In vitro testing for effectiveness of *Paenybacillus polymixa* and MO Plus against *Pyricularia oryzae* (Blast Disease) and *Xanthomonas oryzae* pv. *oryzae* (Bacterial Leaf Blight)

Baharuddin¹, R Jahuddin², A Yani and M Tuwo³,⁴

¹Departement of Plant Pests and Diseases, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia
²Agrotechnology Study Program, Makassar Islamic University, Makassar, Indonesia
³Agricultural Biotechnology Laboratory, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia
⁴Departement of Biology, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar, Indonesia

E-mail: baharuddin@agri.unhas.ac.id

**Abstract.** Efforts to increase rice productivity in Indonesia are still constrained by attacks of plant pest organisms which cause low crop productivity. Diseases that often attack rice plantations include blast and leaf blight disease. Blast is caused by a fungus *Magnaporthe grisea* Cav. While leaf blight is caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo). There are isolates of bacterial that have ability to provide resistance to disease attacks on plants, one of which is *Paenybacillus polymixa*. Based on this study, the aim of this study was to determine the ability of *P. polymixa* isolates in controlling the attack of *Xanthomonas* and fungi *Magnaporthe grisea* in vitro. The research stage is a test of bacterial inhibition of Xoo pathogens in MO Plus. The inhibition zone index in the antagonist test for Xoo pathogens in MO Plus was higher than *P. polymixa*. MO Plus inhibition zone index 1.87 while *P. polymixa* is only 1.20 at 7 days after incubation. *P. polymixa* bacteria have the highest index in inhibiting *P. oryzae*, which is 57.02. While the MO Plus inhibition index is 21.12.

**1. Introduction**

Rice originating from rice (*Oryza sativa* L.) is the main commodity for most agricultural communities in Indonesia and is a staple food consumed by about 90% of Indonesia's population. The need for rice in Indonesia continues to increase as the population increases. Estimates of population growth in 2010, 2015 and 2020 are 235 million, 249 million and 263 million respectively so that the population's rice needs are projected to increase by 32.13 million tons, 34.12 million tons, and 35.97 million tons [1].

While productivity at the provincial level in South Sulawesi is even higher at 52.17 quintals per hectare [2]. The trigger for increasing rice production is because of the increase in harvested area of 540 thousand ha and productivity of 1.20 quintals per ha. The growth of rice harvest area in Java is only around 0.20% per year while outside Java is around 1.76% per year. Likewise, the increase in
rice productivity in Java is only around 0.08% per year while outside Java is around 1.45% per year [3]. The level of productivity of rice plantations can still be improved in various ways including the technical improvement of its cultivation and one of the most important cultivation techniques.

The disease that often attacks rice crops is blast and crackle disease. Blast disease is one of the factors that inhibits rice planting, which is caused by the fungus *Magnaporthe grisea* Cav. Crackle / bacterial leaf blight caused by *Xanthomonas oryzae* pv. oryzae (Xoo) is one of the main diseases in lowland rice in Indonesia and in other rice producing countries, such as Japan, India and the Philippines. This disease began to cause damage to rice plantations in Indonesia during the rainy season of 1948/1949.

There are isolates of bacterial isolates that have the ability to provide resistance to disease attacks on plants. *Paenybacillus polymixa* isolate is one of the antagonistic bacterial isolates that can control several types of plant diseases, especially crackle and blast disease in rice plants, each of which is caused by Xoo bacteria and *Magnaporthe grisea* Cav fungi. Currently there are many useful bacterial formulation products available plants including MO Plus which is a combination of biological fertilizers and microorganisms formulated in liquid form and produced through biotechnology processes to support the needs of organic farming. MO Plus contains a variety of useful microorganisms that can increase crop production and enriched with the bacteria *Bradyrhizobium japonicum*, which forms soybean root nodules to extract nitrogen directly from the air and *Streptomyces* as biological control of plant diseases. Efforts to merge MO Plus with *P. polymyxa* isolates in a consortium of biological product formulations need to control of diseases in rice seedlings and currently have no effect on various rice varieties because each variety is thought to have a different response to isolates. MO Plus and *P. polymyxa* isolates so it was deemed necessary to conduct research related to this.

2. Materials and Methods

2.1 Propagation of Antagonistic Bacteria and Pathogens

In this study the bacteria used were *P. polymixa* antagonists and blight pathogenic bacteria (Xoo), blast fungus (*P. oryzae*) which was the result of isolation from previous researchers and stored as stock in the Biotechnology Laboratory, Research and Development Center, LPPM, UNHAS. MO Plus fertilizer used is the production of CV. Organic Farming Indonesia, South Sulawesi, in collaboration with the Biotechnology Laboratory. Antagonistic bacteria and pathogens were grown propagated on NGA media, whereas *Magnaporthe grisea* fungi were grown and propagated on PDA media.

2.2 In Vitro Antagonist Test

2.2.1. *Xanthomonas oryzae* pv. oryzae Bacteria. The antagonistic test was carried out by growing pathogens and antagonists together in the medium of Nutrient Glucose Agar (NGA) with the following work steps:

Pure isolates of pathogenic bacteria were diluted from 10⁻¹ to 10⁻⁴, after which a suspension of 10⁻⁴ was taken as much as 0.1 ml using a dropper pipette and dripped into NGA media and then flattened using a spatula. Furthermore, pure isolates of antagonists were taken using an ose needle and then put into a test tube containing 2 ml of sterile water, then homogenized using vortex. After that the 0.5 cm paperdisc was dipped into the pathogen bacterial suspension, then the paperdisc was taken with an ose needle and placed into the same medium in the middle, after a day of incubation, Xoo inhibition was observed by measuring the inhibition zone diameter for three days of observation with time intervals two days. The treatment in antagonist test are:

1. Xoo bacteria with *P. polymyxa*
2. Bacteria Xoo with MO Plus
3. Bacteria Xoo (as a Control)
2.2.2. *P. oryzae* Fungi. The antagonist test was carried out on PDA media by growing together between fungus *Magnaporthe grisea* and antagonistic microorganisms:

The testing phase is intended to test the ability to inhibit *P. oryzae* fungus. The antagonist test was carried out using direct opposition between fungi isolates and MO Plus, *P. polymixa*, on PDA media with a distance of 3 cm in the petri dish.

![Diagram](image)

**Figure 1.** Laying of isolates and measurement of fungus colonies to calculate the percentage of inhibitory power by antagonistic bacteria in petri dishes. a) Antagonist isolates b) pathogenic isolates.

With treatment:
1. *Magnaporthe grisea* fungus with *P. polymixa*
2. *Magnaporthe grisea* fungus with MO Plus
3. *Magnaporthe grisea* fungus (as a Control)

Calculation of bacterial inhibition of fungi was carried out using the formula Fokkema et al. (1959):

\[
I = \left( \frac{r_1 - r_2}{r_1} \right) \times 100 \%
\]

Information:

- **I** = percentage of inhibition
- **r1** = radius of development of pathogenic isolates (b) which grows in the opposite direction to the antagonist (a)
- **r2** = radius of development of pathogenic isolates (b) which grows towards the antagonist (a)

2.3 Synergism Test

2.3.1 *Nutrient Glucose Agar* (NGA). This synergism test is carried out by growing the three antagonistic bacteria on NGA media in solid media with observational parameters if the three *P. polymixa* and MO Plus bacteria can grow together in one medium in a petri dish.

2.3.2 *Nutrient Broth* (NB). From the results of the Synergism test on solid media then proceed to liquid media. Uji sinergitas dilakukan dengan memformulasikan bakteri antagonis *P. polymixa* dan MO Plus, yang di tumbuhkan pada media NB. NB media is inserted in the test tube as much as ± 10 ml, then sterilized, after the cold medium has been inoculated according to the combination of treatments. After the combination treatment suspension was 1-5 days old, the suspension was grown on solid media, namely NGA media to be observed by counting the number of bacterial colonies with a time interval of 2 x 24 hours for 13 days. The combination of synergy test treatment is as follows:

- **Treatment 1** = *P. polymixa*
- **Treatment 2** = MO Plus
Treatment 3 = \textit{P. polymixa} + MO Plus

3. Results

3.1 In vitro antagonist test of pathogenic microbes

The antagonist test of isolates was carried out on 2 types of pathogenic microbes, namely \textit{Xoo} bacteria that caused crackle disease, and \textit{Magnaporthe grisea} fungus caused Blast disease. Both diseases cause damage to rice plants.

3.1.1 Antagonistic Test of \textit{Xoo} Bacteria. The antagonistic test for \textit{Xoo} bacteria was shown by the presence of clear zones around the isolates (figure 2). Clear zone is a zone that shows the inhibiting ability carried out by antagonistic bacteria. The results showed MO Plus inhibition zones were higher than \textit{P. polymixa} and controls. The MO Plus inhibition zone index is 1.87 while P. is only 1.20 at 7 days after incubation (DAI) (figure 3).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure2.png}
\caption{The clear zone (arrow) around the isolate indicates an inhibition of the treatment of \textit{Xoo} bacteria.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.7\textwidth]{figure3.png}
\caption{Inhibition Index of Control, MO Plus, and \textit{P. polymixa} Treatment against \textit{Xoo} bacteria.}
\end{figure}
3.1.2 Antagonist Test of P. oryzae Fungus. The antagonistic test of the Magnaporthe grisea fungus isolates was based on the ability of isolates to treat MO Plus antagonist bacteria and P. polymixa bacteria to inhibit Magnaporthe grisea (figure 4).

![Figure 4. Antagonistic test to inhibit the growth of P. oryzae. A = without inhibition (control), inhibition by B = P. polymixa, C = MO Plus.](image)

Figure 4 shows the control (not inhibited), the pathogenic fungus Magnaporthe grisea growing meets petri dishes. Whereas B and C, the fungus is not able to grow to meet the petri dish because it is inhibited respectively by P. polymixa and MO Plus. The results showed that the longer the incubation time, the greater the inhibition index for P. oryzae. At 8 days after incubation, P. polymixa bacteria had the highest index in inhibiting P. oryzae, which was 57.02. While the MO Plus inhibition index is only 21.12 (figure 5).

![Figure 5. Inhibition Index of Control Treatment, MO Plus, and P. polymixa against P. oryzae fungus.](image)

3.1.3 Synergism Test of MO Plus and P. polymixa. Synergism test is done to find out whether the two antagonistic bacteria can be applied in one container, without inhibiting growth between one another. Synergism test on solid media shows MO Plus and P. polymixa able to grow well on Nutrient Glucose Agar (NGA) media. Similarly, if grown together in one petri dish with NGA media, MO Plus and P. polymixa each can grow well (figure 6).
4. Discussion

4.1. In vitro antagonistic test of pathogenic microbes

4.1.1. Antagonistic Test of Xoo Bacteria. The Xoo bacteria causes bacterial leaf blight in rice plants. These bacterial cells grow and multiply very quickly, infecting plants by entering plant tissues through wounds, hydatodes, stomata, or contaminated seeds [4]. Wounds usually start from the edge of the leaves near the shoots, pale green to gray, then turn white to yellow [5]. This pathogen can infect rice plants in all phases of growth, starting from seedlings to before harvest [6]. Pathogens infect the leaf through leaf wounds or natural holes in the form of stomata and damage leaf chlorophyll [7]. This causes the ability of plants in photosynthesis to decrease. If transmission of the disease occurs in the generative phase, the process of filling the grain is less than perfect [6].

The antagonist test with MO Plus and Paenybacillus showed a clear zone formed around the isolates. This is an indicator of the ability of bacterial isolates to inhibit Xoo bacteria. The inhibition index continued to increase throughout the observation, namely 3 DAI (days after incubation), 5 DAI, and 7 DAI. At the last observation (7 DAI), the inhibition index on MO Plus was higher at 1.87 compared to P. polymixa, which was only 1.20. This ability can be caused by MO Plus containing several microorganisms compared to Paenybacillus. A microbial consortium (combination) is more effective than a single application [8]. The ability of a bacterium to fix nitrogen, dissolve phosphorus, produce siderofor compounds, nitrogen cyanide (HCN), chitinase enzymes, proteases and cellulose is a desirable characteristic [9] therefore to obtain bacteria that has the potential to be evaluated various characters. MO Plus is a bacterial formula that has the potential as a biological agent to control bacterial leaf blight.

4.1.2. Antagonistic Test of P. oryzae Fungus. Magnaporthe grisea fungus which causes blast disease in rice plants. This pathogen causes a quite serious attack on rice plants in all stages of growth. Start the seedlings to mature plants. According to [10], high leaf blast attacks can affect plant growth and productive tillers that cause small panicles with a small amount of grain. It can even cause all plants to die before flowering. The attack can reduce results directly. This occurs because the neck of the panicle is rotten and broken so that filling is disrupted and the grain of rice becomes empty.

In this study, MO Plus and Paenybacillus antagonists were tested for pathogenic fungi P. oryzae. The results showed an inhibition of growth of pathogenic fungi compared to controls. In the treatment without bacterial isolates, the fungus grows to meet the petri dish. Whereas with the provision of MO Plus and Paenybacillus, pathogenic fungi grow stunted. This shows the ability and potential of the two antagonistic bacteria to inhibit P. oryzae.

The longer the incubation period the higher the inhibition index of pathogenic fungi P. oryzae. At the last observation, which was 8 DAI, the highest inhibition index was shown by the Paenybacillus bacteria, which amounted to 57.02, whereas MO Plus was only 21.12. Paenybacillus is an antagonistic bacterium. Antagonistic bacteria are bacteria that can suppress disease growth caused by other microorganisms. This bacterium is able to remove antibiotics, siderophore, and other secondary metabolites to inhibit the activity of other microorganisms [11]. Antagonistic microbes are microorganisms that can suppress, inhibit or destroy other microbes. Thus antagonistic microbes have the opportunity to be used as biological agents in controlling microbes that cause plant diseases [12]. The study [13] showed that P. polymixa BRF-1 isolates from soybean rhizosphere produced antifungal peptides which functioned as antagonistic substances.

4.2. Synergism Test of MO Plus and P. polymixa

Synergy tests carried out on NGA solid media showed that MO Plus and P. polymixa can grow together without inhibiting each other. This shows that the two antagonistic bacteria can be used as a formula in making biological control agent products to control blast disease and bacterial leaf blight. If it is antagonistic, one or both of these bacteria will die if isolated in one medium. The mechanism of
suppression of an antagonistic microbe can occur through space and nutrient competition and antibiosis [11]. Synergism test on liquid media (Figure 6) shows that the incubation period did not significantly affect the number of bacterial colonies until the end of the observation (13 DAI). At 10⁻³ dilutions, the number of colonies increased from 1 to 13 DAI, both in MO Plus, *P. polymixa*, and MO Plus + *P. polymixa*. The highest number of colonies was obtained by treatment MO Plus + *P. polymixa*, ie 102.5 CFU in 11 hsi to 111.5 CFU at 13 DAI. This shows no antagonistic activity between the two bacteria. Or it can be said that there is a synergy between the two bacteria. Goto (1992) suggested competition for space and nutrition greatly affected the rate of pathogen inhibition. The occurrence of space and nutritional competition can form dormant structures, namely endospores that are resistant. Space and nutrition competition can affect other microbial growth. Nutrients that are not available in sufficient quantities can cause *Clostridium sp* to form dormant/ inactive spores.

5. Conclusion
The inhibition zone index in the antagonist test for Xoo pathogens in MO Plus is higher than *P. polymixa*. MO Plus inhibition zone index 1.87 while *P. polymixa* is only 1.20 at 7 days after incubation (DAI). Inhibition index for pathogenic fungi *P. oryzae*. *P. polymixa* bacteria have the highest index in inhibiting *P. oryzae*, which is 57.02. While the MO Plus inhibition index is 21.12.

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