UNIVERSITI PUTRA MALAYSIA

BIOLOGICAL CONTROL OF Pyricularia oryzae USING FUNGAL METABOLITES

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BIOLOGICAL CONTROL OF *Pyricularia oryzae* USING FUNGAL METABOLITES

By

ALI ABDULAMEER IDAN

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

October 2017

DEDICATION

I Would Like To Dedicate My Thesis To

My Greatest Parents

My Lovely Brothers

My Dearly Friends

Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

BIOLOGICAL CONTROL OF *Pyricularia oryzae* USING FUNGAL METABOLITES

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ALI ABDULAMEER IDAN

October 2017

Chairman : Associate Professor Kamaruzaman Bin Sijam, PhD
Faculty : Agriculture

Rice blast caused by fungus *Pyricularia oryzea* is one of the most devastating among rice diseases. The disease has been classified as one of the most serious diseases that influence the production of rice since rice fields are seriously damaged, thereby affecting yield and quality. To decrease the use of pesticides, biological control is considered as an alternative sustainable control. *Aspergillus* spp. has been widely found to be useful in biotechnology as a bio control agent. They have a high ability in the production of secondary metabolites. *Aspergillus* spp. was obtained from the rice field soil located in Selangor, Tanjung Karang, Peninsular Malaysia. Isolates UPMZ01 and UPMZ02 were screened against blast pathogen by applying dual culture technique. The antagonism of isolate UPMZ01 was (81.326%) while UPMZ02 gave (70.9%) of inhibition percentage against *P. oryzae*. Culture filtrate was also used to screen the inhibitory effect against the pathogen. The results revealed that isolate UPMZ01 gave the optimum inhibitory percentage of (100%) at all concentrations of fungal secondary metabolites at 14 days. Based on ITS sequencing, the isolate UPMZ01 was identified as *Aspergillus niger* and UPMZ02 as *Aspergillus flavus*. The environmental factors such as pH and temperature influenced the production of secondary metabolites. The optimum condition for *A. niger* to produce efficient antifungal metabolites that gave (100%) PIGR against blast pathogen was at pH 5.0 and 21 to 29ºC. The secondary metabolites compounds were identified by gas chromatography-mass spectrometry (GC-MS). Fifteen compounds were recognized as major compounds. The identified compounds were Oleic Acid, n-Hexadecanoic acid, Hexose, Glycerol, Stearic acid, Tetradecanoic acid, Dodecanoic acid and 5-Hydrxoymethylfurfural. The Liquid chromatography-mass spectrophotometry (LC-MS/MS) analysis revealed four compounds, Succinic acid, Al21 anion, Diphenylarsine and N-Acetylaspartic acid. However, the culture filtrate of *A. niger* UPMZ01 may contribute to control rice blast pathogen.

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

KAWALAN BIOLOGI BAGI *Pyricularia oryzae,* EJEN PENYEBAB PENYAKIT BLASTA PADI DENGAN MENGGUNAKAN KULAT METABOLIT

Oleh

ALI ABDULAMEER IDAN

Oktober 2017

Pengerusi : Profesor Madya Kamaruzaman Bin Sijam, PhD
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Penyahit karah, yang disebabkan oleh kulat *Pyricularia oryzea,* adalah satu daripada penyakit utama bagi tanaman padi. Penyakit ini telah diklasifikasikan sebagai satu penyakit yang serius dalam mempengaruhi pengeluaran padi oleh kerana ia memberi kerosakan kepada tanaman, sekaligus menjejaskan hasil dan kualiti. Bagi mengurangkan penggunaan racun kulat, kawalan biologi telah dianggap sebagai satu kaedah kawalan pilihan yang kekal. Kulat *Aspergillus* spp. adalah penting dalam bidang bioteknologi sebagai ejen kawalan biologi. Kulat tersebut mempunyai keupayaan yang tinggi dalam pengeluaran metabolit sekunder. Dalam kajian ini, *Aspergillus* spp. telah diperolehi daripada tanah sawah dari Tanjung Karang, Selangor, Malaysia. *Aspergillus* spp., UPMZ01 dan UPMZ02 telah diuji terhadap patogen Penyahit dengan menggunakan teknik dwi-kultur. Sifat antagonis *Aspergillus* spp., UPMZ01 telah berjaya memberi kesekatlaluan 81.326% sementara UPMZ02 memberi 70.9% terhadapan *P. oryzae*. Kultur filtrat juga telah digunakan untuk ujian menghalang kesan patogen. Keputusan menunjukkan bahawa kulat *Aspergillus* spp., UPMZ01 memberi kesekatlaluan 100% pada semua kepekatan metabolit sekunder selepas 14 hari. Berdasarkan kepada urutan ITS, peneilan UPMZ01 telah dikenalpasti sebagai *Aspergillus niger* and jenis UPMZ02 sebagai *Aspergillus flavus*. Faktor persekitaran seperti pH dan suhu mempengaruhi pengeluaran metabolit sekunder. Keadaan optimum bagi *Aspergillus niger* mengeluarkan metabolit anti-kulat adalah pada pH 5.0 dan suhu 21 hingga 29º C. Kompaun metabolit sekunder telah dikenalpasti melalui kaedah gas chromatography-mass spectrometry (GC-MS). Lima belas kompaun utama telah dikenalpasti termasuklah asid oleic, asid n-Hexadecanoic, hexose, glycerol, asid stearic, asid tetradecanoic, asid dodecanoic dan 5-Hydrxoymethylfurfural. Kaedah analisis liquid chromatography-mass spectrophotometry (LC-MS/MS) mendapati empat jenis kompaun termasuklah asid succinic, Al21 anion, Diphenylarsine dan asid N-Acetylaspartic. Kultur saringan *A.*

niger UPMZ01 dapat memberi kawalan terhadap patogen untuk mengawal patogen karah padi.

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I certify that a Thesis Examination Committee has met on 5 October 2017 to conduct the final examination of Ali Abdulameer Idan on his thesis entitled "Biological Control of *Pyricularia oryzae* using Fungal Metabolites" 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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LIST OF ABBREVIATIONS

%	Percentage
± SEM	Standard error of means
Mg	Microgram
Mm	Millimeter
mM	Milli molar
µg/ml	Microgram per milliliter
Ml	Microlitre
Mm	Micrometer
min.	minute
cfu/ml	Colony forming unit per millilitre
1x	One time
ºC	Degree centigrade
pH	Logarithm of hydrogen ion activity
approx.	Approximately
rDNA	Ribosomal deoxyribonucleic acid
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
CTAB	Cetyltrimethyl ammonium bromide
EDTA	Ethylenediaminetetra acetic acid
TE	Tris-EDTA buffer solution
Fwd_seq	Forward sequence
Rev_seq	Reverse sequence
GC-MS	Gas Chromatography Mass Spectrometry
LC-MS	Liquid Chromatography Mass Spectrometry
H	hour
ITS	Internal transcribed spacer regions
PCR	Polymerase chain reaction
Bp	Base pair
Kg	Kilogram
L	Liter

M	Molar
PIGR	Percentage inhibition growth redial
MIC	Minimum inhibitory concentration
vv^{-1}	Volume per volume
rpm	Revolution per minute
PDA	Potatoes Dextrose Agar
PDB	Potatoes Dextrose Broth
NaOCl	Sodium hypochlorite
dH_2O	Distilled water
FAO	Food and Agriculture Organization of The United Nations
IRRI	International Rice Research Institute
WHO	World Health Organization
FDA	Food and Drug Administration
No.	Number
NCBI	National centre of biotechnology information
Conc.	Concentration

CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa L.*) is one of the major crops consumed as a source of carbohydrate and energy (Zhang *et al.*, 2007). The major producers of rice are China, India, Bangladesh, Indonesia, Thailand, Vietnam, the Philippine, Myanmar, Japan, and Brazil (IRRI, 2008; FAO, 2013). The nutrition of about six billion people worldwide depends on rice (Maclean *et al.*, 2002). Asians obtain about 35-75 % of their daily calories from rice (Khush, 2005). Rice is one of the main crops planted in Malaysia besides oil palm, rubber, and cocoa. Approximately 72 % of rice grown in Malaysia is in eight storehouse territories that produce two types of crops yearly. In addition, only a small section of the total rice production is grown in the Eastern part of Malaysia as wet paddy under dry land paddy cultivation (Yahya 2001). Rice in Malaysia is considered as the first major crop grown by both private and public sectors. Every Malaysian in average consumes about 82.3 Kg of rice annually. Therefore, rice is regarded as a part of Malaysian diet (Selamat and Ismail 2009).

Rice, like any other crop, has been exposed to many threats such as diseases and pests. The rice blast is a disease which has been classified as one of the most dangerous diseases that affect the cultivation of rice as rice fields are seriously damaged, thereby affecting the production yield and quality (Hayasaka *et al.*, 2008). Rice blast is endemic in about 85 countries (Hajano *et al.* 2011), and in a suitable weather condition, would be very destructive to the rice plants (Scardaci *et al.*, 1997). The loss of yield annually to rice blast amounts to about 10 – 30 % of the global rice production (Tongen *et al.*, 2006), thereby having a great influence on the yield and quality of rice production. In the fields where rice cultivatation is above the ground can be infected by rice blast. The loss of the leaves and the panicles can lead to great losses in terms of yield directly or indirectly (Silva *et al.*, 2009).

In Malaysia, the disease occurs often as a seedling, foliar and panicle blasts. Rice blast can be controlled by using fungicides as well as resistant cultivars, but the use of chemicals have been showed to pose threats and several damages to the environment and can also influence the natural resistance of the host plant based on a single gene (Seebold et al., 2004; Ji et al., 2008). In this case, an urgent need for new methodologies is needed to find compliment strategies to achieve better results in the management of rice blast disease.

The resistance of rice blast disease is a great problem that is posing many difficulties in controling nowadays, although some fungicides have been found to be effective in the control of this disease. However, due to cost, fungicides are not preferred choice as well as not environmentally friendly. Also, it could encourage the growth of other organisms around the treated territories. The use of resistant species could provide a short-term solution. Various rice-growing regions of the world have adopted various strategies of breeding to improve the resistance of rice to various plant diseases. Information on the population of the pathogen is necessary and useful for a successful development of disease resistant rice varieties. The structure of the population is dependent on the genetic differences of the pathogen and can alter depending on the time and zone, as these populations are capable of developing and adjusting in the way they respond to environmental conditions (McDonald and Linde, 2002).

The utilization of bio-agents in biological control is a great alternative to fungicides and chemicals. Biological control has proved to be successful in many countries against plant diseases. Different kinds of fungi such as *Aspergillus, Penicillium, Gliocladium, Trichoderma, Paecilomyces,* and bacteria such as *Pseudomonas, Bacillus, Serratia, Erwinia, Rhizobium, and Paenibacillus* are excellent examples. Among them, *Aspergillus* and *Penicillium* are commonly used for industrial and biotechnological applications. These fungi have been used as extracellular enzyme producers such as glucose oxidase, α-amylase, pectinase, and glucoamylase; organic acids, penicillin, and recombinant proteins.

A soil usually harbors a massive number of unique microorganisms like fungi. The vast majority of fungi produced various chemical compounds to secure and resist other organisms in the same territory and under comparative ecological conditions. It has been approved that this safety resistance is usually joined by a dominant creation of strange bioactive metabolites.

The chemical fungicides have been considered as common natural products, are driven by the fungus and their surroundings that cooperate to produce fundamental and chemical substances so as to promote survival and aggressiveness (Lee, 2010). In the early history of antibiotics, an assortment of bacterial and fungal strains was investigated from temperate local specimens. In particular, fungi supported approximately 38 % of all isolated microbial products and 17 % from unicellular bacterial microorganisms (Berdy, 2005). Hence, as an antimicrobial, fungi have found to be a rich source of unique antibiotics. Meanwhile, fungal bioactive metabolites have been found to be an excellent hotspot of pharmaceutical bioactive components (Hoffmeister, 2007). In fact, fungi are basic in nature and considered as great natural hotspots for antimicrobial operators (Lindequist *et al*., 2005).

The objectives of this research were:

i. To isolate and identify fungi from the soil of rice field and to determine their antagonistic activity against *P. oryzae*.
ii. To determine the effect of different levels of pH and temperature on the production of antimicrobial compounds by *Aspergillus niger*, and to identify the metabolic compounds using GC-MS and LC-MS/MS.

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