UNIVERSITI PUTRA MALAYSIA

DIFFERENTIAL ANALYSIS OF MYCELIAL PROTEINS AND METABOLITES FROM Rigidoporus microporus DURING IN VITRO INTERACTION WITH Hevea brasiliensis Müll.Arg

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FBSB 2021 22

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By

AHMAD FAIZ BIN CHE FISOL

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

January 2021

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

DIFFERENTIAL ANALYSIS OF MYCELIAL PROTEINS AND METABOLITES FROM *Rigidoporus microporus* DURING *IN VITRO* INTERACTION WITH *Hevea brasiliensis* Müll.Arg.

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January 2021

Chairman : Associate Professor Noor Baity binti Saidi, PhD
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Rigidoporus microporus is the fungus accountable for the white root rot disease that is detrimental to the rubber tree, *Hevea brasiliensis*. The pathogenicity mechanism of *R. microporus* and the identity of the fungal proteins and metabolites involved during the infection process remains unclear. It was suspected that the changes in *R. microporus* mycelial protein and metabolite profiles during interaction with the host plant leads to fungal virulence and this study aimed to identify the pathogenicity-related proteins and metabolites of two *R. microporus* isolates during *in vitro* interaction with *H. brasiliensis*. The two *R. microporus* isolates, Segamat (SEG) and Ayer Molek (AM) were used to inoculate *H. brasiliensis* clone RRIM 2025 *in vitro* and the mycelia adhering to the roots of the plant were collected for analysis. Transmission Electron Microscope (TEM) images acquired confirms the hyphae attachment and colonization of the mycelia on the root of the *H. brasiliensis* clones after four days of inoculation. The protein samples were subjected to 2-DE analysis and analyzed using MALDI-ToF MS/MS while the metabolites were extracted using methanol and analyzed using LC-QToF MS/MS. Based on the differential proteomic and metabolomic analyses, fungal pathogenicity may be the result from protein upregulation that are essential for fungal growth such as malate dehydrogenase, fructose 1,6-biphosphate aldolase (FBA) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and an increase in acidic compounds such as terepthalic acid, mefenamic acid and dihydropteroic acid that led to an increase in cell wall degrading enzyme activity. It can be concluded that the pathogenesis of RM might be related to metabolic pathways (e.g., glycolysis and gluconeogenesis) that involves responsive proteins such as FBA and GAPDH which can be the potential biological markers for early detection of the white root rot disease.

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

ANALISIS PERBEZAAN KE ATAS PROTEIN MISELIA DAN METABOLIT DARIPADA *Rigidoporus microporus* KETIKA INTERAKSI *IN VITRO* DENGAN *Hevea brasiliensis* Müll.Arg.

Oleh

AHMAD FAIZ BIN CHE FISOL

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Pengerusi : Profesor Madya Noor Baity binti Saidi, PhD
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Rigidoporus microporus ialah kulat yang bertanggungjawab bagi penyakit akar reput putih yang berbahaya pada pokok getah, *Hevea brasiliensis*. Mekanisma kepatogenan *R. microporus* dan identiti protein dan metabolit kulat yang terlibat ketika proses jangkitan masih tidak jelas. Ia disyaki bahawa perubahan pada protein miselia *R. microporus* dan profil metabolit semasa interaksi dengan tumbuhan hos membawa kepada kevirulenan kulat dan kajian ini bertujuan untuk mengenalpasti protein dan metabolit yang berkaitan dengan patogen dua isolat *R. microporus* semasa interaksi *in vitro* dengan *H. brasiliensis*. Dua isolat *R. microporus*, Segamat (SEG) dan Ayer Molek (AM) telah digunakan bagi menginokulasikan klon *H. brasiliensis* RRIM 2025 *in vitro* dan miselia yang melekat pada akar tumbuhan tersebut diambil bagi tujuan analisis. Imej Mikroskop Elektron Transmisi (TEM) yang diperolehi mengesahkan ikatan hifa dan kolonisasi miselia pada akar klon *H. brasiliensis* selepas empat hari inokulasi. Sampel protein telah dianalisis menggunakan analisis 2-DE dan MALDI-ToF MS/MS manakala metabolit telah diekstrak menggunakan metanol dan dianalisis menggunakan LC-QToF MS/MS. Berdasarkan analisis perbezaan proteomik dan metabolomik, kepatogenan kulat mungkin adalah kesan daripada peningkatan protein yang penting bagi tumbesaran kulat seperti malat dehidrogenase, fruktosa 1,6-bifosfat aldolase, gliseraldehid-3-fosfat dehidrogenase dan peningkatan pada kompoun berasid seperti asid tereptalik, asid mefenamik dan asid dihidropteoik yang membawa kepada peningkatan aktiviti enzim pemecahan dinding sel. Di sini boleh disimpulkan bahawa kepatogenan RM mungkin berkaitan dengan laluan metabolik (e.g., glikolisis dan glikoneogenesis) yang melibatkan protein responsif seperti FBA dan GAPDH yang boleh menjadi penanda bio yang berpotensi bagi pengesanan awal penyakit akar putih.

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ACKNOWLEDGEMENTS

The years of this research has been an emotional and gruesome period but fruitful in many ways for me and several other important people involved in it. Here, I would like to express my sincerest acknowledgement and gratitude to them.

First and foremost, my appreciation goes to my supervisor, Dr. Baity for giving me the opportunity to take on this project albeit of my inadequacy in so many aspects of research. She never lacks the wisdom in guidance and encouragement even when the roads get tough, and I am extremely thankful for her. Another person equally as important is Dr. Jameel who without a doubt contributed the most in terms of thoughts and ideas to realize this project. His experience and knowledge made this research much easier that it should be and even when the road gets tough, he always finds a way around it and I am forever grateful for his presence.

Not to forget, Dr. Safiah and Dr. Linda from Lembaga Getah Malaysia who kickstarted the project and without them this project would have not reach its goals. Also, credits to Dr. Dhilia for helping with supervising and giving insights on the work.

Special thanks as well to all the lab members in Agro-Biotechnology Institute, especially Kak Liza, Kak Nani, Muz and Nadia for their help and support with lab work. Their contribution and presence have made such impact to my work and life as a graduate student a moment to cherish.

Lastly, my gratitude goes to my family and friends for all the moral support they gave along my journey as a postgraduate. My motivation in completing this research came largely from them and mere words is the least I can do to show my appreciation towards them.

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I certify that a Thesis Examination Committee has met on 29 January 2021 to conduct the final examination of Ahmad Faiz bin Che Fisol on his thesis entitled "Differential Analysis of Mycelial Proteins and Metabolites from *Rigidoporus microporus* during *In Vitro* Interaction with *Hevea brasiliensis"* 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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TABLE OF CONTENTS

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LIST OF TABLES

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LIST OF FIGURES

© COPYRIGHT UPM

© COPYRIGHT UPM

LIST OF APPENDICES

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LIST OF ABBREVIATIONS

2DGE	2-dimensional gel electrophoresis
ABC	Ammonium bicarbonate
ACN	Acetonitrile
ANOVA	Analysis of variance
BSA	Bovine serum albumin
CDWE	Cell wall degrading enzymes
DTT	Dithiothreitol
EDTA	ethylenediaminetetraacetic acid
ESI	Electrospray ionization
FBA	Fructose 1,6-biphosphate aldolase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
Hsp70	Heat shock protein 70
IAA	Iodoacetamide
IEF	Isoelectric focusing
IPG	Immobiline drystrips gel
KCl	Potassium chloride
KEGG	Kyoto Encyclopedia of Genes and Genomes
LC	Liquid chromatography
LGM	Lembaga Getah Malaysia
MALDI	Matrix assisted laser desorption ionization
MDH	Malate dehydrogenase

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MS	Mass spectrometry
$NH_4H_2PO_4$	Ammonium dihydrogen phosphate
PDA	Potato dextrose agar
PDC	Pyruvate decarboxylase
PTFE	Polytetrafluoroethylene
RH	Relative humidity
RM	*Rigidoporus microporus*
TEM	Transmission electron microscopy
TFA	Trifluoroacetic acid
TIC	Total ion chromatogram
ToF	Time of flight

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CHAPTER 1

INTRODUCTION

Monoclonal *Hevea brasiliensis* is a tree valued for its latex content. The importance of this material has been greatly emphasized in the production of elastomers which is widely used in water, space and ship technologies (Omorusi, V.I, Eguavoen, O.I, Ogbebor, N.O, Bosah, B.O., Orumwense, K, Ijie, 2014). Malaysia was considered one of the first and biggest countries producing natural rubber, with 46% of the total rubber production comes from Malaysia alone. However, the rubber plantation in Malaysia is affected by the 'white root rot' disease caused by the basidiomycetes fungus *Rigidoporus microporus*. This pathogen is a well-known destructive agent of the rubber trees, responsible for 50% of yield losses in West Africa and was recognized as a significant endemic problem in Indonesia, Malaysia and Thailand (O. N. Ogbebor et al., 2013).

The *Hevea*-fungus interactions involve attacks by *R. microporus* on the root of the *Hevea* tree. There are three stages of disease infection development: permeation, establishment, and decomposing. The mycelium of the pathogen enters the tree through the root system and degrades the host's cell structure from there. The root rot pathogen of *R. microporus* must carry out the penetration and colonization of host's cell wall repeatedly. The disease infection activities are carried out by enzymatic digestion of the tissues characterized by differentiation of specialized structures and after some time, half of the rubber trees in the plantation are lost to the disease (Omorusi et al., 2014).

The detection of the white root rot disease in the early stages is difficult due to the trees are rapidly killed by the fungus which makes the usage of fungicide ineffective. When aboveground symptoms started to show, it already too late as the tree is already dying. Until recently, only little is known about the *Rigidoporus* species at the molecular level. The main problem in controlling the disease infection of the rubber tree is lack of sufficient information about fungus behavior during the interaction with the tree at biological and genetic level.

Recently, due to the availability of the genomic sequences and resources, the study of fungal plant pathogens has increased through functional genomic analysis including proteomics, transcriptomics and metabolomics (K. C. Tan, Ipcho, et al., 2009). Although genomic-based investigation of host-pathogen interactions could give valuable information on the changes on gene expression, the study of changes in protein and metabolite abundance is also as important to identify the essential components during the interaction. This is because there is often poor correlation between transcript, protein, and metabolite abundance (Al-Obaidi et al., 2014).

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Proteomics analysis is a method that can give us a lot of information about fungal pathogenicity by high-throughput studies. This approach allows identification of new fungal virulence factors, characterization of signal transduction signaling and pathways annotation. Using different software, the identification of the proteins will help us in understanding the protein interactions or biochemical pathways and study the fungal lifestyle and life cycles. This information can be used to provide new targets for disease crop diagnosis focused on fungicide design. In this sense, proteomic allows us to identify numerous differential proteins involved in multi-player crosstalk happening between plant and pathogens which could later potentially help us find novel biomarkers and characterize fungal strains in host pathogens interactions.

Meanwhile, metabolomic analysis provide a comprehensive information of biological and biochemical processes by studying the metabolites within the system. Metabolites are the result of reactions; therefore, changes in metabolites can be considered a definitive response of cellular system in biotic and abiotic stresses (Srivastava, 2019). Presently, metabolomics techniques have been widely applied in pathogen-plant interactions; to identify fungi, determine infection mechanisms and detect interactions with host plants. Therefore, having another level of molecular analysis is helpful in filling the knowledge gap of molecular mechanisms of *R. microporus* pathogenicity towards *H. brasiliensis* as it is hypothesized that interaction between fungus and host plant leads to changes in fungal proteins and metabolites, resulting in fungal pathogenicity.

Thus, this study exclusively aims to:

1. To identify the pathogenicity-related mycelial proteins of two different pathogenic isolates of *R. microporus* during *in vitro* interaction with *H. brasiliensis* by proteomic approach.
2. To identify the pathogenic metabolites from two *R. microporus* isolates involved during the *in vitro* interaction with *H. brasiliensis* by metabolomic approach.

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