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Antagonistic Activity of Endophytic Bacterial Consortia Against *Helminthosporium oryzae* Breda de Haan, The Causal Agent of Brown Spot Disease in Rice

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Received November 07, 2025; revised December 22, 2025; accepted December 31, 2025

ABSTRACT

Helminthosporium oryzae is a pathogen responsible for rice damage and losses of up to 45%. A consortium of endophytic bacteria as a biological agent is one of alternative and eco-friendly ways to suppress the development of plant pathogenic fungi. This research aimed to obtain an endophytic bacterial consortium capable of inhibiting the growth of *H. oryzae.* This research used a Completely Randomized Design (CRD) to investigate the efficacy of a consortium of six endophytic bacteria, alongside a control group, with three replications. The treatments included A (control), B (*Serratia marcescens* ULG1E4; *Serratia marcescens* JB1E3), C (*Bacillus sp*. HI; *Serratia marcescens* JB1E3), D (Bacillus sp. HI; Bacillus sp. SJI; Serratia marcescens JB1E3), E (*Serratia marcescens* ULG1E4; *Serratia marcescens* JB1E3; *Serratia marcescens* JB1E2), F (*Bacillus sp.* HI; *Bacillus sp.* SJI), and G (*Bacillus sp.* SJI; *Serratia marcescens* ULG1E4). The ability of endophytic bacterial consortia to suppress the growth of *H. oryzae* was evaluated utilizing dual culture and poisoned media techniques. The parameters observed were the inhibitory capacity of endophytic bacterial consortia, the inhibitory potential of secondary metabolites produced by the endophytic bacterial consortia, as well as the fresh weight and dry weight of the fungus. The results showed that treatment G (*Bacillus* sp. SJI; *Serratia marcescens* ULG1E4), B (*S. marcescens* ULG1E4; *Serratia marcescens* JB1E3), and E (*Serratia marcescens* ULG1E4; *Serratia marcescens* JB1E3; *Serratia marcescens* JB1E2), had a high ability to suppress the growth of *H. oryzae* with suspension inhibition 61,28%, 62,93%, 63,55%, and secondary metabolites inhibition rates of 96,93%, 94,21%, 97,60%.

Keywords: Antibiotic, Competition, Dual culture, Inhibition, Secondary metabolite.

Potensi Konsorsium Bakteri Endofit dalam Menghambat Pertumbuhan *Helminthosporium oryzae* Breda de Haan Penyebab Penyakit Bercak Cokelat Pada Tanaman Padi

ABSTRAK

Helminthosporium oryzae merupakan salah satu patogen penting pada tanaman padi yang menyebabkan kerusakan dan kehilangan hasil hingga 45%. Pemanfaatan konsorsium bakteri endofit sebagai agens hayati adalah salah satu pengendalian yang ramah lingkungan. Penelitian bertujuan untuk mendapatkan konsorsium bakteri endofit yang efektif dalam menekan pertumbuhan *H. oryzae* secara *in vitro.* Penelitian menggunakan Rancangan Acak Lengkap (RAL) terdiri dari 7 perlakuan dalam 3 ulangan. Perlakuan terdiri dari A (kontrol), perlakuan B (*Serratia marcescens* ULG1E4; *Serratia marcescens* JB1E3), perlakuan C (*Bacillus* sp. HI; *Serratia marcescens* JB1E3), perlakuan D (*Bacillus* sp. HI; *Bacillus* sp. SJI; *Serratia marcescens* JB1E3), perlakuan E (*Serratia marcescens* ULG1E4; *Serratia marcescens* JB1E3; *Serratia marcescens* JB1E2), perlakuan F (*Bacillus* sp. HI; *Bacillus* sp. SJI), dan perlakuan G (*Bacillus* sp. SJI; *Serratia marcescens* ULG1E4). Uji kemampuan konsorsium bakteri endofit dalam menekan pertumbuhan *H. oryzae* diuji dengan metode *dual culture* dan peracunan media. Parameter pengamatan meliputi daya hambat konsorsium bakteri endofit, daya hambat metabolit konsorsium bakteri endofit, berat segar dan berat kering jamur. Perlakuan G (*Bacillus* sp. SJI; *Serratia marcescens* ULG1E4), B (*Serratia marcescens* ULG1E4; *Serratia marcescens* JB1E3), dan E (*Serratia marcescens* ULG1E4; *Serratia marcescens* JB1E3; *Serratia marcescens* JB1E2), efektif dalam menekan pertumbuhan *H. oryzae* dengan daya hambat suspensi bakteri 61,28%, 62,93%, 63,55%, dan daya hambat metabolit sekunder 96.93%, 94,21%, 97,60%.

Kata Kunci: Antibiosis, Daya hambat, *Dual culture*, Kompetisi, Metabolit sekunder

INTRODUCTION

Rice (*Oryza sativa* L.) is the primary staple crop in Indonesia, serving as the main source of calories for the population (Minarni & Fadhilah, 2017). *Helminthosporium oryzae*, a pathogenic fungus belonging to the family *Dematiaceae*, is the causal agent of rice brown spot (Surendhar *et al*., 2021). This pathogen can infect rice plants throughout all growth

Cite this as: Resti Z, Nurbailis, Fauzia A. 2025. Antagonistic Activity of Endophytic Bacterial Consortia Against Helminthosporium oryzae Breda de Haan, The Causal Agent of Brown Spot Disease in Rice. Cropsaver: Journal of Plant Protection, 8(2): 129-136. https://doi.org/10.24198/cropsaver.v8i2.67906

stages, causing brown lesions that may coalesce, leading to leaf damage, reduced tillering, decreased grain weight, and fewer panicles (Putri *et al*., 2021; Dariush *et al*., 2020; Sunder *et al*., 2014). The infection may originate from seeds (primary inoculum) or be disseminated by wind (secondary inoculum), with an incubation period of less than 24 hours. Disease development is strongly influenced by environmental factors, such as relative humidity above 89% and optimum temperatures between 25–30°C (Imran *et al*., 2020; Surendhar *et al*., 2021), and is more prevalent in upland areas, especially rainfed fields (Barnwal *et al*., 2013).

Brown spot management strategies include the use of resistant varieties, field sanitation, balanced fertilization, and fungicide application (Asghar *et al*., 2019). However, improper fungicide use may lead to negative consequences such as chemical residues, pathogen resistance, environmental pollution, and risks to human and animal health (Khalili *et al*., 2012). A more sustainable alternative is biological control using endophytic bacteria (Wang *et al*., 2019), which can suppress pathogens either directly through competition and antibiosis or indirectly through inducing systemic resistance in plants (Ryan *et al*., 2008; Van Loon, 2007). Certain bacteria, such as *Bacillus* spp. and *Serratia marcescens*, are known to produce pathogen-inhibiting compounds, including cell wall degrading enzymes effective against fungi such as *Fusarium* sp. and *Alternaria porri* (Flori *et al*., 2020; Nasiroh *et al*., 2015), as well as antifungal metabolites (Jaiganesh *et al*., 2007; Lestari & Aini, 2021).

The efficacy of endophytic bacteria can be enhanced through consortium formation, wherein compatible strains act synergistically (James & Mathew, 2015). Such consortia can employ multiple mechanisms of pathogen suppression, including the increased production of secondary metabolites such as antibiotics, enzymes, and toxins (Pal & Gardener, 2006 in Rahma *et al*., 2018), as well as siderophores that chelate iron, rendering iron unavailable to pathogens (Tanati, 2012). The antagonistic potential of endophytic bacterial consortia has been widely reported, for example, the combination of *Pseudomonas aeruginosa* and *Bacillus cereus* that inhibited *Pyricularia grisea* (rice blast) (Ukhra *et al*., 2016), and *Bacillus* spp. with *Pseudomonas fluorescens* from forest plants that effectively controlled *Fusarium oxysporum* and *Sclerotium rolfsii* (Munif *et al*., 2021). Compatibility-tested consortia have also successfully suppressed *Culvularia oryzae* in rice, such as the combination of *S. marcescens* ULG1E4 with *S. marcescens* JB1E3 (77% inhibition), *Bacillus* sp. HI, *Bacillus* sp. SJI, and *S. marcescens* JB1E3 (72.33%), and *Bacillus* sp. SJI alone (71.33%) (Resti *et al*., 2022). Given their effectiveness against diverse pathogens, endophytic bacterial consortia hold strong potential as a biological control strategy for *H. oryzae*, the causal agent of brown spot in rice. Therefore, this research aimed to obtain endophytic bacterial consortium capable of inhibiting the growth of *Helminthosporium oryzae.*

MATERIALS AND METHODS

The experiment was arranged in a Completely Randomized Design (CRD) with seven treatments (six endophytic bacterial consortia and one control) and three replicates.

Table 1. Endophytic Bacterial Consortium Treatments

Treatment	Consortium
A	Control
B	*Serratia marcescens* ULG1E4; *Serratia marcescens* JB1E3
C	*Bacillus* sp. HI; *Serratia marcescens* JB1E3
D	*Bacillus* sp. HI; *Bacillus* sp. SJI; *Serratia marcescens* JB1E3
E	*Serratia marcescens* ULG1E4; *Serratia marcescens* JB1E3; *Serratia marcescens* JB1E2
F	*Bacillus* sp. HI; *Bacillus* sp. SJI
G	*Bacillus* sp. SJI; *Serratia marcescens* ULG1E4

Preparation of *Helmithosporium oryzae* Isolates

The *Helminthosporium oryzae* isolate was obtained from the rice variety Bujang Marantau grown in Sawah Piai, Solok City, using the direct planting isolation method (Waruwu *et al*., 2016) from symptomatic brown spot leaves. Leaf samples were surface-sterilized (70% ethanol for 30 s, 1% NaOCl for 2 min, followed by three rinses with sterile distilled water) and plated onto PDA medium, incubated for 5 days at room temperature, and purified by transferring hyphal tips to fresh PDA until a pure culture was obtained. Morphological identification was conducted based on colony shape and color (macroscopic) as well as hyphal structure, and conidial shape and pigmentation (microscopic) following Jaiganesh & Kannan (2019) and Manamgoda *et al*. (2014). Pathogenicity tests were performed on healthy Bujang Marantau rice plants aged 30 days after sowing in a greenhouse by spraying 10 mL of a conidial suspension (10^6 conidia/mL) and covering the plants with plastic sheets to maintain humidity. Initial symptoms of yellow-brown oval lesions appeared 7 days after inoculation (Imrani *et al*., 2017).

Preparation of Endophytic Bacteria Consortia

Endophytic bacteria from the collection of Dr. Zurai Resti, S.P., M.P. were **rejuvenated** and purified on NA medium using the quadrant streak method, followed by Gram staining (Schaad *et al*., 2001) and hypersensitive reaction tests on the abaxial surface of tobacco leaves (Klement *et al*., 1990) to confirm non-pathogenicity. For consortium preparation, 48-hour-old pure cultures were grown in nutrient broth in a rotary shaker at 150 rpm, and compatible isolates were combined according to treatment design. The mixture was incubated until reaching a density of 10^8 cells/mL, standardized against McFarland scale 8 (Resti *et al*., 2018). Secondary metabolites were obtained by centrifuging the bacterial suspension at 10,000 × g for 10 min, filtering the supernatant through a 0.22 µm membrane filter, and discarding the pellet (Resti *et al*., 2020).

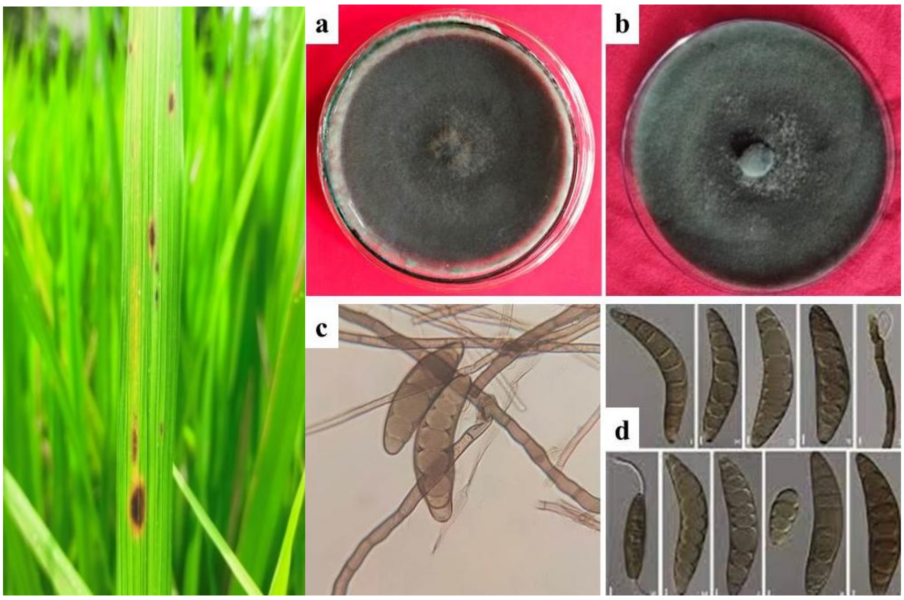

Figure 1. Rice leaves showing brown spot symptoms as the inoculum source of *H. oryzae* from Sawah Piai, Solok City (right); Morphology of *H. oryzae* on PDA medium after 14 days of incubation. (a, b) Macroscopic characteristics of *H. oryzae* on PDA medium (personal collection, Chandana *et al*., 2022), (c, d) Microscopic characteristics of *H. oryzae* at 400× magnification (personal collection, Manamgoda *et al*., 2014)

Inhibitory Test of Endophytic Bacterial Consortium Against *H. oryzae*

The inhibitory potential of the endophytic bacterial consortia against *H. oryzae* was evaluated using the dual culture technique. Sterile 5 mm paper discs were immersed in bacterial suspensions for approximately 3 min, then placed on PDA plates opposite a 5 mm mycelial plug of *H. oryzae* at a distance of 3 cm from the plate center. In the control treatment, the fungal plug was placed 3 cm from the Petri dish edge without bacteria. Plates were incubated at room temperature until fungal colonies in the control reached the plate edge (Sandiase *et al*., 2023).

Secondary Metabolite Assay of Bacterial Consortia Against *H. oryzae*

To assess the inhibitory effect of bacterial secondary metabolites, 1 mL of bacterial supernatant was mixed with 9 mL of PDA at 35 °C, homogenized, poured into Petri dishes, and inoculated with a 5 mm fungal mycelial plug in the center. The control plates contained sterile distilled water instead of supernatant. All plates were incubated at room temperature until control colonies fully covered the plates (Rustam, 2011 in Rahma *et al*., 2018).

Observations began one day after incubation and continued until fungal colonies in the control reached the Petri dish edge. The percentage inhibition of fungal growth by bacterial consortia and bacterial secondary metabolites was calculated following Khalili *et al*. (2012) and Mayadianti *et al* (2020).

$$I = \frac{R1 - R2}{R1} \text{ x } 100$$

Description:

I	=	Percentage of inhibition
R1	=	Radius of pathogen colonies growing on control
R2	=	Radius of pathogen colonies grown under treatment application

Fresh and Dry Weight Measurement of *H. oryzae* Colonies

Fresh and dry weights of *H. oryzae* colonies were recorded after control plates were fully colonized. Agar was dissolved using 10 mL of hot 2.5% HCl (50–75 °C for 1–2 min), and mycelia were filtered through pre-weighed Whatman No. 41 filter paper. The filter paper containing fresh mycelia was weighed to determine fresh weight. For dry weight determination, the mycelia were oven-dried at 60 °C for 48 h until constant weight was reached (Arneti *et al*., 2020).

Fresh and dry weights were calculated following Arneti *et al*. (2020):

BB = BT - BK

Description:

BB = Fresh weight

BK = Fresh weight on control
BT = Fresh weight under treatment application

BB' = BT' – BK'

Description:
BB = Dry weight
BK = Dry weight on control
BT = Dry weight under treatment application

Data Analysis

Data were analyzed using Analysis of Variance (ANOVA), and significant differences among treatments were determined using the Least Significant Difference (LSD) test at the 5% significance level.

RESULT AND DISCUSSIONS

Inhibitory Activity of Endophytic Bacterial Consortia Against *H. oryzae*

The inhibitory activity of the endophytic bacterial consortia against *H. oryzae* varied significantly among treatments (Table 2).

Table 2. Inhibitory activity of endophytic bacterial consortium against *H. oryzae* fungus in vitro

Treatment		Inhibition zone	Inhibition (%)	
E	(*Serratia marcescens* ULG1E4; *Serratia marcescens* JB1E3; *Serratia marcescens* JB1E2)	+	63,55	a
B	(*Serratia marcescens* ULG1E4; *Serratia marcescens* JB1E3)	-	62,93	a
F	(*Bacillus* sp. HI; *Bacillus* sp. SJI)	-	61,79	ab
G	(*Bacillus* sp. SJI; *Serratia marcescens* ULG1E4)	-	61,28	ab
D	(*Bacillus* sp. HI; *Bacillus* sp. SJI; *Serratia marcescens* JB1E3)	-	58,93	bc
C	(*Bacillus* sp. HI; *Serratia marcescens* JB1E3)	-	56,59	c
A	(Control)	-	0,00	d

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

Previous studies have shown that endophytic bacterial consortia exhibit substantial inhibitory potential against plant pathogenic fungi, with inhibition rates exceeding above 50%. Specifically, six tested consortia exhibited inhibition capacities ranging from 56.59% to 63.55%. Treatments C and D showed no statistically significant difference in their antagonistic effect, with inhibition values of 56.59% and 58.93%, respectively, indicating similar fungal responses to both consortia. In comparison, treatments G, F, B, and E demonstrated stronger antagonistic effects than the control treatment, achieving inhibition rates of 61.28%, 61.79%, 62.93%, and 63.55%, respectively, with no significant differences among them. These findings suggest that several consortia are highly effective in limiting pathogen development, although their relative effects may vary depending on the specific bacterial composition.

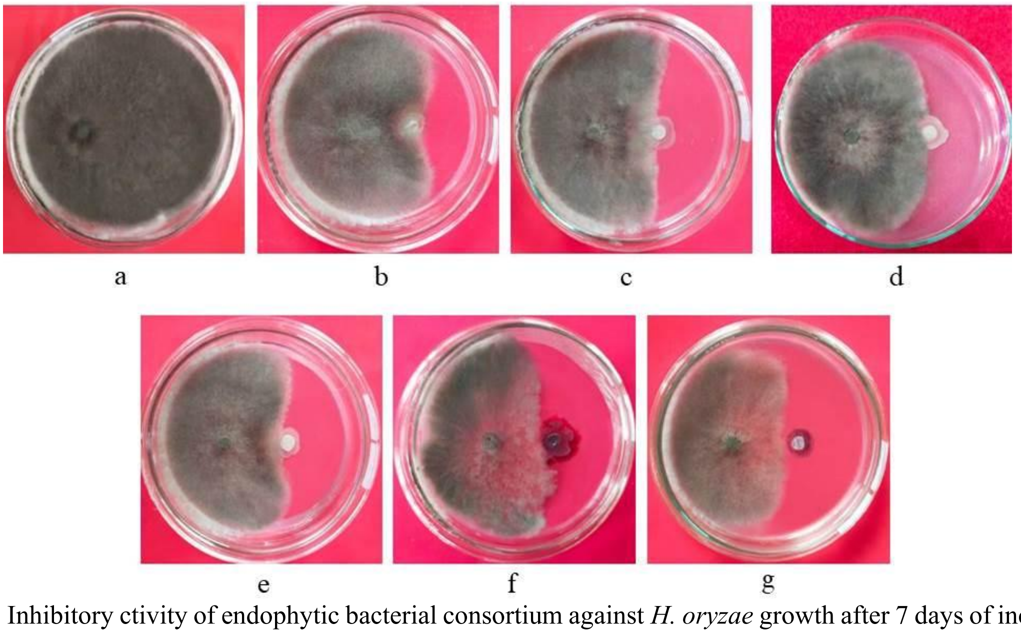

Figure 2. Inhibitory ctivity of endophytic bacterial consortium against *H. oryzae* growth after 7 days of incubation in each treatment. (a). Control, (b). Treatment C, (c). Treatment D, (d). Treatment G, (e). Treatment F, (f). Treatment B, (g). Treatment E.

Further investigations revealed that these consortia not only suppressed fungal colony growth but also caused notable changes in colony morphology. All tested consortia were able to restrict fungal growth, yet the extent and nature of the effects differed across treatments. For instance, treatments B and E not only suppressed colony expansion but also caused noticeable thinning of the fungal colonies. Treatments C, D, and F, however, induced localized thinning specifically in the hyphae that were in direct contact with the endophytic bacterial consortia, suggesting a more targeted antagonistic interaction. Interestingly, treatment G resulted in a different morphological response, where the fungal mycelia appeared thickened rather than thinned. In contrast, the control treatment showed normal colony morphology, with no signs of inhibition or alteration (Figure 2). These variations highlight the complex and diverse mechanisms by which endophytic bacterial consortia can interfere with fungal growth and development, reinforcing their potential as effective biocontrol agents in plant disease management.

Inhibitory Activity of Endophytic Bacterial Secondary Metabolites Against *H. oryzae*

The inhibitory activity of secondary metabolites produced by the bacterial consortia also differed significantly among treatments (Table 3).

Table 3. Inhibitory Activity of Secondary Metabolites Produced by Endophytic Bacterial Consortia Against *H. Oryza* (In-vitro)

	Treatment	Effectiveness (%)
E	(*Serratia marcescens* ULG1E4; *Serratia marcescens* JB1E3; *Serratia marcescens* JB1E2)	97,60 a
G	(*Bacillus* sp. SJI; *Serratia marcescens* ULG1E4)	96,93 a
C	(*Bacillus* sp. HI; *Serratia marcescens* JB1E3)	95,97 a
B	(*Serratia marcescens* ULG1E4; *Serratia marcescens* JB1E3)	94,21 a
D	(*Bacillus* sp. HI; *Bacillus* sp. SJI; *Serratia marcescens* JB1E3)	92,63 ab
F	(*Bacillus* sp. HI; *Bacillus* sp. SJI)	87,73 b
A	(Kontrol)	0,00 c

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

Secondary metabolites produced by endophytic bacterial consortia showed strong antifungal activity, with inhibition rates above 80%. Specifically, the six tested treatments showed inhibition capacities ranging from 87.73% to 97.60%. Among them, treatments D, B, C, G, and E exhibited no statistically significant differences, yet all provided highly effective suppression with inhibition values of 92.63%, 94.21%, 95.97%, 96.93%, and 97.60%, respectively. Treatment F, while still highly effective, showed a comparatively lower inhibition rate of 87.73% (Figure 3). These results indicate that the majority of consortia-derived secondary metabolites are highly potent in restricting fungal development, with several treatments approaching nearly complete inhibition.

In addition to growth suppression, secondary metabolites from the bacterial consortia also induced visible morphological changes in *Helminthosporium oryzae* colonies. The extent of colony suppression varied across treatments, with treatment E producing the smallest colony size, suggesting the strongest growth restriction. Morphological alterations were also observed in fungal colonies, with treatments B, D, E, F, and G causing noticeable thickening of the fungal hyphae. In contrast, treatment C induced thinning of fungal colonies, pointing toward a different mode of antagonistic interaction. Meanwhile, the control colonies exhibited normal growth patterns with no morphological alterations. These observations demonstrate that endophytic bacterial consortia not only limit fungal growth but also influence pathogen morphology, highlighting the multifaceted roles of their secondary metabolites in antifungal activity. Such diverse inhibitory effects suggest that secondary metabolites play a crucial role in the biocontrol potential of endophytic bacteria (Figure 3).

Fresh and Dry Weight of *H. oryzae*

Analysis of variance (ANOVA) revealed that the application of endophytic bacterial consortia had a statistically significant effect on both fresh and dry weights of *Helminthosporium oryzae* compared with the control. However, no significant differences were observed among the six consortia treatments, indicating similar effects on fungal biomass accumulation. The fresh weight of the fungal colonies in treated groups ranged from 1.63 g to 1.28 g, while the corresponding dry weight ranged from 0.04 g to 0.02 g (Table 4). These findings suggest that all tested bacterial consortia were effective in reducing fungal biomass, thereby supporting their potential role as biocontrol agents through suppression of pathogen growth.

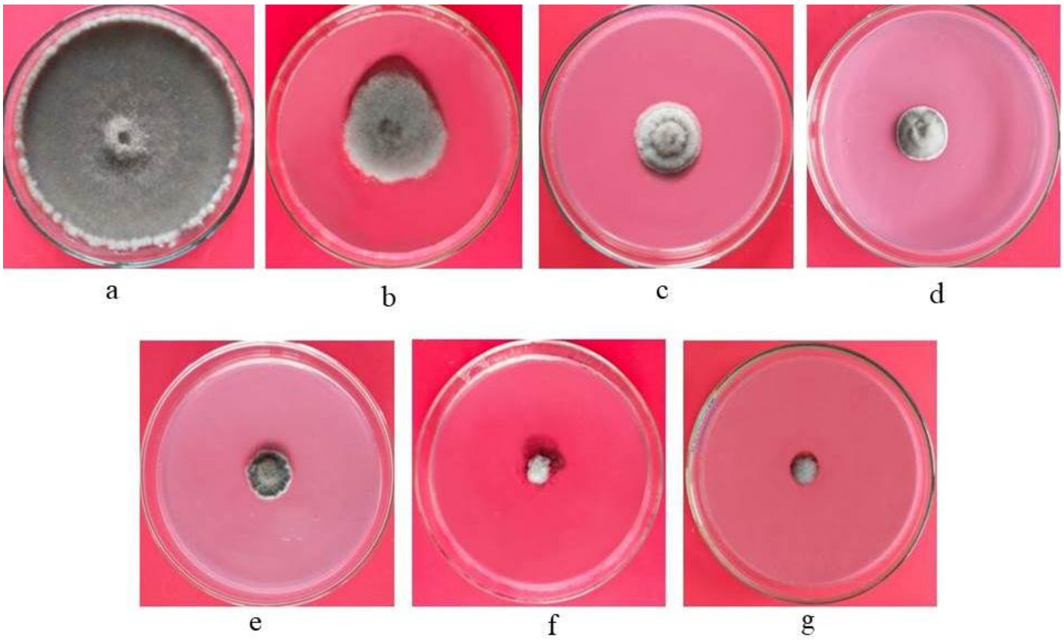

Figure 4. Inhibitory activity of secondary metabolites of endophytic bacterial consortium on the growth of *H. oryzae* 7 days after incubation. (a). Control, (b). Treatment F, (c). Treatment D, (d). Treatment B, (e). Treatment C, (f). Treatment G, (g). Treatment E

Table 4. Effect of endophytic bacterial consortium on fresh weight of *H. oryzae* fungal colonies

Treatment		Fresh weight (gr)	Dry weight (gr)
C	(*Bacillus* sp. HI; *Serratia marcescens* JB1E3)	1,28 a	0,02 a
G	(*Bacillus* sp. SJI; *Serratia marcescens* ULG1E4)	1,35 a	0,02 a
F	(*Bacillus* sp. HI; *Bacillus* sp. SJI)	1,50 a	0,06 a
D	(*Bacillus* sp. HI; *Bacillus* sp. SJI; *Serratia marcescens* JB1E3)	1,59 a	0,15 ab
E	(*Serratia marcescens* ULG1E4; *Serratia marcescens* JB1E3; *Serratia marcescens* JB1E2)	1,61 a	0,02 a
B	(*Serratia marcescens* ULG1E4; *Serratia marcescens* JB1E3)	1,63 a	0,04 a
A	(Control)	4,86 b	0,34 b

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

The six endophytic bacterial consortia tested in this study effectively suppressed the growth of *Helminthosporium oryzae* under in vitro conditions. The most effective treatments were B (*Serratia marcescens* ULG1E4; *S. marcescens* JB1E3), G (*Bacillus* sp. SJI; *S. marcescens* ULG1E4), and E (*S. marcescens* ULG1E4; *S. marcescens* JB1E3; *S. marcescens* JB1E2), showing stronger inhibitory effects than single-strain treatments. These results suggest that bacterial consortia produce more diverse and abundant secondary metabolites, thereby enhancing antagonistic activity. According to Mukherjee et al. (2018), endophytic consortia exhibit higher physiological activity and generate a broader array of metabolites compared to individual isolates. In the present study, inhibition of *H. oryzae* by the consortia ranged from 50–60%, while metabolite extracts achieved inhibition levels above 80%, with several treatments exceeding 90%. Previous findings support this, showing that endophytic bacteria such as *Bacillus* and *Serratia* produce antimicrobial compounds including chitinase, protease, lipase, siderophores, and antibiotics, which contribute synergistically to pathogen suppression (Resti et al., 2017; Jahuddin et al., 2021).

The enhanced effectiveness of treatments B, G, and E is likely due to the complementary activities of the bacterial strains involved. *S. marcescens* was particularly important, as it produces chitinase to degrade fungal cell walls and prodigiosin, a red-pigmented secondary metabolite with antifungal and antiproliferative activity (Samrot et al., 2011; Dalimunthe et al., 2019). Similarly, *Bacillus* sp. contributes additional antagonistic mechanisms, including volatile antifungal compounds and siderophore production, which restrict iron availability to pathogens (Abidin et al., 2015; Neilands & Nakamura, 1991). The synergy between *S. marcescens* and *Bacillus* likely explains the superior suppression observed in consortia compared to single strains. In addition to metabolite-mediated inhibition, the bacterial consortia reduced both fresh and dry fungal biomass, suggesting effective nutrient competition and enzymatic degradation (Resti et al., 2020; Sandiase et al., 2023). Collectively, these results show that endophytic bacterial consortia not only inhibit the growth and development of *H. oryzae* through multifaceted antagonistic interactions but also hold strong potential as biocontrol agents in integrated disease management strategies.

CONCLUSIONS

This study concludes that endophytic bacterial consortia can effectively suppress *H. oryzae* in vitro, with

Treatments B (*S. marcescens* ULG1E4 + JB1E3), G (*Bacillus* sp. SJI + *S. marcescens* ULG1E4), and E (*S. marcescens* ULG1E4 + JB1E3 + JB1E2) showing the highest inhibition (62.93–63.55% for bacterial suspensions; 94.21–97.60% for secondary metabolites). These results highlight their potential as sustainable biocontrol agents in rice disease management, reducing reliance on chemical fungicides (Mukherjee *et al*., 2018; Resti *et al*., 2020; Jahuddin *et al*., 2021).

ACKNOWLEDGEMENT

This research was financially supported by the Faculty of Agriculture, Universitas Andalas. The authors also acknowledge the technical assistance provided by the laboratory analysts, as well as the contribution of Farah Nabila Tores, S.Si., for her assistance in manuscript preparation.

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