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Isolation of Potential *Actinomycetes* from Oil Palm (*Elaeis guineensis*) Plantations as Biological Control of *Ganoderma* Disease

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ABSTRACT

Actinomycetes were isolated from soil in different oil palm plantations. The soil samples were collected from healthy standing palms in areas affected with Ganoderma disease. Four media were used to isolate actinomycetes: Yeast-Malt Extract Agar (ISP2), Starch-inorganic salt Agar (ISP4), Humic acid-vitamin-Agar (HVA) and Yeast Extract-Casamino acid Agar (YCED). A total of 600 isolates of actinomycetes were obtained. Based on morphological characteristics, 568 isolates were Streptomyces and 32 isolates non-Streptomyces. All of the isolates have been tested for antifungal activity against G. boninense in vitro. Results indicated that 81 isolates (out of 600) had shown positive activity towards G. boninense with Percent Inhibition Radial Growth (PIRG) values more than 80%.

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INTRODUCTION

*Actinomycetes i*s one of the major groups of soil population and are widely distributed (Kustner, 1968). Soils, freshwater, lake and river bottoms, manures and compost contain an abundance of these organisms. They are of universal occurrence in nature, living and multiplying in both cold and tropical zones, and have been reported to occur even under the most extreme conditions of the desert. The temperate zones are, however, generally most favorable for their development (Strzelczyk *et al.*, 1969). The *actinomycetes* can be easily isolated. They are gram positive bacteria which will grow as a filament branch (Nanjwade *et al.*, 2010). This group of microorganism had been proved to have a high possibility in producing antibiotic and enzyme. A recent study by several researchers demonstrated that the actinomycetes are important in the

rhizosphere, where they may play an important role in influencing plant growth and controlling the infection of roots by soil-borne pathogenic fungi and bacteria (Hayakawa *et al.*, 2007).

MATERIAL AND METHODS

Samples Collection

Soil samples were collected from four locations in different oil palm plantation in Peninsular Malaysia. The locations consist of four different types of soil: lateritic soil (Kluang, Johor), Peat soil (Teluk Intan, Perak), Coastal soil (Sepang, Selangor) and Inland soil (Bangi, Selangor).

Isolation of *Actinomycetes*

One-g of dried soil was added into 9 ml of sterile distilled water and diluted to1000-fold. A 0.1 ml of the soil suspension was plated onto four isolation agars: Yeast-Malt Extract Agar (ISP2), starch-inorganic salt Agar (ISP4), Humic acid-vitamin-Agar (HVA) (Seong *et al.*, 2001) and Yeast Extract-Casamino acid Agar (YCED) (Crawford *et al.*, 1993), pH 7.2 supplemented with 50 ppm of Nystatin, 50 ppm of cycloheximide and 20 ppm of Nalidixic acid. The plates were incubated for 7 days at 28 °C. The pure culture of each isolate was subculture on Yeast-Malt Extract Agar (ISP2) slants and kept at 4°C until further used. The potential isolates with anti-microbial activity were storage in the deep freeze in 20% glycerol stock for long-term culture preservation.

Classification of *Actinomycetes*

Actinomycetes suspected colonies were isolated onto yeast extract malt extract agar and incubated for 7 days to obtain pure culture. Isolates were preliminary identified through naked eye and stereoscopic microscope. Coloration of aerial mycelium (on the surface of agar) and substrate mycelium (underside of plate) and diffusible pigment were observed and recorded as described by Ho *et al.*, (2002). As description of color is quite subjective, a color chart from Nippon 9000 (1997) was used for standardization.

Screening of *Actinomycetes* Activity Against *G. boninense*

The isolates were screened for bioactivity against *G. boninense* according to the method as described by Tan *et al.* (2002) and Baniasadi *et al.* (2009). The screening was done on Potato Dextrose Agar (PDA) by plating two plugs of *G. boninense* against 7 days-old isolate of actinomycetes. Duplicate test plates were prepared and all plates were incubated at 28°C up to 7 days. The antifungal activity of the actinomycete was determined by calculating the value of the Percent Inhibition of Radial Growth (PIRG) with the following formula); PIRG % = [(A-B) /A] X 100% where, A: Length of mycelia without biocontrol agent, B: Length of mycelia with biocontrol agent (Tan *et al.*, 2002, Rahman et. *al.*, 2007).

RESULTS AND DISCUSSIONS

Isolation of *Actinomycetes*

In this study, four types of isolation media were used to isolate actinomycetes collected from four soil types. Amongst all media used, the ISP2 and YCED were proven to be a good media in isolating actinomycetes. Both of these media contain low levels of organic nutrients (Crawford *et. al.* 1993) compared to ISP4 and HVA. The data obtained proved the fact that actinomycetes

are capable to grow on simple media without requiring any special growth factor. By using these four media, a total of 600 strains were isolated and purified for evaluation on the bioactivity against *G. boninense in vitro.*

Classification of *Actinomycetes*

The actinomycetes can be differentiated from bacteria and fungi based on their morphological appearance on the plate surface. They can be identified with the formation of discrete, floccose, powdery or velvety colonies. The appearance of the growing colonies is results from the aerial mycelium that arises as vertically developing filaments from the substrate mycelium and form a network of aerial hyphae (Srivinasan *et al.,* 1991). Based on surface color of isolate of actinomycetes on ISP2 after 7 days of incubation, they were grouped into three colors: grey, white and brown (Ho *et al.*, 2002) (Table 1; *Figure* 1).

Screening of *Actinomycetes* Activity Against *G. boninense*

All 600 isolates of *actinomycete* were screened for their antifungal activity against *G. boninense in vitro*. A total of 81 isolates showed positive inhibition towards *G. boninense* with more than 80% PIRG values, 148 isolates with 50 to 80% PIRG and 371 isolates with <50% or no activity against *G. boninense* (Table 2).

TABLE 1. CLASSIFICATION OF THE *Actinomycetes* ISOLATES ON YEAST EXTRACT-MALT EXTRACT AGAR (ISP2) BASED ON COLOR OF COLONIES SURFACE AS DESCRIBED BY HO *et al.* (2002)

Color of colonies surface	Number of *Actinomycete* isolates	Percentage (%)
Grey	363	60.5
White	206	34.33
Brown	31	5.17
Total	600	100

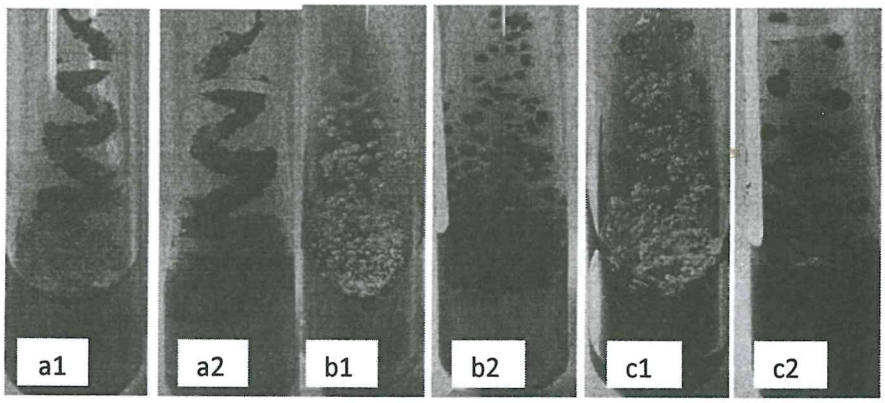

Figure 1. Color of actinomycete *colonies surface observed on Yeast Extract-Malt Extract Agar (ISP2). Notes: grey (a1 and a2); white (b1 and b2) and brown (c1 and c2).*

TABLE 2. THE PERCENT INHIBITION OF RADIAL GROWTH (PIRG) OF ACTINOMYCETES AGAINST *G. boninense IN VITRO*

Total number of isolates tested	PIRG values (%)		
	< 50	50 – 80	> 80
600	371	148	81

CONCLUSION

In conclusion, *Actinomycetes* have been proven to produce a numbered of antimicrobial metabolites. A total of 81 isolates of *Actinomycete* from oil palm plantations had shown a positive activity towards *G. boninense in vitro*. However, a more conclusive study is needed to be conducted in nursery and field to confirm their efficacy as biological control agents (BCAs) against *Ganoderma*.

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