

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENT	iii
ABSTRACT (in English)	iv
ABSTRACT (in Thai)	vi
TABLE OF CONTENTS	x
LIST OF TABLES	xii
LIST OF FIGURES	xiii
ABBREVIATIONS AND SYMBOLS	xvi
CHAPTER I INTRODUCTION	1
CHAPTER II LITERATURE REVIEWS	5
2.1. History and taxonomy	5
2.2. General morphology and serotype	7
2.3. Mode of transmission, pathogenesis and clinical characteristics	10
2.4. Virulence factors	13
2.4.1. Growth at 37°C	13
2.4.2. The production of a polysaccharide capsule	13
2.4.3. Melanin and laccase	14
2.5. Treatment	16
2.6. Mechanism action of polyene	17
2.7. Mechanism action of azoles	20
2.8. Epidemiology and mechanisms of fluconazole resistance in <i>C. neoformans</i>	24
CHAPTER III OBJECTIVES	30
CHAPTER IV MATERIALS AND METHODS	31
4.1. Experimental design	31
4.2. Identification of <i>C. neoformans</i> from clinical and environmental samples	32

	PAGE
4.2.1. <i>C. neoformans</i> isolates	32
4.2.2. Identification	32
4.3. Broth microdilution susceptibility testing	32
4.3.1. Inoculum preparation	33
4.3.2. MIC interpretation	33
4.3.3. Quality control	33
4.4. Screening of <i>C. neoformans</i> fluconazole heteroresistant isolates	34
4.5. Serotype and mating type identification	35
4.6. DNA extraction	36
4.7. Total RNA extraction	37
4.8. DNA sequence analysis	38
4.9. Cloning of the <i>ERG11</i> gene	39
4.10. Nucleotide sequence analysis	40
4.11. Characterization of drug resistant genes (<i>ERG11</i> , <i>AFR1</i> and <i>MDR1</i>) in fluconazole resistant and fluconazole susceptible <i>C. neoformans</i> isolates	41
4.11.1. Reverse transcription-polymerase chain reaction (RT-PCR)	41
4.11.2. Northern blot analysis	42
4.12. Biosafety consideration	43
CHAPTER V RESULTS	44
5.1. Identification of <i>C. neoformans</i>	44
5.2. Antifungal susceptibility testing	45
5.3. Screening for fluconazole heteroresistant isolate	49
5.4. Serotype and mating type identification	52
5.5. Identification of point mutations in the lanosterol 14 α -demethylase (<i>ERG11</i>) gene	55

PAGE

5.5.1. Recognition of the coding frame from H99 (<i>C. neoformans</i> var. <i>grubii</i>)	56
5.5.2. Recognition of the coding frame from CN4901	58
5.5.3. Recognition of the coding frame from CN4969S	60
5.5.4. Recognition of the coding frame from CN4969HR	62
5.5.5. Comparison of the <i>C. neoformans</i> Erg11p to other fungal Erg11p homologs	64
5.6. Characterization of drug resistant genes (<i>ERG11</i> , <i>AFR1</i> and <i>MDR1</i>) in fluconazole resistant and fluconazole susceptible <i>C. neoformans</i> isolates.	67
5.6.1. Reverse transcription-polymerase chain reaction (RT-PCR)	67
5.6.2. Northern blot analysis	70
CHAPTER VI DISCUSSION	71
CHAPTER VII SUMMARY	76
REFERENCES	79
APPENDICES	97
Appendix A: Culture Media	97
Appendix B: Reagents and buffers	100
Appendix C: Properties of amino acid	102
Appendix D: Chromatogram of nucleotide sequence	104
CURRICULUM VITAE	106

LIST OF TABLES

TABLE

PAGE

1. Recommended 48 hour MIC limits for two quality control organisms for broth microdilution	34
2. Primers sequences applied in serotype and mating type identification	35
3. PCR master mix preparation to determine serotypes and mating type of <i>C. neoformans</i> with specific primers for <i>STE20</i> genes (<i>STE20 Aα</i> , <i>STE20 Aα</i> , <i>STE20 Dα</i> and <i>STE20 Dα</i>)	36
4. PCR condition for serotype and mating type identification	36
5. Primers for DNA sequence analysis of <i>ERG11</i> gene	38
6. Primers sequences applied in RT-PCR analysis	42
7. <i>In vitro</i> antifungal susceptibilities of 190 isolates of <i>C. neoformans</i> to amphotericin B, fluconazole, itraconazole and ketoconazole by broth microdilution method.	46
8. The MICs50 and MICs90 of 104 clinical isolates and 86 environmental isolates of <i>C. neoformans</i> to amphotericin B, fluconazole, itraconazole and ketoconazole.	46
9. MIC determination of <i>C. neoformans</i> isolates by broth microdilution	51
10. Serotype and mating type in CN4901, CN4969S and CN4969HR isolates	52
11. Relative gene expression levels by RT-PCR	69

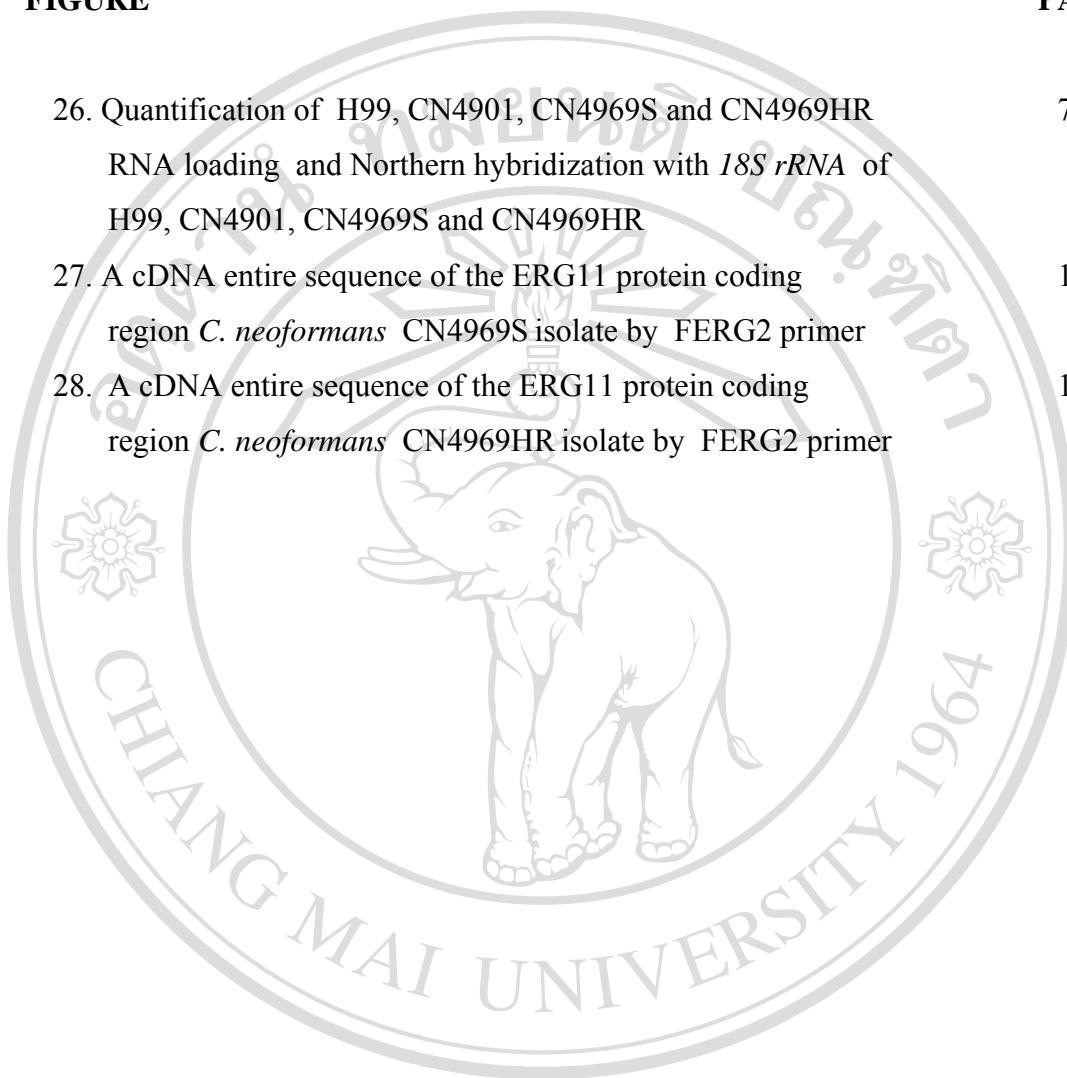
LIST OF FIGURES

FIGURE	PAGE
1. Structure formula of amphotericin B, fluconazole,itraconazole and ketoconazole	19
2. Schematic representation of the interaction between amphotericin B and cholesterol in a phospholipid bilayers	20
3. Mechanism action of azoles	23
4. Main mechanisms of fluconazole resistance	29
5. Schematic representation of roles of <i>ERG11</i> , <i>AFR1</i> and <i>MDR1</i> genes	31
6. Map of the pJET 1.2/blunt vector showing the reading frames and main features	39
7. DNA sequences of multiple cloning site regions	40
8. Identification of <i>C. neoformans</i> from clinical and environmental samples	44
9. The minimal inhibitory concentrations of amphotericin B which inhibits 50% (MIC50) and 90% (MIC90) of <i>C. neoformans</i> isolates	47
10. The minimal inhibitory concentrations of fluconazole which inhibits 50% (MIC50) and 90% (MIC90) of <i>C. neoformans</i> isolates	47
11. The minimal inhibitory concentrations of itraconazole which inhibits 50% (MIC50) and 90% (MIC90) of <i>C. neoformans</i> isolates	48
12. The minimal inhibitory concentrations of ketoconazole which inhibits 50% (MIC50) and 90% (MIC90) of <i>C. neoformans</i> isolates	48
13. The fluconazole-heteroresistant isolate (CN4969HR) on PDA containing 64 µg of fluconazole/ml at 30°C for 72 h	50
14. Susceptibility of CN4969HR isolate to fluconazole determined by the broth microdilution method	50

FIGURE**PAGE**

15. Characteristics of CN4969S (susceptible) and CN4969HR (heteroresistant) isolates in the E-test for fluconazole	51
16. Representative gels of PCR analyse with serotype and mating type specific primer	53
17. The six-frame translation, the nucleotide sequence and deduced amino acid sequence of H99 lanosterol 14 α -demethylase	56
18. The six-frame translation, the nucleotide sequence and deduced amino acid sequence of CN4901 lanosterol 14 α -demethylase	58
19. The six-frame translation, the nucleotide sequence and deduced amino acid sequence of CN4969S lanosterol 14 α -demethylase	60
20. The six-frame translation, the nucleotide sequence and deduced amino acid sequence of CN4969HR lanosterol 14 α -demethylase	62
21. Comparison of standard <i>C. neofmans</i> strains and other organism 14 α -demethylase protein sequences surrounding amino acid substitutions identified in <i>ERG11</i> genes from CN4901, CN4969S and CN4969HR	64
22. Nucleotide sequence analysis of the <i>ERG11</i> cDNA clone plasmid in CN4969S	66
23. Nucleotide sequence analysis of the <i>ERG11</i> cDNA clone plasmid in CN4969HR	66
24. Evaluation of differential expression by RT-PCR of <i>ERG11</i> , <i>AFR1</i> and <i>MDR1</i> genes of H99, CN4901, CN4969S and CN4969HR in two independent experiments	68
25. The relative levels of <i>ERG11</i> , <i>AFR1</i> and <i>MDR1</i> gene expression normalizing with <i>18S rRNA</i> expression in fluconazole-susceptible (H99, CN4901 and CN4969S) and -resistant (CN4969HR) strains in two independent experiments	69

FIGURE	PAGE
26. Quantification of H99, CN4901, CN4969S and CN4969HR RNA loading and Northern hybridization with <i>18S rRNA</i> of H99, CN4901, CN4969S and CN4969HR	70
27. A cDNA entire sequence of the ERG11 protein coding region <i>C. neoformans</i> CN4969S isolate by FERG2 primer	104
28. A cDNA entire sequence of the ERG11 protein coding region <i>C. neoformans</i> CN4969HR isolate by FERG2 primer	105



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
 Copyright[©] by Chiang Mai University
 All rights reserved

ABBREVIATIONS AND SYMBOLS

%	Percent
β -ME	β -mercaptoethanol
$^{\circ}$ C	Degree celcius
μ g	Microgram
μ l	Microliter
μ M	Micromolar
x g	Gravity
g	Gram (s)
AIDS	Acquired Immunodeficiency syndrome
AFRI	Antifungal resistance 1 gene
BLAST	Basic local alignment search tools
bp	Base pair
cDNA	Complementary deoxyribonucleic acid
CFU	Colony forming unit
cm	Centimeter
DEPC	Diethylpyrocarbonate
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
EDTA	Ethylenediamine tetraacetic acid
ERG11	Ergosterol 11 gene or lanosterol 14 α -demethylase gene
et al.	And others
h	Hour (s)
HIV	Human immunodeficiency virus
kDa	Kilodalton
Kb	Kilobase
M	Molar
MDR1	Multidrug resistance 1 gene

mg	Milligram (s)
min	Minute (s)
ml	Milliliter (s)
mm	Millimeter (s)
mM	Millimolar
MOPS	3-[N-morpholino] propanesulfonic acid
mRNA	Messenger ribonucleic acid
MW	Molecular weight
NCBI	National Center for Biological Information
NCCLS	National Center of Committee Laboratory standard
ng	Nanogram
ORF	Open Reading Frame
OD	Optical Density
PCR	Polymerase Chain Reaction
rpm	Revolution per minute
RT-PCR	Reverse Transcription- Polymerase Chain Reaction
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

â€¢ ข้อสันธิหน้าเรียนภาษาอังกฤษใหม่
 Copyright © by Chiang Mai University
 All rights reserved