *O. intermedium* LmB1, and *S. maltophilia* LmB35; B. Negative control.

S. maltophilia produces 2.737 mg/L (Al Banna & Arifuddin, 2021).

Additionally, the availability of nitrogen during grain filling is crucial. Ayaz et al. (2023) emphasized that phosphate-solubilizing endophytes serve dual roles: they suppress pathogens and act as biostimulants by promoting root health, nutrient uptake, and hormone production. Some, like those studied by Huong et al. (2022), also produce gibberellins and auxins, which further enhance growth.

The most effective consortium, consisting of *B. thuringiensis* LmD13, *O. intermedium* LmB1, and *S. maltophilia* LmB35 successfully suppressed the development of sheath blight symptoms and enhanced rice plant growth, resulting in a plant height of 123.33 cm, 22.77 tillers, and a grain weight of 72.78 g.

CONCLUSION

The results demonstrated that the endophytic bacterial consortium effectively controlled sheath blight and enhanced the growth and yield of rice plants. This consortium extended the incubation period to 35.00 days after inoculation and significantly reduced disease incidence, severity, and the area under the disease progress curve (AUDPC) by 22.22%, 0.29%, and 1.01, respectively. Additionally, it increased rice yield, as indicated by the fresh and dry weights of rice grains, which reached 72.78 g and 63.02 g, respectively, compared to the positive control. The consortium of *B. thuringiensis* LmD13, *O. intermedium* LmB1, and *S. maltophilia* LmB35 shows strong potential as a biocontrol agent and growth promoter in rice cultivation.

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AUTHORS' CONTRIBUTIONS

I and ZR conceptualized and designed the experiments. Y and FAHN conducted the experimental work. FAHN and ZR analyzed and interpreted the data. I drafted the manuscript. All authors contributed to refining the research flow, data analysis, interpretation, and manuscript structure. All authors have read and approved the final version of the manuscript.

COMPETING INTEREST

The authors declare no competing interests regarding the publication of this manuscripts.

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