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The Inhibitory Ability of Endophytic Bacteria *Bacillus* sp. BTH-22 and BTH-31a to the Growth of *Xanthomonas oryzae* in Vitro

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ABSTRACT

Xanthomonas oryzae (*Xoo*) is an important pathogenic bacteria in rice plants that causes leaf blight disease. Infection by *Xoo* disrupts photosynthesis activity and the grain-filling process, in addition, this disease reduces crop yields by 15-80%. Control in the field is generally carried out using bactericides and if carried out continuously it will be dangerous for humans and the environment, so biological control using endophytic bacteria is needed. Endophytic bacteria are microorganisms that have a mutualistic symbiosis with the tissue of eggplant (*Solanum melongena*) and can be isolated from the plant tissue. Endophytic bacteria that have been successfully isolated are *Bacillus* sp. Bth-22 and Bth-31a. The purpose of the study was to prove the inhibitory ability of *Bacillus* sp. Bth-22 and Bth-31a to the growth of *Xoo* bacteria on Nutrient Agar (NA) media in the laboratory. The study was conducted using a Completely Randomized Design with 3 treatments, namely sterile water (B0), *Bacillus* sp. Bth-22, (B1) and Bth-31a (B2) with 6 replications. The study was conducted at the Plant Health Laboratory of the Faculty of Agriculture, UPN "Veteran" East Java, located at 7°9′- 7°21′ LS and 112° 36′ – 112° 54′ BT. Data were analyzed using variance (ANOVA) at a 5% error rate. If different results were obtained, further testing was carried out using DMRT (Duncan Multiple Range Test) at a 5% level. Data analysis was performed using IBM SPSS Statistics 24 software. The results of the study showed that inhibition of *Xoo* growth was *Bacillus* sp. Bth-22 (B1) 3.9 cm and Bth-31a (B2) 9.3 cm, the inhibition mechanism is antibiosis as a bacteriostatic.

Keywords: Xanthomonas oryzae, endophytic bacteria, biological control agents

Introduction

Xanthomonas oryzae (*Xoo*) bacteria are one of the important pathogenic bacteria in rice plants that cause leaf blight disease or known as kresek disease. *Xoo* infection causes crop losses of up to 50% di Asia tropics (Kim & Reinke, 2019; Fiyaz et al., 2022), but under conditions of maximum tiller numbers, crop losses decrease by 20-40% (Yasmin et al., 2017) and in Indonesia crop losses due to *Xoo* infection reach 70-80%. Besides that, *Xoo* bacteria generally infect rice plants during the rainy season and cause two symptoms, namely blight which appears in the tillering phase to the ripening phase and kresek appears in plants aged 30 days from the nursery. *Xoo* infection interferes with photosynthesis activity and the grain-filling process, in addition, this disease reduces crop yields by 15-80%, can cause crop failure, and infects plants in the generative and generative phases (Sudir & Yuliani, 2016; Hersaputri, 2023). *Xoo* bacteria on Nutrient Agar (NA) media have characteristics of yellow colonies, round shape, shiny surface, convex, and microscopic observation results have characteristics in the form of bacilli or rods and gram negative (Sayekti et al., 2024). The control of *Xoo* bacteria carried out in the field so far is the use of pesticides that have negative impacts on human health and the environment. Based on this, environmentally friendly control is needed, one of which is by using biological agents of the endophytic bacteria *Bacillus* sp. Bth-22 and Bth-31a. *Bacillus* sp. is a bacteria that is easily isolated from plant roots, acts as an antimicrobial by producing the enzyme chitinase and is able to induce plant resistance

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to pathogens. In addition, it is able to produce other antimicrobial compounds such as surfactin, bacilibactin, bacilysin, and bacillomycin (Senol *et al*., 2014; Lv et al., 2020).

Bacillus sp. Bth-22 and Bth-31a are endophytic bacteria isolated from eggplant stems (*Solanum melongena*) that can control *Fusarium* sp. cause of stem rot disease in corn plants using bioencapsulation formulation and can control and suppress bacterial wilt disease in eggplant plants using bioencapsulation formulation (Akrom *et al*., 2024; Purnawati et al., 2024).

The purpose of the study was to prove the ability of endophytic bacteria *Bacillus* sp. Bth-22 and Bth-31a to the growth of *Xoo* bacteria on NA media *in vitro*.

Material and Methods

The study was conducted using a Completely Randomized Design with 3 treatments, namely sterile water (B0), *Bacillus* sp. Bth-22, (B1) and Bth-31a (B2) with 6 replications. The study was conducted at the Plant Health Laboratory of the Faculty of Agriculture, UPN "Veteran" Jawa Timur, located at 7°9′- 7°21′ LS and 112° 36′ -112° 54′ BT.

Preparation of Bacillus sp. and Xanthomonas sp. isolates

Endophytic bacteria *Bacillus* sp. Bth-22 and Bth-31a on NA media aged 24 hours were purified on new NA media after 24 hours and then used for research. *Xoo* bacteria were isolated from diseased rice plants in Pulungan Village, Sidoarjo which is located at 7° 23′ 30″ S, 112° 46′ 17″ E. The isolation results were grown on sterile NA media (Merck), if they grew, they were purified on new NA media and after 24 hours were used for research.

Antagonism test

Antagonism test using dual culture method. Whatman paper with a diameter of 5 mm was soaked for 1 minute in 10 mL of *Bacillus* sp. suspension (10^8 CFU/mL). Drained for 1 hour and placed on NA media in a Petri dish, incubated for 48 hours at room temperature. The Petri dish was then inverted and dripped with 1 mL of chloroform, inverted again after 2 hours. Xoo suspension (10^8 CFU/mL) as much as 200 µl was added to 4 mL of 0.6% liquid agar and poured into the Petri dish. Observation of the inhibition zone of *Bacillus* sp. on the growth of *Xoo* bacteria was carried out after 24 hours of incubation, using the formula according to Magvirah et al. (2019) (Fig 1).

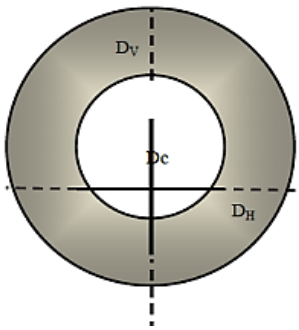

Figure 1 Inhibition zone observation method (Magvirah et al., 2019)

DV : Diameter Vertical
DH : Diameter horizontal
DC : Whatman paper diameter

Inhibition mechanism test

The inhibition zone section is taken 1 loop using an ose needle, inserted into 0.5% peptone, and incubated for 24 hours at room temperature. If the peptone is cloudy, it means that *Xoo* bac-

teria are growing, indicating that *Bacillus* sp. suppresses the growth of *Xoo* bacteria with an antibiosis mechanism as a bacteriostatic, if the peptone is clear, *Xoo* bacteria do not grow, indicating that *Bacillus* sp. kills *Xoo* bacteria with an antibiosis mechanism as a bactericide (Purnawati, 2013).

Results and Discussion

Antagonism test

Bacillus sp. Bth-22 and Bth-31a inhibition to *Xoo* growth (Fig 2 and 3).

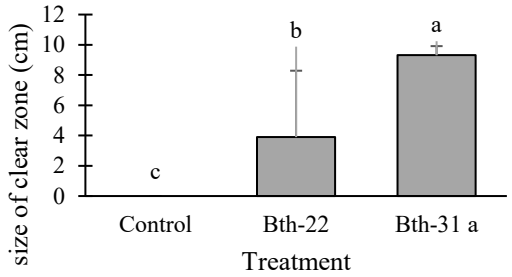

Figure 2 Histogram *Bacillus* sp. inhibition to *Xoo*

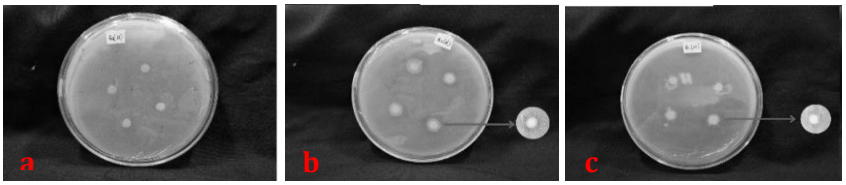

Figure 3. Antagonism test of *Bacillus* sp. to *Xoo*
(a) control, (b) Bth-22, (c) Bth-31a

Based on Fig 2, the inhibition of *Bacillus* sp. Bth-22 to *Xoo* growth is 3.9 cm ± 4.4, the inhibition of *Bacillus* sp. Bth-31a to *Xoo* growth is 9.3 cm ± 0.6 and the inhibition of Bth-31a more higher than Bth-22 while in Fig 3, both of *Bacillus* sp. Bth-22 and Bth-31a inhibited the growth of *Xoo* compared to the control because both *Bacillus* sp. Bth-22 and Bth-31a produce antimicrobial compounds, namely the enzymes chitinase, surfactin, bacilibactin, bacilysin, and bacillomycin (Senol et al., 2014; Lv et al., 2020).

Inhibition mechanism test

The inhibition mechanism of *Bacillus* sp. to *Xoo* is proven by the change of 0.5% peptone from clear to cloudy (Fig. 4) which indicates the antibiosis mechanism as bacteriostatic.

Figure 4. Inhibition mechanism of *Bacillus* sp. to *Xoo*

Based on Fig. 4, the inhibitory mechanism of *Bacillus* sp. to *Xoo* is bacteriostatic, meaning that the endophytic bacteria *Bacillus* sp. are unable to kill *Xoo* but only able to inhibit its growth (Djatmiko et al., 2007).

Conclusion

Endophytic bacteria *Bacillus* sp. Bth-22 and Bth-31a inhibited the growth of *Xoo*, with inhibition sizes is 3.9 cm and 9.3 cm, inhibitory mechanism of *Bacillus* sp. Bth-22 and Bth-31a to *Xoo* is antibiosis as bacteriostatic.

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