



UNIVERSITI PUTRA MALAYSIA

***INVESTIGATION OF METABOLITES PRODUCED BY RICE BLAST
FUNGUS, *Magnaporthe oryzae* DURING APPRESSORIUM
DEVELOPMENT***

AZIAN BINTI MD ZAIN

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FUNGUS, *Magnaporthe oryzae* DURING APPRESSORIUM DEVELOPMENT**

By

AZIAN BINTI MD ZAIN

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

June 2020

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

INVESTIGATION OF METABOLITES PRODUCED BY RICE BLAST FUNGUS, *Magnaporthe oryzae* DURING APPRESSORIUM DEVELOPMENT

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June 2020

Chairman : Mohd Termizi bin Yusof, PhD
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Magnaporthe oryzae is important fungal pathogen that caused serious rice blast disease worldwide. The disease cycle of *M. oryzae* is unique, required appressorium in order to penetrate the host cells. Appressorium is critical structure to cause disease but metabolites produced during appressorium development is poorly understood. To date, there is limited information on fungal metabolites and their functions produced by *M. oryzae* during appressoria development. Therefore, the aim of this study was to determine metabolites produced by *M. oryzae* during appressorium development *in vitro* and revealed important metabolic pathways involved. Untargeted metabolomics of Proton Nuclear Magnetic Resonance (^1H NMR) was used to determine metabolites from *M. oryzae* extracts and any metabolite changes was observed during 0 h, 8 h and 24 h development stage. Spectra of ^1H NMR were analyzed using multivariate data analysis (MVDA) and model validation was studied. Rich numbers of primary metabolites were detected from ^1H NMR spectra and there were 43 metabolites identified putatively based on metabolomics library and previous reports. Partial least square discriminant analysis (PLS-DA) disclosed metabolites pattern among 0 h, 8 h, 24 h and mycelia. Metabolites that showed significant changes ($p < 0.05$) among groups of 0 h, 8 h, 24 h and mycelia including butyrate, leucine, isoleucine, valine, isobutyrate, ethanol, methylmalonate, threonine, lactate, alanine, lysine, arginine, 4-aminobutyrate, glutamate, homoserine, isocitrate, glutamine, choline, glucose, xylose, mannose, glycerol, mannitol, glucitol, tyrosine, sucrose and tryptophan. Orthogonal projections to latent structures discriminant analysis (OPLS-DA) revealed metabolites produced during each time-point of 0 h, 8 h, 24 h. Number of metabolites at 8 h and 24 h were produced the highest compared to 0 h. Then, metabolomics pathway analysis (MetPa 4.0) from Metaboanalyst.ca was used to illustrate metabolic pathways involved during appressorium development. There were eight key metabolic pathways that highly involved during appressorium development including amino acids, carbohydrates and lipid metabolisms. Fungal metabolites produced by *M. oryzae* have potential for

targeted metabolomics to target specific metabolite or pathways required for pathogenicity thus provide opportunity in developing inhibitors for rice blast disease.



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sebagai memenuhi keperluan untuk ijazah Master Sains

**KAJIAN PENGHASILAN METABOLIT OLEH KULAT KARAH PADI,
Magnaporthe oryzae SEMASA PERKEMBANGAN “APPRESSORIUM”**

Oleh

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Magnaporthe oryzae ialah sejenis kulat patogen penyebab penyakit karah padi yang menjangkiti padi di seluruh dunia. Kitaran penyakit karah yang disebabkan oleh *Magnaporthe oryzae* ini unik, di mana ia memerlukan appressorium untuk menembusi sel perumah. Appressorium ialah struktur kritikal yang diperlukan untuk terjadinya penyakit, namun dari sudut penghasilan metabolit semasa perkembangan appressorium, ianya sangat kurang diketahui. Sehingga kini, maklumat mengenai metabolit kulat *M. oryzae* serta fungsi-fungsi penghasilannya semasa perkembangan appressoria amat terhad. Oleh itu, tujuan penyelidikan ini adalah untuk menentukan metabolit yang dihasilkan oleh *M. oryzae* semasa perkembangan appressoria secara *in vitro* dan menerokai laluan metabolik yang terlibat. Kaedah metabolomik tidak sasar daripada Proton Nuklear Magnetik Resonan (^1H NMR) telah digunakan untuk menentukan metabolit yang dihasilkan dari ekstrak *M. oryzae* dan sebarang perubahan metabolit telah dikenalpasti pada setiap peringkat perkembangan iaitu 0 jam, 8 jam dan 24 jam. Spektra ^1H NMR telah dianalisis menggunakan analisa multivariat (MVDA) dan model validasi telah dikaji. Terdapat banyak metabolit “primary” berdasarkan dari spektra ^1H NMR dan sebanyak 43 metabolit telah dikenaplasti secara “putative” hasil carian rujukan metabolomik dan kajian terdahulu. Analisa separa persegi diskriminan (PLS-DA) menunjukkan pola metabolit pada 0 jam, 8 jam, 24 jam dan miselia. Di antara metabolit yang menunjukkan perubahan yang signifikan ($p < 0.05$) antara kumpulan 0 jam, 8 jam, 24 jam dan miselia ialah butirar, leusina, isoleusina, valina, isobutirat, etanol, metilmalonat, treonina, laktat, alanina, lisina, arginina, 4-aminobutirat, glutamat, homoserina, isositrat, glutamina, kolina, glukosa, xilosa, manosa, gliserol, manitol, glusitol, tirosina, sukrosa dan triptofan. Analisa unjuran orthogonal kepada struktur laten diskriminan (OPLS-DA) memberikan gambaran metabolit yang dihasilkan pada setiap masa perkembangan iaitu 0 jam, 8 jam dan 24 jam. Banyak penghasilan metabolit dilihat berlaku pada 8 jam dan 24 jam berbanding 0 jam. Analisa laluan metabolomik (MetPa 4.0) daripada Metaboanalyst.ca digunakan untuk menerangkan laluan metabolik yang terlibat

semasa perkembangan appressoria. Terdapat lapan laluan metabolik penting dikenalpasti terlibat semasa perkembangan appressoria termasuk metabolisme asid amino, metabolisme karbohidrat dan metabolisme lipid. Metabolit-metabolit yang dihasilkan oleh kulat *M. oryzae* ini berpotensi untuk kajian sasaran metabolomik dalam menyasarkan metabolit atau laluan metabolik tertentu yang penting terlibat dalam perkembangan penyakit karah padi sekaligus memberi peluang ke arah perkembangan menghasilkan perencat kepada penyakit karah padi.



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LIST OF ABBREVIATIONS

<i>M. oryzae</i>	<i>Magnaporthe oryzae</i>
h	Hour
m	Mycelia
MPa	Megapascal
µm	Micrometre
°C	Degree Celcius
min	minute
¹ H NMR	Proton Nuclear Magnetic Resonance
MVDA	Multivariate Data Analysis
PCA	Principal Component Analysis
PLS-DA	Partial Least Squares Discriminant Analysis
OPLS-DA	Orthogonal Partial Least Square Discriminant Analysis
TSP	Trimethylsilyl-2,2,3,3-tetradeuteropropionic acid
ppm	chemical shift
VIP	Variable influence on projection
TCA	Tricarboxylic acid cycle/citric acid cycle
s	singlet
d	doublet
dd	double doublet
t	triplet
m	multiplet
q	quartet
J	coupling constant
GABA	gamma-aminobutyrate

CHAPTER 1

INTRODUCTION

Rice blast disease is one of the devastating diseases in agriculture caused by fungal pathogen, *Magnaporthe oryzae*. The rice yield loss about 10-30% worldwide because of rice blast every year with estimation about US\$6 billion sufficient to feed another 60 million people (Pennisi, 2010; Talbot, 2003). As rice is a staple food, the impact of rice blast to human signify a threat to food security considering increase of rice production demands consumed by approximately 23% human population (Wilson and Talbot, 2009). In Malaysia, 10% of Muda Agricultural Development Authority (MADA) crop area approximately more than 60,000 tons of rice was attacked by rice blast in 2016-2017 season and rice price was cut-down by 50% which brought huge losses to the rice production. Susceptible rice crop now at risk as it could be affected easily by this disease (Berita Harian, 2017). Interestingly, the fungus has become intense research subject in years to obtain in-depth understanding of *Magnaporthe oryzae* infection stage, from early infection until it took over the host entirely. Knowledge of how this pathogen could survive in harsh environment and successfully invade host can help in developing reliable disease countermeasure or finding inhibitors to combat fungal disease (Skamnioti and Gurr, 2009; Dunn *et al.*, 2009).

Magnaporthe oryzae formed essential infection structure called appressorium. The appressorium can be induced away from rice leaf (*in vitro*) (Soanes *et al.*, 2012) by providing hydrophobic, hard surface and nutrient-free condition. Conidium undergoes germination to form germ tube and once the germ tube has met its required physical signals, it becomes swollen and differentiates to become appressorium (Wilson and Talbot, 2009). The appressorium accumulates high glycerol level so that enormous turgor pressure can be driven by osmosis (water moves into the appressorium) and presses down the base of appressorium by forming small penetration peg hence develops invasive hyphae from inside host (Fernandez and Orth, 2018). Complex infection mechanisms of *M. oryzae* were controlled by various specific genes, proteins, metabolites, regulators and enzymes activities (Fernandez and Orth, 2018; Jones *et al.*, 2011; Aliferis and Jabaji, 2010; Foster *et al.*, 2003).

Integrated control measures have been implemented to decrease the severity of rice blast but complete eradication is still unachievable. Methods like developing resistant cultivar, chemical control, biological control, nutritional management have been introduced to control the disease (Miah *et al.*, 2017) but comprehensive study is also highly needed for better understanding of *M. oryzae* physiological processes. In addition, metabolites produced by *M. oryzae* during appressorium development and their functions in pathogenicity are still unclear. So, this study focused on metabolites produced by *M. oryzae* to provide information on possible metabolites activated during each time of development stage hence could reveal underlying biochemical mechanisms of *M. oryzae* and allow development of effective control measures. Metabolites production levels

were influenced by time of appressoria development whereby early appressoria development stage produced low number of metabolites compared to appressoria maturation stage. Previous study of *M. oryzae* metabolites unveil several metabolites such as alanine, malate, glutamine and unknown sugar exhibited high levels after 24 h post inoculation (Jones *et al.*, 2011). Targeted gene studies of *M. oryzae* indicated amino acids like arginine, isoleucine, valine and lysine (Zhang *et al.*, 2015; Chen *et al.*, 2014; Du *et al.*, 2013) were essential for appressoria formation, conidia development and pathogenicity.

Fungal metabolites could be identified using a robust technique of NMR-based metabolomics. A combination of advanced instrumentation and analytical software have allowed metabolomics emerged as powerful tools and unbiased for determination of endogenous metabolites derived from living samples such as urine, serum, microbes or biological tissue extract in response to genetic modification or developmental stimuli (Clark and Haselden, 2008). In this study, *M. oryzae* was germinated on artificial surface to produce appressoria under 0 h, 8 h, 24 h time-point development to assess metabolites production and changes of metabolites within 0 h, 8 h and 24 h compared with mycelia. Our study provided new insight of metabolites analytical analysis and possible mechanisms involved during appressoria development in *Magnaporthe oryzae* in hope for better understanding regarding fungal pathogen metabolites.

Hence, the research objectives were to:

1. identify metabolites produced by *Magnaporthe oryzae* during appressoria development stage.
2. determine metabolites associated changes during each development time-point and propose metabolic pathways involved in *Magnaporthe oryzae* during appressoria development stage.

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