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Screening of White-Rot Fungi as Biological Control Agents Against *Ganoderma philippii*

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ABSTRACT

Ganoderma philippii is the causal agent of root rot disease causing economic losses to Acacia plantations. In an effort to control the *Ganoderma* root-rot disease, we isolated and screened white-rot fungi as biological control agents. We collected 107 samples from forestry plantations in Riau Province. The fungi were isolated from rotten wood including trunks and twigs, and fruiting bodies. Out of the 107 samples, 28 from rotten woods and 51 from fruiting bodies were successfully isolated. Screening of the isolated fungi was done on wood block, wood disc, and wood-powder-containing agar. Eleven isolates showed fast growth on wood block and in subsequent second screening in dual culture on wood disc, three isolates showed fast growth and were capable of overgrowing *G. philippii.* The third screening was to examine quantitative growth rate of selected fungal isolates on malt extract agar wood powder (MEA-WP) two isolates were selected. These two isolates have shown potential as biological control agents of the root-rot pathogen, *G. philippii.*

Keywords: *acacia*, dual culture, isolation, root-rot disease, rotten wood

INTRODUCTION

The increasing demand for paper means short-rotation industrial plantations for pulpwood are needed to secure a stable supply of wood chips. The pulpwood plantations that are largely planted in South-East Asia are Acacias, including *Acacia mangium* and *A. crassicarpa*. Total area planted was estimated 2 million hectares (Arisman & Hardiyanto 2006), mostly in Sumatra and Kalimantan, Indonesia (Potter *et al*. 2006). In term of productivity loss, one of the most economically important diseases invading *Acacia* is red root rot. Based on molecular study, *Ganoderma philippii* is known as the main species infecting roots of Acacias, particularly *A. mangium* (Glen *et al*. 2009). Affected roots are covered by wrinkled rhizomorphic skin on the surface and the bark becomes red on outside and white pattern is evident on underside (Irianto *et al*. 2006). Losses due to root rot are estimated 10% in 3 - to 5-year-old plantations. Eyles *et al*. (2008) estimated that the cost for losses of root rot per year was around $US50m. In addition to incorporation of tolerant genotypes (Gafur *et al*. 2015), use of biocontrol agents is seen

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as an economically and environmentally feasible control measure to minimize the losses due to the disease. Despite their potentials, white rot fungi have not been adequately explored as biological control agents that can compete with *G. philippii* for wood resources.

The aim of this study was to collect, isolate, and select white rot fungi in order to obtain isolates that have the potential to control root rot disease caused by *G. philippii.*

MATERIAL AND METHODS

Collection, Isolation and Purification of White-rot Fungi

Fruiting bodies and rotten woods were collected from several forest plantations in Riau. All samples were placed into plastic bags and marked with information such as date, number of samples, and location. The fungi were isolated and purified on potato dextrose wood powder agar (PDA-WP) medium with addition of chloramphenicol.

Surface of each sample was cleaned with brush to remove dirt or soil then sprayed with 70% ethanol. Small pieces from their interiors were placed on the surface of PDA-WP and subsequently incubated at 26 °C for 3-7 days. To purify fungal isolate, the mycelia were transferred into new PDA-WP and incubated at 26 °C. This was repeated until pure and stable cultures were obtained. For stock culture, the fungal isolates were maintained on malt extract wood powder agar (MEA-WP) slant and stored at 25 °C until used.

First Screening on *Acacia Mangium* Wood-block

Acacia mangium blocks of 2.5cm (diameter)x10 cm (length) were obtained from plantations. The wood blocks were completely cleaned with brush. Every single block was placed in an autoclavable plastic bag and sterilized for 30 min at 121 °C.

Fungal isolate was multiplied on PDA-WP at 26 °C for 7-10 days. Two plugs of inocula (0.5 cm in diameter) were taken and placed onto surface of *A. mangium* wood blocks, then sealed, and incubated at 25 °C. Physiological and morphological characteristics of each isolate were assessed, including growth rate and type of mycelium. Treatment was performed in triplicate.

Second Screening on *Acacia Mangium* Wood Disc (Dual Culture)

Isolates obtained from first screening were subsequently screened against *G. philippii* by employing dual culture technique on *A. mangium* wood discs of 14 (width)x12 (length)x1.5cm (thick) obtained from plantations. The wood discs were wiped and cleaned with brush and made intersection of two lines. Every single wood disc was placed in an autoclavable plastic bag and sterilized for 30 min at 121 °C. After cooling, 7-day-old selected fungal isolate and *G. philippii* were placed simultaneously, about 3cm from centre of wood disc at opposite sides and incubated at 25 °C. The growth of selected fungal isolates and *G. philippii* was assessed.

Dual Culture Technique on MEA-WP

The ability to grow of isolates selected from previous secondary screening was determined on dual culture technique on MEA-WP by measuring mycelial extension rate at 25 °C. The mycelial rate of both selected isolates and *G. philippii* was recorded as an average daily increase on colony diameter, which was measured in two orthogonal directions over 10 days. For control, selected isolates or *G. philippii* was inoculated as single cultures. All measurements were performed in triplicate.

RESULTS AND DISCUSSION

Out of the 107 collected samples, 79 isolates were successfully isolated (Table 1). We used three steps screening method to select the potential fungi. First screening was done on *A. mangium* wood block. This substrate has advantages of more similar to natural conditions, easy to prepare, and easier to see growth rate of the fungal isolates. The 79 isolates were subjected to this substrate (Table 2) and growth rate was recorded when fungal isolates covered the whole wood block. We found 11 isolates that gave fast growth.

Eleven fastest growing isolates were selected from first screening and furthered for second screening for interference ability in dual culture technique on *A. mangium* wood disc. Among the selected isolates, three isolates (WFA033, WFA064 and WFA068) were very good in suppressing the growth of *G. philippii* (Figure 1).

The growth of the three selected isolates from secondary screening was quantitatively determined on MEA-WP. Using Petri dish (90mm in diameter), WFA033 and WFA068 grew and completed the dishes on day 7, while WFA064 completed the dishes more than 10 days. On the other hand, *G. philippii* seemed to grow very slowly on MEA-WP (Figure 2). In dual culture test on MEA-WP, WFA033 and WFA068 grew fast and suppressed growth of *G. philippii* (Figures 3, 4). Meanwhile, WFA064 grew slower compared to WFA033 and WFA068 and seemed to trigger the growth of *G. philippii* (Figure 5). As shown in Figure 6, WFA033 and WFA068 inhibited growth of *G. philippii* on MEA-WP.

Biological control of root rot fungi using non- or weak pathogenic fungi can be considered. These biological control agents could break down wood debris faster than the pathogen, occupy the same resource as the pathogen, compete for nutrients, produce inhibitory secondary metabolites, and are able to mycoparasitize the pathogen (Peterson 2006; Eyles *et al*. 2008). These characters are found in WFA033 and WFA068 because during screening on three different types of media, both are able to compete and inhibit growth of *G. philippii.*

The commercial biological agent *Phlebiopsis gigantea* is widely used in northern hemisphere to control *Heterobasidion annosum* that invades conifer plantations (Arlinger *et al*. 2006). *P. gigantea* was screened on malt extract agar, pine wood blocks, and pine wood discs (Sierota 1975; Stalpers 1978). This shows that all these media are suitable and good to grow potential fungi as biocontrol agents. We modified the screening technique used by the previous researchers. The results show that we get two potential white-rot fungi (WFA033 and WFA068) as biological control agents. The same screening approach may be employed to screen for biocontrol agents of other pathogens including *G. boninense*, the causal agent of the basal stem rot disease on oil palm.

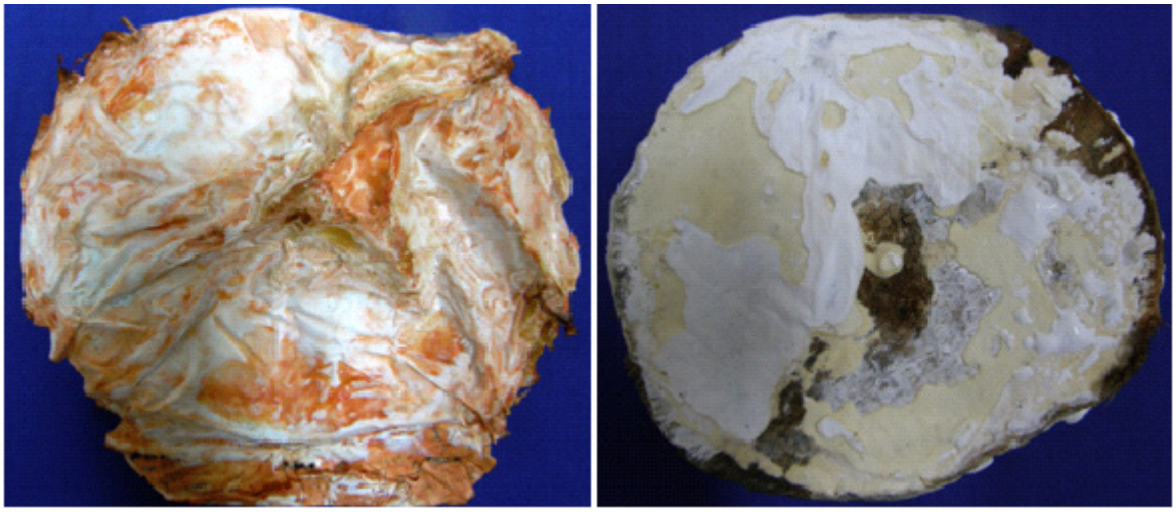
Table 1 Number of isolation samples

Fruiting body	Rotten wood
51	28

Table 2 Growth rate of new fungal isolates on *Acacia mangium* wood block

Number of strains	Growth rate*
11	++++
29	+++
15	++
24	+

* ++++: very fast growth (0-15 days covered wood block)
+++: fast growth (16-30 days covered wood block)
++: medium growth (31-45 days covered wood block)
+: slow growth (> 45 days covered wood block)



a b

Figure 1 a Dual culture of WFA033 and b WFA068 on *Acacia mangium* wood disc. Both overgrew the wood disc and inhibited growth of *Ganoderma philippii*.

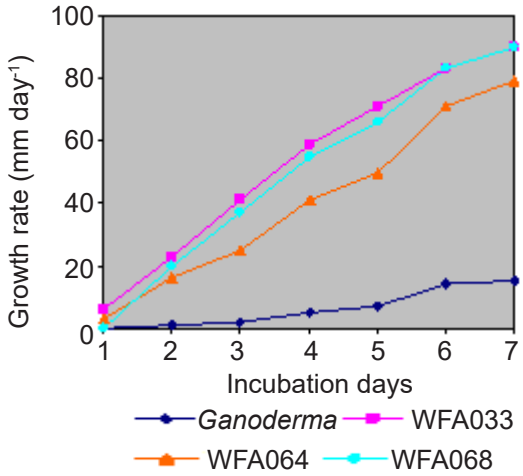


Figure 2 Growth rate of WFA033, WFA064, WFA068 and *Ganoderma philippii* on MEA-WP.

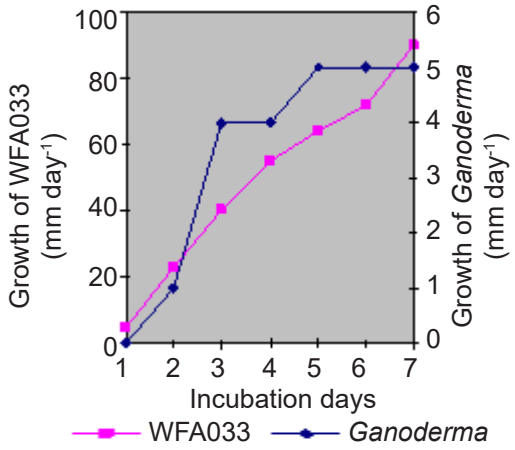


Figure 3 Dual culture of WFA033 against *Ganoderma philippii* on MEA-WP.

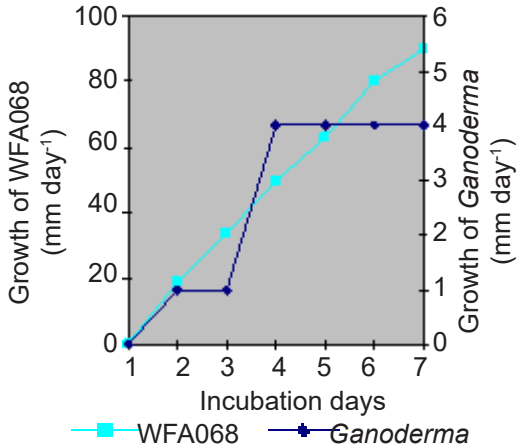


Figure 4 Dual culture of WFA068 against *Ganoderma philippii* on MEA-WP.

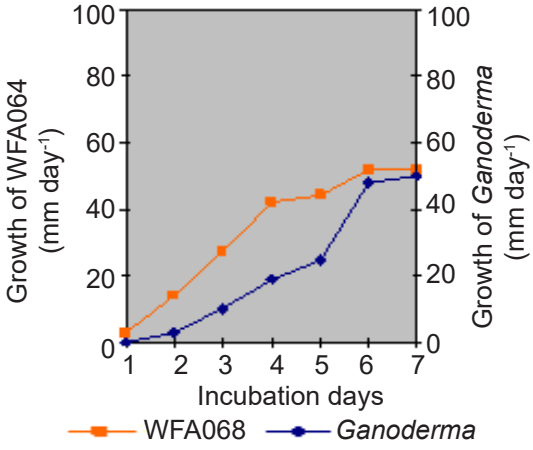


Figure 5 Dual culture of WFA064 against *Ganoderma philippii* on MEA-WP.

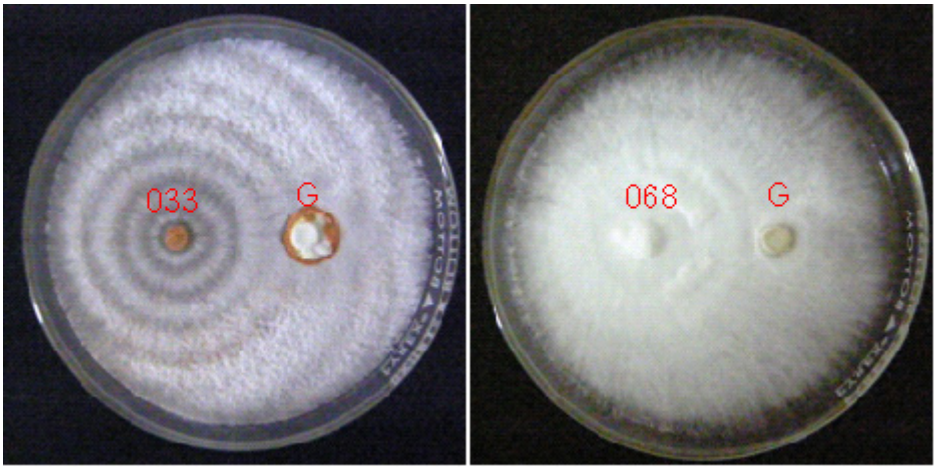


Figure 6 Growth inhibition by WFA033 and WFA068 of *Ganoderma philippii* on MEA-WP.

CONCLUSION

Through screenings on three different types of media (wood blocks, wood discs, and MEA-WP) we found two white-rot fungi (WFA033 and WFA068) as biocontrol agents. Their performances are great in interfering and inhibiting growth of *G. philippii* in all the media used. These two isolates have shown as potential biological control agents of the red root-rot pathogen, *G. philippii*.

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