UNIVERSITI PUTRA MALAYSIA

MORPHOLOGICAL STUDY AND LIGNIN DEGRADATION OF PATHOGENIC GANODERMA SPECIES OF BASAL STEM ROT IN OIL PALM

WONG LING CHIE

FPSM 2013 12

WONG LING CHIE

MASTER OF SCIENCE

2013

MORPHOLOGICAL STUDY AND LIGNIN DEGRADATION OF PATHOGENIC *GANODERMA* SPECIES OF BASAL STEM ROT IN OIL PALM

WONG LING CHIE

MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

2013

© COPYRIGHT UPM

MORPHOLOGICAL STUDY AND LIGNIN DEGRADATION OF PATHOGENIC GANODERMA SPECIES OF BASAL STEM ROT IN OIL PALM

By

WONG LING CHIE

Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in Fulfilment of the
Requirements for the Degree of
Master of Science

October 2013

© COPYRIGHT UPM

All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia

© COPYRIGHT UPM

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

MORPHOLOGICAL STUDY AND LIGNIN DEGRADATION OF PATHOGENIC GANODERMA SPECIES OF BASAL STEM ROT IN OIL PALM

By

WONG LING CHIE

October 2013

Chairman: Joseph Bong Choon Fah
Faculty: Agriculture and Food Sciences, Bintulu

Basal stem rot (BSR) is one of the most serious diseases causing major losses in the oil palm industry in Southeast Asia, especially in Malaysia and Indonesia. Several species of *Ganoderma* have been reported to be pathogenic to oil palm, however, the diversity and differentiation of the *Ganoderma* species were not widely studied and the identity of these species are still unclear leading to inaccurate and inefficient decision-making in disease management. In this study, several isolates of *Ganoderma* were collected in Sarawak, Malaysia and Multiplex Polymerase Chain Reaction was carried out to differentiate the isolates into species level. This was followed by macromorphological, micromorphological and compatibility studies of the *Ganoderma* isolates. Lignin degradation study on the isolates was also carried out. Multiplex PCR-DNA analysis demonstrated positive results for *G. boninense, G. zonatum* and *G. miniatocinctum,* indicating that these three *Ganoderma* species were associated with the basal stem rot disease in Sarawak. Compatibility study had shown that isolates from different palms were not compatible. Morphological studies of the *Ganoderma* isolates showed that hyphae characteristics and spore length can be used to distinguish among the *Ganoderma* species. Therefore an identification key was developed as a guideline on species differentiation of the pathogenic *Ganoderma* species. Isolates of *G. zonatum* G46 and *G. miniatocinctum* G30, demonstrated similar lignin degrading ability as *G. boninense* G25 and G47 whereby *G. zonatum* G46 was capable of degrading the most amount of lignin after a week of infection. From week 1 to week 5, *G. zonatum* G46 was able to degrade more lignin compared to *G. boninense* G25 and G47, indicating that *G. zonatum* could cause more damage to oil palms for long term. Therefore, more researches should emphasize on *G. zonatum* instead of focusing on *G. boninense* alone in order to formulate suitable solutions to control or eliminate BSR caused by the different *Ganoderma* species.

© COPYRIGHT UPM

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

KAJIAN MOFOLOGI DAN DEGRADASI LIGNIN OLEH SPESIES *GANODERMA* YANG PATOGENIK DAN MEMBAWA PENYAKIT REPUT PANGKAL PADA KELAPA SAWIT

Oleh

WONG LING CHIE

October 2013

Pengerusi: Joseph Bong Choon Fah
Fakulti: Sains Pertanian dan Makanan, Bintulu

Penyakit reput pangkal merupakan salah satu penyakit paling serius yang membawa kerugian besar kepada industri kelapa sawit di Asia Tenggara, terutamanya Malaysia dan Indonesia. Beberapa spesies *Ganoderma* telah dilaporkan patogenik terhadap kelapa sawit, walau bagaimanapun, kepelbagaian dan pembezaan spesies *Ganoderma* tidak dikaji dengan mendalam dan identiti spesies masih tidak jelas menyebabkan ketidaktepatan dan ketidakcekapan untuk membuat keputusan dalam pengurusan penyakit. Dalam kajian ini, beberapa pencilan *Ganoderma* telah diperolehi dari Sarawak, Malaysia dan Multiplex PCR-DNA telah dijalankan untuk membezakan pencilan tersebut sehingga peringkat spesies. Ini diikuti dengan kajian makromorfologi, mikromorfologi dan keserasian pencilan *Ganoderma* tersebut. Kajian degradasi lignin juga dijalankan. Analisis Multiplex PCR-DNA menunjukkan keputusan positif untuk *G. boninense, G. zonatum* and *G. miniatocinctum,* justeru itu, menunjukkan bahawa tiga spesies *Ganoderma* tersebut mempunyai kaitan dengan penyakit reput pangkal di Sarawak. Kajian keserasian menunjukkan bahawa pencilan daripada pokok kelapa sawit yang berbeza adalah tidak serasi. Kajian morfologi pencilan *Ganoderma* menunjukkan bahawa ciri-ciri hifa dan panjang spora boleh digunakan untuk membezakan spesies *Ganoderma* tersebut. Maka, satu kekunci pengenalpastian telah dibangun untuk memberi garis panduan untuk membezakan spesies *Ganoderma* yang patogenik. Isolat *G. zonatum* G46 dan *G. miniatocinctum* G30 telah menunjukkan keupayaan degradasi lignin yang sama dengan *G. boninense* G25 dan G47 di mana *G. zonatum* G46 berupaya mendegradasikan paling banyak amaun lignin selepas satu minggu dijangkiti. Dari minggu pertama hingga minggu kelima, *G. zonatum* G46 berupaya mendegradasikan lebih banyak lignin berbanding dengan *G. boninense* G25 dan G47 di mana ia menunjukkan bahawa *G. zonatum* berupaya menyebabkan lebih banyak kerosakan terhadap kelapa sawit dalam jangka masa panjang. Oleh itu, lebih banyak kajian perlu ditumpukan kepada *G. zonatum* daripada menfokus kepada *G. boninense* sahaja untuk merangka langkah pengawalan yang sesuai atau untuk menghapuskan penyakit reput pangkal yang disebabkan oleh beberapa spesies *Ganoderma*.

© COPYRIGHT UPM

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to those who were involved in the completion of this study.

First of all, I would like to thank my Supervisor Associate Professor Dr Joseph Bong Choon Fah for his advices, guidance and supports. He had also given ample of encouragements throughout the study. My appreciation is also extended to Dr. Idris Abu Seman who had given support and guidance, not to mention providing materials and facilities for the Multiplex PCR-DNA study. I would also like to express my sincere gratitude to Dr. Wong Sing King for giving guidance and providing facilities for lignin studies. My sincere gratitude is also extended to Associate Professor Dr. Rajan Amarthalingam who had given advices and support during the study.

I would like to express my heartiest gratitude to Miss Hoe Pik Kheng, Mr. Chan Seow Phan and Mr. Lim Guan Miaw who have been continuously lending out warmth hands and giving abundance of support and encouragements during the high and low moments throughout the study. My journey to the completion of the study had been filled with joy and laughters with them around and I would treasure these memories to the end of time.

Not to forget, sincere thanks to Miss Diana Bong Siaw Then, Miss Nyam Vei Ting, Miss Yii Jing Ye and Mr. Kon Thian Woei who had given assistance especially during sampling trips. I would also like to express sincere gratitude to Mr. Lau Hsien Loong who had given support and guidance in lignin studies. Sincere thanks are also extended to Miss Lee Huei Hong, Miss Neoh Pei Nee and Miss Weng Bong Siew Then for their advices and assistance in the molecular study.

Last but not least, sincere thanks to my family for providing continuous support and care throughout the study.

© COPYRIGHT UPM

I certify that a Thesis Examination Committee has met on 25 October 2013 to conduct the final examination of Wong Ling Chie on her thesis entitled "Morphological Study and Lignin Degradation of Pathogenic Ganoderma Species of Basal Stem Rot in Oil Palm" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Examination Committee are as follows:

Japar Sidik Bujang, PhD
Professor
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia (Bintulu Campus)
(Chairman)

Zainal Abidin bin Mior Ahmad, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Jugah Kadir, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Latiffah Zakaria, PhD
Senior Lecturer
University Sains Malaysia
Malaysia
(External Examiner)

NORITA OMAR, PhD
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 17 February 2014

© COPYRIGHT UPM

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Joseph Bong Choon Fah, Ph.D
Associate Professor
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia
Bintulu Sarawak Campus
(Chairman)

Idris Abu Seman, Ph.D
Head of Ganoderma and Diseases Research of Oil Palm Unit
Malaysia Palm Oil Board
(Member)

© COPYRIGHT UPM

_____
BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hearby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by University Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: _____

© COPYRIGHT UPM

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- Supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature:	_____	Signature:	_____
Name of Chairman of Supervisory Committee:	_____	Name of Member of Supervisory Committee:	_____

© COPYRIGHT UPM

TABLE OF CONTENTS

© COPYRIGHT UPM

© COPYRIGHT UPM

LIST OF TABLES

© COPYRIGHT UPM

LIST OF FIGURES

© COPYRIGHT UPM

© COPYRIGHT UPM

LIST OF ABBREVIATIONS

Ha	Hectare
Kg	Kilogram
g	Gram
mg	Milligram
mL	Milliliter
µL	Microliter
mM	millimolar
rpm	round per minute
%	Percentage
°C	Degree Celcius
UV	Ultra violet
TSC	Total solid content
MPOB	Malaysia Palm Oil Board
EFB	Empty fruit bunch
FFB	Fresh fruit bunch
BSR	Basal Stem Rot
ELISA	Enzyme-linked immunosorbent Assay
ITS	Internal Transcribe Spacer
RAPD	Random Amplified Polymorphism DNA
PCR	Polymerase Chain Reaction
MPCR	Multiplex Polymerase Chain Reaction
DNMRT	Duncan New Multiple Range Test
DPO	Dual Priming Oligonucleotide
bp	base pair
8-MOP	8-methoxypysoralen
WRF	White rot fungi
LME	Lignin modifying enzyme
LiP	Lignin peroxidase
MnP	Manganese peroxidase
GSM	*Ganoderma* Selective Media

© COPYRIGHT UPM

CHAPTER 1

INTRODUCTION

In the last three decades, the oil palm industry in Malaysia has experienced rapid growth and has emerged as one of the most important source of vegetable oils and fats in the world. Compared to other oil-producing crops, oil palm is considered as the most productive oil crop in the world. Therefore, the wellbeing of the oil palm industry is extremely crucial as it contributes tremendously to the economics of oil-producing countries.

Although oil palm is a valuable commodity, there are obstacles and factors that are posing great threats to the oil palm industry. The major obstacle in order to achieve crop sustainability in oil palm plantation is basal stem rot (BSR), a disease which irremediably causes palm deaths. *Ganoderma boninense*, a type of white rot fungi capable of degrading the lignin component in wood, is the predominant *Ganoderma* species which is responsible for the BSR occurrence and development (Breton *et al.*, 2006; Paterson, 2006; Paterson, 2007a) causing economic losses to oil palm industry in various regions around the world especially Southeast Asia (Flood *et al.,* 2000; Corley and Tinker, 2003; Pilotti, 2005; Breton *et al.*, 2006; Rees *et al*., 2007). Many researchers agreed on the fact that *Ganoderma boninense* causes the most destructive disease in oil palms, especially in Malaysia and Indonesia (Gurmit, 1990; Susanto *et al.*, 2005; Idris *et al*., 2006). In order to control the BSR disease, sufficient knowledge on the pathogen is crucial, especially on the identity of the pathogen, mode of infection and spread of the disease.

Ganoderma boninense was not the only pathogen that has been causing the BSR disease; in fact, studies had proven that there are several *Ganoderma* species that are capable of causing BSR in oil palm (Idris *et al*., 2000). However, non-pathogenic *Ganoderma* species were also found in oil palm plantations, growing on dead trunks or stumps, therefore leading to confusion in the identification of pathogenic *Ganoderma* species. These confusions can cause significant losses to the oil palm industry due to inaccurate and unsuitable disease management. Therefore, the development of a fast method to accurately identify the pathogen is extremely crucial in order to initiate prompt actions for disease management of the specific pathogen.

In order to successfully formulate a method to control the BSR disease, the knowledge on how the *Ganoderma* species infect and spread in the oil palm is extremely important. *Ganoderma* species, a type of white rot fungi, is well-known to be capable of degrading lignin components via secretion of various extracellular ligninolytic enzymes (D'Souza *et al.*, 1999; Ali *et al*., 2004; Elissetche *et al.*, 2007; Revankar and Lele, 2007). The understanding of these ligninolytic enzymes and the degree of damage that it leads to is important in order to assess the development of the disease and formulate a solution to curb the BSR disease.

Due to the *Ganoderma* complex, identification of *Ganoderma* species which are pathogenic to oil palms had been difficult and unconvincing. As *Ganoderma* species was proven to be heterogenic in numerous studies (Utomo and Niepold, 2000; Zakaria *et al.*, 2005), variations might occur even within the same species both

© COPYRIGHT UPM

genetically and morphologically. Therefore, studies had been done to narrow down the characteristics of these species that were capable of identifying and differentiating between the pathogenic *Ganoderma* species in a shorter time in order to facilitate the disease management of BSR caused by these *Ganoderma* species.

Therefore, this study was conducted to (1) isolate and identify *Ganoderma* species pathogenic to oil palm via Multiplex PCR DNA analysis (2) distinguish between pathogenic *Ganoderma* species through morphological study (3) evaluate the lignin degradation in oil palm by the *Ganoderma* species.

© COPYRIGHT UPM

REFERENCES

Adaskaveg, J.E., Gilbertson, R.L. and Blanchette, R.A.. 1990. Comparative studies of delignification caused by Ganoderma species. *Applied and Environmental Microbiology* 56(5): 1932-1943.

Adaskaveg, J.E. and Gilbertson, R.L. 1987. Vegetative incompatibility between intraspecific dikaryotic pairings of *Ganoderma lucidum* and *Ganoderma tsugae*. *Mycologia* 79(4): 603-613.

Adaskaveg, J.E. and Gilbertson, R.L. 1988. Basidiospores, pilocyctidia and other basidiocarp characters in several species of the *Ganoderma lucidum* complex. *Mycologia* 80(4): 493-507.

Ahmad, Z., Saman, H.M. and Tahir, P.M. 2010. Oil palm trunk fiber as biowaste resource for concrete reinforcement. *International Journal of Mechanical and Materials Engineering (IJMME)* 5 (2): 199-207

Alamu, OJ., Akintola, T.A., Enweremadu, C.C. and Adeleke, A.E. 2008. Characterization of palm-kernel oil biodiesel produced through NaOH-catalysed transesterification process. *Scientific Research and Essay* 3(7): 308-311.

Ali, S.R.A., Yaacob, N.S., Idris, A.S. and Wahid, M.B. 2004. Oil palm cellulose and lignin degradation of different *Ganoderma* sp. based on ASTM standard rotting experiment. In International Conference on Pests and Diseases of Importance to the Oil Palm Industry. 19-19 May 2004. Malaysian Oil Palm Board, Kuala Lumpur pp. 2-15.

Arantes, V. and Milagres, A.M.F. 2007. The synergistic action of ligninolytic enzymes (MnP and laccase) and Fe3+-reducing activity from white-rot fungi for degradation of Azure B. *Enzyme and Microbial Technology* 42: 17-22.

Ariffin, D. and Idris, A.S. 1991. A selective medium for the isolation of *Ganoderma* from diseased tissue. In Proceeding of the 1991 PIPOC International Palm Oil Conference – Progress, Prospects and Challenges Towards the 21st Century (Model I - Agriculture). Malaysian Oil Palm Board, Malaysia pp. 517-519.

Ariffin, D., Idris, A.S., Singh, G. 2000. Status of *Ganoderma* in oil palm. In *Ganoderma* Diseases of Perennial Crops, ed. Flood, J., P.D. Bridge, and M. Holderness pp. 49–68. Wallingford, UK: CAB International.

Berger, A., Rein, D., Kratky, E., Monnard, I., Hajjaj, H., Meirim, I., Piguet-Welsh, C., Hauser, J., Mace, K. and Niederberger, P. 2004. Cholesterol-lowering properties of *Ganoderma lucidum in vitro*, *ex vivo,* and in hamsters and minipigs. *Lipids in Health and Disease* 3: 2

Breton, F., Hassan, Y., Hariadi, Lubis, Z. and Franqueville, H.D.. 2006. The Development of an Early Screening Test for Basal Stem Rot Tolerance in Oil Palm Progenies. *Journal of Oil Palm Research* (Special edition) pp.24-36.

© COPYRIGHT UPM

Chang and Miles, 2004. Mushrooms: Cultivation, nutritional value, medicinal effect and environmental impact. Kuala Lumpur: CRC Press.

Chase, A.R. and Broschat, T.K. 1993. Diseases and disorders of ornamental palms. St. Paul: The American Phytopathological Society Press.

Chun, J.Y., Kim, K.J., Hwang, I.T., Kim, Y.J., Lee, D.H., Lee, I.K. and Kim, J.K. 2007. Dual priming oligonucleotide system for the multiplex detection of respiratory viruses and SNP genotyping of CYP2C119 gene. *Nucleic Acids Research* 35(6): e40.

Corley, R.H.V. and Tinker, P.B.H.. 2003. The Oil Palm (4th ed.). Blackwell Science Ltd.,Oxford.

D'Souza, T.M., Merritt, C.S. and Reddy, C.A. 1999. Lignin-modifying enzymes of the white-rot basidiomycete *Ganoderma lucidum*. *Applied and Environmental Microbiology* 65(12): 5307-5313.

Datta, A., Bettermann, A. and Kirk, T.K. 1991. Identification of a specific manganese peroxidase among ligninolytic enzymes secreted by during wood decay. *Applied and Environmental Microbiology* 57(5): 1453-1460.

Dosoretz, C.G., Chen, H.C. and Grethlein, H.E. 1990. Effect of environmental conditions on Extracellular Protease activity in lignolytic cultures of *Phanerochaete chrysosporium*. *Applied and Environmental Microbiology* 56(2): 395-400.

Edwards, M.C. and Gibbs, R.A. 1994. Multiplex PCR: advantages, development and applications. *Genome Research* 3: S65-S75.

Elissetche, J.P., Ferraz, A., Freer, J. and Rodríguez, J. 2007. Enzymes produced by *Gnaoderma australe* growing on wood and submerged cultures. *World Journal of Microbiological Biotechnology* 23: 429-434.

Flood, J., Husan, Y., Turner, P.D. and O'Grady, E.B. 2000. The spread of *Ganoderma* from infective sources in the filed and its implications for management of the disease in oil palm. In *Ganoderma* Diseases of Perennial Crops, ed. Flood, J., P.D. Bridge, M. Holderness pp 101-112. Wallingford, UK: CAB International.

Fonseca, M.I., Shimizu, E., Zapata, P.D. and Villalba, L.L. 2009. Laccase-producing ability and the inducing effect of copper on laccase production of white rot fungi native from Misiones (Argentina). *Enzyme and Microbial Technology* 46: 534-539

Glen, M. 2006. The use of DNA techniques to identify fungi. In *Heart rot and root rot in tropical Acacia plantations* ed. Potter, K., A. Rimbawanto and C. Beadle. ACIAR proceedings 124: 46-54.

Gurmit, S.1990. Ganoderma - The Scourge of oil palms in the coastal areas (Peninsular Malaysia). *Planter* 67 (786): 421-444.

© COPYRIGHT UPM

Güzeldağ, G. and Çolak. 2007. Molecular Identification of *Ganoderma Lucidum* from Turkey. *International Journal of Agriculture and Biology* 9 (5): 767-770.

Harris, J.L. 1986. Modified method for fungal slide culture. *Journal of Clinical Microbiology* 24(3): 460-461.

Hayyan, A., Alam, M.Z., Mirghani, M.E.S., Kabbashi, N.A., Hakimi, N.I.N.M., Siran, Y.M. and Tahiruddin, S. 2010. Production of biodiesel from sludge palm oil by esterification process. *Journal of Energy and Power Engineering* 4(1): 11-17

Ho, Y.H. and Nawawi, A. 1985. *Ganoderma boninense* from basal stem rot in oil palm (*Elaeis guineensis*) in Peninsular Malaysia. *Pertanika* 8(3): 425-428.

Ho, Y.H. and Nawawi, A. 1986. Isolation, growth and sporophore development of *Ganoderma boninense* from oil palm in Malaysia. *Pertanika* 9(1): 69-73.

Ho, Y.H., Latiffah, Z. and Khairuddin, H. 1993. Spore production of *Ganoderma boninense* Pat. and the effect of pH, light and dryness on germination. *Journal of Plant Protection in the Tropics* 10 (1): 51-58.

Hofrichter, M. 2002. Review: Lignin conversion by manganese peroxidase (MnP). *Enzyme and Microbial Technology* 30: 454-466.

Hormiga, J.A., Vera, J., Frias, I. and Darias, N.V.T. 2008. Growth and ligninolytic system production dynamics of the *Phanerochaeten chrysosporium* fungus, a modeling and optimization approach. *Journal of Biotechnology* 137: 50-58.

Hseu, RS., Wang, H.H., Wang, H.F. and Moncalvo, J.M. 1996. Differentiation and grouping of isolates of the Ganoderma lucidum complex by random amplified polymorphic DNA-PCR compared with grouping on the basis of internal transcribed spacer sequences. *Applied and Environmental Microbiology* 62(4): 1354-1363.

Hood, I.A. 2006. The mycology of the Basidiomycetes. In *Heart rot and root rot in tropical Acacia plantations* ed. Potter, K., A. Rimbawanto and C. Beadle. ACIAR proceedings 124: 34-45.

Idris, A.S., Ariffin, D., Swinburne, T.R. and Watt, T.A. 2000. The identity of Ganoderma species responsible for BSR disease of oil palm in Malaysia – Pathogenicity Test. MPOB Information Series MPOB TT No. 77b.

Idris, A.S., Yamaoka, M., Hayakawa, S., Basri, M.W., Noorhasimah, I. and Ariffin, D. 2003. PCR technique for detection of *Ganoderma*. MPOB Information Series MPOB TT No. 188.

Idris, A.S., Kushairi, D. Ariffin, D. and Basri, M.W.. 2006. Technique for inoculation of oil palm germinated seeds with Ganoderma. MPOB Information Series MPOB TT No. 314.

Idris, A.S. and Rafidah, A.R. 2008. Polyclonal antibody for detection of *Ganoderma.* MPOB Information Series MPOB TT No. 405.

© COPYRIGHT UPM

Idris, A.S., Rajinder, S., Madihah, A.Z. and Wahid, M.B.2010. Multiplex PCR-DNA kit for early detection and identification of *Ganoderma* species in oil palm. MPOB Information Series MPOB TS No. 73.

Jawad, N., Syed Khaleel Ahmad and Farrukh, N. 2008. Palm biodiesel as alternative green renewable energy for the energy demands of the future. In International conference on construction and building technology. 16-20 June 2008. ICCBT 2008-F-(07) pp. 79-94.

Jo, W.S., Park, H.N., Cho, D.H., Yoo, Y.B. and Park, S.C. 2011. Detection of extracellular enzyme activities in *Ganoderma neo-japonicum*. Mycobiology 39 (2): 118-120.

Karakousis, A., Tan, L., Ellis, D., Alexiou, H. and Wormald, P.J. 2006. An assessment of the efficiency of fungal DNA extraction methods for maximizing the detection of medically important fungi using PCR. *Journal of Microbiological Methods* 65: 38-48.

Kim, H.K., Shim, M.Y., Seo, G.S. and Kim, H.G. 2002. Comparison of characteristics of *Ganoderma* according to geographical origins (III): Classification between species of genus *Ganoderma* using dikaryon-monokaryon mating. *Mycobiology* 30(2): 61-64.

Kinge, T.R. and Mih, A.M. 2011. *Ganoderma ryvardense* sp. nov. associated with basal stem rot (BSR) disease of oil palm in Cameroon. *Mycosphere* 2(2): 179-188.

Kondo, R., Kurashiki, K. and Sakai, K. 1994. In vitro bleaching of hardwood kraft pulp by extracellular enzymes excreted from white rot fungi in a cultivation system using a membrane filter. *Applied and Environmental Microbiology* 60(3): 921-926.

Latiffah, Z., Harikrishna, K., Tan, S.G., Tan, S.H., Abdullah, F. and Ho, Y.H. 2002. Restriction analysis and sequencing of the ITS regions and 5.8S gene of rDNA of *Ganoderma* isolates from infected oil palm and coconut stumps in Malaysia. *Annals of Applied Biology* 141: 133-142.

Latiffah, Z., Kulaverasingham, H., Tan, S.G., Abdullah, F. and Ho, Y.W. 2005. Random amplified polymorphic DNA (RAPD) and Random amplified microsatellite (RAMS) of *Ganoderma* from infected oil palm and coconut stumps in Malaysia. *Asian Pacific Journal of Molecular Biology and Biotechnology* 13 (1): 23-34.

Latiffah, Z., Ali, N.S., Salleh, B. and Zakaria, M. 2009. Molecular analysis of *Ganoderma* species from different hosts in Peninsula Malaysia. *Journal of Biological Sciences* 9 (1): 12-20.

Latiffah, Z. and Ho, Y.W. 2005. Morphological characteristics and somatic incompatibility of *Ganoderma* from infected oil palm from three inland estates. *Malaysian Journal of Microbiology* 1 (2): 46-52.

© COPYRIGHT UPM

Levin, L., Forchissin, F. and Ramos, A.M. 2002. Copper induction of lignin-modifying enzymes in the white rot fungus *Trametes trogii*. *Mycologia* 94 (3): 377-383.

Levin, L., Papinutti, L. and Forchiassin, F. 2004. Evaluation of Argentian white rot fungi for their ability to produce lignin-modifying enzymes and decolorize industrial dyes. *Bioresource Technology* 94: 169-176.

Levin, L., Herrmann, C. and Papinutti, V.L. 2008. Optimization of lignocellulolytic enzyme production by white-rot fungus *Trametes trogii* in solid-state fermentation using response surface methodology. *Biochemical Engineering Journal* 39: 207-214.

Lim, H.P. and Fong, Y.K. 2005. An insight into spore dispersal of *Ganoderma boninense* on oil palm. *Mycopathologia* 159: 171-179.

Lin, Z.B. and Zhang, H.N. 2004. Anti-tumor and immunoregulatory activities of Ganoderma lucidum and its possible mechanisms. *Acta Pharmacologica Sinica* 25(11): 1387-1395.

Lu, J., Qin, J.Z., Chen, P., Chen, X., Zheng, Y.Z. and Zhao, S.J. 2012. Quality difference study of six varieties of *Ganoderma lucidum* with different origins. *Frontiers in Pharmacology* 3:57.

Miller, R.N.G., Holderness, M., Bridge, P.D., Chung, G.F. and Zakaria, M.H. 1999. Genetic diversity of *Ganoderma* in oil palm plantings. *Plant Pathology* 48: 595–603.

Moncalvo, J.M., Wang, H.H. and Hseu, R.S. 1995a. Phylogenetic relationships in *Ganoderma* inferred from the internal transcribed spacers and 25S ribosomal DNA sequences. *Mycologia* 87 (2): 223-238.

Moncalvo, J.M., Wang, H.H. and Hseu R.S. 1995b. Gene phylogeny of the *Ganoderma lucidum* complex based on ribosomal DNA sequences. Comparison with traditional taxonomix characters. *Mycological Research* 99 (12): 1489-1499.

Moncalvo, J.M. 2005. Molecular systematic of Ganoderma: What is Reishi? *International Journal of Medicinal Mushroom* 7(3): 353-354.

MPOB 2012. Oil palm statistics. Economics and industrial development division, bepi.mpob.gov.my, accessed on 20 January 2013.

Murill, W.A. 1902. The Polyporaceae of North America. I. The Genus *Ganoderma*. *Bulletin of the Torrey Botanical Club* 29 (10): 599-608.

Murugesan, K., Kim, Y.M., Jeon, J.R. and Chang, Y.S. 2009. Effect of metal ions on reactive dye decoloration by laccase from *Ganoderma lucidum*. *Journal of Hazardous Materials* 168: 523-529.

Nawawi, A. and Ho, Y.W. 1990. Effect of temperature and pH on growth pattern pf *Ganoderma boninense* from oil palm in Peninsular Malaysia. *Pertanika* 13 (3): 303-307

© COPYRIGHT UPM

Nerud, F. and Misurcova, Z. 1996. Distribution of lignin enzymes in selected white rot fungi. *Folia Microbiology* 41 (3): 264-266.

Nusaibah, S.A, Rajinder, S. and Idris, A.S. 2010. Somatic incompatibility and AFLP analysis of four species of *Ganoderma* isolated from oil palm. *Journal of Oil Palm Research* 22: 814-821.

Nyahongo G.S., Gomes, J., Gübitz, G.M., Zvauya, R., Read, J. and Steiner, W. 2002. Decoloration of textile dyes by laccases from a newly isolated strain of *Trametes modesta*. *Water Research* 36: 1449-1456.

Onuorah, E.O. 2005. Properties of fiberboards made from oil palm (*Elaeis guineensis*) stem and/or mixed tropical hardwood sawmill residues. *Journal of Tropical Forest Science* 17(4): 497-507.

Park, Y.J., Kwon, O.C., Son, E.S., Yoon, D.E., Han, W., Yoo, Y.B. and Lee, C.S. 2012a. Taxonomy of *Ganoderma lucidum* from Korea based on rDNA and partial β-Tubulin gene sequence analysis. *Mycobiology* 40(1): 71-75.

Park, Y.J., Kwon, O.C., Son, E.S., Yoon, D.E., Han, W., Nam, J.Y., Yoo, Y.B. and Lee, C.S. 2012b. Genetic diversity analysis of *Ganoderma* species and development of a specific marker for identification of medicinal mushroom *Ganoderma lucidum*. *African Journal of Microbiology Research* 6(25): 5417-5425.

Paterson, R.R.M. 2006. *Ganoderma* - A therapeutic fungal biofactory. *Phytochemistry* 67:1985-2001.

Paterson, R.R.M. 2007a. *Ganoderma* disease of oil palm-A white rot perspective necessary for integrated control. *Crop Protection* 26 (9): 1369-1376.

Paterson, RRM. 2007b. Internal amplification controls have not been employed in fungal PCR hence potential false negative results. *Journal of Applied Microbiology* 102(1): 1-10.

Paterson RRM., Sariah, M., Zainal Abidin, M.A. and Lima, N. 2008. Prospects for inhibition of lignin degrading enzymes to control *Ganoderma* white rot of oil palm. *Current Enzyme Inhibition* 4: 172-179.

Pigott, J. 1990. Growing oil palms: An illustrated guide. Malaysia: United Selangor Press Sdn Bhd.

Pilotti, C.A., Sanderson, F.R. and Aitken, E.A.B. 2003. Genetic structure of a population of *Ganoderma boninense* on oil palm. *Plant Pathology* 52: 455-463.

Pilotti, C.A. 2004. Morphological variation and host range of two *Ganoderma* species from Papua New Guinea. *Mycopathologia* 158: 251-265.

Pilotti, C.A. 2005. Stem rots of oil palm by *Ganoderma boninense*: Pathogen biology and epidemiology. *Mycopathologia* 159: 129-137.

© COPYRIGHT UPM

Pledger, D.N. and Young, T.W.K. 1973. Basidiospore form in the British species of *Ganoderma* Karst. *Kew Bulletin* 28(3): 351-364.

Raghukumar, C., D'Souza, T.D., Thorn, R.G. and Reddy, C.A. 1999. Lignin-modifying enzymes of *Flavodon flavus*, a basidiomycete isolated from a coastal marine environment. *Applied and Environmental Microbiology* 65(5): 2103-2111.

Rajendran, L., Kandan, A., Karthikeyan, G., Raguchander, T. and Samiyappan, R. 2009. Early detection of *Ganoderma* causing basal stem rot in coconut plantations. *Journal of Oil Palm Research* 21: 672-635.

Rees, R.W., Flood, J., Hasan, Y. and Cooper, R.M. 2007. Effects of inoculum potential, shading and soil temperature on root infection of oil palm seedlings by the basal stem rot pathogen *Ganoderma boninense*. *Plant Pathology* 56: 862-870.

Rees RW., Flood, J., Hassan, Y., Potter, U. and Cooper, R.M. 2009. Basal stem rot of oil palm (*Elaeis guineensis*); mode of root infection and lower stem invasion by *Ganoderma boninense*. *Plant Pathology* 58: 982-989.

Revankar, M.S., and Lele, S.S. 2007. Synthetic dye decoloration by white rot fungus, *Ganoderma* sp. WR-1. *Bioresource Technology* 98: 775-780.

Sapparat, M.C.N., Mocchiutti, P., Liggieri, C.S., Aulicino, M.B., Caffini, N.O., Balatti, P.A. and Martinez, M.J. 2008. Ligninolytic enzyme ability and potential biotechnology applications of the white-rot fungus *Grammothele subargentea* LPSC no. 436 strain. *Process Biochemistry* 43: 368-375.

Sariah, M., Hussein, M.A., Miller, R.N.G. and Holderness, M. 1994. Pathogenicity of *Ganoderma boninense* tested by inoculation of oil palm seedlings. *Plant Pathology* 43: 507-510.

Shamala, S., Chris, D., Sioban, O. and Idris, A.S. 2006. Preliminary studies on the development of monoclonal antibodies against mycelia of *Ganoderma boninense*, the causal pathogen of basal stem rot of oil palm. *Malaysian Journal of Microbiology* 2(1): 30-34.

Sliva C.M.M.S., De Melo, I.S. and De Oliveira, P.R. 2005. Ligninolytic enzyme production by *Ganoderma* spp. *Enzyme and Microbial Technology* 37: 324-329.

Sliva D. 2003. *Ganoderma lucidum* (Reishi) in Cancer Treatment. *Integrative Cancer Therapies* 2(4): 358-364.

Slivova V., Valachovicova, T., Jiang, J.H. and Sliva, D.. 2004. *Ganoderma lucidum* inhibits invasiveness of breast cancer cells. *Journal of Cancer Integrative Medicine* 2(1):25-30.

Songulashvili, G., Jimenez-Tobon, G., Jaspers, C. and Penningckx, M.J. 2011. High production of laccase by *Ganoderma lucidum* 447 in submerged cultivation on ethanol production residue supplemented with Cu^{2+}. *Mycosphere* 2(4): 507-513.

© COPYRIGHT UPM

Sundram, K., Sambanthamurth, R. and Tan, Y.A. 2003. Palm fruit chemistry and nutrition. *Asia Pacific Journal of Clinical Nutrition* 12(3): 355-362.

Stamets, P. 2000. Growing gourmet and medicinal mushroom (3rd ed.). pp. 119-367. Berkeley, California: Ten Speed Press.

Stewart, P., Kersten, P., Wymelenberg, A.V., Gaskell, J. and Cullen, D. 1992. Lignin peroxidase gene family of *Phanerochaete chrysosporium*: Complex regulation by carbon and nitrogen limitation and identification of a second dimorphic chromosome. *Journal of Bacteriology* 174(15): 5036-5042.

Swamy, J. and Ramsay, J.A. 1999. The evaluation of white rot fungi in the decoloration of textile dyes. *Enzyme and Microbial Technology* 24:130-137.

Susanto, S., Sudharto, P.D. and Purba, R.Y. 2005. Enhancing biological control of basal stem rot disease (*Ganoderma boninense*) in the oil palm plantations. *Mycopathologia* 159: 154-157.

Teerapatsakul C., Abe, N., Buske, C., Kongkathip, N., Jareonkitmongkol, S. and Chitradon, L. 2007. Novel laccases of *Ganoderma* sp. KU-Alk4, regulated by different glucose concentration in alkaline media. *World Journal of Microbiological Biotechnology* 23: 1559-1567.

Teh, B.S.C., Goh, K.J. and Kharirun, N.K. 2010. Physical changes to oil palm empty fruit bunches (EFB) and EFB Mat (Ecomat) during their decomposition in the field. *Pertanika Journal of Tropical Agricultural Science* 33(1): 39-44.

Toyama, N. and Ogawa, K. 1974. Comparative studies on cellulolytic and oxidizing enzyme activities of edible and inedible wood rotters. *Mushroom Science IV(Part I)* 9: 745-760.

Turner, P.D. and Bull, R.A. 1967. Diseases and disorders of oil palm in Malaysia. Kuala Lumpur: The Incorporated Society of Planters.

Turner, PD. 1981. Oil Palm Diseases and Disorders. Oxford, New York: Melbourne Oxford University Press.

Utomo, C. and Niepold, F. 2000. Development of diagnostic methods for detecting *Ganoderma*-infected oil palm. *Journal of Phytopathology* 148: 507-514.

Vares, T. and Hatakka, A. 1997. Lignin-degrading activity and ligninolytic enzymes of different white –rot fungi: Effects of manganese and malote. *Canadian Journal of Botany* 75: 61-71

Verheye, W. 2010. Growth and production of oil palm. In Land use, land cover and soil sciences. ed. Verheye, W. Encyclopedia of Life Support Systems (EOLSS). Oxford, UK:UNESCO-EOLSS Publishers. (http://www.eolss.net)

Wang, DM., Wu, S.H., Su, C.H., Peng, J.T., Shih, Y.H. and Chen, L.C. 2009. *Ganoderma multipelium*, the correct name for '*G. lucidum*' in tropical Asia. *Botanical Studies* 50: 451-458.

© COPYRIGHT UPM

Wang, X.C., Xi, R.J., Wang, D.M. and Yao, Y.J. 2012. The species identity of the widely cultivated *Ganoderma*, '*Ganoderma lucidum'* (Ling-Zhi), in China. PLoS ONE 7(7):e40857.

Watchtel-Galor, S., Tomlinson, B. and Benzie, I.F.F. 2004. *Ganoderma lucidum* ('Lingzhi'), a Chinese medicinal mushroom: biomarker responses in a controlled human supplementation study. *British Journal of Nutrition* 91: 263-269.

Wijesekera, H.T.R., Wijesundera, R.L.C. and Rajapakse, C.N.K. 1996. Hyphal interactions between *Trichoderma viride* and *Ganoderma boninense* Pat., the cause of coconut root and bole rot. *Journal of the Natioanl Science Council of Sri Lanka* 24(3): 217-219.

Woo, C.Y.P., Ngan, H.Y.A., Chui, H.K., Lau, K.P.S. and Yuen, K.Y. 2010. Agar block smear preparation: a novel method of slide preparation for preservation of native fungal structures for microscopic examination and long-term storage. *Journal of Clinical Microbiology* 48(9): 3053-3061.

Worall, J.J. 1997. Somatic incompatibility in basic. *Mycologia* 89(1):24-36.

Wu, X.Y., Guan, H.G., Li, J.Z., Guo, J.Z. and Hou, B.Y. 2012. Evaluation of antitumour action of *Ganoderma lucidum* extract in hepatocarcinoma mice. *African Journal of Pharmacy and Pharmacology* 6(41): 2884-2887.

Wuyep, P.A., Khan, A.U. and Nok, A.J. 2003. Production and regulation of lignin degrading enzymes from *Lentinus squarrosulus* (mont.) Singer and *Psathyrella atriumbonata* Pegler. *African Journal of Biotechnology* 2(11): 444-447.

Xiong, X.P., Wen, X.H., Bai, Y. and Qian, Y. 2008. Effect of culture conditions on ligninolytic enzymes and protease production by *Phanerochaete chrysosporium* in air. *Journal of Environmenttal Science (China)* 20(1): 94-100.

Yamanaka, R., Soares, C.F., Matheus, D.R. and Machado, K.M.G. 2008. Lignolytic enzymes produces by *Trametes villosa* CCB176 under different culture conditions. *Brazilian Journal of Microbiology* 39: 78-84.

Yap, Y.H.T., Nurul, FA. and Mahiran, B.. 2011. Biodiesel Production via Transesterification of Palm Oil Using NaOH/Al2O3 Catalysts. *Sains Malaysiana* 40(6): 587-594

Zahari, W. M., Abu Hassan, O., Wong, H.K. and Liang, J.B. 2003. Utilization of Oil Palm Frond - Based Diets for Beef and Dairy Production in Malaysia. *Asian-Australian Journal of Animal Science* 16(4): 625-614.

Zakaria, L., Kulaveraasingham, H., Tan, S.G., Abdullah, F. and Ho, Y.W. 2005. Random amplified polymorphic DNA (RAPD) and Random amplified microsatellite (RAMS) of *Ganoderma* from infected oil palm and coconut stumps in Malaysia. *Journal of Biological Biotechnology* 13: 23-24.

© COPYRIGHT UPM

Zakaria, L., Ali, N.S., Salleh, B. and Zakaria, M. 2009. Molecular analysis of *Ganoderma* species from different hosts in Peninsula Malaysia. *Journal of Biological Sciences* 9: 12-20.

© COPYRIGHT UPM

BIODATA OF STUDENT

Wong Ling Chie was born in Sibu, Sarawak on 7th February 1984. She did her primary education in S.R.B. St. Anthony, Bintulu and proceeded to secondary education in S.M.K. Bandar where she obtained her Penilaian Menengah Rendah (PMR) and Sijil Peperiksaan Malaysia (SPM). She then went to S.M.K. Bintulu to obtain Sijil Tinggi Peperiksaan Malaysia (STPM).

She was admitted to Universiti Putra Malaysia Bintulu, Sarawak Campus and obtained First Class in Bachelor Degree in Bioindustry Sciences. She then pursued a Master of Science in Plant Pathology.

Her interest is pursuing research on plant pathology in the future.

© COPYRIGHT UPM

LIST OF PUBLICATIONS

1. Wong, L.C., C.F.J. Bong and A.S. Idris. 2012. *Ganoderma* species associated with Basal Stem Rot Disease of oil palm. *American Journal of Applied Sciences* **9(6):** 879-885.

2. Wong, L.C., C.F.J. Bong and A.S. Idris. 2010. Differentiation of *Ganoderma* spp. causing Basal Stem Rot in oil palm. 15th Biological Sciences Graduate Congress (BSGC), 15-17th December 2010, Institute of Biological Sciences, University of Malaya.

3. Wong, L.C. and C.F.J. Bong. 2008. Studies on growth performance and quantification of (1-3),(1-6)-β-glucan for two strains of *Ganoderma lucidum* and one strain of *Ganoderma tropicum*. 4th USM-Life Sciences Postgraduate Conference, Universiti Sains Malaysia.

© COPYRIGHT UPM