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Cover Page Footnote

The authors would like to thank the staff, researchers, assistant and all related parties who has helped carry out this research in the laboratory.

Potential of Indigenous Methanotrophic Bacteria as a Biological Control Agent Against *Xanthomonas oryzae* pv. *oryzae* Causing Diseases on Rice

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Abstract

Methanotrophic bacteria inhabit the rhizosphere and potentially inhibit the growth of pathogens. Therefore, they have potential utility as biological control agents. This study aimed to analyze 10 isolates of indigenous Methanotrophic bacteria with the potential to act as biological control agents for *Xanthomonas oryzae* pv. *oryzae*. Analysis of antagonistic activity was conducted by the direct inhibition method on media using the following parameters: the time to formation of a clear zone, the diameter of the clear zone, and the index of inhibition. The study recommended two isolates as biological control agents that can be combined to inhibit the growth of *Xanthomonas oryzae* pv. *oryzae*.

Keywords: bacteria, biological control, diseases, methanotrophic, rice

Introduction

Bacterial leaf blight (BLB) is one of the most widespread diseases in rice. BLB is an important problem in several countries, including Indonesia. The yield losses caused by BLB reached 70%–80% in Indonesia, 74%–81% in India, and 20%–50% in Japan [1]. BLB attacks cause crop failure, reaching 21%–36% during the rainy season and 18%–28% in the dry season [2].

BLB is a disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). This pathogen can infect rice at all phases of growth, from the nursery to the harvest, by infecting the stomata and damaging the chlorophyll. These bacteria are spread by irrigation water in rice fields and most likely inhabit the rhizosphere on rice

The rhizosphere is an excellent habitat for microbes because plant roots release organic materials that stimulate both pathogenic and antagonistic microbes. Methanotrophic bacteria can be used as bioremediation agents, as they are known to reduce methane gas emissions. In addition, they can be used as biological control agents against *Xoo*. Some rhizosphere bacteria can act as both a biological control agent and a biofertilizer [3]. Furthermore, there is great opportunity for biological control of plant diseases because the organisms are already available in nature, and their activity can be stim-

ulated with a modification of the environment as well as the host plant [4].

BLB disease control measures have been carried out with various technologies, including the development of resistant varieties. However, it is constrained by the ability of the pathogen to form a new, more resistant strain. In addition, pesticides in the form of antibacterial chemicals have been developed, but they have negative effects on human and animal health and the environment. Therefore, the use of a biological control agent in the form of antagonistic bacteria is very appropriate for BLB control without damaging the environment. Biological control agents for aquatic organism are live microorganisms that are associated with and beneficial to the host plant [5].

The use of bacteria as a biological control agent supports BLB control; therefore, this study aims to analyze the potential of indigenous methanotrophic bacteria as biological control agents against *Xoo*

Materials and Methods

The bacteria used are a collection of 10 indigenous methanotrophic isolates [6]. Isolates were cultivated on Nitrate Mineral Salt media [7]. The pathogenic bacterium used was previously isolated *Xoo*. The isolates

were cultured on solid media. The isolates and the pathogen were diluted to a concentration of 10^8 CFU/ml on Nutrient Broth media and subsequently incubated on a shaker for 24 hours.

Analysis of antagonistic activity was performed directly by the inhibition method on growth media. Both indigenous methanotrophic bacteria and the pathogen were grown together on Nutrient Agar. The establishment of a clear zone was observed by incubation with antimicrobial susceptibility test disks (diameter: 5 mm). This treatment was repeated three times for each test. Observation parameters included the time to formation of a clear zone, the diameter of the clear zone, and the index of inhibition.

The index of inhibition was scored according to the diameter of the inhibition zone in the following categories: very strong (>3.0) with the symbol (++++); strong (2.0 to 2.9) with the symbol (+++); moderate (1.0 to 1.9) with the symbol (++); weak (0.1 to 0.9) with the symbol (+); and absent (0,0). The index of inhibition (II) is calculated with the following formula:

$$IB = \frac{DCZ - DTD}{DTD} \quad (1)$$

Description:

IB = the index of inhibition

DTD = the diameter of test disks (5 mm)

DCZ = the diameter of clear zone (cm)

Results and Discussion

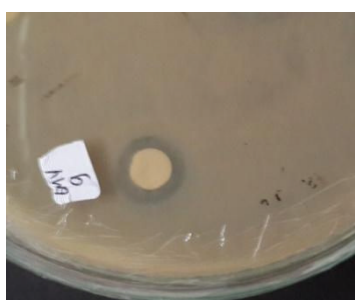
All isolates (Table 1) could act as antagonists against *Xoo*. The antagonistic activity of bacterial isolates is characterized by the formation of a clear zone on the growth medium (Figure 1), indicating an antibiotic mechanism of action.

Zuraidah [8] stated that isolates that potentially inhibit the growth of *Xoo* were marked with the formation of a clear zone. Thus, it could be said that indigenous methanotrophic bacteria produce antibiotic compounds that effectively inhibit the growth of pathogenic *Xoo*. Based on the findings of research carried out by Nonci et al. [9], indigenous methanotrophic bacteria can produce

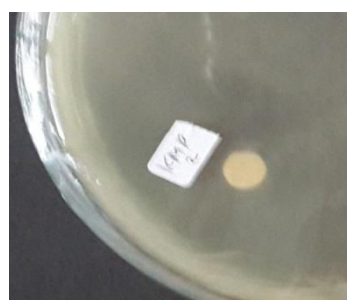
Table 1. Results for Analysis of Antagonistic Activity

Isolate Code	Analysis of antagonistic activity			
	Diameter of clear zone (cm)	Start Time of Formatting clear zone (days)	Barriers of Index value	Category
GMV 1	0.9	4	0.8	+
GMV 3	1.2	5	1.4	++
GMV 9	1.2	2	1.4	++
GMR 1	1.0	5	1.0	++
GMR 8	0.6	5	0.2	+
GMP 2	0.8	5	0.6	+
GMP 4	1.5	5	2.0	+++
TMV 3	1.0	3	1.0	++
TMV 5	0.8	3	0.6	+
TMP 5	0.8	3	0.6	+

Description Category; + = weak, ++ = moderate, +++ = Strong



(a)



(b)

Figure 1. (A) Isolate GMV 9: Clear Zone is Formed (B) Isolate Control: Clear Zone is not Formed

H₂S, a toxic gas compound. Furthermore, Whipps [10] explained that the antibiotic compound inhibits the growth of pathogens through direct contact between antagonists and pathogens. The clear zones of isolates GMP 4 and GMR 8 were found to have the greatest (1.5 cm) and smallest (0.6 cm) diameters, respectively. This indicates that GMP 4 produced stronger antimicrobial compounds than GMR 8.

The fastest time to clear zone formation was found with GMV 9: 2 days after incubation. The slowest was found on GMV 3, GMR 1, GMR 8, GMP 2, and GMP 4: 5 days after incubation. The time taken to form a clear zone depends on the ability of the isolate to compete with the pathogen for nutrients in the medium, as well as the ability of bacteria to release secondary metabolic compounds that inhibit pathogen growth. The ability of bacteria as biological agents is associated with the ability to compete for food and produce secondary metabolic compounds, such as antibiotics, siderophores, and extracellular enzymes [11].

The highest index of inhibition was found for the GMP 4 isolate and the lowest was found for the GMR 8 isolate. Analysis of antagonistic activity was also carried out in another study, producing different values of the inhibition index [12]. Those differences are thought to be caused by genetic differences between isolates. Bargabus *et al.* [13] stated that the antagonistic ability of each isolate is determined by its genetic background.

The strongest inhibition index (++++) was demonstrated by the GMP 4 isolate, followed by moderate inhibition (++) shown by the isolates GMV 9, GMR 1, and TMV 3, and lastly, GMV 1, GMR 8, GMP 2, TMV 5, and TMP 5 comprised the weak inhibition category (+).

Indigenous methanotrophic bacteria that can inhibit the growth of *Xoo* are ideal for use as biological control agents because they are environmentally friendly. These bacteria are abundantly available in the rhizosphere and, therefore, easily acquired. Biological control of pathogens using microorganisms associated with the rhizosphere and organic material is an efficient and environmentally friendly method [14].

The rate of the formation of a clear zone and the inhibition index are different for each isolate. This difference is due to their capability to adapt to the environment and their resistance to toxic compounds produced by pathogenic bacteria. Certain combinations of microbes may suppress diseases by a synergistic mechanism of action.

Conclusion

This study found the following two isolates to be potential biological control agents: GMV 4 had the shortest time to formation of a clear zone, and GMV 9

had the largest index of inhibition. Both isolates can be combined to inhibit the growth of *Xoo* on rice.

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