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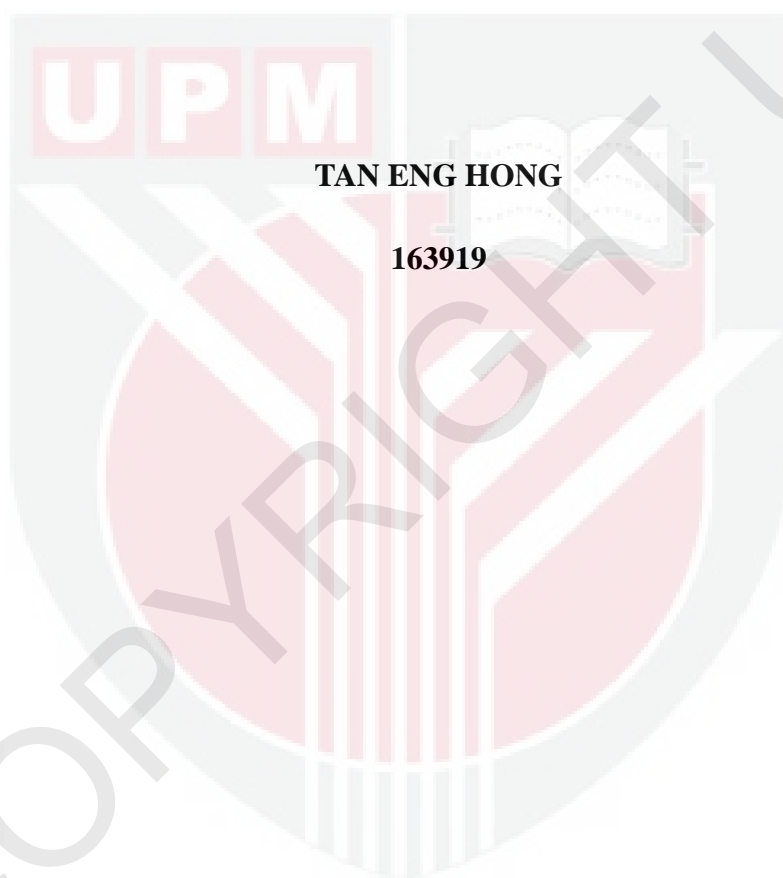
**INVESTIGATION ON CELLULOLYTIC AND PROTEOLYTIC ACTIVITIES  
OF THE ENDOPHYTIC FUNGI ISOLATED FROM MEDICINAL PLANTS**

**TAN ENG HONG**

**FBSB 2015 133**

**Investigation on Cellulolytic and Proteolytic Activities of the Endophytic**

**Fungi Isolated from Medicinal Plants**



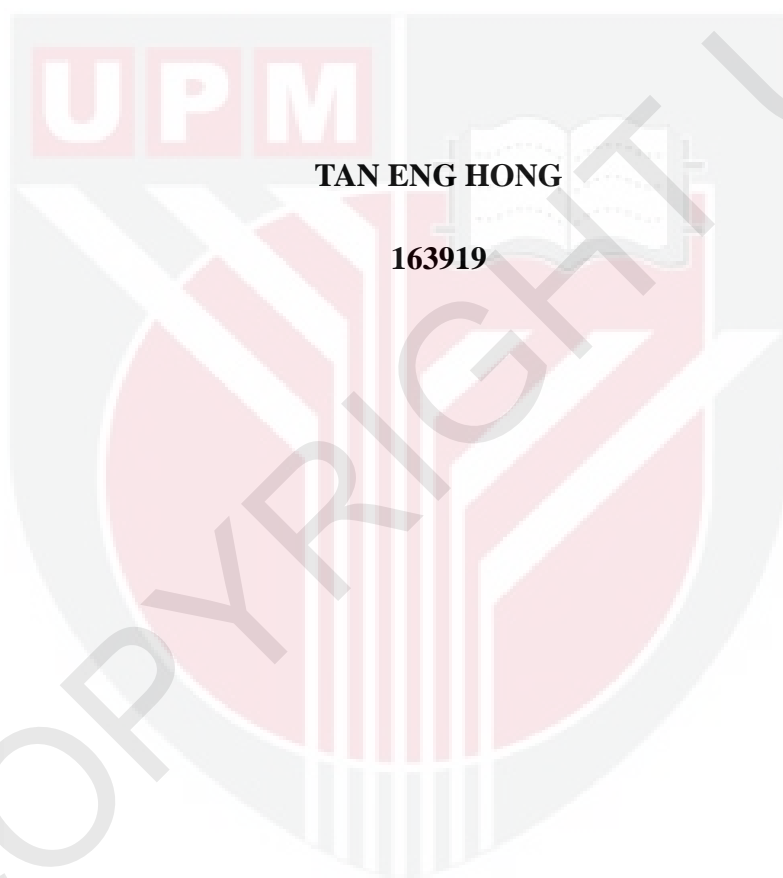
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**Dissertation submitted in partial fulfillment of the requirement for the course  
BMJ 4999 Project in the Department of Microbiology  
Universiti Putra Malaysia  
JUNE 2015**

**Investigation on Cellulolytic and Proteolytic Activities of the Endophytic**

**Fungi Isolated from Medicinal Plants**



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**2015**

## PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk “Investigation on Cellulolytic and Proteolytic Activities of the Endophytic Fungi Isolated from Medicinal Plants” telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh Tan Eng Hong (163919) sebagai syarat untuk kursus BMY 4999 projek.

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## ABSTRACT

Endophytic fungi are the fungi that reside in the plant internal tissues. They are an excellent source of bioactive natural compounds such as antimicrobial, antiviral and anticancer compounds. They are also an alternative source other than the commercial bacteria to produce extracellular enzymes to meet the high industrial demand. Scientists found out that there are more than a million of fungal species still yet to be identified. Novel fungal species are believed to be discovered among the under-explored endophytic fungi. This study was to isolate and identify endophytic fungi from 10 medicinal plants, screen fungal isolates for their cellulolytic and proteolytic abilities and determine the cellulase and protease activities using spectrophotometric methods. From the study, fungal isolates IS02, IS03, IS05, IS07 and IS10 showed positive results in cellulolytic screening while IS06, IS08, IS09 and IS10 showed positive results in proteolytic screening. Cellulase producers (IS07 and IS03) and protease producers (IS06 and IS09) which recorded the highest and second highest Index of Relative Enzyme Activity were subjected to spectrophotometric assays. IS03 and IS07 showed their highest cellulase activities on day 10 which were 0.0055 U/mL and 0.0093 U/mL respectively. IS06 and IS09 showed their highest protease activities on day 10 which were 0.0263 U/mL and 0.0212 U/mL respectively. IS06 was suspected to be *Penicillium* sp. whereas IS07 was suspected to be *Aspergillus* sp.. Non-spore formers IS03 and IS09 were failed to identify using microscopic method.

## ABSTRAK

Kulat endofitik adalah kulat yang tinggal di tisu dalaman tumbuh-tumbuhan. Mereka adalah sumber yang sangat baik untuk memperolehi sebatian bioaktif semula jadi seperti antimikrob, antivirus dan antikanser sebatian. Mereka juga adalah sumber alternatif selain daripada bakteria komersial untuk menghasilkan enzim ekstrasellular untuk memenuhi permintaan industri yang tinggi. Para saintis mendapati bahawa terdapat lebih daripada satu juta spesies kulat yang masih belum dikenal pasti. Spesies kulat baru dipercayai dapat ditemui di kalangan kulat endofitik yang kurang diterokai. Kajian ini adalah untuk memencilkan dan mengenalpasti kulat endofitik daripada 10 tumbuh-tumbuhan ubatan, skrin kulat dipencilkan untuk kebolehan selulolitik dan proteolitik mereka dan menentukan aktiviti-aktiviti selulase dan protease dengan kaedah spektrofotometri. Dalam kajian ini, kulat IS02, IS03, IS05, IS07 dan IS10 menunjukkan keputusan positif dalam saringan selulolitik manakala IS06, IS08, IS09 dan IS10 menunjukkan keputusan positif dalam saringan proteolitik. Pengeluar selulase (IS07 dan IS03) dan pengeluar protease (IS06 dan IS09) yang mencatatkan Indeks Relatif Enzim Aktiviti tertinggi dan kedua tertinggi diteruskan dengan asai spektrofotometri. IS03 dan IS07 menunjukkan aktiviti selulase tertinggi pada hari 10 iaitu 0.0055 U/mL dan 0.0093 U/mL masing-masing. IS06 dan IS09 menunjukkan aktiviti protease tertinggi mereka pada hari 10 iaitu 0.0263 U/mL dan 0.0212 U/mL masing-masing. IS06 disyaki adalah *Penicillium* sp. sedangkan IS07 disyaki adalah *Aspergillus* sp.. IS03 dan IS09 yang tidak menghasilkan spora gagal untuk dikenal pasti dengan kaedah mikroskopik.

## ACKNOWLEDGEMENT

I am really grateful that I managed to complete my final year project within the time given. I would like to express the deepest appreciation to my supervisor, Dr. Mohd Termizi Yusof. This project cannot be completed without the persistent guidance and encouragement from him. I would like to thank the lab assistants of Preparation Room, Faculty of Biotechnology and Biomolecular Sciences especially Mr. Hussein and Mdm. Sharifah for their help. In addition, a thank you to Ms. Nor Azzah Binti Mohd Salleh and Ms. Nadila Binti Hanafee, master students in Science of Microbiology, Faculty of Biotechnology and Biomolecular Sciences for their assistance and suggestions. Last but not least, I would like to express my gratitude to my friends who supported me along the way.

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## LIST OF ABBREVIATIONS

%	percentage
°C	degree celsius
β	beta
μg	microgram
μm	micrometer
μmol	micromole
BYDV	barley yellow dwarf virus
C <sub>6</sub> H <sub>5</sub> OH	phenol
cm	centimeter
CMC	carboxymethylcellulose
CMCase	carboxymethylcellulase
CPE	cytopathic effect
DNS	3,5-Dinitrosalicylic acid
FeSO <sub>4</sub> .7H <sub>2</sub> O	iron(II) sulfate heptahydrate
g	gram
g/mol	molar mass (gram/mole)
GRAS	generally regarded as safe
K <sub>2</sub> HPO <sub>4</sub>	dipotassium phosphate
KCl	potassium chloride
KOCOCH(OH)CH(OH)COONa.4H <sub>2</sub> O	potassium sodium tartrate tetrahydrate

L	liter
M	molar (moles/liter)
MgSO <sub>4</sub> .7H <sub>2</sub> O	magnesium sulfate heptahydrate
mg/mL	milligram per milliliter
min	minute
mL	milliliter
N	normal (equivalent/liter)
Na <sub>2</sub> CO <sub>3</sub>	sodium carbonate
Na <sub>2</sub> SO <sub>3</sub>	sodium sulfite
NaNO <sub>3</sub>	sodium nitrate
NaOH	sodium hydroxide
nm	nanometer
No.	number
PDA	potato dextrose agar
PDB	potato dextrose broth
psi	per square inch
p-value	probability value
rDNA	ribosomal deoxyribonucleic acid
rpm	rotations per minute
sp.	species
TCA	trichloroacetic acid

U	enzyme unit
U/mL	enzyme unit per milliliter
v/v	volume per volume
w/v	weight per volume





## CHAPTER 1

### INTRODUCTION

Endophytic fungi that live asymptotically in the plant tissues can be found in almost all species of the plants. Their existences are known to improve plant growth, protect plant against diseases and confer stress tolerance in plant (Malinowski & Belesky, 2006). They are considered as an excellent source of bioactive natural compounds such as antimicrobial, anticancer and antiviral compounds because they occupy a wide range of ecological niches in different kinds of plants (Ahmed et al., 2012).

There are not more than 100,000 known fungal species. Scientists believe that there could be more than a million of fungal species remain unidentified in the world (Blackwell, 2011). It is possible that new fungal species could be discovered through isolation of endophytic fungi from plants. For examples, novel endophytic fungal species *Pestalotiopsis* sp. and *Hypocrea lixii* were recently isolated from plants (Bagyalakshmi et al., 2012; Zhao et al., 2013).

Enzymes of microbial origin are of great importance in industries, medical field and molecular biology. Fungal enzymes are of particular interest among the other enzymes produced by non-pathogenic microorganisms because fungi are easy to cultivate and they produce exoenzymes of high industrial potency (Walsh, 2002). Endophytic fungi are seldom utilized in industries as a biotechnological

source to produce enzymes such as cellulase, lipase and protease in large scale (Bhagobaty & Joshi, 2012). Therefore these under-explored groups of fungi could be a new source to obtain valuable extracellular enzymes to meet the high industrial demand.

The stability of fungal enzymes is known to be higher compared to enzymes of other microbial origins (Sunitha et al., 2013). Fungi are more efficient than bacteria to colonize and break down large particles because they possess networks of hyphae that capable to absorb nutrients along the distributed networks and focus the release of extracellular enzymes at the growing tips (Frey et al., 2003). Hence endophytic fungi are undoubtedly an alternative source other than the commercial bacteria to lead to a continuous, reproducible and cost-effective yield of extracellular enzymes.

In this study, I aimed to isolate and identify endophytic fungi from 10 medicinal plants, screen fungal isolates for their cellulolytic and proteolytic abilities and determine the cellulase and protease activities using spectrophotometric methods.

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