

ENDOPHYTIC BACTERIAL CONSORTIA AS BIOLOGICAL CONTROL OF BACTERIAL LEAF BLIGHT AND PLANT GROWTH PROMOTER OF RICE (*ORYZA SATIVA* L.)

Zurai Resti*, Yenny Liswarni, Martinius

Study Program of Plant Protection, Faculty of Agriculture, Andalas University, Padang, Indonesia.

*Corresponding author

Email: zurairesti@agr.unand.ac.id

Abstract. *The consortia of endophytic bacteria with various mechanisms (competition, antibiotics, induction of resistance, and others), simultaneously, are more effective in controlling pathogens and increasing plant growth. The purpose of this study was to obtain endophytic bacterial consortia capable of suppressing of bacterial leaf blight and promoting the growth of rice plants. The study consisted of three experimental stages. The first stage was the test on the antibiosis ability of the endophytic bacterial consortia to suppress of pathogenic bacteria *Xanthomonas oryzae* pv. *oryzae* (Xoo) using the Kirby Bauer method. The second stage was the test on the ability of endophytic bacterial consortia to promote the growth of rice seedlings. The experiment was arranged in a Completely Randomized Design (CRD) which consisted of six combinations of endophytic bacterial consortia and 15 replications within each combination. The endophytic bacterial consortia were introduced by soaking the seeds, and the observations were made on the plant height, number of leaves, root length, fresh weight, and dry weight of rice seedlings. Meanwhile, the third stage was the test on the ability of endophytic bacterial consortia to suppress of bacterial leaf blight (BLB) diseases and to promote the rice plant growth. The experiment was arranged in a Completely Randomized Design (CRD) with six combinations of endophytic bacterial consortia and five replications within each combination. The endophytic bacterial consortia were introduced by soaking the roots of the seedlings. The observations were made on the incidence of disease, disease severity, number of leaves, plant height, and number of tillers. The results showed that all endophytic bacterial consortia had antibiosis abilities. The best endophytic bacterial consortia for controlling bacterial leaf blight and promoting the growth of rice seedlings and plants were the C (*Bacillus* sp SJI; *Bacillus* sp HI) and D (*Bacillus* sp SJI; *S.marcescens* isolate JB1E3) consortia.*

Keywords: *antibiosis; bacillus sp; bacterial leaf blight; endophytic bacterial consortia; serratia marcescens*

1. Introduction

Rice (*Oryza sativa* L.) is one of the important food crops in Indonesia. This is due to the fact that most Indonesians consume rice as a staple food. As a major carbohydrate supplier, rice productivity must be increased to meet the needs of the Indonesian people. However, the production of rice is always fluctuating due to certain constraints in the rice-producing centers. These obstacles include climate anomalies such as erratic rainfall as well as the pathogen attacks, such as *Xanthomonas oryzae* pv. *oryzae* causing bacterial leaf blight (BLB), *Pyricularia oryzae* causing blast disease, *Culvularia oryzae* and *Helminthosporium oryzae* causing leaf spot, *Deschlera oryzae* causing brown spots, tungro virus, rice grassy stunt virus, and rice ragged stunt virus (Semangun, 2004).

One of the practices used by farmers to control pathogens that attack rice plants is using chemical pesticides. However, excessive application of pesticides adversely affects non-target microbes and humans. Therefore, we need environmentally friendly technology. The recommended technique, which is considered as environmentally friendly for diseases in rice plants, is biological control. Biological control is based on the use of antagonistic microbes that can be direct (competition, hyper-parasite, and antibiosis) or indirectly through the induction of plant resistance. The use of endophytic microbes in increasing plant resistance to several types of pathogens is widely studied. Schulz & Boyle (2006) explained that, generally, endophytic colonization without the appearance of disease symptoms in plants occurs due to an antagonistic balance between plant defense responses and the level of endophytic virulence.

Endophytic bacteria can act as biocontrol agents, which suppress the development of pathogens, several types of nematodes, and insects through direct or indirect mechanisms. Direct mechanism is performed by producing antimicrobial compounds, (Wang *et al.*, 2010), siderophore, and lytic enzymes (Lugtenberg & Kamilova, 2009), by competing in obtaining iron, nutrients, and space, and through parasitism. Indirectly mechanism is performed through the mechanism of systemic resistance induction in host plants. Induced systemic resistance (ISR) is the interaction of certain bacteria with roots that allows the plant to develop resistance to potential pathogens (Bakker *et al.*, 2007).

As plant growth promoters, endophytic bacteria can act as biological fertilizers, rhizoremediators, phytostimulators, and protectors from abiotic and biotic stress (Induced Systemic Tolerance). Endophytic bacteria improve the availability of nutrients for their host through nitrogen fixation and phosphate dissolving ability (Lugtenberg & Kamilova, 2009), provide Fe through siderophores and produce phytohormones such as IAA, gibberellins, and cytokinins (Miller & Berg, 2009).

Endophytic bacteria have advantages over other biocontrol agents due to their presence in plant tissues, so they are able to withstand biotic and abiotic pressures (Hallmann *et al.*, 1997). Besides playing role as biocontrol agents, several types of endophytic bacteria also function as plant growth promoters, such as *Burkholderia cepacia*, *P. fluorescens*, and *Bacillus* sp (Kloepper *et al.*, 1999). *Burkholderia* sp. PsJN strain is able to promote the growth of grapes (*Vitis vinifera* L.) (Compant *et al.*, 2005). *Bacillus* sp can induce the resistance of Shallot plants to bacterial leaf blight disease through increased plant defense enzymes (Resti *et al.*, 2016). *Bacillus lentimorbus* Dutky and *Bacillus cereus* are effective in controlling rust in coffee leaves (Shiomi *et al.*, 2006).

Endophytic bacterial consortia can provide various control mechanisms (competition, antibiotics, induction of endurance, and others) simultaneously, enabling them to be more effective

in controlling pathogens (James & Mathew, 2015). Furthermore, according to Kumar *et al.*, (2016) the combination of microorganisms in the consortium can control various plant pathogens more effectively. Bacteria have more than one beneficial effect on the host, with different disease suppression mechanisms. Combining strains with different disease suppression mechanisms can control pathogens more effectively.

The screening for endophytic bacteria from shallots plants against bacterial leaf blight resulted in 6 isolates that are potential as biological control and plant growth promoter. The endophytic bacteria are *B. cereus* P14, *B. cereus* Se07, *Bacillus* sp HI, *Bacillus* sp SJI, *Serratia marcescens* isolate ULG1E2, and *Serratia marcescens* isolate JB1E3. Singly introduced, these bacteria have the effectiveness of disease suppression and increasing yield of 28.32 - 64.30% and 50.65 - 214.85%, respectively (Resti *et al.*, 2013). However, the effectiveness of those bacteria, when introduced as consortia, needs to be studied. This endophytic bacterial consortium will probably give more effective results because each bacterium has the potential to be quite effective in a single introduction. For this reason, a deeper study on the endophytic bacteria consortium, as a biological controller for plant pathogens, needs to be carried out. The objective of this research was to obtain endophytic bacterial consortia capable of controlling pathogens and promoting the growth of rice plants.

2. Methods

2.1. Preparation of Endophytic Bacterial Consortia

Endophytic bacteria from different species and strains from the collection of (Resti *et al.*, 2013) were rejuvenated using the scratch method. Endophytic bacteria of the genus of *Bacillus* were rejuvenated in the TSA medium and those of the genus of *Serratia* in the NA medium. Bacteria were scratched on the medium and incubated for 48 hours, then the Gram and HR testing were performed to verify the collection of bacteria.

Pure culture of endophytic bacterial isolates was cultured in the NA medium, and 48 hour bacterial culture colony was placed on the slide and mixed with one drop of 3% KOH solution. If the results of the mixture are thick, it shows that the isolate is Gram-negative. On the contrary, if it is watery, it means that it is Gram-positive (Schaad *et al.*, 2001).

The endophytic bacterial suspension (10^8 cells/ml) was infiltrated to the lower surface of tobacco leaves using a syringe and incubated for 2x24 hours. If necrotic does not occur within 2x24 hours, it means that the bacteria are HR negative (Klement *et al.*, 1990).

2.2. Propagation of Endophytic Bacterial Consortia

Endophytic bacteria that are compatible were cultured in the NB medium. The consortium was made by combining all possible compatible combinations which were then cultured in the NB

medium and incubated on a rotary shaker for 48 hours, at a speed of 150 rpm at room temperature. The consortium was prepared with a population of 10^8 cfu/ml. The treatments used in the experiment was the endophytic bacterial consortia as presented in Table 1.

Table 1. Endophytic bacterial consortium treatment

Code	Endophytic Bacteria Strain
A	Control
B	<i>S.marcescens</i> isolate ULG1E4; <i>S. marcescens</i> isolate JB1E3
C	<i>Bacillus</i> sp. SJI; <i>Bacillus</i> sp HI
D	<i>Bacillus</i> sp SJI; <i>S.marcescens</i> isolate JB1E3
E	<i>Bacillus</i> sp. SJI ; <i>Bacillus</i> sp. HI ; <i>S. marcescens</i> isolate JB1E3
F	<i>S.marcescens</i> isolate ULG1E4; <i>S. marcescens</i> isolate JB1E3; <i>Bacillus</i> sp HI

2.3. Antibiosis Test of Endophytic Bacterial Consortia Against *Xoo*

The antibiosis test was performed using the Kerby Bauer method (1959). A consortium of endophytic bacteria, according to the treatment, was bred in the NB medium for 3 x 24 hours. The bacterial culture was then centrifuged at 7000 rpm for 10 minutes. The supernatant was extracted and filtered using 0.22 µl membrane syringe filters. Sterile disc paper was soaked in the supernatant for 10 minutes and dried. A sterile petri dish containing *Wakimoto* medium was inoculated with *Xoo* population of 10^6 cells/ml. Paper discs that already contained a supernatant endophytic bacterial consortium were placed on the surface of the *Wakimoto* medium, and it was incubated for 3 x 24 hours at room temperature. The presence of a clear zone/inhibition zone indicates that a consortium of endophytic bacteria is capable of producing antibiotic compounds. The inhibition zone diameter was measured. The difference in the diameter of the inhibition zone shows the difference in the ability to produce antibiotics.

2.4. Test of Endophytic Bacterial Consortium as Biological Control of Bacterial Leaf Blight and Growth Promoter of Rice Seedlings and Plants

Experiment on the ability of endophytic bacterial consortia to improve the growth of rice seedlings, which was arranged in a Completely Randomized Design (CRD) consisting of six treatments and 15 replications. Experiment on the ability of endophytic bacterial consortia to suppress the development of BLB in rice plants *in planta*, which was arranged in a Randomized Complete Block Design (RCBD) consisting of six treatments and 15 replications (for the nursery phase) and six treatments and five replications (during the planting phase).

2.5. Preparation of Seeds and Growing Media

Germination and growing media used were a mixture of sterilized soil and manure (2: 1 v / v). Sterilization was done by heating a mixture of soil and manure at a temperature of 100°C for 1 hour, then cooled. Sterile media was then placed on the seedbed for sowing. Meanwhile, for planting (growing media), the soil was placed in a 5 kg plastic pot and arranged on the land with a spacing of 10 x 10 cm. The rice cultivar used was Cisokan, which is susceptible to the disease.

2.6. Planting Preparation

The plastic pots were placed in paddy fields (wetland paddy fields in Tanah Sirah Piai Nan XX District), whose surroundings were also planted with rice. Inoculation occurred naturally. The cultivation techniques were adapted to the habits of local farmers.

2.7. Introduction of Endophytic Bacterial Consortium

The rice seeds were sterilized with NaOCl 2 % about 1 minute, then rinsed with sterile distilled water. Furthermore, the seeds were immersed in 50 ml of endophytic bacterial consortium suspension with a population density of 10^8 cells/ml for 15 minutes, dried and planted on the seedbed (Resti *et al.*, 2013). The seedling were kept until for 25 days, then transferred to the plastic pots. Before being planted, the roots of the rice seedlings were immersed in 250 ml of endophytic bacterial consortium suspension with a population density of 10^8 cells/ml for 2 hours (Nandakumar *et al.*, 2001). For the control treatment, the seeds were immersed in sterile water.

Pathogen inoculation occurred naturally, from seedling to harvest. All types of pathogens that infect rice were observed, both from the group of fungi and bacteria.

2.8. The Growth of Rice Seedlings

The seedling growth was observed starting a week after planting to 30 days after planting, at intervals once a week. The variables observed were seedling height, number of seedling leaves, seedling root length, seed fresh weight, and seed dry weight.

2.9. Disease Incidence (%) (Resti *et al.*, 2013)

Disease incidence was observed from the appearance of the first symptoms. It was calculated according to the formula as follows (1):

$$\text{Incidence of disease} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100\% \quad (1)$$

2.10. Disease Severity (%)

The severity of the disease was calculated based on the scale of disease symptoms that appear, which is BLB caused by *Xoo*. The observations were carried out every week after symptoms appeared on five leaves in 5 tested plants in each treatment unit. The intensity of BLB was calculated using the method of Abbot (1925) in Khairuni *et al.*, (2014) by using the formula as follows (2):

$$I = (A / B) \times 100\% \quad (2)$$

Remarks:

I = disease intensity (%)

A = the length of leaf showing blight symptoms

B = overall length of sample leaf

2.11. The Growth of Rice Plants

The variables observed included plant height and number of tillers, which were observed every week starting from a week after planting until constant growth was achieved.

2.12. Data Analysis

All data obtained were statistically analyzed using Analysis of Variance (ANOVA). The difference between treatments was analyzed using the Least Significant Difference test (LSD) at the level of 5%.

3. Results and Discussion

3.1. Antibiosis Test of Endophytic Bacterial Consortia Against *Xoo*

The results of the antibiosis test of endophytic bacterial consortia are shown in Table 2. All endophytic bacterial consortia could produce antibiotics that were indicated by the inhibition zone produced. The difference in the diameter of the inhibition zone distinguishes the consortium's ability to produce antibiotics.

Table 2. The antibiosis ability of consortium endophytic bacteria against *Xoo*

Endophytic bacterial consortia	Clear zone (mm)
D	22.75 a
E	22.00 a
F	19.50 a
B	13.75 b
C	10.00 b
A	0.00 c

The numbers followed by the same lowercase letters are not significant according to the LSD at a 5% level.

3.2. Test of endophytic bacterial consortia as growth promoter of rice seedlings in the nursery

The results of the observation on the seedling height, number of leaves, root length, and fresh weight and dry weight of rice seedlings are shown in Table 3. The introduction of endophytic bacterial consortia could enhance the growth of rice seedlings up to 30 days after sowing. Seedling height, number of leaves, root length, and fresh weight and dry weight of rice seedlings introduced with endophytic bacterial consortia were statistically significantly different.

The D endophytic bacterial consortium (*Bacillus* sp SJI; *S. marcescens* isolate JB1E3) significantly increased the height of the rice seedlings compared to other consortium treatments, with a seedling height of 55,400 cm (Figure 1). The C endophytic bacterial consortium (*Bacillus* sp SJI; *Bacillus* sp HI) significantly increased the number of leaves of the rice seedlings compared to other treatments, with reaching 11,733 leaves. Meanwhile, there was no significant difference observed in the root length of the rice seedlings between control, C, D, and E consortium treatments. The endophytic bacterial consortia of D (*Bacillus* sp SJI; *S. marcescens* isolate JB1E3) and E (*Bacillus* sp SJI; *Bacillus* sp HI; *S. marcescens* isolate JB1E3) produced the highest values

of rice seedling fresh weight compared to other consortia, with a fresh weight of 2,720 g and 2,773 g, respectively. Meanwhile, the highest values of rice seedling dry weight were observed in the seedlings introduced with endophytic bacterial consortium of C (*Bacillus* sp SJI; *Bacillus* sp HI), with a dry weight of 0.607 g.

Table 3. Height, number of leaves, root length, fresh weight and dry weight of rice seedlings introduced with a consortium of endophytic bacteria (30 Das)

Endophytic bacterial consortia	Seedling Growth				
	Height (cm)	Number of leaves (helai)	Root length (Cm)	Fresh weight (g)	Dry weight (g)
A	53.600 ab	11.267 a	14.667 a	2.473 ab	0.407 cd
B	50.467 b	8.867 b	11.867 b	1.926 bc	0.267 e
C	52.600 ab	11.733 a	12.467 ab	2.340 ab	0.607 a
D	55.400 a	10.533 ab	13.267 ab	2.720 a	0.467 bc
E	51.200 b	10.733 ab	13.133 ab	2.773 a	0.560 ab
F	50.667 b	10.333 ab	11.133 b	1.540 c	0.353 de

The numbers followed by the same lowercase letters are not significant according to the LSD at a 5% level

3.3. Test of Endophytic Bacterial Consortia as Biological Control of Bacterial Leaf Blight Disease in Rice Plants

The symptoms of bacterial leaf blight disease were in the form of chlorosis along the midrib, starting from the leaf blade. The introduction of endophytic bacterial consortium in rice plants could suppress the attack of bacterial leaf blight in rice plants. The percentage of the incidence of bacterial leaf blight is shown in Figure 1. The ability of the C endophytic bacterial consortium (*Bacillus* sp SJI; *Bacillus* sp HI) to suppress the incidence of bacterial leaf blight in rice plants was significantly different from that of other consortia and control treatment. The introduction of the C endophytic bacterial consortium (*Bacillus* sp SJI; *Bacillus* sp HI) also suppressed the severity of bacterial leaf blight, with the percentage of disease severity of 6.78%, which was significantly different from control but not significantly different from other treatments (Figure 1).

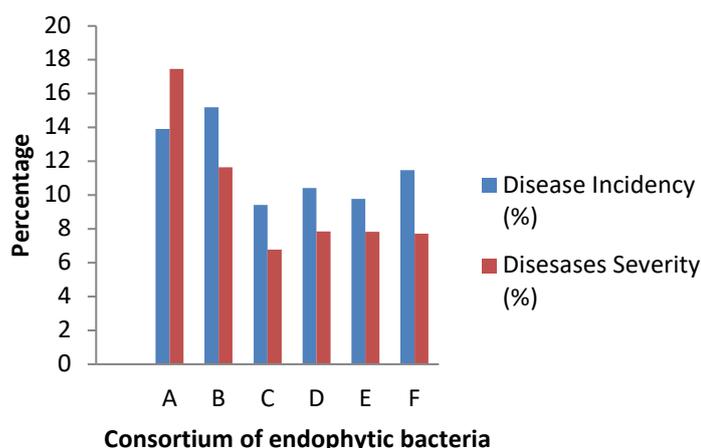


Figure 1. Incidence and severity of BLB disease in rice plants introduced by endophytic bacterial consortium (60 Dat)

3.4. Test of Endophytic Bacterial Consortia as Growth Promoter Of Rice Plant

The growth of rice plants introduced with the endophytic bacterial consortia is shown in Table 4. The endophytic bacterial consortium of B (*S. marcescens* isolate ULG1E4 ; *S. marcescens* isolate JB1E3), C (*Bacillus* sp SJI; *Bacillus* sp HI), D (*Bacillus* sp SJI; *S. marcescens* isolate JB1E3), and E (*Bacillus* sp SJI; *Bacillus* sp SJI; *Bacillus* sp HI; *S. marcescens* isolate JB1E3) were able to significantly increase rice plant height compared to control treatment. The comparison of plant height in endophytic bacterial consortium processing with control is shown in Figure 2. The introduction of rice plants with D endophytic bacterial consortium of D (*Bacillus* sp SJI; *S. marcescens* isolate JB1E3) could significantly increase the number of leaves and the number of rice tillers.

3.5. Discussion

The endophytic bacterial consortia were able to suppress the development of *Xoo* in vitro. The ability of antibiosis was indicated by the presence of inhibition zones (clear zones) formed around the pathogenic bacterial colonies. The greater diameter of the inhibition zone formed, the greater ability of the consortium to inhibit *Xoo*. The highest antibiosis ability was observed in the endophytic bacterial consortium of D (*Bacillus* sp SJI; *S. marcescens* isolate JB1E3), E (*Bacillus* sp SJI; *Bacillus* sp HI; *S. marcescens* isolate JB1E3), and F (*S. marcescens* isolate ULG1E4; *S. marcescens* JB1E3; *Bacillus* sp HI).

Table 4. Plant height, number of leaves and number of tillers introduced by endophytic bacterial consortium (60 Dat)

Endophytic bacterial consortia	Plant Growth		
	Plant height (cm)	Number of leaves	Number of tillers
A	74.80 b	147.40 b	44.29 b
B	84.00 a	165.80 ab	50.40 ab
C	82.90 a	157.80 ab	45.00 ab
D	82.70 a	170.40 a	51.80 a
E	83.90 a	158.20 ab	47.40 ab
F	78.60 ab	170.80 a	50.40 ab

The numbers followed by the same lowercase letters are not significant according to the LSD at a 5% level

The introduction of endophytic bacterial consortia also could improve seedling growth, reduce the BLB diseases, and enhance rice growth. The introduction of endophytic bacterial consortium of D (*Bacillus* sp SJI; *S. marcescens* isolate JB1E3) was able to significantly increase the seedling height (55.4 cm), the number of seedling leaves (11.73), and seedling fresh weight (2.71 g) (Table 3). The endophytic bacterial consortium of C (*Bacillus* sp SJI; *Bacillus* sp HI) were able to suppress BLB diseases in rice plants, to increase the seedling dry weight, and to increase the plant height compared to control. Furthermore, the endophytic bacterial consortium of D (*Bacillus* sp SJI; *S. marcescens* isolate JB1E3) were able to increase the plant height, the number of leaves, and the number of tillers.

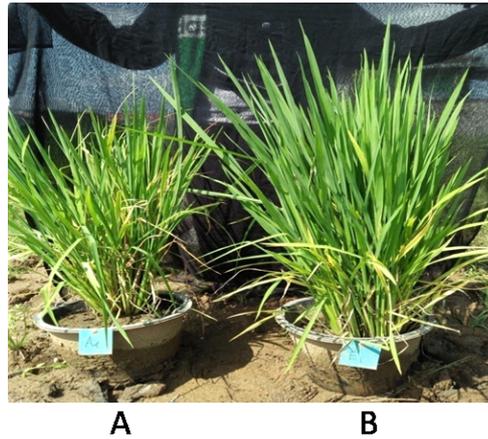


Figure 2. Comparison of rice plant growth (60 Dat) A. Control, B. Treatment of endophytic bacterial consortium D (*Bacillus* sp SJI; *S. marcescens* isolate JB1E3)

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The ability of endophytic bacterial consortia to improve seedling growth (height, number of leaves, fresh weight and dry weight) and to enhance rice plant growth (height, number of leaves and number of tillers) is due to their ability to stimulate plant growth. The consortia used were a mixed culture of the genus *Bacillus* and *S. marcescens*. According to Resti *et al.* (2017), endophytic bacteria of *Bacillus* sp HI, *Bacillus* sp SJI, and *S. marcescens* are able to produce IAA. Besides that, *S. marcescens* is also able to dissolve phosphate. Endophytic bacteria of the genus *Bacillus* have the characteristic as biocontrol because they can produce antibiotics and colonize the shallots root tissue to a population of 3.2×10^5 to 6.2×10^5 cfu/g (Resti *et al.*, 2018a). This endophytic bacterial consortium has also been introduced to chili seeds and was able to suppress the growth of *Ralstonia solanacearum* bacteria and enhance the growth of chili seedlings and plants (Resti *et al.*, 2018b). According to Yasmin *et al.* (2016), five antagonistic bacteria, namely *Pseudomonas* spp E227, E233, Rh323, *Serratia* sp. Rh269 and *Bacillus* sp Rh219 were able to increase root length (41%) and plant dry weight (60%) compared to controls. All isolates were also able to dissolve phosphate (82-116 μ g/ml) and produce IAA (0.48-1.85 mg/ml).

The endophytic bacterial consortium of C (*Bacillus* sp SJI; *Bacillus* sp HI) was able to suppress BLB disease in rice plants. The introduction of the consortia of *Bacillus* sp SJI

and *Bacillus* sp HI reduced the incidence of BLB by 32.35% and reduced the severity of the disease by 61.15%. This ability is caused by the consortium consisting of two types of *Bacillus* sp bacteria that can produce compounds that can inhibit plant pathogens such as antibiotics, siderophore, and salicylic acid. According to Resti *et al.*, (2017), *Bacillus* sp SJI and *Bacillus* sp HI were able to produce salicylic acid, respectively, 14.67 ppm/ml and 14.40 ppm/ml. Besides that, *Bacillus* sp HI also produced siderophore. Furthermore, according to Yasmin *et al.* (2016), *Pseudomonas* spp. E227, E233, Rh323, *Serratia* sp. Rh269 and *Bacillus* sp. Rh219 showed the antagonistic ability to *Xoo* with inhibition zones of 1 - 19 mm, and the five bacteria were also capable of producing siderophore.

The suppression of BLB disease in rice plants in the field was also due to the influence of the induction mechanism of plant resistance introduced with endophytic bacterial consortia. The endophytic bacterial consortia of *Bacillus* sp SJI and *Bacillus* sp HI can affect the resistance of rice plants to BLB. According to Resti *et al.* (2016), the introduction of endophytic bacteria isolates PU2E2 and SN1E4 to shallots was able to increase the activity of the peroxidase enzyme in the roots and leaves of the shallots, showing the resistance to the disease. Based on molecular identification, the two endophytic bacterial isolates are bacteria from the species *Bacillus* sp. Nagendran *et al.* (2013) stated that the introduction of *B. subtilis* (FZB 24) to rice seeds was able to suppress *Xoo* attacks and to reduce the incidence of BLB disease by 2.80%. The introduction of *B. subtilis* (FZB 24) increases the activity of the enzyme peroxidase, polyphenol oxidase, phenylalanine lyase, and the accumulation of phenol compounds in rice plants.

The introduction of endophytic bacterial consortia is more effective in suppressing disease and increasing the growth of rice plants because more than one type of endophytic bacteria found have different mechanisms of suppressing disease and promoting plant growth. Combining bacteria from the *Serratia* group (which can produce IAA and dissolve phosphate), with *Bacillus* (which can produce siderophore and activate plant defense enzymes), will be more effective compared to a single introduction of the endophytic bacteria either at suppressing disease or increasing plant growth.

4. Conclusion

All endophytic bacterial consortia have antibiosis abilities. The best endophytic bacterial consortium in controlling bacterial leaf blight and improving the growth of rice seedlings and plants is the consortia of C (*Bacillus* sp SJI; *Bacillus* sp HI) and D (*Bacillus* sp SJI; *S. marcescens* isolate JB1E3).

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