

TABLE OF CONTENTS

	Page
Acknowledgements	iii
Abstract (English)	v
Abstract (Thai)	x
List of tables	xxiii
List of illustrations	xxvii
Abbreviations and symbols	xxxii
Chapter 1 General introduction	1
Chapter 2 Literature review	
2.1 Distribution and habitats of Zingiberaceae	6
2.2 Definition and classification of Zingiberaceae	7
2.3 Importance of Zingiberaceae	9
2.4 Biology and biodiversity of fungi	14
2.5 Fungi in Thailand	19
2.6 Fungi described from Zingiberaceae	22
2.7 Life strategies of fungi	32
2.8 Endophytic fungi	
2.8.1 Definition of endophytes	33

TABLE OF CONTENTS (CONTINUED)

	Page
2.8.2 Biological role of endophytes	34
2.8.3 Ecology of endophytes	42
2.9 Relationships among endophytic, saprobic and pathogenic fungi	44
2.10 Importance and biotechnological potential of fungi	45
2.11 Measurement of fungal biodiversity	49
2.12 Molecular characterization of fungi	53
2.13 Antimicrobial agents	57
 Chapter 3 Endophytic fungi from Zingiberaceae	
3.1 Introduction	63
3.2 Materials and methods	
3.2.1 Sample selection	64
3.2.2 Surface sterilization and isolation of endophytes	64
3.2.3 Identification	66
3.2.4 Statistical analyses	66
3.3 Results	
3.3.1 Isolate prevalence and intensity	67
3.3.2 Tissue specificity	
3.3.2.1 Leaf age analysis	71

TABLE OF CONTENTS (CONTINUED)

	Page
3.3.2.2 Pseudostem, rhizome and leaf (vein and intervein) analysis	71
3.3.3 Composition of endophytic assemblages	73
3.3.4 Effect of tissue type on the endophytic assemblages	82
3.3.5 Host species and site level patterns	83
3.3.6 Determination of adequacy of sampling size	83
3.4 Discussion	
3.4.1 Isolate prevalence and intensity	85
3.4.2 Tissue specificity	
3.4.2.1 Leaf age analysis	86
3.4.2.2 Pseudostem, rhizome and leaf (vein and intervein) analysis	87
3.4.3 Composition of endophytic assemblages	88
3.4.4 The role of endophytes in Zingiberaceae	90

Chapter 4 Saprobes on Zingiberaceae

4.1 Introduction	92
4.2 Materials and methods	
4.2.1 Sample collection	93
4.2.2 Examination of samples	94
4.2.3 Statistical analysis	95

TABLE OF CONTENTS (CONTINUED)

	Page
4.3 Results	
4.3.1 Determination of adequacy of sample size	96
4.3.2 Fungal taxonomic composition	98
4.3.3 Effect of site, type of sample (forest ground and standing plant) and tissue type (leaf and pseudostem) on fungal communities	117
4.3.4 Effect of host on fungal communities	120
4.3.5 Effect of season on fungal communities	122
4.4 Discussion	
4.4.1 Biodiversity and host specificity	123
4.4.2 Comparison of fungi on Zingiberaceae	125
4.4.3 Tissue specificity	126
4.4.4 Abundance of anamorphic fungi	128
4.4.5 Plant pathogens	129
Chapter 5 Pathogens, pathogenicity tests and antagonistic tests	
5.1 Introduction	130
5.2 Materials and methods	
5.2.1 Collection of zingiberaceous diseases	131
5.2.2 Isolation of zingiberaceous pathogens	131

TABLE OF CONTENTS (CONTINUED)

	Page
5.2.3 Pathogenicity tests	132
5.2.4 Antagonistic tests	133
5.3 Results	
5.3.1 Collection of zingiberaceous diseases	135
5.3.2 Pathogenicity tests	139
5.3.3 Antagonistic tests	142
5.4 Discussion	
5.4.1 Pathogens on Zingiberaceae	146
5.4.2 Can endophytes and saprobes of Zingiberaceae become pathogens?	147
5.4.3 Are zingiberaceous fungi antagonistic to other fungi?	148

Chapter 6 Molecular and morphological identification

A. Pyricularia

6.1A Introduction	151
6.2A Materials and methods	
6.2.1A Fungal isolates and morphology	153
6.2.2A Extraction of genomic DNA	155
6.2.3A PCR amplification and sequencing	156
6.2.4A DNA sequence alignment and phylogenetic analysis	157

TABLE OF CONTENTS (CONTINUED)

	Page
6.3A Results	
6.3.1A DNA extraction, sequencing and alignment	158
6.3.2A Molecular phylogeny	158
6.4A Discussion	
6.4.1A Molecular phylogeny and relationships of <i>Pyricularia</i> and related genera	164
6.4.2A Anamorph-teleomorph connections	167
<i>B. Myrothecium</i>	
6.1B Introduction	170
6.2B Materials and methods	
6.2.1B Fungal isolates and morphology	171
6.2.2B DNA extraction, PCR amplification and sequencing	172
6.2.3B DNA sequence alignment and phylogenetic analysis	172
6.3B Results	
6.3.1B Taxonomy	175
6.3.2B Molecular phylogeny	179
6.4B Discussion	181

TABLE OF CONTENTS (CONTINUED)

	Page
Chapter 7 Antimicrobial activity and enzyme production from zingiberaceous fungi	
7.1 Introduction	184
7.2 Materials and methods	
7.2.1 Preliminary screening of zingiberaceous fungi for enzyme production	186
7.2.2 Evaluation of antimicrobial production	
7.2.2.1 Test organisms and preparation of assay plates	187
7.2.2.2 Screening for antimicrobial production	188
7.2.2.3 Extraction and concentration	189
7.2.2.4 Bioassay	189
7.2.2.5 Taxonomy of the selected strain	190
7.2.2.6 Optimization of antimicrobial production	190
7.2.2.7 Characterization of bioactive compounds from <i>Chaetomium globosum</i> (CMUZE0132)	192
7.3 Results	
7.3.1 Screening of zingiberaceous fungi for enzyme production	196
7.3.2. Screening for antimicrobial production	199

7.3.3 Taxonomy of <i>Chaetomium globosum</i> (CMUZE0132)	200
--	-----

TABLE OF CONTENTS (CONTINUED)

	Page
7.3.4 Optimization of antimicrobial production	206
7.3.5 Characterization of bioactive compound from <i>Chaetomium globosum</i> (CMUZE0132)	210
7.4 Discussion	
7.4.1 Secondary metabolites from fungi	213
7.4.2 Potential of zingiberaceous fungi for enzyme production	214
7.4.3 Potential of zingiberaceous fungi for antimicrobial production	215
Chapter 8 General discussion and conclusion	
8.1 Diversity of endophytic and saprobic fungi in Zingiberaceae	220
8.2 The relationships among endophytic, saprobic and pathogenic fungi from Zingiberaceae	223
8.3 Potential of zingiberaceous fungi to produce bioactive compounds	224
8.4 Molecular and morphological characterization of fungi from Zingiberaceae	225

TABLE OF CONTENTS (CONTINUED)

	Page
References	227
Appendix A	297
Appendix B	302
Appendix C	304
Appendix D	310
Curriculum Vitae	311

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
 Copyright © by Chiang Mai University
 All rights reserved

LIST OF TABLES

Table	Page
2.1 Ethnomedicinal uses of Thai zingiberaceous plants (Chuakul, 2003).	11
2.2 Differences between the three domains (Campbell <i>et al.</i> , 2003).	15
2.3 Most significant morphological and biochemical features of the main groups of fungi (modified from Jennings and Lysek, 1996; Nicklin <i>et al.</i> , 1999).	16
2.4 Principal niches and microhabitats for fungi in a tropical forest (Hawksworth <i>et al.</i> , 1996).	18
2.5 Number of fungi described from the Zingiberaceae worldwide (Bussaban <i>et al.</i> , 2004).	23
2.6 Index of fungi described from the Zingiberaceae (Braun, 2001; Bussaban <i>et al.</i> , 2002, 2003a, b; Chen <i>et al.</i> , 2002).	24
2.7 Endophytic fungi reported for various hosts worldwide.	35
2.8 Classification of antibiotics by mechanisms of action.	58
3.1 Isolate prevalence, intensity, multiple infection and number of taxa of endophytes isolated from Zingiberaceae at each site in the wet or dry season.	69
3.2 A comparison of the total fungal taxa recovered from zingiberaceous species.	74

LIST OF TABLES (CONTINUED)

Table	Page
3.3 Relative frequencies (RF) and isolate prevalences (IP) of endophytes from <i>Alpinia malaccensis</i> occurring at isolate prevalences of >1 %.	76
3.4 Relative frequencies (RF) and isolate prevalences (IP) of endophytes from <i>Amomum siamense</i> occurring at isolate prevalences of >1 %.	77
3.5 Relative frequencies (RF) and isolate prevalences (IP) of endophytes from <i>Etltingera littoralis</i> occurring at isolate prevalences of >1 %.	78
3.6 Relative frequencies (RF) and isolate prevalences (IP) of endophytes from <i>Etltingera elatior</i> occurring at isolate prevalences of >1 %.	79
3.7 Relative frequencies (RF) and isolate prevalences (IP) of endophytes from <i>Alpinia galanga</i> occurring at isolate prevalences of >1 %.	80
3.8 Relative frequencies (RF) and isolate prevalences (IP) of endophytes from <i>Zingiber officinale</i> occurring at isolate prevalences of >1 %.	81
4.1 Collection details of saprobic fungi study.	93
4.2 A comparison of the total fungal taxa recovered from zingiberaceous species.	100
4.3 Overall percentage occurrence and frequency of fungal taxa recovered from <i>Amomum siamense</i> .	106
4.4 Overall percentage occurrence and frequency of fungal taxa recovered	110

from *Alpinia malaccensis*.

LIST OF TABLES (CONTINUED)

Table	Page
4.5 Overall percentage occurrence and frequency of fungal taxa recovered from <i>Etilingera littoralis</i> .	112
4.6 Overall percentage occurrence and frequency of fungal taxa recovered from <i>Etilingera elatior</i> .	114
4.7 Overall percentage occurrence and frequency of fungal taxa recovered from <i>Alpinia galanga</i> .	115
4.8 Overall percentage occurrence and frequency of fungal taxa recovered from <i>Zingiber officinale</i> .	116
4.9 Similarity of fungal taxa compositions between Zingiberaceae.	121
5.1 Diseases identified from wild and cultivated Zingiberaceae.	138
5.2 Results of pathogenicity testing of fungal taxa isolated as endophytes, saprobes or pathogens, and inoculated onto tissue cultured gingers (<i>Curcuma</i> sp.).	140
5.3 Percentage inhibition of radial growth of <i>Pyricularia costina</i> by endophytic and saprobic fungi after dual growth on PDA for 13 days.	143

- 6.1 The sources of *Pyricularia* isolates and allied genera used for ITS1-5.8S-ITS2 rDNA sequence analysis. 154

LIST OF TABLES (CONTINUED)

Table	Page
6.2 The PCR product size (bp) and GenBank sequence accession numbers of ITS1-5.8S-ITS2 of <i>Pyricularia</i> and allied fungi.	160
6.3 Isolates used in the phylogenetic analyses or morphological studies.	174
6.4 Comparison of morphological characters of <i>Myrothecium</i> isolated from monocotyledonous plants in Thailand.	178
7.1 Qualitative activities of cellulase, mannanase and protease produced by selected zingiberaceous fungi.	197
7.2 Qualitative antimicrobial activity of zingiberaceous fungi cultured in fermentation media F1 and F2.	201
7.3 The inhibition zone (> 7 mm diameter) against test organisms of zingiberaceous fungi cultured in fermentation media F1 and F2.	202
7.4 Inhibition zones of active fractions eluted from HP20 resin and silica gel columns.	212
7.5 R _f values on TLC chromatograms of the active compounds eluted from	212

HP20 resin and silica gel columns.



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright © by Chiang Mai University
All rights reserved

LIST OF ILLUSTRATIONS

Figure	Page
1.1 Wild and cultivated zingiberaceous plants selected for the present study.	3
1.2 Schematic presentation of the relationships between chapters of the thesis.	5
2.1 Distribution of Zingiberaceae.	6
2.2 General structure of the Zingiberaceae, exemplified by <i>Zingiber officinale</i> Rosc.	8
2.3 Tree diagram of order Zingiberales based on morphological and molecular analyses (Kress <i>et al.</i> , 2001).	9
2.4 Universal phylogenetic tree in rooted form, showing the three domains: Archaea, Bacteria, and Eukarya.	15
2.5 Major factors involved in biodiversity.	50
2.6 Principle of PCR amplification.	54
2.7 Disk diffusion test.	60
2.8 Broth dilution procedure.	60
3.1 Zingiberaceous samples: vein (a), intervein (b), pseudostem (c) and rhizome (d) on malt extract agar containing Rose Bengal and streptomycin sulfate, with fungal hyphae growing out from the tissues (e–h).	65
3.2 Boxplots comparing the overall isolate prevalences of endophytes recovered from wild and cultivated zingiberaceous plants.	68

LIST OF ILLUSTRATIONS (CONTINUED)

Figure	Page
3.3 Boxplots comparing the ranked medians of the numbers of isolates recovered from leaf, pseudostem and rhizomes, from wild and cultivated zingiberaceous plants.	72
3.4 Species area curves for endophytic fungi collected from Zingiberaceae.	84
4.1 Sampling leaves and pseudostems from fallen (left) and standing (right) dead plants.	94
4.2 Species area curves for saprobic fungi collected from Zingiberaceae.	97
4.3 Shannon indices (H') comparing the species diversity of zingiberaceous plants.	99
4.4 Three dimensional correspondence analyses for the fungal compositions of Zingiberaceae.	119
4.5 Three dimensional correspondence analyses for the fungal compositions of Zingiberaceae.	121
5.1 Surviving <i>Curcuma</i> plants transplanted to plastic pots after pathogenicity test.	133
5.2 Percentage inhibition in dual culture test.	134
5.3 Disease symptoms found on Zingiberaceae.	137
5.4 Disease symptoms on tissue cultured gingers (<i>Curcuma</i> sp.).	141

LIST OF ILLUSTRATIONS (CONTINUED)

Figure	Page
5.5 Reisolation of fungi from disease lesions on tissue cultured gingers (<i>Curcuma</i> sp.).	141
5.6 <i>Fusarium</i> sp. (CMUZE0396) (a) and <i>Papulaspora</i> sp. (CMUZE0116) (b) showing inhibition in growth of <i>Pyricularia costina</i> on PDA.	145
5.7 <i>Pyricularia costina</i> in dual culture plate inhibited by <i>Fusarium</i> sp. (CMUZE0396) showed distorted mycelium (arrowed).	145
6.1 Conidial secession: rhexolytic (involving the circumscissile splitting of the periclinal wall of the cell below the basal conidial septum rather than the septum itself) and schizolytic (involving a splitting of delimiting septum).	152
6.2 One of 90 equally most parsimonious trees inferred from a heuristic search of the ITS1-5.8S-ITS2 rDNA sequence alignment of 41 isolates of <i>Pyricularia</i> and related genera.	163
6.3 Diagrammatic representation of conidiophores, conidiogenous cells and conidia of <i>Myrothecium cinctum</i> isolated from Zingiberaceae (a), <i>Licuala longecalycata</i> (b), <i>Musa acuminata</i> (c) and <i>M. pandanicola</i> from	177

Pandanus penetrans (d).

LIST OF ILLUSTRATIONS (CONTINUED)

Figure	Page
6.4 One of six most parsimonious trees inferred from a heuristic search of the ITS1-5.8S-ITS2 rDNA sequence alignment of 34 isolates of <i>Myrothecium</i> and related genera.	180
7.1 Measuring of R _f values of bioactive compounds in bioautograph (left) and band (arrowed) appearing under UV light (right) on TLC plate. d. distance from origins of active compounds, D. distance of solvent.	195
7.2 <i>Chaetomium globosum</i> strain CMUZE0132: a, colony on PDA, b. ascomata with dense lateral and terminal hairs, c. spiral terminal hairs, d. lemon-shaped ascospores.	205
7.3 Phylogenetic tree (from branch and bound search) showing the relationships of endophytic <i>Chaetomium globosum</i> (CMUZE0132), related species of the same genus and other taxa based on ITS rDNA sequences.	206
7.4 Effect of C/N-sources on antimicrobial production by <i>Chaetomium globosum</i> (CMUZE0132) against <i>Penicillium avellaneum</i> .	207

- 7.5 The inhibition zones of antimicrobial agent produced by *Chaetomium globosum* (CMUZE0132) cultured in F1 medium containing different C/N-sources against *Penicillium avellaneum*. 208

LIST OF ILLUSTRATIONS (CONTINUED)

Figure	Page
7.6 Antimicrobial production by <i>Chaetomium globosum</i> (CMUZE0132) at different pH levels and temperatures, against <i>Penicillium avellaneum</i> .	209
7.7 Antimicrobial production by <i>Chaetomium globosum</i> (CMUZE0132) over time, against <i>Penicillium avellaneum</i> .	209
7.8 Zone of growth inhibition of <i>Candida albicans</i> and <i>Penicillium avellaneum</i> by ethyl acetate extract of <i>Chaetomium globosum</i> (CMUZE0132) in DMSO at different concentrations.	210

ABBREVIATIONS AND SYMBOLS

cm	centimeter
CMA	corn meal agar
g	gram
mg	milligram
min	minute
ml	milliliter
mm	millimeter
mM	millimolar
No.	number
PDA	potato dextrose agar
rpm	rotation per minute
sec	second
UV	ultraviolet
w/v	weight by volume
v/v	volume by volume
°C	degree Celsius
α	alpha
β	beta
μg	microgram
μl	microliter
μm	micrometer