

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENT	iii
ABSTRACT (in English)	iv
ABSTRACT (in Thai)	vi
TABLE OF CONTENTS	viii
LIST OF TABLES	xi
LIST OF FIGURES	xii
ABBREVIATIONS AND SYMBOLS	xiv
CHAPTER 1 INTRODUCTION	1
CHAPTER 2 LITERATURE REVIEWS	6
2.1 <i>Histoplasma capsulatum</i>	6
2.1.1 History and taxonomy	6
2.1.2 General morphology and characteristics	7
2.1.3 Sexual reproduction	9
2.1.4 Epidemiology and ecology	11
2.1.5 Pathogenesis	13
2.1.6 Diagnosis	13
2.1.7 Treatment	18
2.1.8 Isolation of <i>H. capsulatum</i> from soil	2

2.2	<i>Cryptococcus neoformans</i>	21
2.2.1	History and taxonomy	21
2.2.2	General morphology and characteristics	22
2.2.3	Sexual reproduction	23
2.2.4	Epidemiology and ecology	24
2.2.5	Pathogenesis	26
2.2.6	Diagnosis	28
2.2.7	Treatment	32
	CHAPTER 3 OBJECTIVES	33
	CHAPTER 4 MATERIALS AND METHODS	34
4.1	Experimental design	34
4.2	Sample collection	35
4.3	Material processing	42
4.4	DNA extraction	42
4.5	Detection of <i>Cryptococcus neoformans</i> in soil contaminated with avian and bat droppings by culture and nested-PCR	43
4.5.1	Isolation of <i>C. neoformans</i> by culture method	43
4.5.2	Detection of <i>C. neoformans</i> by nested PCR	44
4.5.3	Nucleotide sequence analysis of <i>C. neoformans</i>	46
4.5.4	Sensitivity test of PCR conditions of <i>C. neoformans</i>	47
4.5.5	Specificity test of PCR condition <i>C. neoformans</i>	48
4.6	Detection of <i>H. capsulatum</i> in soil contaminated with avian and bat droppings by nested-PCR	48

4.6.1	Detection of <i>H. capsulatum</i> by nested PCR	48
4.6.2	Nucleotide sequence analysis of <i>H. capsulatum</i>	49
4.6.3	Sensitivity test of PCR conditions of <i>H. capsulatum</i>	49
4.6.4	Specificity test of PCR condition <i>H. capsulatum</i>	50
CHAPTER 5 RESULTS		52
5.1	To detect <i>Cryptococcus neoformans</i> in soil contaminated with avian and bat droppings by culture and nested-PCR	52
5.1.1	Detection of <i>C. neoformans</i> by culture and nested PCR	52
5.1.2	Sensitivity test of PCR condition to detect <i>C. neoformans</i>	52
5.1.3	Specificity test of PCR condition to detect <i>C. neoformans</i>	53
5.2	To detect <i>Histoplasma capsulatum</i> in soil contaminated with avian and bat droppings by nested-PCR	61
5.2.1	Detection of <i>H. capsulatum</i> by nested PCR	61
5.2.2	Sensitivity test of PCR condition to detect <i>H. capsulatum</i>	61
5.2.3	Specificity test of PCR condition to detect <i>H. capsulatum</i>	62
CHAPTER 6 DISCUSSION		70
CHAPTER 7 SUMMARY		74
REFERENCES		77
APPENDIX		93
CURRICULUM VITAE		97

LIST OF TABLES

Table		Page
1	List of 267 soil contaminated with avian and bat dropping samples	35
2	Biochemical characteristics of <i>C. neoformans</i>	44
3	Oligonucleotide primers for 18S rDNA gene of <i>C. neoformans</i>	45
4	PCR master mix preparation for the detection of <i>C. neoformans</i>	46
5	Oligonucleotide primers for 100 kDa like protein gene of <i>H. capsulatum</i>	51
6	PCR master mix preparation for the detection of <i>H. capsulatum</i>	51
7	Detection of <i>C. neoformans</i> from soil contaminated with avian and bat droppings by culture and nested PCR	60
8	Detection of <i>H. capsulatum</i> in soil contaminated with avian and bat droppings by nested PCR	68
9	Nucleotide sequencing analyses of PCR products	69

LIST OF FIGURES

Figure	Page
1 <i>Histoplasma capsulatum</i>	10
2 Asexual reproduction and sexual reproduction of <i>Histoplasma capsulatum</i>	11
3 India ink preparation of <i>C. neoformans</i> and the teleomorph stages of <i>C. neoformans</i> var. <i>neoformans</i>	24
4 Schematic representation of the detection of <i>C. neoformans</i> and <i>H. capsulatum</i> in soil contaminated with avian and bat droppings by nested PCR	34
5 <i>C. neoformans</i> cultured on Sabouraud dextrose agar, L-dopa agar and india ink preparation of yeast cells of <i>C. neoformans</i>	54
6 The carbon assimilation and fermentation tests for identification of <i>C. neoformans</i>	55
7 Representative gels of PCR products analysed with Fungus I, Fungus II and CrypI, CrypII primers	56
8 Sensitivity of polymerase chain reaction (PCR) using <i>C. neoformans</i> specific primers	57
9 Ability to detect <i>Cryptococcus neoformans</i> cells in soil samples by nested PCR	58
10 Specificity of PCR assay with DNA from various pathogenic organisms by using Fungus I-Fungus II and Cryp I-Cryp II primers	59

11	Representative gel of PCR analyses with Hc I, Hc II and Hc III, Hc IV (bat guano samples)	63
12	Representative gel of PCR analyses with Hc I, Hc II and Hc III, Hc IV (chicken dropping samples)	64
13	Sensitivity of polymerase chain reaction (PCR) using <i>H. capsulatum</i> specific primers	65
14	Ability to detect <i>Histoplasma capsulatum</i> cells in soil suspensions by nested PCR	66
16	Specificity of PCR assays with DNA from various pathogenic organisms by using Hc I - Hc II and Hc III - Hc IV primers	67

ABBREVIATIONS AND SYMBOLS

%	Percent
°C	Degree celcius
µg	Microgram
µl	Microliter
µM	Micromolar
x g	Gravity
g	Gram (s)
AIDS	Acquired immunodeficiency syndrome
BLAST	Basic local alignment search tools
bp	Base pair
DNA	Deoxyribonucleic acid
CFU	Colony forming unit
cm	Centrimeter
CNS	central nervous system
dNTP	Deoxyribonucleotide triphosphate
EDTA	Ethylenediamine tetraacetic acid
et al.	And others
h	Hour (s)
HIV	Human immunodeficiency virus
kDa	Kilodalton

Kb	Kilobase
M	Molar
mg	Milligram (s)
min	Minute (s)
ml	Milliliter (s)
mm	Millimeter (s)
mM	Millimolar
mRNA	Messenger ribonucleic acid
MW	Molecular weight
ng	Nanogram
OD	Optical Density
PCR	Polymerase Chain Reaction
rpm	Revolution per minute
s	Second
SDA	Sabouraud dextrose agar
SDS	Sodium dodecyl sulfate
Taq	<i>Thermus aquaticus</i>
TAE	Tris-acetate buffer
U	Unit (s)
UV	Ultraviolet
V	Volt