Antifungal Study of *Bacillus Subtilis* BR2 on *Ganoderma* sp., The Cause of Basal Stem Rot Disease in Oil Palm Plants

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ABSTRACT— ***Bacillus subtilis BR2 is antagonistic bacteria has the ability to inhibit the growth of several fungal pathogens such as Fusarium oxysporum, Helminthosporium maydis, Pyricularia oryzae, and Rhizoctonia solani, respectively as cause wilt in watermelon plants, leaf spot in corn plants, blast in rice, and leaf blight of rice plants. The research aims to determine the ability of B. subtilis BR2 suppress the growth of fungi Ganoderma sp. causing basal stem rot of oil palms. The study was conducted at the level of in vitro, by testing inhibition of B. subtilis BR2 on solid and liquid medium. The results showed that isolate of B. subtilis BR2 able to suppress the growth of fungi Ganoderma sp., with percentage of 80% inhibition in a solid medium and 78-97% in a liquid medium. Thus, isolates of B. subtilis BR2 has antibiosis mechanism in addition to mechanisms other antagonist to inhibit the growth of fungi Ganoderma sp.***

Keywords— *Bacillus subtilis*, palm oil, stem rot, *Ganoderma* sp.

1. INTRODUCTION

Oil palm plantations are the mainstay of agricultural crops in Indonesia. Oil palm planted area nationally nearly 10.5 million hectares, with the major oil palm plantation areas are in Riau Province (2.19 million ha), North Sumatra (1.34 million ha), Central Kalimantan (1.1 million ha) and South Sumatra (1.06 million ha) [1]. Oil palm plantation sector has been able to absorb a lot of labor, increase the income and welfare of the community, and making Indonesia the world's major manufacturers CPO (crude palm oil).

The extent of oil palm plantations in Indonesia also contain the potential threat of explosion of pests and diseases. This is because the characteristics of the cultivation of oil palm is usually grown in monoculture with a very wide expanse of planting, planting monoculture with the same age of the plant, and the use of production inputs such as varieties that have relatively uniform, insecticides, herbicides and inorganic fertilizers continuously. These conditions will lead to outbreaks of diseases such as occurred in Ireland in 1845-1860, emerging epidemic disease late blight (leaf spot) on potato caused by the fungus *Phytophtora infestans* [2].

Basal stem rot disease caused by the fungus *Ganoderma* sp. is one of the major diseases that cause great losses in oil palm plantations, particularly in Indonesia and Malaysia [3]. In some oil palm plantations, the disease can cause damage up to 80% [4]. Symptoms of the disease usually seen after 6-12 months after infection [5]. Plants infecting will rot and fall before the productive period ends. Previously the disease was reported only in older age palms and currently found on young stages palms as well [6].

The use of antagonistic bacteria, such as *B. subtilis* BR2 to control basal stem rot disease in oil palm plants is one of the techniques in biological control. The use of other antagonist microbes, such as *Trichoderma harzianum* also been treated against the cause of basal stem rot in oil palm plants [7]. This control technique has several advantages over chemical control techniques, such as: environmentally friendly, the control can be permanent, can reproduce themselves, and it is relatively easy to implement.

This study aims to determine the potential of the use of *B. subtilis* BR2 in vitro level for suppressing the growth of the fungus *Ganoderma* sp., the cause of basal stem rot in oil palm plants.

2. MATERIALS AND METHODS

Preparation antagonist and pathogen isolates

Antagonistic bacteria, *B. subtilis* BR2 (BR2 isolate), constitute the author's own collection of isolates isolated from the roots of grasses in the area Ciampea, Bogor district. These isolates have been known to have the ability to

inhibit the growth of several fungal pathogens such as *Fusarium oxysporum* (causing wilt disease in watermelon plants), *Helminthosporium maydis* (causing leaf spot disease on corn), *Pyricularia oryzae* (cause of the blast disease in rice plants), and *Rhizoctonia solani* (causes sheath blight in rice plants) [8].

Based on the results of 16S rRNA sequences turns characterization of the isolate identified as *Bacillus subtilis* [9]. As for some morphological and biochemical characters of the isolate of *B. subtilis* BR2 presented in Table 1. Meanwhile the fungus isolate causing stem rot disease of coconut palm (*Ganoderma* sp.) obtained from the collection of the Laboratory of Mycology, Department of Plant Protection, Bogor Agricultural University.

Table 1: Some characters morphological and biochemical of BR2 isolate

Characters	BR2 isolate
Morphology of koloni:	
- Size (mm)	4-5
- Form	Irregular
- Elevation	Flat
- Edge	Wave
- Color	White milk
- Surface	Slippery
Morphology of cell	Rod
Gram reaction	+
Size of cell (μm)	0,25 x 1,75
Endospora	+ (terminal)
Motile	No
Biochmistry:	
- Chitinase test	+
- Catalase test	+
- Citric test	-
- Decarboxylase (lysine)	Non enterobacter
- H_2S Production	-
- Indole test	-
- Methyl red test	+
- Motility	-
- Nitrate reduction	+
- hydrolysis of urea	-
- Voge-proskauer	-
- Glucose	+
- Sucrose	+
- Dextrose	-
- Sorbitol	-
- Mannitol	+
- Phytotoksicity	-
- Production of siderophores	+
- Dissolving phosphate	+

Remarks: + = reaction, - = no reaction
Source: [8] and [10].

BR2 isolate inhibition in solid medium (PDA)

Testing inhibition against pathogenic isolates BR2 (*Ganoderma* sp.) were calculated using a dual culture on PDA medium. Pieces colony fungus Ganoderma sp. with a diameter of 0.5 cm were transferred to PDA medium at a distance of 3 cm from the edge of the petri dish. As a treatment, in the opposite direction with a distance of 3 cm from the edge of the petri dish is moved as much as 10 mL suspension culture isolates BR2 (population 10^6 cells/ml) or isolates of *Escherichia coli* DH5α lacking the gene encoding antibiosis, not expressing antibiotic compounds (collection Laboratorum of Bacteriology, Department of Plant Protection, Bogor Agricultural University). Tests performed 5 replications. The percentage inhibition of the growth of fungal isolates BR2 *Ganoderma* sp. calculated using the formula:

$$\text{Inhibition (\%)} = \frac{R1 - R2}{R1} \text{ I\%}$$

Remarks: R1 = the radius of the growth of the fungus to the edge of the petri dish
R2 = The radius of the growth of the fungus to antagonist bacteria

Inhibition of BR2 isolate in a liquid medium (PDB)

Colony pieces of pathogenic fungus (*Ganoderma* sp.) with diameter 0.5 cm inserted into the 250 ml Erlenmeyer flask which already contains 50 ml of medium potato dextrose broth (PDB). For treatment added 1.5 ml suspension of BR2 isolate culture (10^6 cells/ml) or isolate of *E. coli* DH5α. As for controls added 1.5 ml sterile distilled water. Each treatment was repeated as many as 5 replicates. The preparation was incubated at room temperature for 10 days. The effect of treatment on the growth of the fungus *Ganoderma* sp. is determined by measuring the weight of the wet and the dry weight of the fungus colony on the last day of incubation. Data were tabulated and analyzed using analysis of variance

3. RESULTS AND DISCUSSION

Result

BR2 isolate inhibition in solid medium (PDA)

BR2 isolate were able to inhibit the growth of the fungus *Ganoderma* sp. The growth of the fungus *Ganoderma* sp. up to 80% inhibited by treatment BR2 isolates. In contrast inhibition of the growth of *Ganoderma* sp. does not occur by the treatment and control DH5α isolates (Table 2 and Figure 1). This proves that BR2 isolate has antifungal properties against fungus *Ganoderma* sp. causing basal stem rot disease in oil palm plants.

Table 2: Inhibition of BR2 isolate on the growth of fungi *Ganoderma* sp.

Treatments	Inhibition (%)
BR2 isolate culture	80
DH5α isolate culture	0
Control	0

Figure 1: Apperance of the growth *Ganoderma* sp. on a solid medium (lefti) and a liquid medium (right) after treated by BR2 isolate or DH5α

Inhibition of BR2 isolate on a liquid medium (PDB)

BR2 isolate was also suppress the growth of fungi *Ganoderma* sp. in a liquid medium. Emphasis growth of the fungus *Ganoderma* sp. by BR2 isolate indicated by the reduced weight of the wet and the dry weight of the fungus colonies were obtained. The wet weight and dry weight of colonies of fungus *Ganoderma* sp. obtained from BR2 isolate treatment respectively 0.4 g and 0.11 g (Table 3). Thus occurred the inhibition of the growth of the fungi *Ganoderma* sp. by BR2 isolate of 97% and 78%, respectively based on the wet weight and dry weight of fungi colonies.

Table 3: Wet weight and dry weight of mycelium fungus *Ganoderma* sp. after incubated for 10 days in a liquid medium for each treatment

Treatments	Wet weight (g)	Dry weight (g)
BR2	0,40 a	0,11 a
DH5α	7,70 b	0,46 b
Control	7,71 b	0,50 b

The numbers in the same column followed by the same letter is not significant according DNMRT test at 5%

Discussion

BR2 isolate consistently inhibited the growth of the fungus *Ganoderma* sp. either in a solid medium or in a liquid medium. Inhibition of the growth of the fungus *Ganoderma* sp. due BR2 isolates produce antifungal compounds. These results are consistent with the result of test from [8] that BR2 isolate properties chitinolytic and antagonist against the fungus *Rhizoctonia solani*, *Fusarium oxisporum*, and *Helminthosporium maydis*, respectively as a cause of disease sheath blight in rice plants, causing wilt disease on melon plants, and causes leaf spot disease in maize. Inhibition of the BR2 isolate on the fungus around 36-52%. The same thing was reported [11] that *Bacillus subtilis* RB14-C can suppress the growth of fungi *R. solani* and some other soil microbes.

Several studies using microbial antagonists for the control of *Ganoderma* sp. demonstrated success. [12] reported that the bacterial BK17 isolates quite effective in suppressing the growth of the basal stem rot cause of oil palm caused by *Ganoderma boninense* Pat. but not yet reported the results of the identification of bacterial isolates BK17. [9, 10] informs that based on 16S rRNA sequence analysis, morphology, biochemistry, and character growth, the BR2 isolate which has antifungal properties against fungus *Ganoderma* sp. identified as *Bacillus subtilis*. Meanwhile research carried out by the use of other microbes [13] who got the fungus *Trichoderma* T38 and T39 have antifungal properties against some isolates of *Ganoderma*. The use of other antagonist microbes, such as *Trichoderma harzianum* also been treated against *Ganoderma* causing basal stem rot in oil palm plants [7].

Antifungal compounds can be produced by BR2 isolate either in a solid medium or in a liquid medium. The efficacy of antifungal compounds produced by BR2 isolate in liquid medium reported [14], that the antifungal activity of BR2 isolate increased to 72 hours the first, following the increase in the bacterial cell growth. Results of the study of [15] concluded that the growth of bacterial cells *Acinetobacter baumanni* antagonist LCH001 closely correlated with antifungalnya activity. Furthermore, reported [14] that the antifungal compounds in BR2 isolate can be extracted using a solvent ethyl acetate extract inhibition of almost 78% against the fungus *R. solani*. The value of minimum inhibitory concentration (MIC) of ethyl acetate extract of 10 mg/l (10^{-3}%). However, the stability of the antifungal compounds is strongly influenced by temperature, ie the higher the temperature, the activity of antifungal compounds also decreased. Instead stability antifungal compounds are relatively stable in acidic, neutral and alkaline [16].

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5. CONCLUSIONS

From these results it can be concluded that the *Bacilus subtilis* BR2 isolate can suppress the growth of fungi *Ganoderma* sp. causing basal stem rot of oil palm plant. Inhibition of the growth of the fungus *Ganoderma* sp. by *B. subtilis* BR2 isolate in vitro around 78-97%.

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