UNIVERSITI PUTRA MALAYSIA

COMPARATIVE STUDIES ON GANODERMA (KARST.) FROM INFECTED OIL PALM AND COCONUT STUMPS WITH SPECIAL REFERENCE TO THEIR MORPHOLOGICAL, MOLECULAR AND ISOZYME CHARACTERISTICS

LATIFFAH ZAKARIA

IB 2002 1

COMPARATIVE STUDIES ON *GANODERMA* (KARST.) FROM INFECTED OIL PALM AND COCONUT STUMPS WITH SPECIAL REFERENCE TO THEIR MORPHOLOGICAL, MOLECULAR AND ISOZYME CHARACTERISTICS

By

LATIFFAH ZAKARIA

Thesis Submitted in Fulfilment of the Requirement for the Degree of
Doctor of Philosophy in the Institute of Bioscience
Universiti Putra Malaysia

January 2002

Abstract of the thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Doctor of Philosophy

A COMPARATIVE STUDY ON *GANODERMA* (KARST.) FROM INFECTED OIL PALM AND COCONUT STUMPS WITH SPECIAL REFERENCE TO THEIR MORPHOLOGICAL, MOLECULAR AND ISOZYME CHARACTERISTICS

By

LATIFFAH BT. ZAKARIA

January 2002

Chairman: Prof. Dr. Ho Yin Wan

Faculty: Institute of Bioscience

The basal stem rot of oil palm caused by *Ganoderma* is the most serious disease infecting oil palm in South-east Asia. It is believed that coconut stumps which are colonized by *Ganoderma* can act as sources of inoculum for infection to healthy palms through root contact. However, it is not known whether the *Ganoderma* infecting oil palm and those colonizing coconut stumps are the same species. Therefore, a comparative study was conducted to determine the similarities and differences between *Ganoderma* isolates from infected oil palm and coconut stumps, using a multidisciplinary approaches in which morphological, biochemical (intracellular and extracellular enzyme systems) and molecular characteristics (RAPD, RAMS, RFLP and direct sequencing of the ITS regions + 5.8S gene of rDNA) were analysed.

Based on the morphological characteristics of the basidiomata, *Ganoderma* from infected oil palm and coconut stumps conformed to the description of *G. boninense* in Steyaert's classification system for *Ganoderma* (1967 and 1975). The growth on various media and at different temperatures, and the cultural characteristics of the isolates from infected oil palm and coconut stumps were similar with no significant difference observed between the two groups of *Ganoderma*. However, the isolates were somatically incompatible with one another, which indicated that they were genotypically distinct individuals and not clones of a genotypic individual.

The isozyme profiles from intracellular and extracellular enzyme systems, and the DNA profiles from RAPD, RAMS and RFLP of the ITS regions + 5.8S gene revealed that *Ganoderma* isolates from infected oil palm and coconut stumps were very variable. Nucleotide sequences of the ITS regions + 5.8S gene of rDNA from a limited number of isolates also showed that the isolates from both groups of *Ganoderma* were very variable. The Southern hybridization of RAMS gel showed that labelled probes from oil palm and coconut hybridized to the common bands of 1.2 kb by from primer $(CGA)_5$ and 1.4 kb band by primer $(ACA)_5$ which indicated that the bands of the same molecular sizes are likely to be homologous.

Cluster analysis based on data from biochemical and molecular characters, and phylogenetic analysis of the nucleotide sequence showed that the oil palm isolates and the coconut isolates did not cluster separately which indicated that isolates of both groups of *Ganoderma* are closely related.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

KAJIAN PERBANDINGAN *GANODERMA* (KARST.) DARIPADA KELAPA SAWIT DAN TUNGGUL KELAPA MENGGUNAKAN CIRI-CIRI MORFOLOGI, MOLEKUL DAN ISOZIM

Oleh

LATIFFAH ZAKARIA

Januari 2002

Pengerusi: Profesor Dr. Ho Yin Wan

Fakulti : Institut Biosains

Penyakit reput pangkal batang yang disebabkan oleh kulat *Ganoderma* merupakan penyakit yang paling serius menjangkiti pokok-pokok kelapa sawit di Asia Tenggara. Penyakit ini dipercayai berjangkit daripada tunggul kelapa di mana ianya sebagai sumber inokulum, boleh menjangkiti pokok-pokok kelapa sawit yang sihat melalui persentuhan akar. Walaubagaimanapun, tidak diketahui samada *Ganoderma* yang menjangkiti kelapa sawit dan yang terdapat pada tunggul kelapa terdiri daripada spesis yang sama. Oleh itu, satu kajian perbandingan telah dijalankan untuk mengenalpasti persamaan dan perbezaan di antara isolat-isolat *Ganoderma* daripada kelapa sawit dan tunggul kelapa menggunakan pelbagai kaedah seperti ciri-ciri morfologi basidiomata, biokimia (menggunakan sistem enzim intrasel dan ekstrasel) dan molekul (menggunakan analisis RAPD, RAMS, RFLP dan jujukan 'ITS region + gen 5.8S').

Berdasarkan ciri-ciri morfologi basidiomata, didapati ciri-ciri *Ganoderma* daripada kelapa sawit dan tunggul kelapa menyerupai ciri-ciri *G. boninense* yang terdapat pada sistem klasifikasi *Ganoderma* oleh Steyaert (1967 dan 1975). Pertumbuhan miselium pada pelbagai media dan suhu, serta ciri-ciri kultur pertumbuhan isolat-isolat kelapa sawit dan kelapa menunjukkan persamaan dimana tiada perbezaan yang bererti di antara kedua-dua kumpulan *Ganoderma* tersebut.

Profil isozim daripada sistem enzim intrasel dan ekstrasel dan profil DNA daripada analisis RAPD, RAMS, RFLP dan jujukan 'ITS regions + gen 5.8S', menunjukkan terdapatnya variasi pada isolat-isolat kelapa sawit dan kelapa. Variasi juga dapat dilihat pada jujukan 'ITS regions + gen 5.8S' pada beberapa isolat kelapa sawit dan kelapa. 'Southern Hybridization' dari gel analisis RAMS menunjukkan probe daripada kelapa sawit dan kelapa menghibridasi kepada jalur-jalur yang mempunyai size molekul yang sama iaitu jalur 1.2 kb daripada primer $(CGA)_5$ dan jalur 1.4 kb daripada primer $(ACA)_5$, menunjukkan jalur yang mempunyai size molekul yang sama adalah homologus .

Analisis cluster daripada kaedah pencirian biokimia dan molekular, serta analisa filogenetik jujukan DNA menunjukkan isolat-isolat kelapa sawit dan kelapa tidak berkelompok secara berasingan. Oleh itu, daripada analisis cluster isolat-isolat *Ganoderma* daripada kelapa sawit dan kelapa mempunyai hubungan yang rapat.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks and gratitude to the chairman of the supervisory committee, Prof. Dr. Ho Yin Wan, for her invaluable advice, guidance, constant encouragement and endless support throughout the duration of the study, and for her critical comments and constructive suggestions during the preparation of my thesis.

I also wish to express my appreciation and sincere gratitude to the supervisory committee members, Prof. Dr Tan Soon Guan, Dr. Faridah Abdullah and Prof. Madya Dr. Harikrishna Kulaveerasingam for their advice, guidance and helpful suggestions throughout the course of my study and in the preparation of my thesis.

I am also grateful to Encik Khairudin Hashim and En. Suzaini from Golden Hope Plantations Berhad for their kind assistance in collecting *Ganoderma* fruiting bodies in the field and for providing some of the stock cultures.

My heartfelt appreciations are extended to my colleagues and staff at the Institute of Bioscience, particularly, Lee Weng Wah, Dr. Faridah Qamarruzaman and Dr. Tan Siang Hee from Genome Centre for their advice and assistance in analyzing the sequence data; and Khairul Kamar Bakri, Madam Haw Ah Kam and Jivanathan Arumugam from the Digestive Microbiology Centre for their technical assistance and co-operation.

To my post-graduate friends at the Digestive Microbiology Centre, Kala, Vicky, Pak Darlis, Chin Chin, Wan, Lee, Thongsuk, and Sidieg, thank you for your friendship, support and laughter which contributed to the successful accomplishment of this work

Finally, very special thanks are due to my family, Abah, Cik, Acun, Kema, for their constant support, encouragements, understanding and sacrifices which inspired and motivated me during the duration of my study.

TABLE OF CONTENTS

		Page
ABSTRACT		II
ABSTRAK		IV
ACKNOWLEDGEMENTS		VI
APPROVAL SHEETS		VIII
DECLARATION FORM		X
TABLE OF CONTENTS		XI
LIST OF TABLES		XV
LIST OF FIGURES		XIX
LIST OF ABBREVIATIONS		XXXIII
CHAPTER		
1	INTRODUCTION	1
2	LITERATURE REVIEW	6
	2.1. Taxonomy of *Ganoderma*	6
	2.1.1. Morphological Characteristics	8
	2.1.2. Isozyme Profiles	9
	2.1.3. Molecular Characteristics	13
	2.1.4. Other Additional Characteristics	15
	2.2. Pathogenicity of *Ganoderma* Species that Cause the Basal Stem Rot of Oil Palm	16
	2.2.1. The Pathogen	16
	2.2.2. Pathogenicity Tests	18
	2.2.3. Inoculum Size and Age	20
	2.3. Occurrence of Basal Stem Rot of Oil Palm	21
	2.4. Disease Symptoms and Histopathology	22
	2.5. Control of the Basal Stem Rot	25
	2.5.1. Cultural Practice	25
	2.5.2. Fungicide Treatment	27
	2.5.3. Surgery	28
	2.5.4. Breeding for Resistance	28
	2.6. *Ganoderma* on Coconut	28
	2.7. Molecular-based Characterisation Techniques Used in Fungal Identification and Taxonomy	30
	2.7.1. Random Amplified Polymorphic DNA (RAPD)	31
	2.7.2. Random Amplified Microsatellite DNA (RAMS)	33
	2.7.3. Restriction Fragment Length Polymorphism (RFLP)	35
	2.7.4. Analysis of ITS Regions of Ribosomal DNA (rDNA)	36
3	GENERAL METHODOLOGY	39
	3.1. Collection of *Ganoderma* Basidiomata	39
	3.2. *Ganoderma* Isolates	40
	3.3. Preparation of Cultures for Molecular Analysis	45
	3.4. Extraction of Genomic DNA	45
	3.4.1. DNA-zol – Genomic DNA Isolation Reagent	45

		Page
	3.4.2. Phenol-Chloroform Extraction Method.....	46
	3.5. DNA Quantification.....	47
	3.6. Similarity Matrix and Cluster Analysis of Isozyme, RAPD and RAMS.....	48
4	BASIDIOMA MORPHOLOGY, MYCELIAL GROWTH CHARACTERISTICS AND SOMATIC INCOMPATIBILITY OF *GANODERMA* ISOLATES FROM INFECTED OIL PALM AND COCONUT STUMPS.....	50
	4.1. Introduction.....	50
	4.2. Materials and Methods.....	51
	4.2.1. Morphological Studies.....	51
	4.2.2. Growth on Different Cultural Media.....	52
	4.2.3. Growth on Different Temperatures.....	54
	4.2.4. Somatic Incompatibilty Study.....	55
	4.2.5. Data Analysis.....	55
	4.3. Results.....	56
	4.3.1. Morphological Characteristics of Basidiomata.....	56
	4.3.2. Growth on Various Media.....	66
	4.3.3. Growth on Various Temperatures.....	73
	4.3.4. Somatic Incompatibility Study.....	76
	4.4. Discussion.....	81
5	ISOZYME ANALYSIS OF *GANODERMA* ISOLATES FROM INFECTED OIL PALM AND COCONUT STUMPS USING POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE) AND ISOELECTRIC FOCUSING (IEF).....	91
	5.1. Introduction.....	91
	5.2. Materials and Methods.....	92
	5.2.1. Intracellular Isozymes.....	92
	5.2.2. Extracellular Isozymes.....	99
	5.2.3. Cluster Analysis of Isozyme Data Obtained from PAGE...	102
	5.3. Results.....	103
	5.3.1. Intracellular Isozymes using PAGE.....	103
	5.3.2. Intracellular Enzymes using IEF.....	148
	5.3.3. Extracellular Isozymes.....	161
	5.4. Discussion.....	174
6	RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD), LONG PRIMER POLYMORPHIC DNA (LP-RAPD) AND RANDOM AMPLIFIED MICROSATELLITE DNA (RAMS) ANALYSES OF *GANODERMA* ISOLATES FROM INFECTED OIL PALM AND COCONUT STUMPS.....	185
	6.1. Introduction.....	185
	6.2. Materials and Methods.....	185

		Page
	6.2.1. Random Amplified Polymorphic DNA (RAPD).....	187
	6.2.2. Long Primer Random Amplified Polymorphic DNA (LP - RAPD).....	190
	6.2.3. Random Amplified Polymorphic Microsatellite DNA (RAMS).....	192
	6.2.4. Analysis of Data.....	193
	6.3. Results.....	193
	6.3.1. Random Amplified Polymorphic DNA.....	193
	6.3.2. Long Primer Random Amplified Polymorphic DNA....	214
	6.3.3. Random Amplified Polymorphic Microsatellite DNA....	215
	6.4. Discussion.....	232
7	ANALYSIS OF COMMON BANDS FROM RANDOM AMPLIFIED POLYMORPHIC DNA (RAMS) ANALYSIS USING SOUTHERN HYBRIDIZATION.....	245
	7.1. Introduction.....	245
	7.2. Materials and Methods.....	245
	7.2.1. Southern Blotting.....	246
	7.2.2. Southern Hybridization of RAMS gel.....	248
	7.2.3. Southern Hybridization of Genomic DNA.....	257
	7.3. Results.....	264
	7.3.1. Southern Hybridization of RAMS gel.....	264
	7.3.2. Southern Hybridization of Genomic DNA.....	282
	7.4. Discussion.....	282
8	RESTRICTION ANALYSIS OF PCR-AMPLIFIED (RFLP/PCR) INTERNAL TRANSCRIBED SPACER REGIONS OF RIBOSOMAL DNA (rDNA) AND DIRECT SEQUENCING OF THE ITS REGIONS.....	289
	8.1. Introduction.....	289
	8.2. Materials and Methods.....	290
	8.2.1. Isolates used and DNA Extraction Methods.....	290
	8.2.2. Polymerase Chain Reaction Amplification of the ITS Regions+5.8S gene of rDNA.....	290
	8.2.3. Restriction Analysis of ITS Regions+5.8S gene.....	291
	8.2.4. Direct Sequencing of Amplified ITS Regions+5.8S gene...	293
	8.2.5. Sequence Analysis.....	297
	8.3. Results.....	299
	8.3.1. Size of PCR-Amplified ITS Regions+5.8S gene.....	299
	8.3.2. Restriction Analysis of PCR-Amplified ITS Regions+5.8S..	301
	8.3.3. Data Analysis of RFLP/PCR Restriction Patterns of ITS Regions + 5.8S gene.....	319
	8.3.4. Sequence Analysis.....	324
	8.4. Discussion.....	332
9	GENERAL DISCUSSION AND CONCLUSIONS.....	341
	9.1. General Discussion	341

	Page
9.2. Conclusions	351
BIBLIOGRAPHY……	352
APPENDICES……..	372
VITA……	401

TABLE OF CONTENTS

		Page
ABSTRACT		II
ABSTRAK		IV
ACKNOWLEDGEMENTS		VI
APPROVAL SHEETS		VIII
DECLARATION FORM		X
TABLE OF CONTENTS		XI
LIST OF TABLES		XV
LIST OF FIGURES		XIX
LIST OF ABBREVIATIONS		XXXIII
CHAPTER		
1	INTRODUCTION	1
2	LITERATURE REVIEW	6
	2.1. Taxonomy of *Ganoderma*	6
	2.1.1. Morphological Characteristics	8
	2.1.2. Isozyme Profiles	9
	2.1.3. Molecular Characteristics	13
	2.1.4. Other Additional Characteristics	15
	2.2. Pathogenicity of *Ganoderma* Species that Cause the Basal Stem Rot of Oil Palm	16
	2.2.1. The Pathogen	16
	2.2.2. Pathogenicity Tests	18
	2.2.3. Inoculum Size and Age	20
	2.3. Occurrence of Basal Stem Rot of Oil Palm	21
	2.4. Disease Symptoms and Histopathology	22
	2.5. Control of the Basal Stem Rot	25
	2.5.1. Cultural Practice	25
	2.5.2. Fungicide Treatment	27
	2.5.3. Surgery	28
	2.5.4. Breeding for Resistance	28
	2.6. *Ganoderma* on Coconut	28
	2.7. Molecular-based Characterisation Techniques Used in Fungal Identification and Taxonomy	30
	2.7.1. Random Amplified Polymorphic DNA (RAPD)	31
	2.7.2. Random Amplified Microsatellite DNA (RAMS)	33
	2.7.3. Restriction Fragment Length Polymorphism (RFLP)	35
	2.7.4. Analysis of ITS Regions of Ribosomal DNA (rDNA)	36
3	GENERAL METHODOLOGY	39
	3.1. Collection of *Ganoderma* Basidiomata	39
	3.2. *Ganoderma* Isolates	40
	3.3. Preparation of Cultures for Molecular Analysis	45
	3.4. Extraction of Genomic DNA	45
	3.4.1. DNA-zol – Genomic DNA Isolation Reagent	45

		Page
	3.4.2. Phenol-Chloroform Extraction Method.....	46
	3.5. DNA Quantification.....	47
	3.6. Similarity Matrix and Cluster Analysis of Isozyme, RAPD and RAMS.....	48
4	BASIDIOMA MORPHOLOGY, MYCELIAL GROWTH CHARACTERISTICS AND SOMATIC INCOMPATIBILITY OF *GANODERMA* ISOLATES FROM INFECTED OIL PALM AND COCONUT STUMPS.....	50
	4.1. Introduction.....	50
	4.2. Materials and Methods.....	51
	4.2.1. Morphological Studies.....	51
	4.2.2. Growth o n Different Cultural Media.....	52
	4.2.3. Growth on Different Temperatures.....	54
	4.2.4. Somatic Incompatibilty Study.....	55
	4.2.5. Data Analysis.....	55
	4.3. Results.....	56
	4.3.1. Morphological Characteristics of Basidiomata.....	56
	4.3.2. Growth on Various Media.....	66
	4.3.3. Growth on Various Temperatures.....	73
	4.3.4. Somatic Incompatibility Study.....	76
	4.4. Discussion.....	81
5	ISOZYME ANALYSIS OF *GANODERMA* ISOLATES FROM INFECTED OIL PALM AND COCONUT STUMPS USING POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE) AND ISOELECTRIC FOCUSING (IEF).....	91
	5.1. Introduction.....	91
	5.2. Materials and Methods.....	92
	5.2.1. Intracellular Isozymes.....	92
	5.2.2. Extracellular Isozymes.....	99
	5.2.3. Cluster Analysis of Isozyme Data Obtained from PAGE...	102
	5.3. Results.....	103
	5.3.1. Intracellular Isozymes using PAGE.....	103
	5.3.2. Intracellular Enzymes using IEF.....	148
	5.3.3. Extracellular Isozymes.....	161
	5.4. Discussion.....	174
6	RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD), LONG PRIMER POLYMORPHIC DNA (LP-RAPD) AND RANDOM AMPLIFIED MICROSATELLITE DNA (RAMS) ANALYSES OF *GANODERMA* ISOLATES FROM INFECTED OIL PALM AND COCONUT STUMPS.....	185
	6.1. Introduction.....	185
	6.2. Materials and Methods.....	185

		Page
	6.2.1. Random Amplified Polymorphic DNA (RAPD).....	187
	6.2.2. Long Primer Random Amplified Polymorphic DNA (LP - RAPD).....	190
	6.2.3. Random Amplified Polymorphic Microsatellite DNA (RAMS).....	192
	6.2.4. Analysis of Data.....	193
	6.3. Results.....	193
	6.3.1. Random Amplified Polymorphic DNA.....	193
	6.3.2. Long Primer Random Amplified Polymorphic DNA....	214
	6.3.3. Random Amplified Polymorphic Microsatellite DNA....	215
	6.4. Discussion.....	232
7	ANALYSIS OF COMMON BANDS FROM RANDOM AMPLIFIED POLYMORPHIC DNA (RAMS) ANALYSIS USING SOUTHERN HYBRIDIZATION.....	245
	7.1. Introduction.....	245
	7.2. Materials and Methods.....	245
	7.2.1. Southern Blotting.....	246
	7.2.2. Southern Hybridization of RAMS gel.....	248
	7.2.3. Southern Hybridization of Genomic DNA.....	257
	7.3. Results.....	264
	7.3.1. Southern Hybridization of RAMS gel.....	264
	7.3.2. Southern Hybridization of Genomic DNA.....	282
	7.4. Discussion.....	282
8	RESTRICTION ANALYSIS OF PCR-AMPLIFIED (RFLP/PCR) INTERNAL TRANSCRIBED SPACER REGIONS OF RIBOSOMAL DNA (rDNA) AND DIRECT SEQUENCING OF THE ITS REGIONS.....	289
	8.1. Introduction.....	289
	8.2. Materials and Methods.....	290
	8.2.1. Isolates used and DNA Extraction Methods.....	290
	8.2.2. Polymerase Chain Reaction Amplification of the ITS Regions+5.8S gene of rDNA.....	290
	8.2.3. Restriction Analysis of ITS Regions+5.8S gene.....	291
	8.2.4. Direct Sequencing of Amplified ITS Regions+5.8S gene...	293
	8.2.5. Sequence Analysis.....	297
	8.3. Results.....	299
	8.3.1. Size of PCR-Amplified ITS Regions+5.8S gene.....	299
	8.3.2. Restriction Analysis of PCR-Amplified ITS Regions+5.8S..	301
	8.3.3. Data Analysis of RFLP/PCR Restriction Patterns of ITS Regions + 5.8S gene.....	319
	8.3.4. Sequence Analysis.....	324
	8.4. Discussion.....	332
9	GENERAL DISCUSSION AND CONCLUSIONS.....	341
	9.1. General Discussion	341

	Page
9.2. Conclusions	351
BIBLIOGRAPHY.....	352
APPENDICES.....	372
VITA.....	401

LIST OF FIGURES

Figure		Page
1	An inoculated oil palm seedling showing basidiomata of *G. boninense* at the stem base (from Ho and Khairudin, 1995).....	19
2	*Ganoderma* basidiomata on infected oil palm trunks.....	40
3	Map of Malaysia showing the locations of the oil palm estates and three coconut areas in Banting where *Ganoderma* basidiomata were collected.....	42
4	Very young *Ganoderma* basidiomata (white buttons) at the oil palm stem base.....	59
5	Young basidioma (Y) with thick, white growing margin and old basidioma (O) attached at the base of an infected oil palm stem.....	59
6	Base of an infected oil palm stem with attached *Ganoderma* basidiomata covered with a layer of brown spore deposit on their upper surface.....	61
7	A sessile fabellate (fan-shaped) basidioma (from infected oil palm) with laccate reddish brown, corrugated dorsal surface and yellow margin.....	61
8	A slightly imbricate basidioma (from infected oil palm) with a yellowish brown, slightly laccate dorsal surface and a yellow margin.....	62
9	A stipitate basidioma (from infected oil palm) with a laccate, corrugated, dark brown dorsal surface and brown margin.....	62
10	Variations in colour, shape and size of *Ganoderma* basidiomata from oil palm. Note that some basidiomata are stipitate, while others are sessile.	63
11	Morphological variations of *Ganoderma* basidiomata with laccate, reddish brown dorsal surface. I – 4 : basidioma from infected oil palm. 5 : basidioma from coconut stumps.....	64
12	A dissected basidioma (from infected oil palm) showing the context layer (C), pore tube layer (PT) and margin (M).....	65

		Page
13	A sessile basidioma (from coconut stump) with a slightly laccate, yellowish-brown dorsal surface and a brown margin. Arrow shows the attachment to the palm tissue, not a stipe…….……	65
14	A sessile basidioma (from coconut stump) with slightly laccate, brown dorsal surface and a yellow margin …….....	66
15	Cultures of *Ganoderma* isolate (1.EG01) and coconut (2.CNDDE2) on MA after 7 days of incubation……..……..	70
16	Cultures of *Ganoderma* isolate from oil palm isolate (EG01) and coconut (CNDDE2) on MA after about 12 days showing the formation of brown, buckled crust on the under surface of the medium……...	71
17	Mycelial growth of *Ganoderma* isolates from oil palm (EG) and coconut (CN) at various temperatures……..	74
18	Self-pair *Ganoderma* isolates between EGJS1 and EGJS1, of oil palm. The mycelia of the two colonies will grow together and eventually form a single colony…….	78
19	Self-pair *Ganoderma* isolates, CNBt64 and CNBt64, of coconut. The mycelia of the two colonies will eventually form a single colony……	78
20	Strong interaction between *Ganoderma* isolates, EGL5 and EGPK5, of oil palm showing a clear zone between the two interacting isolates……..	79
21	Strong interaction between *Ganoderma* isolates, OP65 and CNKK4, of oil palm and coconut showing a thin line of mycelium and sparse zone separating the two interacting isolates…….	79
22	Strong interaction between *Ganoderma* isolates, OP114 and EGJS1, of oil palm showing a thin dense, raised line of mycelium between the two interacting isolates……...	80
23	Strong interaction between *Ganoderma* isolates, CNUB01 and CNBt64 of coconut showing a clear zone and a thick, dense line of mycelium between the two interacting isolates…….	80
24	ADH electrophoretic phenotype of *Ganoderma* isolates from infected oil palm and coconut stumps……..	104

		Page
25	ADH banding patterns of *Ganoderma* isolates from oil palm and coconut isolates showing electrophoretic phenotype of ADH1…….	104
26	PER electrophoretic phenotype of *Ganoderma* isolates from infected oil palm and coconut stumps……..	105
27	PER banding patterns of *Ganoderma* isolates from oil palm and coconut showing electrophoretic phenotype PER1……...	105
28	GOT electrophoretic phenotype of *Ganoderma* isolates from infected oil palm and coconut stumps……..	106
29	GOT banding patterns of *Ganoderma* isolates from oil palm and coconut (in duplicates)……..	106
30	ACO electrophoretic phenotypes of *Ganoderma* from infected oil palm and coconut stumps……..	107
31	ACO banding patterns of *Ganoderma* isolates from oil palm and coconut showing electrophoretic phenotypes of ACO1 and ACO2..	108
32	HK electrophoretic phenotypes of *Ganoderma* isolates from infected oil palm and coconut stumps……..	109
33	HK banding patterns of oil palm isolates and coconut isolates showing electrophoretic phenotypes of HK1 and HK2……..	110
34	MDH electrophoretic phenotypes of *Ganoderma* from infected oil palm and coconut stumps……...	111
35	MDH banding patterns of oil palm and coconut isolates showing electrophoretic phenotype of MDH1…….	112
36	ME electrophoretic phenotypes of *Ganoderma* from infected oil palm and coconut stumps……..	113
37	ME banding patterns of oil palm isolates and coconut isolates showing electrophoretic phenotype of ME2…….	114
38	HBDH electrophoretic phenotypes of *Ganoderma* isolates from infected oil palm and coconut stumps……...	115

		Page
39	HBDH banding patterns of oil palm isolates and coconut isolates (in duplicates) showing electrophoretic phenotypes of HBDH1, HBDH2 and HBDH3.....	116
40	SKDH electrophoretic phenotypes of *Ganoderma* from infected oil palm and coconut stumps.....	117
41	SKDH banding patterns of oil palm isolates and coconut isolates showing electrophoretic phenotype of SKDH2.....	118
42	ACP electrophoretic phenotypes of *Ganoderma* from infected oil palm and coconut stumps.....	119
43	ACP banding patterns oil palm and coconut isolates showing electrophoretic phenotypes of ACP1 and ACP2.....	120
44	AKP electrophoretic phenotypes of *Ganoderma* isolates from infected oil palm and coconut stumps.....	121
45	AKP banding patterns of oil palm isolates and coconut isolates showing electrophoretic phenotypes of AKP1 and AKP2.....	122
46	PGM electrophoretic phenotypes of *Ganoderma* isolates from infected oil palm and coconut stumps.....	123
47	PGM banding patterns of oil palm isolates and coconut isolates (in duplicates) showing electrophoretic phenotypes of PGM1, PGM3, PGM5, PGM6 and PGM8.....	125
48	PGM banding patterns of oil palm isolates and coconut isolates (in duplicates) showing electrophoretic phenotypes of PGM1, PGM3, PGM5, PGM6 and PGM8.....	125
49	IDH electrophoretic phenotypes of *Ganoderma* isolates from infected oil palm and coconut stumps.....	126
50	IDH banding patterns of oil palm isolates and coconut isolates (in duplicates) showing electrophoretic phenotypes of IDH2, IDH3, IDH4 and IDH5.....	127
51	PEP D electrophoretic pheotypes of *Ganoderma* from infected oil palm and coconut stumps.....	129

		Page
52	PEP D banding patterns of oil palm isolates and coconut isolates showing electrophoretic phenotypes of PEP D1,PEP D2 and PEP D8.....	131
53	PEP D banding patterns of oil palm isolates and coconut isolates showing electrophoretic phenotypes of PEP D1, PEP D2, PEP D3, PEP D5 and PEP D6.....	131
54	LAP electrophoretic phenotypes of *Ganoderma* isolates from infected oil paim and coconut stumps.....	133
55	LAP banding patterns of oil palm isolates and coconut isolates showing electrophoretic phenotypes of LAP2, LAP4, LAP5, LAP6, LAP8, LAP9, LAP10, LAP12.	135
56	ES electrophoretic phenotypes of *Ganoderma* isolates from infected oil palm and coconut stumps (ES1 – ES14).....	136
57	ES electrophoretic phenotypes of *Ganoderma* isolates from infected oil palm and coconut stumps (ES15 – ES28).....	137
58	ES banding patterns of oil palm isolates and coconut isolates (in duplicates) showing electrophoretic phenotypes of ES6, ES8, ES15, ES17, ES18, ES20 and ES26.....	139
59	ES banding patterns of oil palm isolates (in duplicates) showing electrophoretic phenotypes of ES5, ES9, ES12, ES13, ES14, ES19 and ES22.....	139
60	Dendrogram from UPGMA analysis using Simple Matching Coefficient based on isozyme bands of *Ganoderma* isolates from infected oil palm and coconut stumps.....	143
61	Principal Component Analysis of data from 16 isozyme for *Ganoderma* isolates from infected oil palm and coconut stumps.....	147
62	ADH zymogram of *Ganoderma* isolates at a pH range of 3.5 – 9.5..	149
63	ADH zymogram of *Ganoderma* isolates from infected oil palm at a pH range of 3.5 – 9.5..... .	150
64	ADH zymogram of *Ganoderma* isolates at a pH range of 5.5 – 8.5.....	150