



Antagonism Activity of Phosphate Solubilizing Bacteria Against *Ganoderma philippii* and *Fusarium oxysporum* of Acacia Plants

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Abstract

Ganoderma philippii and *Fusarium oxysporum* 0148c are the primary pathogenic fungi that causes root rot and damping-off in young acacia plants. The best treatment to date is the use of biological control agents. Phosphate solubilizing bacteria (PSB) isolated from acid soil is a bacterial isolate classified as plant growth-promoting bacteria (PGPB). PGPB has an indirect function as a biocontrol agent for fungal pathogens. This study aimed to determine the potential of PSB isolate EF.NAP 8 in inhibiting *G. philippii* and *F. oxysporum* 0148c from acacia plants. The method used is a dual culture antagonism test and observation of abnormal hyphae after the antagonism process. The results showed that the isolate EF.NAP 8 inhibited *G. philippii* by 34.44% and *F. oxysporum* 0148c by 33.33%. The abnormality of hyphae after antagonistic activity results in hyphal malformations such as hyphae lysis and hyphae coiling. The antagonistic activity of PSB EF.NAP 8 isolate is one of part of the ability of a bacterium classified as PGPB in the form of biocontrol activity against pathogenic fungi. This provides information regarding the opportunity to utilize EF.NAP 8 as a candidate agent for controlling fungal pathogens on acacia plants.

Keywords: acacia, *F. oxysporum*, *G. philippii*, plant growth-promoting bacteria, phosphate solubilizing bacteria

1. INTRODUCTION

Control of pathogenic fungi on plantations, especially industrial plants, is essential. Pathogenic fungi can damage plant growth and production, and control can be done using chemicals or biologically. The use of bacteria as agents for controlling pathogenic fungi in plants is currently a priority. One of which is pathogenic fungi that attack acacia plants such as *Ganoderma philippii* and *Fusarium oxysporum*. *G. philippii* is a fungal pathogen that infects young plants *Acacia mangium* and *Eucalyptus pellita*, causing root rot in both types of plants [1]. Root rot caused by *G. philippii* is an essential disease in acacia plants. The threat of this disease increases in the planting of *A. mangium* [2]. *Acacia* spp. including plant species widely developed for Industrial Plantation Forests in several HTI areas in Sumatra and Kalimantan, developed by PT. Arara Abadi, Riau. Acacia plants

are susceptible to root diseases, causing an increase in the area of attack and damage caused from time to time. *A. mangium* plants in South Sumatra, Riau, East Kalimantan, and several other areas were attacked by root rot disease [3]. Besides *G. philippii*, *F. oxysporum* is also a significant problem in acacia seedlings. This pathogenic fungus causes sprouting or root rot during the seedling process. If it is not appropriately handled in the early stages of its growth, it will spread throughout the planting area [4]. Various studies have been reported regarding the attack of *F. oxysporum* on various types of acacia plants such as *A. koa* [5], *A. nilotica* [6], even capable of causing the germination of *A. mangium* to fall six days after the germination process [7]. This disease control strategy is currently being developed, particularly in applying microbes to biological control agents [8][9].

In 2020, Asril et al. [10] has succeeded in isolating phosphate solubilizing bacteria from acid soil in the Institut Teknologi Sumatera area, one of which is EF.NAP 8 isolate. Phosphate solubilizing bacteria is one of the criteria for bacteria classified as plant growth-promoting bacteria (PGPB). PGPB provide many benefits to host plants directly or indirectly. The direct role of PGPB in plants includes dissolving phosphate, nitrogen, other minerals and hormone production, while the indirect mechanism is by suppressing the growth of plant pathogens [11]. PGPB has been known as a plant disease biocontrol agent. *Bacillus* species are

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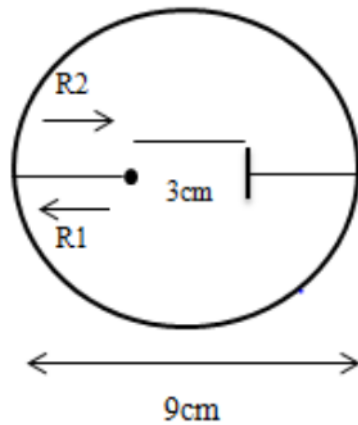


Figure 1. Schematic of dual culture antagonism testing .

antagonistic against fungal pathogens such as *F. oxysporum*. *Bacillus amyloliquefaciens* is a phosphate-solubilizing bacterium that can inhibit the growth of *F. oxysporum* by producing secondary antifungal metabolites, 1-aminocyclopropane carboxylic acid deaminase, chitinase and cellulase enzymes [12]. Chitinase enzyme was able to inhibit the attack of *F. oxysporum* on chilli sprouts [13], *G. boninense* in oil palm plantations [14] and *G. philippii*, which causes the red root of acacia plants [15]. Another species, *B. megaterium*, as a biocontrol agent, *Ralstonia solanacearum*, can produce plant growth promoters (PGP) components, phosphate solubilization [16]. This study aims to test the inhibitory ability of the fungal pathogenic isolate of phosphate solubilizing bacteria EF.NAP 8 and to determine the production of other secondary metabolites that are part of the ability of the PGPB group of bacteria.

2. MATERIALS AND METHODS

2.1 Materials

The material used is phosphate solubilizing bacteria isolated from the Institut Teknologi Sumatera acid soil, which is coded EF.NAP 8 [10]. Bacterial isolates were inoculated on Nutrient Agar from Merck supplemented with 5% $\text{Ca}_3(\text{PO}_4)_2$ medium and incubated at 30 °C. Isolates of pathogenic fungi *F. oxysporum* 0148c and *G. philippii* were collected from PT. Arara Abadi, Riau. Pathogenic fungi isolates were inoculated on Potato Dextrose Agar (PDA) media from Hi-Media and incubated at 28 °C.

2.2 Methods

2.2.1 Preparation of Bacterial Isolate and Antagonism Testing

Phosphate solubilizing bacteria isolates EF.NAP 8 were subjected to gram staining and biochemical tests include motility test in Sulfide Indole Motility (SIM) medium, catalase, oxidase, MR-VP, citrate, and urease test before antagonism testing. The method used to test antagonism against fungal pathogens using the dual culture method. Pathogenic fungi in circular blocks measuring 6 mm were grown on the test PDA media. Meanwhile, the test bacteria isolates were grown on PDA media with a streak technique of 3 cm. The distance between the bacterial isolates and the test fungus was 3 cm each (petri dish diameter = 9 cm) (Figure 1). The treatment test was repeated three times. The antagonism test treatment was observed for nine days and incubated at room temperature (28 °C). The percentage of inhibition (P) of the pathogenic fungus was calculated by the formula: $[100\% \times (R1-R2)/R1]$, where r1 is the length of mycelium growth of the pathogenic fungus towards the edge of the petri (3 cm) and R2 is the length of the mycelium towards the bacterial streak (3 cm) [17][18].

2.2.2 Abnormal Hyphae Observation

Microscopic observation of the abnormal hyphae structure of pathogenic fungi was carried out by observing the tip of the mycelium in the zone of inhibition after the antagonism test. The mycelium ends of the pathogenic fungi *F. oxysporum* and *G. philippii* from PDA media were cut into block squares and placed on a glass object. Hyphae abnormalities were observed under a microscope (Light Binocular Microscope, Olympus, Japan)

Table 1. Characteristics of EF.NAP 8 isolates.

No	Test	Results
1	Gram stain	Gram negative
2	Cell shape	Bacil
3	Motility	Motile
4	Catalase	+
5	Oxidase	+
6	Indole	-
7	MR-VP	-
8	Citric	+
9	Urease	+

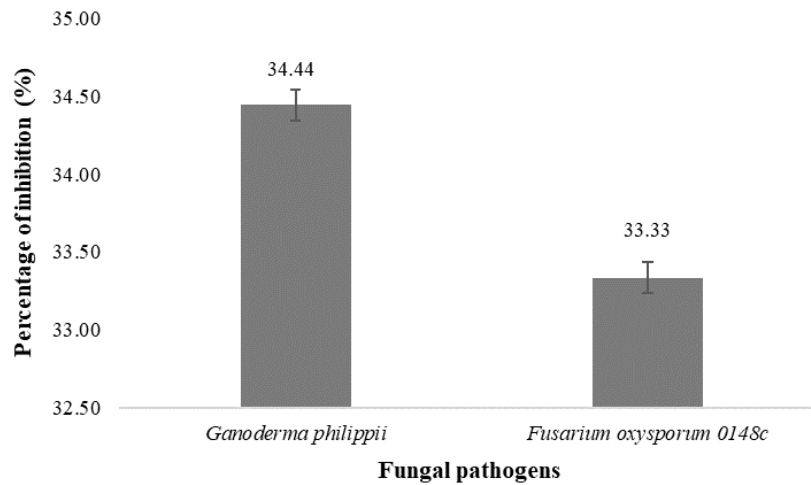


Figure 2. Percentage of inhibition of EF.NAP 8 isolate against fungal pathogens after nine days of incubation.

include bending of hyphae tips, hyphae coiling, hyphae lysis, hyphae splitting, branching, and dwarf hyphae [19].

3. RESULTS AND DISCUSSIONS

3.1 Characteristic of Isolate and Ability of Its Antagonistic Activity

Based on the results of gram staining and bacterial biochemical tests, isolate EF.NAP 8 was a gram-negative rod-shaped bacterium and was motile. These bacteria can also produce catalase and oxidase enzymes and are classified as aerobic bacteria. EF.NAP 8 isolate was also able to use citrate as a carbon source and produce urease (Table 1). Based on the results of the antagonism test of EF.NAP 8 isolates against *G. philippii* and *F. oxysporum* 0148c showed that the phosphate solubilizing bacteria isolates from acid soil could inhibit the growth of both types of fungal pathogens

with varying percentages of inhibition. EF.NAP 8 isolate inhibited both types of fungi by 34.44% and 33.33%, respectively (Figure 2).

These results indicate that the bacterial isolate EF.NAP 8 can be a biological control agent for acacia plant pathogenic fungi. The percentage of inhibition in vitro was carried out as an initial indication to determine the ability of EF.NAP 8 phosphate solubilizing bacteria isolates in inhibiting fungal pathogens. The ability of phosphate solubilizing bacteria EF.NAP 8 in inhibiting both types of fungal pathogens is thought to be due to the ability of PGPB indirectly to produce antibiotics and lytic enzymes [20]. EF.NAP 8 isolate formed a strong inhibition zone against fungal pathogens. As a result, the growth of *G. philippii* and *F. oxysporum* 0148c was inhibited to above 30%. The percentage of inhibition of EF.NAP 8 isolates against *F. oxysporum* was higher than the inhibition of *B. amyloquefaciens* PCfS against *F. oxysporum*,



Figure 3. Antagonism of EF.NAP 8 isolates against *G. philippii* and *F. oxysporum* 0148c on the ninth day of incubation.

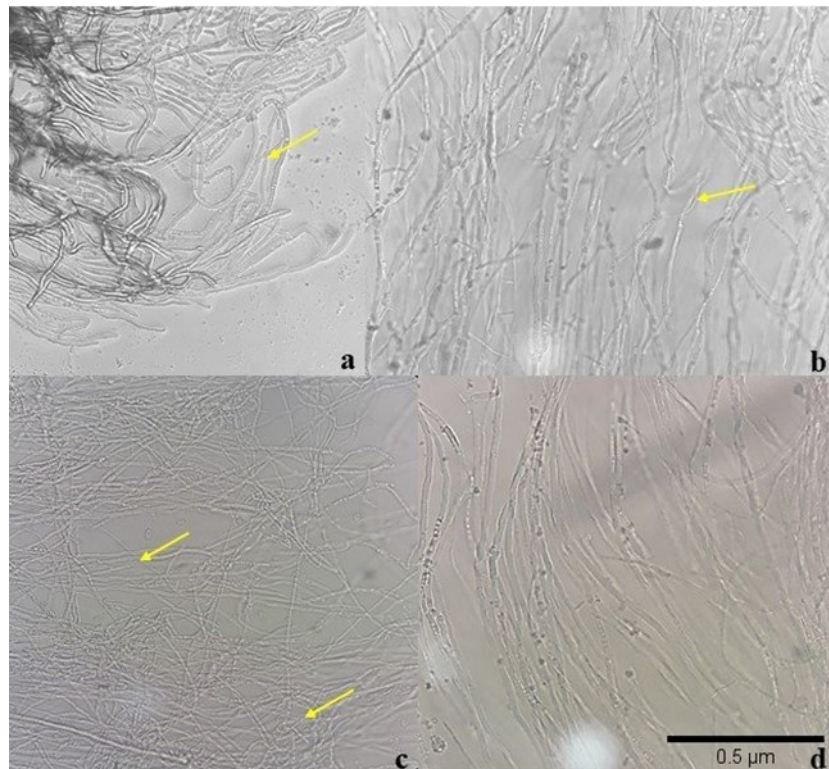


Figure 4. Abnormality of *G. philippii* hyphae after the antagonism process, (a) coiled hyphae, (b), (c) lysis hyphae, (d) normal hyphae.

which was only able to inhibit 28% [12]. Various antibiotics produced by *Pseudomonas* PGPB have been identified, including the compounds ampicillin, 2,4-diacetylphloroglucinol (DAPG), oomycin A, phenazine, pyoluteorin, pyrrolnitrin, tensin, tropolone and cyclic lipopeptides [21]–[23].

The synthesis of various antibiotics/antifungals is the main characteristic of PGPB, which is most often associated with the ability of bacteria to prevent the proliferation of plant pathogens, especially fungi [23][24]. In addition to antibiotic/antifungal activity, bacteria can produce enzymes such as chitinase, cellulase, β -1,3 glucanase, protease and lipase, which can partially degrade cell walls of pathogenic fungi. PGPB can synthesize one or more enzymes that are often found to have biocontrol activity against various fungal pathogens such as *F. oxysporum*, *Rhizoctonia solani*, *Botrytis cinerea*, *Phytophthora* sp. and *Phytium ultimum* [25]. The cell wall components of *Ganoderma* spp and *F. oxyporum* hyphae are dominated by chitin and glucan [13][14][26] so that the lytic enzyme of chitinase and glucanase can easily degrade the cell wall components of pathogenic fungi. In addition, competition for nutrients and niches is one of the ways in the process of inhibiting pathogenic fungi [11]. Although it is difficult to demonstrate directly,

competition for nutrients can be observed in the test of EF.NAP 8 isolates against *G. philippii*. In this case, isolate EF.NAP 8 experienced significant growth. It can be seen that the growth of the isolate on the part that was scratched on the medium was getting thicker, the growth was getting wider and longer compared to the growth in the test with *F. oxysporium* 0148c (Figure 3).

3.2 Hyphae Abnormal Formation

Based on microscopic observation of abnormal hyphae structure of *G. philippii* and *F. oxysporum* 0148c after the antagonism test, it was shown that the inhibition of EF.NAP 8 bacterial isolates against both fungal pathogens were in the form of inhibition of mycelium and thinning of hyphae walls. Due to antagonistic activity, hyphae undergo changes in shape or malformations such as coiled hyphae, coiled hyphae and hyphae lysis (Figures 4 and 5). Hyphal abnormalities were dominated by the formation of lysed hyphae, both in *G. philippii* and in *F. oxysporum* 0148c. The presence of hyphae malformations of pathogenic fungi is thought to be due to a hyperparasitism mechanism of bacterial isolates against pathogenic fungi so that the fungal cell walls are degraded. The contact between bacteria and fungal pathogens causes isolates of

antagonistic bacteria to produce compounds or secondary metabolites in the form of antimicrobials, causing damage to the hyphae of pathogenic fungi [27]. Compounds produced by bacterial isolates will cause shortening, swelling of hyphae or other forms of malformations [28][29]. In addition to secondary metabolites, lytic enzymes such as chitinase and glucanase also play a role in the process of necrosis and hyphae lysis [14]. Bacterial lysis activity is one of the mechanisms that have implications for disease biocontrol. In order to address the shortcomings of microscopic analysis, we propose the performance of molecular analysis techniques on the different strains [30]–[34].

4. CONCLUSIONS

Phosphate solubilizing bacteria isolated from acid soil EF.NAP 8 had antagonistic activity against acacia plant pathogenic fungi, namely *G. philippii* and *F. oxysporum* 0148c, with inhibition percentages of 34.44% and 33.33%, respectively. Inhibition of the fungus is indicated by the presence of hyphae abnormalities such as hyphae lysis, hyphae coiling and coiling as a form of indication of antagonistic activity in the form of production of metabolic compounds or lytic enzymes. This antagonistic activity provides information about the opportunity to use EF.NAP 8 isolate as a candidate for biological control agent for acacia plant

pathogens. Molecular fingerprinting presents a powerful tool to seek the outmost information regarding antagonist effect.

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Figure 5. The abnormality of the hyphae of *F. oxysporum* 0148c after the antagonism process, (a) coiled hyphae, (b) twisted hyphae, (c-e) lysis hyphae, (f) normal hyphae.

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