



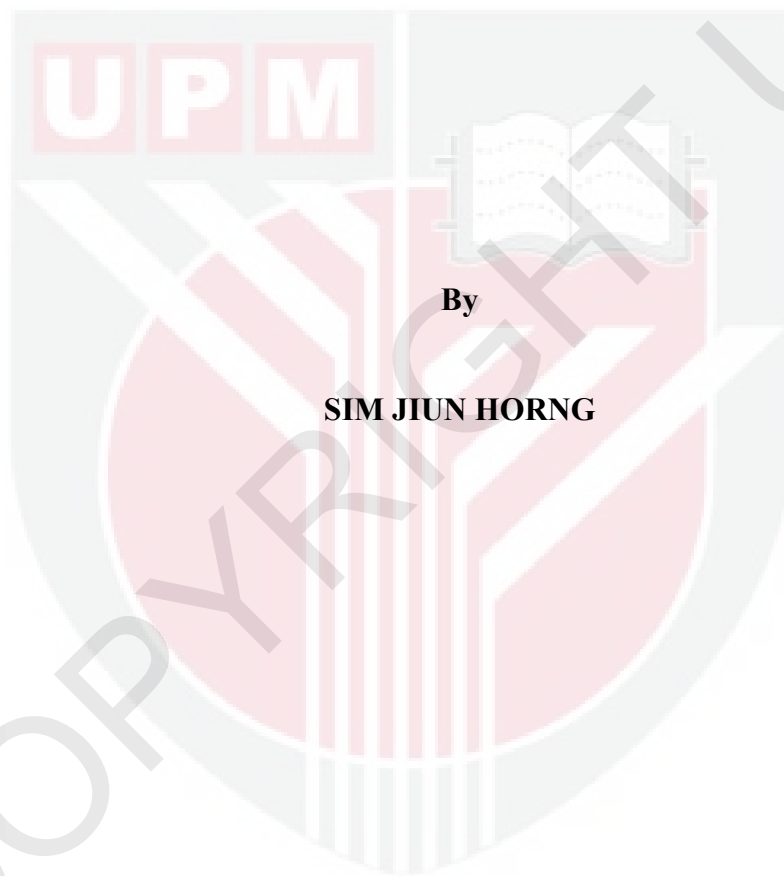
UNIVERSITI PUTRA MALAYSIA

**MOLECULAR DIVERSITY OF ENDOPHYTIC FUNGI ISOLATED FROM
DIFFERENT SECTIONS OF LOCAL FRUIT TREES.**

SIM JIUN HORNG

FPSK(m) 2010 22

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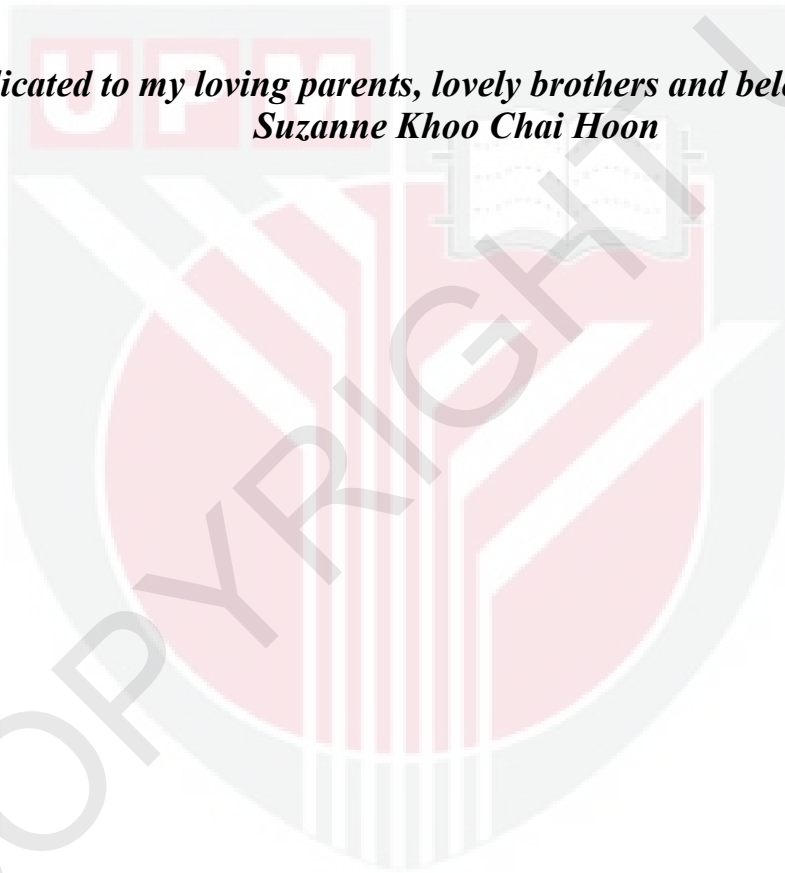
By

SIM JIUN HORNG

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

July 2010

*Dedicated to my loving parents, lovely brothers and beloved partner
Suzanne Khoo Chai Hoon*



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

**MOLECULAR DIVERSITY OF ENDOPHYTIC FUNGI ISOLATED FROM
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By

SIM JIUN HORNG

July 2010

Chair: Associate Professor Cheah Yoke Kqueen, PhD

Faculty: Medicine and Health Sciences

Over the past three decades, endophytic fungi have attracted increasing attention among taxonomists, agronomists, ecologists and evolutionary scientists. Endophytic fungi are fungi species that live within a plant for at least part of its life without causing apparent disease. Virtually every plant studied to date harbors at least one species of endophytic fungi. It now appears that an enormous, relatively untapped source of microbial diversity is represented by endophytic fungi.

Scientific literature had highlighted that the tropical plants of the world are the main source of these microbial endophytes. Malaysia as an agriculture based country; the rich soil allows for many fruits to grow in abundance and throughout the year. Fruit has become one of the major export items that contribute millions income to our country. Since tropical fruit plays an important role as food and as an economic commodity, there is a need to clearly understand the microorganisms that co-exist within the fruit. In order to study the potential endophytic fungi that exhibit in our

local fruits, 27 common local fruits have been chosen for this study. The results indicated that all of the targeted local fruits harbor at least one endophytic fungal.

Different media are believed to be amenable for the isolation of different endophytes. Therefore, three types of media namely PDA, SCA and ACT were included in this study. Potato Dextrose Agar (PDA) showed the highest recovery rate (39%) as compared to Starch Casein Agar (SC) (37%) and Actinomycetes Agar (ACT) (24%). After a series of isolation and purification processes, DNA of the isolated fungi was extracted using the conventional sodium dodecyl sulphate (SDS) method. In order to characterize the isolated fungi sample, molecular techniques based on the internal transcribe sequence (ITS) and random amplified polymorphic DNA (RAPD) were applied.

Ribosomal DNA (rDNA) is the most conserved region in the genome, with capabilities of phylogenetic divergence. The ITS region is now perhaps the most widely sequenced DNA region in fungi. It is typically the most useful tool for molecular systematics at the species level, and even within species. All isolated endophytic fungal DNA samples were subject to ITS primer set (ITS-1 and ITS-4) screening and all of the samples of DNA were able to generate distinct banding after the polymerase chain reaction.

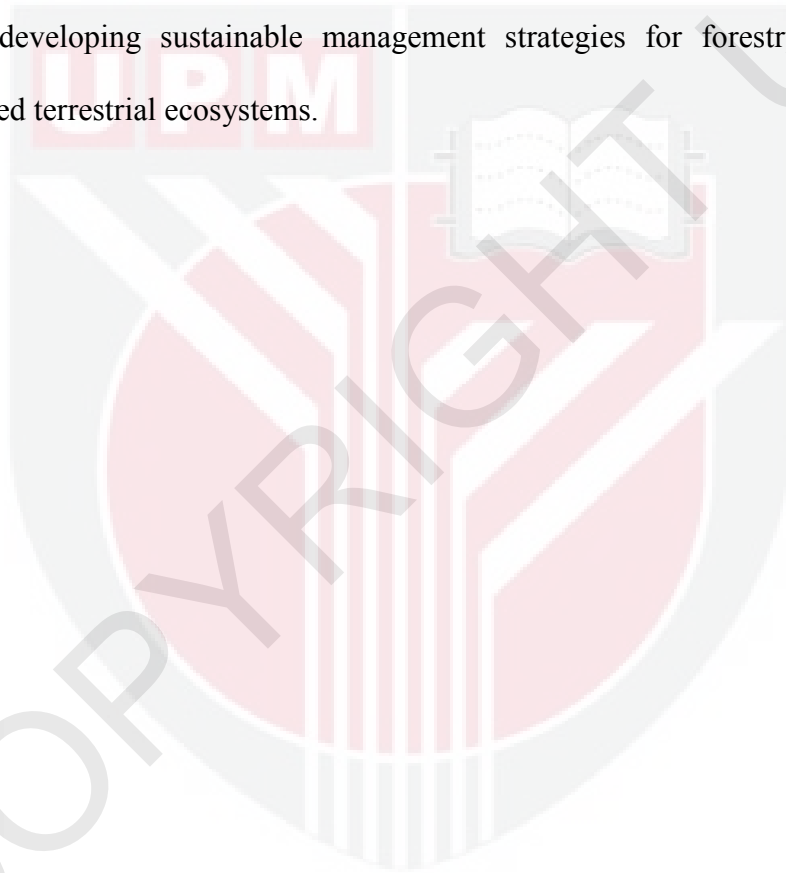
Obtained bands were further subjected to direct sequencing to gain relative coding nucleotide sequence. The gene sequences obtained were aligned using NCBI Blast search and analyzed by the use of bioinformatics software BioEdit and Bionumerics ver 5.0. The results revealed that the endophytic fungi isolates were attributed to 23

different genera and all of the ITS region sequences of endophytic fungal isolates. The accession numbers of all the sequences were generated from Genbank database. In order to study the phylogenetic relationship between the isolates, an analysis based on the comparison of neighbor neighbouring sequences of ITS region were performed. Results of the analysis have showed that all the local isolates share a high percentage of similarity in their ITS region sequences and tend to cluster together as compared to the available sequences from the genome.

RAPD is an amplification based technique that use arbitrary primer to detect the changes in the DNA sequence which enable the discrimination of the samples according to their comparative DNA sequences. A total of 20 different arbitrary primers were screened in the RAPD analysis, and only three arbitrary primers (OPO 6, OPO10 and OPO16) that gave reproducible and discriminatory banding pattern were selected. Results exhibited comparable high level of genetic diversity based on the isolated endophytic fungi which come from the same source. Besides, result in this study also revealed that composite analysis of all three selected primers provide better discrimination ability to distinguish between unrelated strains.

A biomolecular characterisation was performed after the identification of the isolates using well diffusion antimicrobial assay against 14 bacterial cultures. A total of 53 isolates displayed antimicrobial activity against at least one tested microorganism. Among all the isolates, sample 62 which was identified as *Botryosphaeria* sp. gave the most significant inhibiting results against growth of all tested microorganisms.

Conclusion, research based on endophyte-local fruit interactions has strengthened and pooled understanding of diversity, basic biology and ecology of the endophytic fungi combined with tropic interactions and multi-species co-evolution. It also leads to novel ways to discover endophytic fungi's role in the ecosystem especially in fruit. While more comprehensive understanding of microbial communities has theoretical interest, it provides valuable knowledge of causes and consequences of microbial mediated interactions in terrestrial ecosystems which may have economical value when developing sustainable management strategies for forestry, and restoring damaged terrestrial ecosystems.



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

**KEPELBAGAIAN CIRI-CIRI MOLEKUL KULAT ENDOFIT YANG
DIPENCILKAN DARIPADA BERBAGAI BAHAGIAN POKOK BUAH-
BUAHAN TEMPATAN**

Oleh

SIM JIUN HORNG

Julai 2010

Pengerusi: Profesor Madya Cheah Yoke Kqueen, PhD

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Tiga puluh tahun yang lalu, kulat endofit telah menarik perhatian pakar-pakar tasonomi, ahli ilmu tanah, ahli ekologi dan saintis evolusi. Kulat endofit adalah kulat yang hidup di dalam tumbuhan sekurang-kurang sebahagian hayatnya tanpa menyebabkan sebarang penyakit. Hakikatnya, setiap tumbuhan yang telah dikaji memiliki sekurang-kurangnya satu spesies kulat endofit. Kini, kulat endofit melambangkan sebahagian besar alam mikrob yang belum diketahui.

Petikan saintifik pernah menitikberatkan tumbuh-tumbuhan tropika sebagai salah satu sumber untuk kulat endofit. Sebagai negara berasaskan pertanian, Malaysia merupakan sumber berbagai buah-buahan tropika. Buah-buahan tempatan telah menjadi bahan eksport yang memperolehi keuntungan tinggi setiap tahun. Memandangkan buah-buahan tropika mempunyai peranan yang penting dalam ekonomi negara, pemahaman mikroorganisma yang hidup bersama di dalam buah-buahan adalah mustahak. Kajian ini terdiri daripada kulat endofit yang dipencilkan

daripada 27 pokok buah-buahan umum. Keputusan kajian ini menunjukkan kesemua buah-buahan terdapat sekurang-kurangnya satu kulat endofit hidup bersama dengan buah-buahan yang dihasilkan di kebun Malaysia.

Menurut definisi, kulat endofit merupakan kulat yang hidup serapat bersama dengan tisu tumbuhan. Untuk memencilkan kulat endofit daripada tisu tumbuhan, kulat-kulat dan bakteria yang berada di permukaan tisu tumbuhan hendaklah dimusnahkan dan seterusnya memencilkan dengan media pemilihan. Dalam kajian ini, media PDA (39%) merupakan media yang paling efisien bagi memencilkan kulat endofit jika dibandingkan dengan SC (37%) dan ACT (24%). Selepas kulat endofit tulen diperolehi, DNA diekstrakan dengan menggunakan kaedah Sodium lauryl sulfate (SDS). Random amplified polymorphic DNA (RAPD) dan internal transcribe sequence (ITS) adalah kaedah molekul untuk pencirian kulat endofit yang diperolehi.

"rDNA" merupakan kawasan yang paling terpelihara di seluruh genom dan berkeupayan dijadikan sebagai salah satu tempat analisa genetik. "ITS" merupakan lingkungan DNA yang paling banyak dikaji dalam kulat dan sangat berguna dalam ujikaji molekul yang melibatkan pembahagian spesies. Keseluruhan sampel kulat endofit telah berjaya menghasilkan jalur unik selepas amplifikasi oleh PCR atas dasar ITS (ITS-1 and ITS-4).

Jalur-jalur yang diperolehi dihantar ke pusat rangkaian genetik kod untuk mendapatkan kod nukleotida sebenar. Rangkaian gen yang diperolehi dalam kajian ini dijajarkan dengan NCBI BLAST dan perisian komputer bioinformatik BioEdit serta Bionumerics versi 5.0. Hasilan ujikaji menunjukkan kulat endofit yang

diperolehi boleh dibahagikan kepada 23 genera berasingan. Selain daripada itu, semua kod nukleotida sebenar bagi kulat endofit telah pun diserahkan ke "Genbank" dan kod pengenalan masing-masing telah pun diperolehi. Untuk mengaji hubungan filogenetik di antara kulat endofit, satu ujikaji berdasarkan perbandingan kod nukleotida sebenar "ITS" telah dilaksanakan, dan keputusannya menunjukkan kesemua kulat endofit yang terdapat di Malaysia memiliki peratusan persamaan yang tinggi dalam jujukan "ITS" dan sentiasa terkumpul dalam kluster yang sama.

"RAPD" merupakan teknik yang berdasarkan amplifikasi PCR untuk mengesan perubahan jujukan DNA dan seterusnya membolehkan diskriminasi sampel berdasarkan perbandingan jujukan DNA. Sejumlah dua puluh "primer" rambang telah dicuba untuk menghasilkan jalur-jalur unik, tetapi hanya tiga (OPO-06, OPO-10 dan OPO-16) dipilih berdasarkan keputusan yang dapat diulang dan corak jalur yang berdiskriminasi. Keputusan kajian ini menunjukkan keanekaragaman genetik yang tinggi walaupun sampel tersebut berasal dari sumber yang sama. Meskipun, keputusan juga menunjukkan kombinasi analisa serentak bagi ketiga-tiga "primer" menghasilkan kebolehan diskriminasi yang lebih baik khasnya mengasingkan strain yang tidak berkaitan serta keputusan yang lebih bermakna jika dibandingkan dengan penggunaan keputusan dari satu primer.

Pencirian biomolekul telah dijalankan terhadap kulat endofit selepas identifikasi dengan menggunakan kaedah penyebaran anti-mikrob menerusi perigi untuk empat belas kultur bakteria. Kira-kira lima puluh tiga kulat endofit menunjukkan penindasan terhadap sekurang-kurangnya satu bakteria. Sample 63 yang dikenapasti

sebagai *Botryosphaeria* sp. berupaya menghasilkan profil penindasan terhadap semua mikroorganisma yang digunakan dalam kajian ini.

Kesimpulannya, uijkaji yang berdasarkan buah-buahan tempatan dan kulat endofit telah meningkatkan pemahaman dalam pelbagai sektor umpamanya kepelbagaian dalam biologi asas, ekologi kulat endofit bersama denagn interaksi tropika dan evolusinya dengan kepelbagaian spesis yang lain. Selain daripada itu, uijkaji ini berjaya menunjukkan secara komprehensif kulat endofit yang hidup didalam buah-buahan tempatan negara kita. Dengan pengetahuan tersebut, punca dan akibat interaksi mikrob dengan ekosistem darat yang mempunyai nilai tinggi dapat diteliti dengan lebih mendalam selain strategi pengurusan pembangunan hutan dan pemulihan ekosistem darat yang tercemar.

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I certified that an Examination Committee has met on 26th July 2010 to conduct the final examination of Sim Jiun Horng on his Master of Science thesis entitled “Molecular Diversity of Endophytic Fungi Isolated From Different Sections of Local Fruit Trees” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1981. The Committee recommends that the student be awarded the Master of Science.

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Date: 23 February 2011

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

SIM JIUN HORNG

Date: 26 July 2010



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