

## Exploration of Rice Plant Endophyte Bacteria to Suppress *Xanthomonas oryzae* Bacterial Leaf Blight Disease

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### Abstract

**Background:** Rice is a major food commodity for Indonesian. According to BPS (2022) rice production in 2021 decreased 0.43%. The decline caused by various factors, one of which is bacterial leaf blight disease by the pathogenic bacteria *Xanthomonas oryzae*. Control of *X. oryzae* mostly uses pesticides, but this control has a negative impact on the environment, therefore it is necessary to develop endophytic bacteria (EB) that can inhibit the growth of *X. oryzae* and can act as PGPR. This study was to determine the EB ability in healthy rice stem tissue to suppress bacterial leaf blight disease caused by the bacteria *X. oryzae*. **Methodology:** This research was were used isolation from infected plant for *X. oryzae* and from healthy plant for EB, data was analyzed using the corresponding formula. **Findings:** found 10 isolates of EB that could inhibit the *X. oryzae* growth as seen from the inhibition zone produced in the antagonist test. The EB isolates produced the largest inhibition zones such as BE18 (23.3 mm, very strong category), BE16 (13.3 mm, strong category), and BE3 (11.6 mm, strong category). The 4 isolates with bactericidal inhibition mechanisms, namely isolates (BE11, BE15, BE16, and BE18), 6 isolates with bacteriostatic inhibition mechanisms, namely isolates (BE3, BE5, BE6, BE7, BE12, and BE13). **Contribution:** The availability of EB present in the tissues of healthy rice stems is still relatively high and can be used to suppress the growth of pathogenic bacterium *X. oryzae*. This result also supported by the beneficial properties of EB, which can enhance plant resistance

**Keywords:** Endophyte bacteria; Rice plant; *X. oryzae*



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### INTRODUCTION

Rice is a major food commodity for Indonesian citizens that plays a strategic role in the national economy. Zafar & Jianlong (2023) stated that rice is a food crop that produces rice which is the staple food and source of energy for humans. The large increase in population has resulted in an increase in food needs, so that rice production

must be increased. According to data of [BPS \(2022\)](#), rice production in 2021 decreased by 233.91 thousand tons or 0.43 % from 54.65 million tons to 54.42 million tons of dry milled rice. The limiting factor that caused the decrease in rice production was the attack of the *X. oryzae* pathogen which causes bacterial leaf blight disease. [Wahyudi et al., \(2011\)](#) stated that bacterial blight is generally caused by the pathogenic bacteria *X. oryzae*. This disease can cause yield losses of 35.8 % ([Kurniawati et al., 2015](#)). Control of bacterial leaf blight disease caused by *X. oryzae* bacteria has been widely carried out, one of which is the use of New Superior Varieties (VUB) such as Conde and Angke. The problem is, Conde and Angke still have weaknesses including seeds that are difficult to obtain and low resistance to *X. oryzae* if planted in a monoculture pattern.

Control of bacterial leaf blight disease in addition to using resistant varieties also uses biological agent control techniques to suppress attacks by the *X. oryzae* pathogen. Control using biological agents has a positive value because it is effective and safe for the environment. Research on biological agents to suppress bacterial leaf blight disease caused by *X. oryzae* bacteria has been widely carried out. [Alan et al., \(2016\)](#) succeeded in isolating *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Trichoderma* sp which play a role in triggering rice growth and controlling bacterial leaf blight disease.

Endophytic bacteria are bacteria that are not pathogenic but have properties that are beneficial to plants. Endophytic bacteria are currently widely used as biological agents because they produce antimicrobial compounds, plant growth regulators, fix nitrogen, and mobilize phosphate which play a role in stimulating and resilient plants ([Hartanti, 2020](#)). The antibacterial properties of endophytic bacteria are twofold, namely bactericidal and bacteriostatic.

Biological control using endophytic bacteria has advantages including being able to provide plant resistance so that it is not attacked by diseases caused by pathogens. Endophytic bacteria can be found mostly in plant root tissue ([Ali et al., 2024](#)). The role of endophytic bacteria is very large, one of which is being able to increase plant growth by producing or providing elements to meet plant needs, one of the elements needed by plants is phosphate ([Khan et al., 2009](#)). Therefore, research on the exploration of endophytic bacteria in rice plants was carried out in order to obtain endophytic bacteria in healthy rice stem tissue that can be used to suppress bacterial leaf blight disease caused by *X. oryzae* bacteria.

## **METHOD**

### **Endophytic Bakteria and *Xanthomonas oryzae* Preparation**

#### **Endophytic Bacteria**

Endophytic bacterial isolation samples were taken from healthy rice plant stem tissue. Cut healthy rice stems with a length of 3-4 cm, then washed using sterile distilled water. After that, the stem surface was sterilized by soaking the rice stems in 70 % alcohol for 1 minute and 1 % NaOCl for 3 minutes. Then rinsed using sterile distilled water for 1 minute. The sterile stems were divided into two parts using a sterile knife aseptically and then placed on Nutrient Agar (NA) media in an inverted position and then incubated for 24 hours. Observe the growth of endophytic bacterial colonies and

then purify them on new NA media. The endophytic bacterial isolates to be tested amounted to 20 isolates.

#### *Xanthomonas oryzae*

Rice plant samples with symptoms of bacterial leaf blight disease came from Sedati Village, Sidoarjo, East Java. Symptoms of bacterial leaf blight disease that can be identified start from the appearance of gray spots on the edges of the leaves. Isolation of *X. oryzae* bacteria was carried out by cutting the leaves of rice plants with the border of the diseased and healthy leaves. The cut leaves were surface sterilized using 1 % NaOCl solution for 3 minutes then rinsed using sterile water. The sterilized leaves were then crushed using a mortar. The crushed leaves were put into a test tube by adding 1 ml of distilled water to obtain an extract of 1 ml of suspension, then put into a test tube containing 9 ml of distilled water for dilution, vortexed to homogenize the suspension then inoculated into NA media. The Koch postulate test was carried out using healthy rice plants to determine whether the isolates found showed symptoms of damage caused by *X. oryzae*.

#### *X. oryzae* Postulate Koch Test

This Koch's postulate test was carried out by preparing healthy rice seedlings and cutting the leaves (0.5–2 cm in length) using scissors that had been dipped in a suspension of *X. oryzae* bacteria with a concentration of  $10^8$  CFU/mL for 10 seconds (Srinivas et al., 2024). Observations were conducted for approximately one week, during which the symptoms that appeared had to match those of the previously observed bacterial leaf blight disease. Once similar symptoms appeared, re-isolation was performed. The result of the re-isolation must be bacterial colonies with characteristics identical to the original isolate (Dewi et al., 2015).

#### Antagonist Test

The antagonist test was conducted in vitro using the Disc diffusion technique using filter paper with a diameter of 0.5 cm. The antagonist test was conducted by pouring a suspension of *X. oryzae* with a population density of  $10^8$  CFU/mL into a petri dish (diameter 9 cm) containing NA media. The filter paper to be used for the antagonist test of endophytic bacteria against the pathogenic bacteria *X. oryzae* was previously soaked in a suspension of endophytic bacteria with a population density of  $10^8$  CFU/mL. Then the filter paper was dried on sterile tissue so that it was not too wet when placed on the NA media in the petri dish. The dried filter paper was inoculated into a petri dish containing a suspension of *X. oryzae* on NA media using tweezers and incubated for 48 hours. Next, observe the clear zone that is formed. Isolates that produce clear zones will be entered into the following formula refers to (Tjiptoningsih, 2020).

$$\text{Inhibition zone : } \frac{(a-c)+(b-c)}{2} \dots\dots\dots (1)$$

According to [Jamilatun \(2020\)](#), the inhibition zone formed by biological agents can be categorized as follows, < 5 mm (Weak), 5 - 10 mm (Medium), 11 - 20 mm (Strong), > 20 mm (Very Strong).

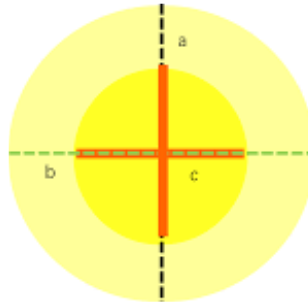


Figure 1. Inhibitory zone

### Inhibitory mechanism

The antibiotic mechanism test of endophytic bacteria was carried out by taking agar from the clear zone formed in the antagonist test using a scalpel. The agar media in the clear zone was put into a test tube containing 10 ml of sterile distilled water and crushed using an ose needle. Then the test tube was shaken for 10 minutes until it became cloudy. Take one ose and grow it using the streak method in a petri dish that already contains NA media. Then incubation was carried out for 7 days and observed whether or not there was growth of the pathogen *X. oryzae* on NA media ([Zinidin, 2022](#)).

### Data Analysis

The data obtained from the observation results were analyzed based on the category of inhibition zones formed in the antagonistic test of endophytic bacteria with *X. oryzae* in vitro. The inhibition zones of endophytic bacteria are categorized into 4 categories, namely Weak (<5 mm), Moderate (5-10 mm), Strong (11-20 mm), Very Strong (> 20 mm). The experiment used Completely Randomized Design (CRD) with a replicant of 3.

## RESULT AND DISCUSSION

### *X. oryzae* Exploration and Isolation

Isolation of *X. oryzae* was carried out from rice plant samples with BLB symptoms obtained in the rice fields of Sedati village, Sidoarjo, East Java. The environmental conditions in the field were humid and there were rice plants suspected of being infected with *X. oryzae* (figure 2.A) with symptoms of damage showing gray rice leaf tips, drying leaves, symptoms developing to the edges of the leaves turning brownish yellow in length so that only the green color remains on the rice leaf veins, this is in accordance with the symptoms of damage caused by rice leaf blight bacteria (*X. oryzae*). [Wahyudi et al., \(2011\)](#) stated that the symptoms of damage caused by *X. oryzae* are brownish yellow lines starting from the base of the leaf to the tip of the leaf, young plants will experience drought due to this bacteria so that the leaves have a texture like crackles.



**Figure 2.** *X. oryzae* exploration (A) Rice plants affected by *X. oryzae*; (B) colony shape; (C) cell shape (100x); (D) Koch postulate test results

The isolation results showed that the *X. oryzae* colony was yellow (Figure 2.B) and microscopic observations showed that the cells were rod-shaped (Figure 2.C) and Koch's postulate test showed the same symptoms of damage as those in the field (Figure 2.D). This is in accordance with the statement of [Wahyudi et al., \(2011\)](#) namely that the *X. oryzae* colony was yellow with bacillus-shaped cells measuring 0.7 - 2.0  $\mu\text{m}$  which usually appeared standing alone or in groups.

### Endophyte bacteria exploration and isolation

Endophytic bacteria (EB) exploration was carried out by taking healthy rice plant stems. [Pradana et al., \(2015\)](#) stated that endophytic bacteria can be found in the soil or plant tissue of endophytic bacteria which in nature can be mutualistic or antagonistic to their host plants. The results of endophytic bacterial isolation obtained 20 isolates of endophytic bacteria which were then subjected to soft rot tests to determine the pathogenic properties of endophytic bacteria. Ten out of the twenty bacterial isolates were endophytic bacteria classified as non-pathogenic.

Table 2. shows the results of isolation and identification of endophytic bacteria as a whole have the same morphology, the only difference is in the color of the colony, namely having a colony color of cream and milky white, all isolates have a bacillus/rod cell shape with blue/positive gram staining. [Desriani et al., \(2014\)](#) stated that there are various types of endophytic bacteria that can be found in the soil and in plant tissue. The color of the colony varies from white, cream, yellow, green, blue, red, and orange, the endophytic bacteria found can be suspected of being *Bacillus* spp. *Pseudomonas* spp.

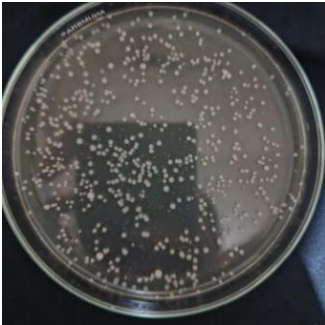
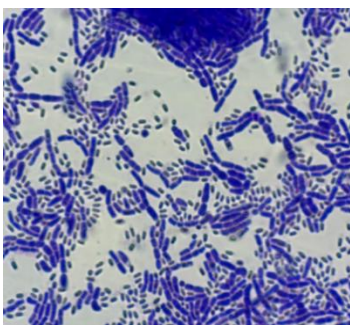


*Micrococcus* spp., *Staphylococcus* spp. and several species from the *arthobacter* spp. and *Curtobacterium* spp. groups.


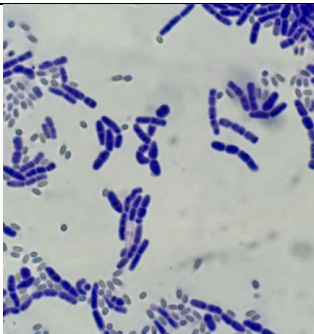
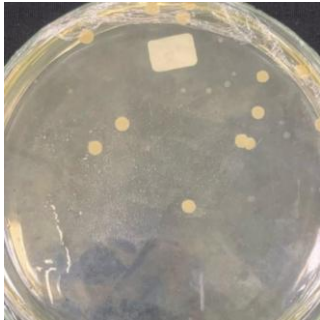
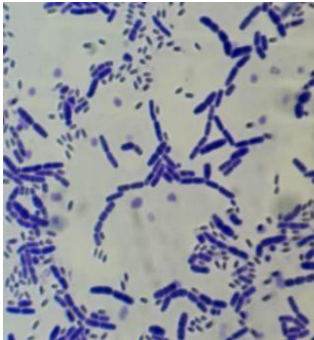
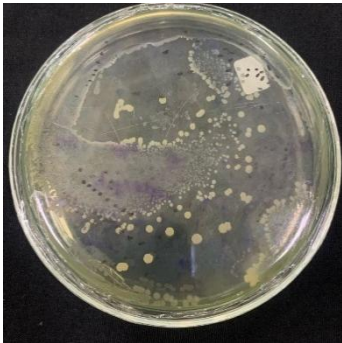
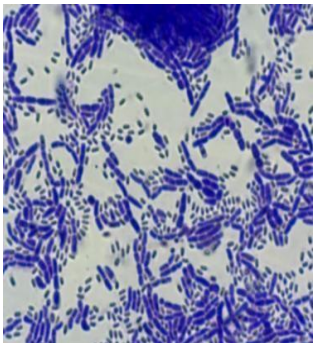
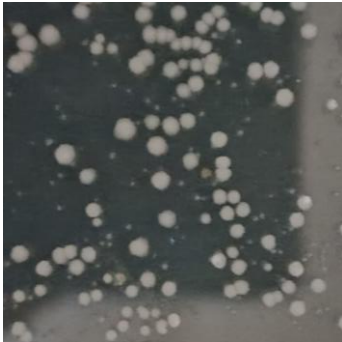
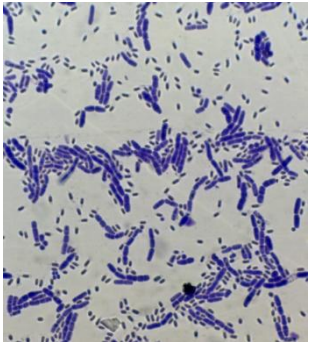
**Table 1.** Soft Rot Test Result

Isolate code	Description
BE1	Pathogen
BE2	Pathogen
BE3	Non pathogen
BE4	Pathogen
BE5	Non pathogen
BE6	Non pathogen
BE7	Non pathogen
BE8	Pathogen
BE9	Pathogen
BE10	Pathogen
BE11	Non pathogen
BE12	Non pathogen
BE13	Non pathogen
BE14	Pathogen
BE15	Non pathogen
BE16	Non pathogen
BE17	Pathogen
BE18	Non pathogen
BE19	Pathogen
BE20	Pathogen


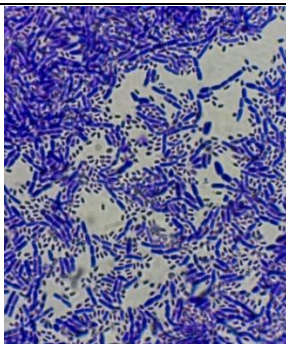

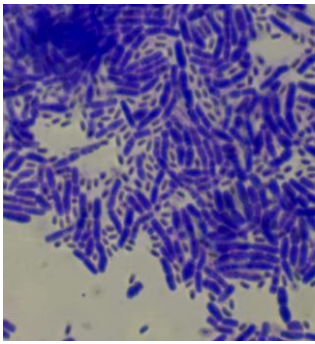
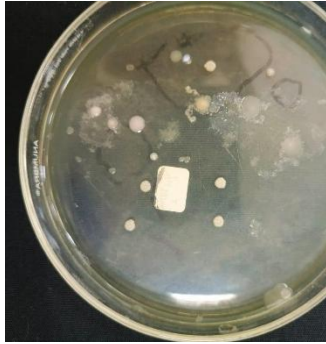
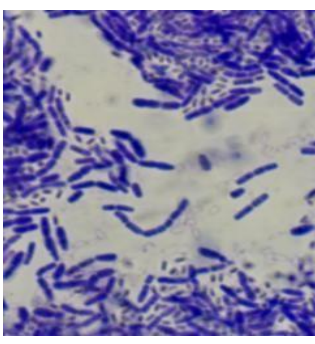
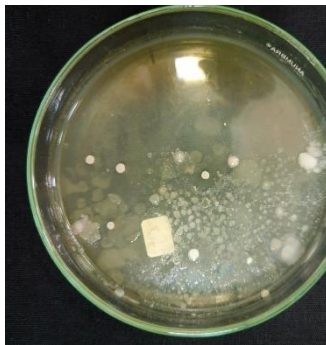
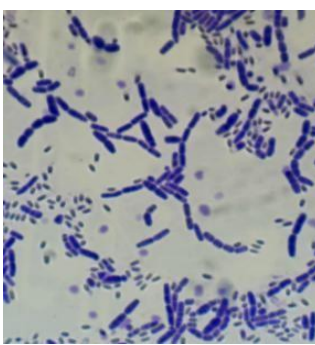
**Tabel 2.** Endophytic Bacteria Identification result

Macroscopic	Microscopic	Description
		1). Gram staining: Blue / + 2). Catalase test: no bubbles / - 3). Koh test: no slime / + 4). Cell shape: rod 5). Colony morphology: white, round colonies with smooth edges

**Tabel 2.** Endophytic Bacteria Identification result

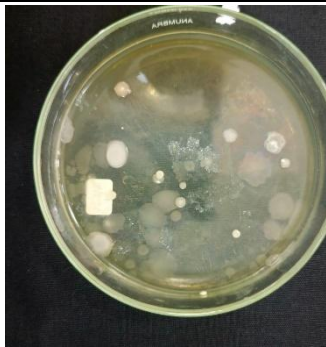
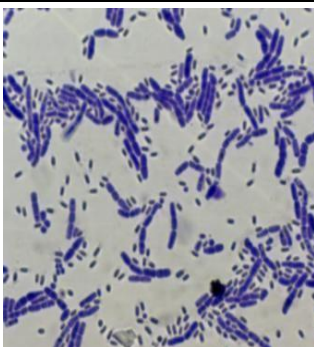
Macroscopic	Microscopic	Description
		<ol style="list-style-type: none"> <li>1). Gram staining: Blue / +</li> <li>2). Catalase test: no bubbles / -</li> <li>3). Koh test: no slime / +</li> <li>4). Cell shape: rod</li> <li>5). Colony morphology: white, round colonies with smooth edges</li> </ol>
Isolate BE5	Cell Structure (100x)	
		<ol style="list-style-type: none"> <li>1). Gram staining: Biru / +</li> <li>2). Catalase test: no bubbles / -</li> <li>3). Koh test: no slime / +</li> <li>4). Cell shape: rod</li> <li>5). Colony morphology: cream-colored, round colonies with smooth edges</li> </ol>
Isolate BE6	Cell Structure (100x)	
		<ol style="list-style-type: none"> <li>1). Gram staining: Blue / +</li> <li>2). Catalase test: no bubbles / -</li> <li>3). Koh test: no slime / +</li> <li>4). Cell shape: rod</li> <li>5). Colony morphology: cream-colored, round colonies with smooth edges</li> </ol>
Isolate BE7	Cell Structure (100x)	
		<ol style="list-style-type: none"> <li>1). Gram staining: Blue / +</li> <li>2). Catalase test: no bubbles / -</li> <li>3). Koh test: no slime / +</li> <li>4). Cell shape: rod</li> <li>5). Colony morphology: cream-colored, round colonies with smooth edges</li> </ol>
Isolate BE11	Cell Structure (100x)	

**Tabel 2.** Endophytic Bacteria Identification result

Macroscopic	Microscopic	Description
		<ol style="list-style-type: none"> <li>1). Gram staining: Blue / +</li> <li>2). Catalase test: no bubbles / -</li> <li>3). Koh test: no slime / +</li> <li>4). Cell shape: rod</li> <li>5). Colony morphology: cream-colored, round colonies with smooth edges</li> </ol>
Isolate BE12	Cell Structure (100x)	
		<ol style="list-style-type: none"> <li>1). Gram staining: Blue / +</li> <li>2). Catalase test: no bubbles / -</li> <li>3). Koh test: no slime / +</li> <li>4). Cell shape: rod</li> <li>5). Colony morphology: cream-colored, round colonies with smooth edges</li> </ol>
Isolate BE13	Cell Structure (100x)	
		<ol style="list-style-type: none"> <li>1). Gram staining: Blue / +</li> <li>2). Catalase test: no bubbles / -</li> <li>3). Koh test: no slime / +</li> <li>4). Cell shape: rod</li> <li>5). Colony morphology: cream-colored, round colonies with smooth edges</li> </ol>
Isolate BE15	Cell Structure (100x)	
		<ol style="list-style-type: none"> <li>1). Gram staining: Blue / +</li> <li>2). Catalase test: no bubbles / -</li> <li>3). Koh test: no slime / +</li> <li>4). Cell shape: rod</li> <li>5). Colony morphology: cream-colored, round colonies with smooth edges</li> </ol>
Isolate BE16	Cell Structure (100x)	



**Tabel 2.** Endophytic Bacteria Identification result

Macroscopic	Microscopic	Description
		1). Gram staining: Blue / + 2). Catalase test: no bubbles / - 3). Koh test: no slime / + 4). Cell shape: rod 5). Colony morphology: cream-colored, round colonies with smooth edges
Isolate BE18	Cell Structure (100x)	

### Antagonistic test between *X. oryzae* and endophytic bacteria

Endophytic bacterial antagonist test was conducted to determine the ability of endophytic bacteria to inhibit the growth of *X. oryzae*. Based on the observation results, it is known that there is a clear zone around the Whatman filter paper. The clear zone began to appear 24 hours after application, then the clear zone increased in diameter until 48 hours. The data on the diameter of the clear zone obtained was processed to obtain the inhibitory power. The antagonist results are known to be included in the category of a very strong inhibition zone in isolate (BE18) with an average diameter of the inhibition zone of (23.3 mm). The category of strong inhibition zone in isolates (BE3 and BE16) with an average diameter of (11.6 mm and 13.3 mm). The category of moderate inhibition zone in isolates (BE11 and BE12) with an average diameter of (8.6 mm and 5 mm). The weak inhibition zone category is owned by (BE5, BE6, BE7, BE13, and BE15) with an average diameter of (4.6 mm, 4.3 mm, 4 mm, 3.3 mm, and 4.4 mm) (Table 3) so it can be concluded that all BE found are able to inhibit the growth of *X. oryzae*.

**Table 3.** Antagonistic Tests Results of endophytic bacteria on the growth of *X. oryzae*

Inhibition zone diameter		
Isolate	Diameter (mm)	Description
BE3	11.6	Strong
BE5	4.6	weak
BE6	4.3	weak
BE7	4	weak
BE11	8.6	Medium
BE12	5.0	Medium
BE13	3.3	Weak
BE15	4.3	Weak
BE16	13.3	Strong
BE18	23.3	Very Strong

### Inhibition mechanisme

Based on the observation results, the inhibition mechanism possessed by endophytic bacteria is in the form of bacteriostatic and bactericidal antibiotics inhibition mechanisms. Table 4 shows the time of emergence of *X. oryzae* colonies, isolates BE3, BE5, BE6, BE7, BE12, and BE13 have bacteriostatic inhibition mechanisms where on average *X. oryzae* colonies appear on day 6 after being grown on NA media while isolates BE11, BE15, BE16, and BE18 show bactericidal inhibition properties because after being grown on NA media there was no growth of *X. oryzae* bacteria until day 8. Research by [Parida et al., \(2016\)](#) showed that *X. oryzae* isolation requires an incubation period of around 2-3 days to be able to grow a perfect colony while *X. oryzae* colonies that were treated require an incubation period of around 6 days so that it can be concluded that the inhibition mechanisms of endophytic bacteria found are bacteriostatic and bactericidal

**Table 3.** Antibiotic Mechanism of Endophytic Bacteria Againts *X. oryzae*

Isolate	Inhibitory mechanism	Colony sighting on - (days)
BE3	Bacteriostatic	5
BE5	Bacteriostatic	6
BE6	Bacteriostatic	6
BE7	Bacteriostatic	4
BE11	Bactericide	-
Be12	Bacteriostatic	5
BE13	Bacteriostatic	3
BE15	Bactericide	-
BE16	Bactericide	-
BE18	bactericide	-

The inhibition mechanism is a method of APH bacteria in inhibiting the growth of pathogens, [Iqlima et al., \(2017\)](#) stated that the bacterial inhibition mechanism consists of competition for space and food, antibiotic production, and induction of plant resistance. The inhibition mechanism can be divided into 2 types, namely bactericidal and bacteriostatic, Research by [Serdani et al., \(2018\)](#) showed that endophytic bacteria found in rice plants have bacteriostatic inhibition abilities in inhibiting the growth of *X. oryzae*. Research by Handayani (2015) showed that endophytic bacteria found from jamblang plant leaves (*Syzygium cuminil*) have bacteriostatic and bactericidal inhibition abilities against pathogenic bacteria.

### CONCLUSION

10 isolates of endophytic bacteria were obtained that could inhibit the growth of *X. oryzae* bacteria. The largest inhibition zones were obtained from isolates BE18, BE16, and BE3, which were 23.3 mm (very strong category), 13.3 mm (strong category), and 11.6 mm (strong category). The inhibition mechanisms produced were 6 isolates that

were bacteriostatic (BE3, BE5, BE6, BE7, BE12, and BE13) and 4 isolates that were bactericidal (BE11, BE15, BE16, and BE18). Suggestion: It is advisable to conduct an in vivo field test to determine its ability to directly inhibit and its ability as a PGPR (Plant Growth Promoting Rhizobium). The availability of endophytic bacteria (EB) present in the tissues of healthy rice stems is still relatively high and can be used to suppress the growth of the pathogenic bacterium *X. oryzae*. This is also supported by the beneficial properties of EB, which can enhance plant resistance.

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