An endophytic fungus with potential as a biocontrol agent of *Ganoderma boninense*

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Introduction

Ganoderma is a white rot fungus capable of degrading lignin. Oil palm is a significant crop in Malaysia and is prone to the basal stem rot (BSR) disease caused by this fungus resulting in economic losses (Paterson, 2007). The detection of this disease was confirmed by the external symptoms and the appearance of the *Ganoderma* fruiting body. At this time more then 50% of the basal stem were already rotted. Chemical control of this disease can be expensive, ineffective and have negative impact on both enviromental and human health. The use of biological control such as endophytic fungi as the sustainable long-term solution are suggested. Endophytes are microorganisms that establish non pathogenic interaction with the plant. Endophytes obtain nourishment, water and physical protection from host plant against biotic adversities and in return they produce secondary metabolites such as alkaloids, antibiotics or toxins that may be toxic to pathogenic fungi (Schulz, 2006). Therefore the endophytic fungi of the oil palm on its own can be the new source of biological control agents for BSR disease. The objective of this study is to screen for an endophytic fungus from healthy oil palm roots that has antagonistic properties against *G. boninense*.

Material & Methods

Isolation of endophytes from oil palm roots. Oil palm seedlings were subjected to three treatments, which were: *G. boninense* and simultaneous application of *Trichoderma harzianum* mulch **(+T),** *G. boninense* only **(+G)** and inoculation of *Glomus* sp. (arbuscular mycorrhizal fungi) **(+GL)**. Roots of oil palm seedling from different treatments were collected separately at 8 to 10 weeks after treatment. Roots were cleaned under running tap water to wash away dirt from the root surfaces. The roots were then cut into 1cm segments and surface-sterilised in 70% ethanol for about 40 seconds and then rinsed in sterile distilled water. The root segments were dried on sterile filter paper and placed onto Potato Dextrose Agar (PDA) culture plates at five segments per plate. The plates were then sealed and incubated at room temperature (25 - 28 °C). The growths of the mycelia from the root segments were observed daily. Only mycelia that grew from the tip of the root segments were subcultured. Subcultures were done until pure culture obtained.

In vitro screening of isolated endophytes against *G. boninense* by dual plating bioassay. Dual plate bioassays were conducted to evaluate the *in vitro* antagonistic activity of oil palm root endophytes against the pathogen *G. boninense* using isolate PER 71 obtained from MPOB. Mycelia disc of pathogen and endophyte were placed 4 cm apart in Petri dish containing PDA. Both were plated at the same time. Two replicate plates and control (for pathogen) were also prepared. The percentage inhibition of radial growth (PIRG) calculated base on the formula: **PIRG= (X-Y/X) x 100%** Where **X**= the radius of fungal pathogen on the control plate and **Y**= the radius of fungal pathogen on the treatment plate.

In vitro volatile bioassay against *G. boninense*. Endophytic fungi that recorded antagonistic activity of PIRG > 80% against the PER 71 during the dual plate screenings were selected for further tests. The media used for the volatile test was PDA. PDA was prepared in universal bottles and each bottle contain 20ml of agar. The media were sterilized by autoclaving at 121 °C for 15 minutes and poured into plate. Each culture plate contains 20 ml of PDA. Plugs of the oil palm root endophyte and the *G. boninense* mycelia (5 mm in diameter) were inoculated separately at the centre of the PDA . The Petri dish covers of both cultures were removed and the plate containing the PER 71 was placed on top of the endophytes plate giving a head space of 1.5 cm distance between the media. The two portions were joined together with hot glue. For control, *G. boninense* inoculated plate was similarly arranged but the bottom plate contained uninoculated PDA. Three replicates were

prepared for control and experimental endophytes tested. Plates were then incubated at 28 °C. PIRG value was assessed with the same formula above.

Results & Discussion

From the isolation, endophyte populations were highest from [+G] treatment and lowest from the [+GL] treatment. As many as 41 endophyte isolates were obtain from the [+G] treatment, 31 endophytes from [+T] treatment and 17 isolates from [+GL] treatment. The results of the dual culture screening showed that 12 of the 89 endophytes screened recorded PIRG values of more than 80%. The top five isolates were M26, G3, M23, GT32 and M18 with PIRG values of 98.83%, 97.83%, 97.50%, 93.67% and 87.17% respectively. The selected five were further tested for inhibited growth by volatile bioassay. Only three isolates produced volatiles that inhibited the growth of PER 71 at PIRG > 50%. They were isolates M26 (67.47%), M23 (64.68%) from [+GL] treatment and G3 (51.16%), [+G] treatment. From the results of both bioassays, isolate M26 can be the potential biocontrol agent of the disease.

Conclusion

Highest endophytic fungi were from [+G] treatment. However,endophytic fungus M26 with good potential as biocontrol agent was from [+GL] treatment.

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