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Control of bacterial leaf blight disease in several varieties of rice plants (*Oryza sativa* L.) by using bacteria of *Paenibacilus polymyxa* Mace

Sopialena[1]*, Suyadi[1], R Jannah[2] and D Tantiani[1]
[1]Faculty of Agriculture, University of Mulawarman
[2]Departement of Agriculture, East Kutai District
Jl. Pasir Balengkong, Kampus Gunung Kelua, Samarinda, East Kalimantan-Indonesia

*Email : sopialena88@gmail.com

Abstract. Bacterial Leaf Blight Disease caused by bacteria *Xanthomonas oryzae* pv. *Oryzae* is an endemic disease found in all rice plantation in Indonesia. The bacteria infects plants from the vegetative to generative phase and is able to reduce production by up to 30%. Most of farmers control the disease by using chemical pesticides that cause pollution to the environment. While this study offers control by using biological pesticides with the bacteria *Paenibacilus polymyxa* Mace. The research aims to control Bacterial Leaf Blight Disease using *P. polymyxa* Mace in several varieties of rice plants (*Oryza sativa* L.) as well as to obtain effective concentrations of *P. polymyxa* Mace Bio agent to control Bacterial Leaf Blight Disease. Experiments were conducted using Split-plot design with 3 (three) replication. The main plot was the Concentration of *P. polymyxa* Mace consist of 4 levels, they were P0 =Control/Untreated, P1=2.5 mL.L-1 water, P2=5 mL.L-1 water, P3=7.5 mL.L-1 water, and as a Sub Plot was a Variety (V) consist of 3 levels, they were V1=Cibogo Variety, V2=Mekongga Variety, V3=Ciherang Variety. The results showed that that the Mekongga variety is the most resistant variety to the pathogen *Xanthomonas oryzae* pv. Oryzae. The use of *P. Polymyxa* Mace as biological agents is effective to control Bacterial Leaf Blight caused by the pathogen *X. oryzae* pv. Oryzae on several rice plant varieties (*Oryza sativa* L.), and the best concentration of *P. Polymyxa* Mace to control Bacterial Leaf Blight disease was 5 mL.L-1 water.

1. Introduction

Bacterial Leaf Blight (BLB) caused by the pathogen *Xanthomonas oryzae* pv. oryzae (*Xoo*) is one of the main diseases of rice in Indonesia and other rice producing countries in Asia. This disease was first reported in Fukuoka Prefecture, Japan in 1884. This bacterium is a disease-causing agent that has a wide distribution in tropical and sub-tropical regions [1]. Bacterial Leaf Blight is caused by the pathogen *X. oryzae* pv *oryzae* (*Xoo*), this bacterium is systemic and infects plants at various growth phases [2]. Symptoms start from the edges of the leaves, are greyish in colour and after a while the leaves become dry. If the attack occurs during flowering. The grain filling process is disrupted, resulting in the grain being partially full or completely empty. In such circumstances, up to 50% to 70% of the yield will be lost. [3].

BLB disease will develop well at a temperature of 25 - 34°C, with a relative humidity above 70%. This is generally observed when strong winds and heavy rain occur continuously, so that bacteria can easily spread through the droplets of infected plant lesions [4]. As a result of this disease attack, production can be reduced by up to 75% [5]. The pathogen *Xoo* is easy to produce new pathotypes, so a resistant variety that is planted repeatedly appears to lose its resistance. To preserve resistant varieties, variety rotation in planting is still recommended. [6].

In Indonesia, until now 12 strains of *Xoo* have been found with different virulence levels and strains IV and VIII are known to dominate the symptoms of Leaf Blight in rice plants in Indonesia [7]. Pesticides are often used to control disease in the field in Indonesia because they are more practical. Pesticides, on the other hand, pollute the environment and harmful to human health. Meanwhile, fungi such as *Trichoderma* sp., *Beuveria* sp., and other fungi are commonly used as biological controls. [8].

This study was intended to fill the knowledge gap regarding biological control using bacteria. The bacteria used to control BLB Disease are *Paenibacilus polymyxa* Mace. This study specifically was aimedto determine the resistance variety of Rice plant to BLB disease; the efficacy of the use of *P. polymyxa* as biological agents and the appropriate concentration to control BLB disease caused by *Xoo* on several rice plant varieties (*Oryza sativa* L.).

2. Research Methods

This research was conducted in Lempake Village, North Samarinda District. East Kalimantan, Indonesia. The materials used in the study consisted of: 3 rice seed varieties (Cibogo, Ciherang and Mekongga), *P. polymyxa* Mace Biological Agent, and *Xoo* Isolate. The research was arranged in a split-plot design) with 3 (three) replications. The experimental plot measuring 3 m x 4 m using a spacing of 25 cm x 25 cm, where the main plot was the biological agent *P. polymyxa* (P) consisting of 4 levels, they were P0 = Control / / without P. *polimyxa* biological agent; P1 = 2.5 $mL.L^{-1}$ water; P2 = 5 $mL.L^{-1}$ water and P3 = 7.5 mL.L-1 water. As subplots, the variety (V) consists of 3 levels, they were V1 = Cibogo variety; V2 = Mekongga variety and V3 = Ciherang variety. Each treatment combination was replicated 3 times. so that there were 4 x 3 x 3 = 36 experimental unit groups. The location of 36 experimental units at the test site was determined by a lottery based on the effects of randomization (random).

2.1. Activities in the laboratory

Sampling of diseased leaves of bacterial leaf blight was carried out at rice plantations in rice production centers. The samples were taken to the Plant Protection Laboratory of Mulawarman University for isolation of (*Xoo*) bacteria. Isolation of bacteria from leaves, carried out by the leaf washing method, namely by cutting / slicing 3 leaves symptomatic between healthy and diseased part of leaves, about 1 mm wide, then washed with sterile distilled water. The washing water was collected in an Erlenmayer glass, diluted to a 10-3 dilution, then approximately 1 ml was taken and incubated in a Petri dish containing Nutrient Agar (NA) medium. Incubation was carried out in the laboratory at room temperature. Single colonies, typical of *Xoo* bacteria, were transferred to NA medium slant, and then inoculated on rice varieties in the field and then identified their development.

2.2. Activities in the field

Plowing, harrowing, and leveling were undertaken 4 weeks before planting to prepare the field for planting. A hand tractor was used in the first process, and human labor was used in the second. After preparing the filed, soil samples were taken for analysis at Mulawarman University's Soil Laboratory..

After the seeds have been sown for 15-21 days, they were planted. Seedlings were planted 2-3 stems per hole, with a spacing of 25 x 25 cm^2. The replicates were separated by 40 cm.

2.3. Application of biological agent P. polymyxa mace

The first stage involved soaking the seeds in 10 mL.L-1 water and then draining them before sowing them. Subsequent applications were conducted in the field with the concentration according to the test treatment at 14, 28, and 42 days after planting (DAP). Since a solution is needed for 500-600 liters of rice plants per hectare, the P. *polymyxa* biological agent solution required for the research field is 82 mL.L-1 water. During the analysis, up to 0.25 liters per application in the field was used. To prevent bacterial damage caused by sunlight, application was performed in the afternoon from 15.00-17.30. [9].

2.4. Pathogen inoculation of X. oryzae pv oryzae (Xoo)
Isolates that have been cultured in the Unmul HPT laboratory were inoculated on rice plants by spraying *Xoo* isolate on rice plants at the beginning of planting, which was 10 days after planting and just before the primordia stage, which was 43 DAS. In order to ovoid exposure to too high a temperature, inoculationwas carried out in the late afternoon, around 15.00-17.30 [9].

2.5. Observation parameters
Observation of disease severity was carried out at 2 weeks after inoculation (WAI) in the morning to determine the reaction of each variety by measuring leaf length and symptoms. Disease severity is the ratio of the length of the symptoms to the length of the inoculated leaves. Variety resistance reactions were grouped according to disease severity as a result of the last observation. Disease severity less than 11% is classified as resistant = R, severity of more than 11% is classified as susceptible = S [10]

Observation of the intensity of BLB (*X. oryzae* pv oryzae) on leaves in the field. Observations were made at 4 WAI and then observed every week. Observations were made 8 times after inoculation. The calculation of disease intensity uses the formula according to [11] as follows:

$$\mathrm{DI} = \frac{\mathrm{a}}{\mathrm{b}} \times 100\%$$

where: DI: Disease Intensity (%)
a : Length of bacterial leaf blight symptoms (cm)
b : Overall leaf length (cm)

2.6. Data analysis
The data obtained were analyzed using the ANOVA. The treatment effect was evaluated based on the calculated F value at the 5% confidence level. The differences between treatments were tested using the LSD significant difference test at the 5% confidence level [13].

3. Results and Discussion

3.1. Bacterial Leaf Blight Disease Intensity in Cibogo Variety (V1) at 5-8 weeks after inoculation.
The average intensity of Bacterial Leaf Blight (HDB) caused by the pathogen *X. oryzae* pv *oryzae* (*Xoo*) on Cibogo varieties when observed 5-8 WAI, can be seen in Table 1.

Table 1. Average Disease Intensity of Cibogo Variety (V1) at 5-8 WAI

Treatment	Disease Intensity (%)			
	5	**6**	**7**	**8**
P0	0.77b	1.90b	3.17b	4.54b
P1	0.61a	1.62b	2.73b	3.89b
P2	0.53a	1.06a	1.58a	2.10a
P3	0.50a	1.00a	1.49a	1.98a

Notes: numbers followed by the same letter in the same column, means that they are not significantly different in the 5% LSD test

Observation of BLB disease during the fifth to eighth week showed that the use of *P. polymyxa* biological agents in rice plants of Cibogo variety with a concentration of 5 mL.L-1 water (P2) was not significantly different from a concentration of 7.5 mL L-1 water (P3), but significantly different at the

concentration of 2.5 mL.L-1 water (P1) and significantly different at the concentration of 0 mL.L-1 water (P0 / without treatment). Concentrations of 5 mL.L-1 water (P2) and 7.5 mL.L-1 water (P3) were both able to suppress the development of acterial leaf blight disease caused by the pathogen *X. oryzae* pv *oryzae* (*Xoo*). Symptoms of BLB disease caused by pathogen Xoo, on the other hand, may grow on plant leaves using 2.5 mL.L-1 water (P1) and on plant leaves that have not been treated with P.*polymyxa* biological agents (P0 / Control).). The environmental conditions at field were temperature of 28^0C, humidity of 82% and rainfall 10 mm / day. Pathogen *Xoo* was still able to cause further symptoms in plant leaves treated with concentration of 2.5 mL.L-1 water (P1) and without treatment (P0) despite low rainfall. On the other hand, *Xoo* pathogen is only able to show very low follow-up symptoms at concentrations. . The highest BLB disease intensity was found in plant leaves without *P.polymyxa* biological agent treatment (P0/Control), namely 4.54%, while the lowest BLB disease intensity was found in plant leaves using a concentration of 7.5 mL L-1 water (P3) namely 1.98%.

3.2. Bacterial Leaf Blight Disease Intensity in Mekongga Varieties (V2) at 5 - 8 WAI

The average intensity of Bacterial Leaf Blight caused by the pathogen *X. oryzae* pv *oryzae* (*Xoo*) on Mekongga varieties when observed 5-8 WAI, can be seen in Table 2.

Table 2. Average Disease Intensity in Mekongga Varieties (V2) at 5-8 WAI Observation

Treatment	Disease Intensity (%)			
	5	**6**	**7**	**8**
P0	1.59b	2.92b	4.07b	5.31b
P1	1.57b	2.85b	3.98b	5.05b
P2	0.53a	1.06a	1.58a	2.10a
P3	0.47a	0.94a	1.40a	1.86a

Notes: numbers followed by the same letter in the same column, means that they are not significantly different in the 5% LSD test

Observations of BLB disease at week five to week eight showed that the use of *P.polymyxa* biological agents at a concentration of 7.5 mL L-1 water (P3) was not significantly different from a concentration of 5 mL L-1 water (P2), but significantly different against a concentration of 0 mL.L-1 water (P0 / without treatment) and plants using *P.polymyxa* biological agent with a concentration of 2.5 mL.L-1 water (P1). The highest BLB disease intensity was found in plant leaves that did not use *P.polymyxa* biological agent (P0 / Control), namely 5.31%, while the lowest BLB was found in plant leaves using a concentration of 7.5 mL. L-1 water (P3) is 1.86%., The use of *P.polymyxa* biological agents with a concentration of 7.5 mL.L-1 water (P3) and a concentration of 5 mL. L-1 water (P2) tended to be able to suppress the development of BLB disease in the Mekongga variety (V2), whereas Plant leaves that use a concentration of 2.5 mL.L-1 water (P1) and plant leaves that do not use *P.polymyxa* (P0) biological agents tended to be less able to suppress the development of this BLB disease. This can be seen from the increase in the percentage of BLB disease intensity caused by the pathogen *X. oryzae* pv oryzae (*Xoo*) at a concentration of 2.5 mL.L-1 water (P1) and those not using *P.polymyxa* biological agents (P0 / Control). Environmental conditions (temperature 28^0C, humidity 82% and rainfall 10 mm / day) during observation were thought to affect the development of BLB disease caused by pathogen *Xoo*. Environmental conditions that strongly support the development of pathogens *Xoo* in the field, were only able to infect fewer plant leaves using a concentration of 5 mL L-1 water (P2) and a concentration of 7.5 mL L-1 water (P3), this is evidenced by the decreasing intensity of BLB disease in these leaves.

3.3. Bacterial Leaf Blight disease intensity in Ciherang Varieties (V3) at 5-8 WAI.
The mean HDB disease intensity caused by the pathogen *X. oryzae* pv oryzae (Xoo) in Cibogo variety when observed at 5-8 WAI, can be seen in Table 3.

Table 3. Average Disease Intensity in Ciherang Varieties (V3) at 5-8 WAI

Treatment	Disease Intensity (%)			
	5	6	7	8
P0	0.60a	1.63a	2.81a	4.09a
P1	0.84a	1.72a	2.53a	3.35a
P2	0.52b	1.03b	1.53b	2.03b
P3	0.56b	1.11b	1.65b	2.19b

Notes: numbers followed by the same letter in the same column, means that they are not significantly different in the 5% LSD test

Observations from week five to week eight showed that the use of *P.polymyxa* biological agent at a concentration of 7.5 mL.L-1 water (P3) was not significantly different from the concentration of 5 mL.L-1 water (P2), but significantly different from concentration 2 , 5 mL.L-1 water (P1) and a concentration of 0 mL.L-1 water (P0 / Control). The highest BLB disease intensity was found in plant leaves that did not use *P.polymyxa* biological agents (P0 / Control), namely 4.09%, while the lowest BLB disease intensity was found in plant leaves using a concentration of 5 mL L-1 water (P2) namely 2.03%. In this week of negligence observations the use of *P.polymyxa* biological agents with a concentration of 7.5 mL.L-1 water (P3) and a concentration of 5 mL.L-1 water (P2) tended to be able to suppress the development of BLB disease in the Ciherang variety (V3) , however, concentrations below that tend to be less able to suppress the development of HDB disease. This can be seen from the increase in the percentage of BLB disease intensity caused by pathogen *Xoo* at a concentration of 2.5 mL.L-1 water (P1) and not using *P.polymyxa* biological agents (P0 / Control).

Based on the results of observations of the fifth day to the eighth day of observation on each variety, it shows that the varieties used in this study were categorized as resistant to BLB disease caused by pathogens *Xoo* with inoculation of pathogens *Xoo* 2 (two) times in the field because they have an average disease intensity below. 5%. The use of *P.polymyxa* biological agents at a concentration of 5 mL.L-1 water (P2) and a concentration of 7.5 mL L-1 water (P3) tends to be able to suppress the development of Bacterial Leaf Blight in each variety in the field, however, it is less effective because the development of the pathogen *X. oryzae* pv oryzae (*Xoo*) is strongly supported by the environment (temperature, humidity). Rainfall in this season was categorized as light because it was below 20 mm / day, but the land used during the research was an irrigated rice fields so that humidity was always maintained. Water plays a role in determining the humidity of the environment that is the habitat of all organisms including the pathogen *Xoo*, according to the statement [14]. The spread of disease can take place rapidly through friction between leaves, carried by wind, and water (inundated land, splashing rainwater, flooding, and from channels. irrigation). [15] also stated that almost 50% of the spread of disease was caused by environmental factors such as water, wind and interactions with injured leaves.

The varieties used in this study were classified as resistant and slightly resistant to the pathogenic symptoms of *X. oryzae* pv oryzae (*Xoo*). The use of resistant varieties is highly recommended, this is in line with the statement [16] which states that the use of resistant varieties is an effective, inexpensive and environmentally friendly control method. The use of resistant varieties is also the most common and easy control method that farmers can do [17]. Mekongga variety (V2) tends to be able to suppress the symptoms of Bacterial Leaf Blight (HDB) due to the use of *P. polymyxa*

biological agents with a concentration of 7.5 mL.L-1 water (P3). This concentration is a concentration above the standard use of 5 mL L-1 water (P2), although the concentration of 5 mL L-1 water (P2) is able to suppress the development of HDB disease every week but is less able to suppress BLB disease at the start the initial initiation of pathogen *Xoo* in the field.

The antagonist bacteria *P. polymyxa* Mace used in this study contained a lot of bacteria per mL. The density of *P. polymyxa* Mace bacteria in 1 mL with 10-3 dilution was 73.33 x 103 cfu. The density of *P. polymyxa* has been tested at the Unmul HPT Laboratory with a 10-3 dilution resulting in (Deuteronomy 1 = 104, Deuteronomy 2 = 74, Deuteronomy 3 = 42) so that it produces an average of 73.33 so the total density of the bacteria is 73.33 x 103 cfu (Figure 1).

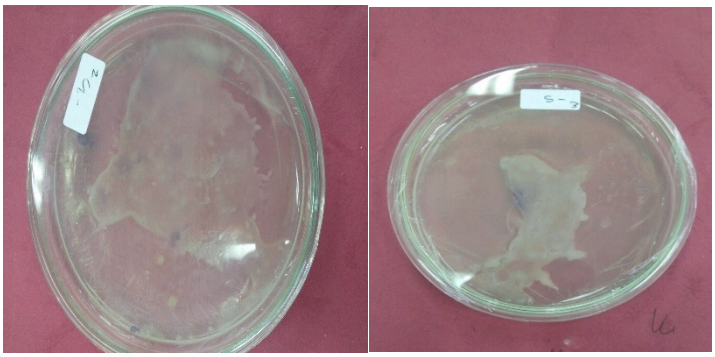

Figure 1. Colony of *Paenibacillus polymyxa* mace

The compounds contained in *P. polymyxa* function as plant biological control antibiotics, *P. polymyxa* also produces many hydraulic enzymes that are antagonistic to control plant pathogens, the relationship between plant roots and *P. polymyxa* bacteria is actually interdependent in increasing root biocontrol activity in the soil. . Interestingly *P. polymyxa* is active against various fungi and bacteria but does not interfere with / against arbuscular mycorrhizae / root fungi [18]. The density of antagonistic bacteria / biological agents of *P.polymyxa* should be able to suppress the development of pathogenic bacteria *X. oryzae* pv oryzae (*Xoo*), this is evidenced by the lack of development of Bacterial Leaf Blight on leaves using a concentration of 5 mL L-1 water (P2) and 7.5 mL.L-1 water (P3). The conclusion that can be drawn from this observation is that *P. polymyxa* biological agents are less able to compete with pathogens *Xoo*, because the environment is very supportive of the development of pathogens *Xoo* and reduces the working power of these biological agents. This is supported by the statement [19] that these biological agents are often poorly applied on a commercial scale, although initially their abilities are very promising. The reason is that these agents are often unable to adapt to new environments or are less able to compete with microorganisms that have inhabited the environment for a long time. Also, maintenance of storage over a long period of time tends to destabilize the agent.

[20] stated that each variety has different resistance genes or susceptibility reactions to different races of pathogens, virulence genes or a virulence. In a study conducted by [21] also stated that plant resistance between one variety and another is different, this is because one plant species has a different number of resistance genes. The number of genes that determine virulence or avirulence varies, as well as the number of resistance or susceptibility genes from one plant to another, in line with the results of research [22] suggesting that differences in the development of HDB disease symptoms for each variety are determined by the virulence of the pathogen *X. oryzae* pv oryzae (*Xoo*) is also influenced by genetic factors of the plant itself. [23] also stated that the control of Bacterial Blight using antibiotics, forecasting, sanitation, and combination of antagonists is still not satisfactory because the

high diversity of pathotypes *Xoo* is caused by the environment, the varieties used and the *Xoo* pathogen are prone to gene mutations.

4. Conclusion

The results showed that the Mekongga variety is the most resistant variety to the pathogen *X. oryzae* pv. Oryzae. The use of *P. polymyxa* Mace as biological agents is effective to control Bacterial Leaf Blight caused by the pathogen *X. oryzae* pv. Oryzae on several rice plant varieties (*Oryza sativa* L.), and the best concentration of *P. polymyxa* Mace to control Bacterial Leaf Blight is 5 mL.L-1 water.

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