UNIVERSITI PUTRA MALAYSIA

GENETIC CHARACTERIZATION OF *GANODERMA* sp. USING INTERFERTILITY AND MOLECULAR METHODS

HUSRITA BINTI HUSSIN

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Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Science, Universiti Putra Malaysia

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Abstract of the Thesis Submitted to the Senate of Universiti Putra Malaysia in Fulfilment of the Requirement for the Degree of Master of Science.

GENETIC CHARACTERIZATION OF *GANODERMA* sp. USING INTERFERTILITY AND MOLECULAR METHODS

By

HUSRITA HUSSIN

August 2009

Chairman: Professor Faridah Abdullah, PhD

Faculty : Science

Laccate polypores of the genus *Ganoderma* are wide spread but species identification cannot be easily done based only on traditional methods. The species of *Ganoderma boninense* is of economic importance to Malaysia as it causes the disease Basal Stem Rot (BSR) of oil palms. In this study, 53 *Ganoderma* were isolates from different hosts. Which are 23 isolates from infected oil palms (*Elaeis guineensis*), 12 from various non-*Elaeis* palmae hosts, 18 *Ganoderma* from non-palm woody hosts and 2 non-*Ganoderma* as an outgroup specimens were used to conduct interfertility studies, Random Amplified Microsatellite DNA (RAMS) and Internal Transcribe Spacer (ITS1). The interfertility studies showed that 34 laccate *Ganoderma* that collected from various palmae hosts determined as *G. boninense* whereas another 12 laccate *Ganoderma* samples and all 7 non-laccate *Ganoderma* specimens were non-

boninense. The compatible dikaryotic pairs were further validated through sporophore induction studies of which all those tested as *G. boninense* produced viable fruiting bodies. Molecular studies using Random Amplified Microsatellite (RAMS) generated a dendrogram of two major clusters. Cluster I, all the laccate, non-laccate *Ganoderma* and two non-*Ganoderma* samples which are determined as non-*boninense G.* by interfertility study were grouped together except two isolates which is determined as *G. boninense* (FA3026 and FA5014). This showed that RAMS not totally support interfertility studies. Clusters II consists of all laccate *Ganoderma* which are determined as *G. boninense* in interfertility study. The dendrogram constructed from gene sequence data of ITS 1 region of the rDNA produced three major clusters. Major Cluster I consisted of outgroups samples PLP, while Major Cluster II was WRR. Major Cluster III separated into two sub-cluster IIIA and IIIB, sub-cluster IIIA were consisted of all laccate *Ganoderma* samples and this sub-cluster were separated into two, 34 samples are determined as *G. boninense* and 12 samples are non-*boninense G.* Sub-cluster IIIB consisted of 7 non-laccate *Ganoderma* and determined as non-*boninense G.* BLAST analysis showed that all 34 determined as *G. boninense*, 4 were *G. neojaponicum*, 2 isolates were *G. formosanum*, 3 were *G. lucidum*, 2 *G. tsugae*, 2 *G. resinaceum*, 2 *G. cupreum*, 3 *G. adspersum* and 4 isolates as a *G. australe*. This investigation found that ITS not only analysed at genus level but also able to identified at species level. The phylogenetic analysis by ITS regions showed agreement with the interfertility data but not with RAMS analysis. The clustering of *Ganoderma* isolates in ITS and RAMS are difference because different approaches were use. With a different types of primers used in the two different DNA-based methods, the banding sites in fungal genome would also be different

which could lead to different clustering of the isolates in the cluster analysis. In this study found that *G. boninense* is a single species which causes the basal stem rot disease on oil palm in Malaysia. *G. boninense* also can effect to non-palmae woody host, which found on *Caesalpinia sappan*.

Abstrak Tesis Yang Dikemukan Kepada Senat Universiti Putra Malaysia Sebagai Memenuhi Keperluan Untuk Ijazah Sarjana Sains

CIRI-CIRI GENETIK *GANODERMA* sp. MENGGUNAKAN KESERASIAN PENGAWANAN DAN KAEDAH MOLEKULAR

Oleh

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Ogos 2009

Pengerusi: Profesor Faridah Abdullah, PhD

Fakulti : Fakulti Sains

Spesies *Ganoderma* berwarna kemerahan dengan permukaan dorsal berkilat banyak ditemui tetapi sehingga kini pengenalpastian spesiesnya tidak mudah dilakukan hanya dengan berasaskan kaedah tradisional. Spesies *Ganoderma boninense* mempengaruhi kepentingan ekonomi di Malaysia kerana ia menyebabkan penyakit reput pangkal batang kelapa sawit. Di dalam kajian ini, sebanyak 53 isolat *Ganoderma* yang dikutip dari pelbagai perumah. Sebanyak 23 isolat dikutip dari pokok kelapa sawit yang dijangkiti (*Elaeis guineensis*), 12 isolat *Ganoderma* dari pokok spesies palma, 18 isolat *Ganoderma* dari pokok berkayu dan 2 isolat bukan *Ganoderma* dijadikan sebagai sampel luaran untuk dijalankan kajian keserasian pengawanan, ‘random amplified microsatellite DNA (RAMS) dan ‘internal transcribe spacer (ITS1)’. Ujian keserasian pengawanan

menunjukkan sebanyak 34 isolat *Ganoderma* kemerahan yang telah dikutip dari pelbagai perumah palma adalah *G. boninense* manakala 12 isolat *Ganoderma* kemerahan dan 7 isolat bukan *Ganoderma* kemerahan merupakan bukan *G. boninense*. Pasangan dikarion yang mengawan akan dilakukan pengesahan lanjut dengan melakukan aruhan sporofor untuk menghasilkan jasad buah dan didapati semua pasangan yang mengawan menghasilkan jasad buah. Kajian molekular menggunakan 'random amplified microsatellite' (RAMS) menghasilkan dendrogram yang terdiri daripada dua major kluster. Kluster I mengandungi semua *Ganoderma* kemerahan, bukan *Ganoderma* kemerahan dan dua isolat bukan *Ganoderma* yang mana semuanya dikenalpasti sebagai bukan *G. boninense* oleh ujian keserasian pengawanan kecuali dua isolate yang dikenalpasti sebagai *G. boninense* (FA3026 dan FA5014) terkelompok bersama. Ini menunjukkan RAMS tidak sepenuhnya menyokong keputusan ujian keserasian pengawanan. Kluster II mengandungi semua *Ganoderma* kemerahan yang mana telah dikenalpasti sebagai *G. boninense* oleh ujian keserasian pengawanan. Dendrogram yang terbina dari data jujukan gen lokus ITS 1 rDNA menghasilkan tiga major kluster. Major kluster I mengandungi isolat luar (bukan *Ganoderma*) iaitu PLP, manakala major kluster II pula WRR. Major kluster III membahagi kepada dua sub-kluster iaitu IIIA dan IIIB, sub-kluster IIIA terdiri daripada semua isolat *Ganoderma* kemerahan dan sub-kluster ini membahagi lagi kepada dua, sebanyak 34 isolat dikenalpasti sebagai *G. boninense* dan 12 isolat bukan *G. boninense*. Sub-kluster IIIB terdiri daripada 7 isolat *Ganoderma* bukan kemerahan dan dikenalpasti sebagai bukan *G. boninense*. Analisis BLAST menunjukkan 34 isolat adalah *G. boninense*, 4 adalah *G. neojaponicum*, 2 isolat adalah *G. formosanum*, 3 adalah *G. lucidum*, 2 adalah *G. tsugae*, 2 *G. resinaceum*, 2 *G. cupreum*, 3 *G. adspersum* dan

4 isolat *G. australae*. Kajian ini mendapati kajian ITS tidak hanya dapat menganalisis organisms pada tahap genus tetapi juga sehingga ke tahap spesies. Analisis filogenetik lokus ITS menyetujui keputusan ujian keserasian pengawanan tetapi tidak menyokong keputusan RAMS. Pengklusteran isolate *Ganoderma* oleh analisis ITS dan RAMS adalah berbeza kerana berbeza pendekatan yang digunakan. Dengan perbezaan jenis jujukan primer yang digunakan oleh kedua-dua kaedah DNA ini, jalur-jalur yang dihasilkan juga akan berbeza yang mana menyebabkan pengkelasan isolate di dalam analisis pengklusteran juga berbeza. Kajian ini mendapati *G. boninense* merupakan spesies tunggal yang menyebabkan penyakit reput pangkal batang kelapa sawit di Malaysia. *G. boninense* juga menjangkiti perumah bukan palma iaitu pokok berkekayu yang mana dijumpai pada pokok *Caesalpinia sappan*.

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I certify that an Examination Committee has met on 29th August 2008 to conduct the final examination of Husrita binti Hussin on her degree of Master of Science thesis entitled 'Genetic Characterisation of *Ganoderma* sp. Using Interfertility And Other Molecular Methods' in accordance with Universiti Pertanian Malaysia (Higher degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the student be awarded the degree of Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilement of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Date : 16 October 2009

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that is has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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HUSRITA BINTI HUSSIN

DATE: 18 August 2009

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BSR	Basal Stem Rot
bp	Base pairs
µg	Microgram
ITS	Internal Transcribed Spacer
M	Molar
µl	Microliter
ml	Millimeter
NTSYS-pc	Numerical Taxonomy and Multivariate Analysis System
PCR	Polymerase Chain Reaction
PAUP	Phylogenetic Analysis Using Parsimony
PDA	Potato Dextrose Agar
RAMS	Random Amplified Microsatellite DNA
RAPD	Random Amplified Polymerase DNA
RFLP	Restriction Fragment Length Polymerase
rpm	Revolutions per minutes
UPGMA	Unweight Paired Group Matching Analysis
V	Volt
W	Watt
IFFPRI	Institute of Forestry and Forest Research Product, Japan
MPOB	Malaysian Palm Oil Board
FRIM	Forestry Research Institute Malaysia
UPM	Universiti Putra Malaysia
BRE	Building Research Establishment
TFRI	Taiwan Forest Research Institute
sp.	Species
NaOCl	Natrium hipochloride
TBE	Tris Borate EDTA
Tris HCl	Tris(hydroxymethyl)aminutesomethanehydrochloride
EDTA	EDTA, Disodium Salt (Dihydrate)
SDS	Sodium Dodecyl sulfate
$NaCl_2$	Natrium Chlorida

CHAPTER 1

INTRODUCTION

The disease basal stem rot of oil palm (*Elaeis guineensis* Jacq.) caused by *Ganoderma* in Malaysia was first recorded in Malaysia by Thompson in 1931. Turner (1981) also believed that several species of *Ganoderma* or various strains of the same species may associate with oil palms in Malaysia. Turner (1981) reported the number of *Ganoderma* species associated with basal stem rot as fifteen. However, Ho and Nawawi (1985) reported that basal stem rot was caused by only one single species, and that is *G. boninense*, based on morphological characteristics of the sporosphores. The name *G. boninense* was given by Patouillard in 1887 from describing the collection of *Ganoderma* from the Bonin, Nouka-hiva and Marquesa Islands in the Pacific Oceans. He described them without establishing formal taxonomic distinctions (Commonwealth Agricultural Bureaux, 1975).

The taxonomy of the whole *Ganoderma* genus is somewhat problematic at present. This is due to a number of reasons, including the number of heterogeneous forms and a large number of criteria upon which different classification approaches have been based on. Species identification, which is usually based on morphological characteristics, can be misleading and have resulted in the description of over 250 species, with frequent synonymy as a result. The situation is further complicated by the description of a number of species complexes by various authors. As a consequence, the identification and

distribution of tropical species of *Ganoderma* remains unclear. A comprehensive revision of the taxonomy has been recommended by a number of authors for several decades (e.g. Steyaert, 1975b). Corner (1983) reviewed Steyaert's classification systems for *Ganoderma*, and made conclusion that gradation occurred in all morphological features used to describe species. A number of authors have thus concluded that the use of basidiomatal morphology, spore characteristics and cutis anatomy alone were insufficient for delimitation of species (Atkinson, 1908; Haddow, 1931; Furtado, 1965, Steyaert, 1980; Furtado 1981; Bazzalo & Wright, 1982; Corner, 1983 and Adaskaveg and Gilbertson, 1986), but their studies failed to define unambiguously the observed structure.

Murrill (1902, 1908, and 1915) considered host specificity, geographical distribution and macromorphology of basidiomes as primary taxonomic characters. As stated by Moncalvo & Ryvarden (1997), among many described species, several are represented by one or few collections, and the type specimens have sometimes been lost or lack of modern descriptions.

The method for an early diagnosis of basal stem rot (BSR) by sampling diseased material of oil palms with a wood drill have been developed: (a) a colorimetric method, using ethylenediaminutesetetraacetic acid (EDTA) to detect *G. lucidum* in coconut where the causal agent of the 'Thanjavur' wilt disease was used by Natarajan *et al*,. (1986) and (b) the use of semiselective media for cultivating *Ganoderma* on agar plates (Darus *et al*,. 1991). However, all conventional methods are time consuming and of a rather low accuracy.