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COMPARATIVE PROTEOMIC ANALYSIS OF Ganoderma SPECIES DURING In Vitro INTERACTION WITH OIL PALM ROOT

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By

SITI NAHDATUL ISNAINI BINTI SAID HUSSIN

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfilment of the requirements of the Degree of Master of Science

May 2017

I dedicate my MSc thesis to my supervisors; Dr. Jameel and Dr. Baity. I am so blessed of having these two amazing persons who made my MSc journey to a success. It was lucky for me to be destined as the first graduating post-graduate student of both of you. May Allah shower His countless blessings on both of you and may this be a good start of your supervision experience!

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Amin.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

COMPARATIVE PROTEOMIC ANALYSIS OF *Ganoderma* SPECIES DURING *IN VITRO* INTERACTION WITH OIL PALM ROOT

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SITI NAHDATUL ISNAINI BINTI SAID HUSSIN

May 2017

Chair : Noor Baity Binti Saidi, PhD
Faculty : Biotechnology and Biomolecular Sciences

Basal Stem Rot (BSR) in oil palm caused by *Ganoderma* spp. is a deadly disease affecting oil palm plantation yield and global cooking oil supply. A pathogenic species, *Ganoderma boninense* is claimed as the main causal agent of BSR while *Ganoderma tornatum* is regarded as non-pathogenic and unable to infect living palms. Insufficient information on the infection mechanism and immature early detection strategy of the pathogen are among the disease control limitations. The existing molecular studies on the oil palm-*Ganoderma* interaction mainly focused on the response of the plant towards the fungus infection while the information on the pathogen responses is still a scarce. Therefore, in this study, response of the fungus at the infective stage during interaction with oil palm at the molecular level was investigated. An optimized protein extraction protocol for 2-Dimensional Electrophoresis (2-DE) gel analysis of *Ganoderma* spp. was developed and a comparative proteomic analysis were conducted to investigate the changes in the dikaryotic mycelial protein expression of the pathogenic *G. boninense* and non-pathogenic *G. tornatum* during *in vitro* interaction with oil palm root. The phenol/ammonium acetate in methanol was shown to be the most effective protein extraction method for 2-DE proteomic studies of *Ganoderma* spp. mycelia. Scanning Electron Microscope (SEM) images obtained confirmed the hyphae attachment and colonisation of both species on the oil palm root surface after 72 h of inoculation. Comparative proteomic analysis showed that the mycelial proteins from oil palm root exhibited different expression profiles when compared to the mycelia grown on Potato Dextrose Agar (PDA). Proteins differentially expressed in both species may have either direct or indirect link to virulence and pathogenicity, metabolism, growth and maintenance of both *Ganoderma* species. During the interaction, proteins with potential contribution to fungal pathogenicity such as enolase, alpha-aminoadipate reductase, carboxypeptidase, dienelactone hydrolase, glutamine synthetase (GS) and guanine nucleotide binding proteins (G proteins) were upregulated in *G. boninense w*hile bZIP protein, triose-phosphate-isomerase, redoxin and peroxiredoxin were mutually expressed in both species. Identification of these proteins during the interaction with oil palm roots may provide fundamental information for

further investigation on specific roles of the identified proteins towards *Ganoderma* infection mechanism and facilitate selection of potential markers for early detection of BSR in the future.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

PERBANDINGAN ANALISIS PROTEOMIK BAGI SPESIS-SPESIS *Ganoderma* KETIKA INTERAKSI *IN VITRO* DENGAN AKAR KELAPA SAWIT

Oleh

SITI NAHDATUL ISNAINI BINTI SAID HUSSIN

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Pengerusi : Noor Baity Binti Saidi, PhD
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Penyakit Busuk Pangkal Batang (BSR) dalam kelapa sawit yang disebabkan oleh spesis *Ganoderma* adalah penyakit yang memberi kesan kepada hasil perladangan kelapa sawit dan bekalan minyak masak global. *Ganoderma boninense* dianggap sebagai agen utama penyebab BSR manakala *Ganoderma tornatum* dianggap sebagai tidak patogenik dan tidak mampu menjangkiti pokok kelapa sawit yang masih hidup. Maklumat yang tidak mencukupi mengenai mekanisme jangkitan dan strategi pengesanan awal patogen yang belum matang adalah antara batasan kawalan penyakit tersebut. Kajian-kajian molekul ke atas interaksi kelapa sawit-*Ganoderma* yang ada kebanyakannya tertumpu pada tindak balas kelapa sawit terhadap jangkitan kulat manakala maklumat mengenai tindak balas patogen masih kurang. Maka, dalam kajian ini, tindak balas yang berlaku pada peringkat molekul di dalam *Ganoderma* semasa interaksi dengan kelapa sawit telah dikaji. Protokol pengekstrakan protein yang optimum bagi analisis gel Elektroforesis Dua Dimensi (2-DE) untuk spesis *Ganoderma* telah dibangunkan dan analisis perbandingan proteom telah dijalankan untuk mengkaji perubahan dalam corak pengekspresan protein oleh *G. boninense* dan *G. tornatum* semasa interaksi *in vitro* dengan akar kelapa sawit. Protokol pengekstrakan protein fenol/ammonium asetat dalam metanol muncul sebagai kaedah paling optimum untuk kajian proteomik 2-DE bagi miselium *Ganoderma*. Analisis imej Mikroskop Elektron Imbasan (SEM) mengesahkan pelekatan dan penyelaputan hifa kedua-dua spesis pada permukaan akar kelapa sawit 72 j selepas inokulasi. Profil pengekspresan yang dipamerkan oleh protein miselium pada akar kelapa sawit adalah berbeza berbanding profil miselium yang tumbuh pada Agar Dekstrosa Kentang (PDA). Protein-protein yang mempunyai perbezaan pengekspresan mungkin mempunyai kaitan secara langsung atau tidak langsung dengan kevirulenan dan patogenisiti, metabolisma, pertumbuhan dan penyelenggaraan bagi kedua-dua spesies *Ganoderma.* Semasa interaksi, protein-protein yang berkemungkinan mempunyai potensi sumbangan tehadap patogenisitas kulat seperti enolase, reduktase alfa-aminoadipate, karboksipeptidase, dienelaktone hydrolase, glutamin sintetase (GS) dan protein guanine nukleotida mengikat (G protein) telah mengalami peningkatan ekspresi dalam *G. boninense* manakala bZIP protein, triose-fosfat-isomerase, redoksin and

peroksiredoksin diekspreskan oleh kedua-dua spesis kulat. Pengenalpastian protein-protein ini semasa interaksi dengan akar kelapa sawit boleh memberikan maklumat asas untuk penyiasatan lanjut mengenai peranan khusus protein yang dikenal pasti terhadap mekanisme jangkitan *Ganoderma* dan memudahkan pemilihan penanda potensi untuk pengesanan awal BSR pada masa akan datang.

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I certify that a Thesis Examination Committee has met on 11 May 2017 to conduct the final examination of Siti Nahdatul Isnaini binti Said Hussin on her thesis entitled "Comparative Proteomic Analysis of *Ganoderma* Species During *In Vitro* Interaction with Oil Palm Root" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

2-ME	2-Mercaptoethanol
ACN	Acetonitrile
ABC	Ammonium bicarbonate
NH_4HCO_3	Ammonium dihydrogen phosphate
NH_4H_2PO4	Ammonium phosphate monobasic hplc
et al.	And others *(et alli*)
BSR	Basal Stem Rot
BSA	Bovine Serum Albumin
2-DE	Two-dimensional electrophoresis
Da	Dalton
DTT	Dithiothreitol
dpi	Dots per inch
EDTA	Ethylenediaminetetraacetic acid
g	Gravity
hpi	Hour post inoculation
HCl	Hydrochloric acid
IAA	Iodoacetamide
IEF	Isoelectric focusing
kDa	Kilo Dalton
kV	Kilo Volt
LCMS	Liquid Chromatography–Mass Spectrometry
MS/MS	Mass Spectrometer/ Mass Spectrometer
MALDI-TOF	Matrix Assisted Laser Desorption/ Ionization- Time of Flight
mA/gel	Mili ampere per gel
NCBI	National Center for Biotechnology Information
1-DE	One-dimensional gel electrophoresis
ppm	Part per million
PAGE	Polyacrylamide gel electrophoresis
PVPP	Polyvinyl polypyrrolidone
PSD	Post source decay
$K_3Fe(CN)_6$	Potassium ferricyanide
p	*p*-value
RH	Relative humidity
rpm	Revolution per minute
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium Dodecyl Sulphate- Polyacrylamide gel electrophoresis
NaS_2O_3	Sodium thiosulfate
Spp.	species
TOF	Time of flight
TCA	Trichloroacetic acid
TFA	Trifluoroacetic acid
v/v	Volume per volume
w/v	Weight per volume

CHAPTER 1

INTRODUCTION

Basal Stem Rot (BSR) disease caused by a white rot fungus, *Ganoderma boninense* (*G. boninense*) is the most serious problem in oil palm (*Elaeisguineensis Jacq*) plantations in South East Asia, especially Malaysia and Indonesia (Idris, 2009; Chong, 2012). The annual loss due to *Ganoderma* disease was reported between RM225 million to RM1.5 billion (Arif *et al*., 2011) and distressingly, it can cause up to 50% economic loss (Singh *et al*., 2013; Assis *et al*., 2016).

G. boninense, *G. zonatum* and *G. miniatotinctum* have been reported to be responsible for BSR disease of oil palm in Malaysia, while *G. tornatum* was reported as non-pathogenic as it only infects dead trunks of oil palms (Wong *et al*., 2012, Idris and Rafidah 2008). Many studies identified *G. boninense* as the most aggressive pathogen known to infect oil palm since it causes both Basal Stem Rot (BSR) and Upper Stem Rot (USR) (Rakib *et al*., 2016). The ability of *G. boninense* to cause disease is attributed to its dikaryotic mycelia which are formed as a result of mating between two monokaryons (Jing *et al.,* 2015, Chan *et al.,* 2011; Morrow and Fraser, 2009; Lim and Fong, 2005).

Although many methods have been taken to control BSR, to this day no acceptable method successfully eliminates the disease (Sahebi *et al.*, 2016; Husharian *et al*., 2013). The control attempts are hampered by the difficulties to detect *G. boninense* at the early stage of infection (Naher *et al*., 2013; Sariah *et al.,* 2011) due to confusion with species identification between *G. zonatum* and *G. boninense* (Pilotti, 2005) and huge variability in life cycles and modes of interaction of *G. boninense* with their host plants (Husharian *et al*., 2013; Ralf *et al*., 2011). Thus, identification of *G. boninense* at early stage of infection and understanding on its biology is important for development of efficient BSR control method and good plantation management (Maxime *et al*., 2015; Tee *et al*.; 2014). In line with this, in recent years the focus on *Ganoderma* research was more on early detection of the fungal species (Sahebi *et. al*., 2016; Maxime *et al*., 2015; Lim *et al.,* 2014; Tee *et al.,* 2014; Dutse *et al*. 2013; Dutse *et al*. 2012) .

Studies of host-pathogen interaction in general are significantly supported by the availability of genome sequences and resources for variety of 'omics' analyses (Tan *et al*., 2009). Comparative proteomics has been employed in a numbers of oil palm studies revealing alterations in proteins involved in photosynthesis, signalling, carbohydrate metabolism, and immunity and defence during interaction with *G. boninense* (Jeffery *et al.,* 2015, Al-Obaidi *et al*., 2016a, Al-Obaidi *et al.,* 2014; Al-Obaidi *et al.,* 2013; Syahanim *et al.* 2013). However, the studies were focussed on responses of the host plants while information related to adaptation, survival and pathogenicity of the pathogen itself is still lacking.

Hence, a comparative proteomics profiling of mycelial protein expression of pathogenic and non-pathogenic *Ganoderma* species during early interaction with oil palm would be relevant to provide insights into the events occurring at the subcellular level and proteins regulating the responses. It was hypothesized that a significant number of virulence-related proteins and specific proteins exclusively expressed are differentially expressed in *G. boninense* during its interaction with oil palm at the early time points. To prove the hypothesis, two-dimensional protein electrophoresis combined with MALDI-TOF/TOF MS/MS analyses were employed to elucidate the protein expression profiles of *G. boninense* and *G. tornatum* for the identification of putative virulence-related factors during early development of BSR disease. The protein profiles of both *Ganoderma* species growing on the PDA and on the oil palm root surface were compared and analysed as early as 72 h and 120 h post interaction.

This project was carried with the following specific objectives:

1. To develop an optimized protein extraction protocol for 2-DE gel analysis of *Ganoderma* species,
2. To compare the profile of mycelial protein for the identification of putative virulence-related factors during in vitro interaction between *G. boninense* and *G. tornatum* with oil palm root.

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