UNIVERSITI PUTRA MALAYSIA

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MD. SHAFIQUZZAMAN SIDDIQUEE

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By

MD. SHAFIQUZZAMAN SIDDIQUEE

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

November 2007

DEDICATIONS

Dedicated especially to my elder brother Mr. Md. Shamsuzzaman Siddiquee (Shahin),
for his financial support,
guidance and encouragement during the writing of this thesis and
to my sister-in-law Ms. Jesmin Hossain and niece Samarah Siddiquee and all those
individuals who behind the scenes
make me possible to complete my study successfully.

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

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November 2007

Chairman: Professor Faridah Abdullah, PhD

Faculty: Science

Trichoderma is a genus of soil-borne fungus with abundant reports on its success as biological control agents of a variety of plant pathogens. Antagonistic assessment by dual culture technique showed that 18 out of 48 selected *T. harzianum* isolates successfully inhibited the mycelial growth of the pathogen *Ganoderma boninense* (isolate: PER71) at 47.86 to 72.06% with the strongest inhibitor exhibited by strain FA30. Eight samples produced effective volatile antifungal compounds which suppressed the growth of PER71 at 24.528 to 58.70 % over 6 days. When the 10 samples were assayed for the production of non-volatile antifungal compounds, whereby showed the inhibitory effects of 18.35 to 40.16% over 6 days. Strain FA30 was the best inhibitor isolate not only by dual culture inhibition technique, but was also the best producer of volatile and non-voltile inhibitor compounds, at 58.70 and 40.16% respectively.

The identifications of species of *Trichoderma* worldwide are currently deduced from micro-morphological descriptions which is tedious and prone to error. This study undertook a molecular approach, using isozyme electrophoresis, random amplified microsatellite (RAMS) analysis and gene sequence of the internal transcribed spacer-1 (ITS 1) region of the ribosomal DNA of selected *Trichoderma* isolates.

Electrophoretic variation of nine isozyme systems of 47 isolates from 3 species of *Trichoderma* namely, *T. harzianum, T. aureoviride* and *T. longibrachiatum* were studied. The UPGMA cluster analysis of the isozyme data showed the putatively identified *T. harzianum* to be distinctly separated from the outgroup sample of *T. longibrachiatum*, whereas *T. aureoviride* showed a closer genetic relationship to the *T. harzianum* populations. No distinct conclusion could be drawn from the dendrogram as the level of separation between *T. harzianum* and *T. aureoviride* and may not necessarily indicate a difference at the species level. A second molecular approach used was to extract DNA and characterise the sample for their Random Amplified Microsatelite DNA (RAMS) profile. The RAMS generated dendrogram showed that besides the distinct *T. longibrachiatum*, 2 other lineages were evident by UPGMA analysis. Again the level of taxonomic difference could not be determined. However, no clear separation was obtained by the dendrogram generated by the neighbor-joining (NJ).

The third approach was to putatively sequence the samples using the internal transcribed spacer 1 (ITS 1) region of the rDNA. The nucleotide sequences were multiple aligned and compared against the ex-type strains sequences from the NCBI and *Tricho*BLAST Genbank database. Results showed that 25 out of the 26 putatively identified *T.*

harzianum were in agreement with the genome of the *T. harzianum* ex-type strain while the single exception belonged to *T. virens* instead. The 9 putative *T. aureoviride* were misidentifications where 7 were *T. harzianum* and 2 were *T. virens* based on the Genbank database. The single strain of *T. longibrachiatum* (IMI: 375055) was in agreement with the ex-type strain. This study showed that conventional identification of *T. harzianum*, despite being done under the best possible care and condition, can still lead to incorrect identifications. Molecular studies by isozyme analysis did not give confident level of separation at the species level. The dendrogram based on UPGMA from RAMS analysis supported the ITS 1 gene sequence analysis but it could not confirm the specific species level. The ITS 1 region study showed that the gene sequences of *Trichoderma* samples were the most accurate technique for identification, with a bootstrap stability at 100% and a homology of 98-100%.

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

PERHUBUNGAN GENETIK ANTARA TIGA SPESIS *TRICHODERMA* DAN KESAN PERENCATAN *T. HARZIANUM* (RIFAI) TERHADAP *GANODERMA BONINENSE*

Oleh

MD. SHAFIQUZZAMAN SIDDIQUEE

November 2007

Pengerusi: Profesor Faridah Abdullah, PhD

Fakulti: Sains

Trichoderma adalah sejenis kulat yang hidup dan berasal dari tanah yang sehingga kini telah banyak dikaji dan dilaporkan mengenai kejayaannya sebagai agen kawalan biologi bagi berbagai kulat patogen tumbuhan. Teknik dual kultur telah menunjukkan 18 daripada 48 isolat *T. harzianum* berjaya menghalang pertumbuhan miselia patogen *Ganoderma boninense* (PER71) pada 47.86 hingga 72.06% dengan penghalangan tertinggi telah ditunjukkan oleh strain FA30. Lapan sampel telah menghasilkan sebatian volatil antikulat yang berkesan dalam menindas pertumbuhan patogen pada 24.528 hingga 58.70 % dalam tempoh masa enam hari. Apabila 10 sampel tersebut telah diuji untuk penghasilan sebatian bukan volatil antikulat, didapati semua strain menunjukkan kesan penghalang sebanyak 18.35% hingga 40.16% dalam masa 6 hari. Strain FA30 adalah isolat terbaik bukan sahaja bagi teknik dual kultur malahan terbaik bagi menghasilkan sebatian penghalang volatil dan bukan volatil, pada 58.70 and 40.16%.

Pengenalpastian spesies *Trichoderma* secara meluasnya pada masa ini ditentukan daripada deskripsi mikromorfologi agregrat spesis. Pada kebiasaannya, teknik ini rumit dan cenderung kepada ralat. Menenusi penyelidikan ini pendekatan molekular telah diambil, dengan menggunakan elektroforesis isozim, analisis 'random amplified microsatellite' (RAMS) dan pencirian gen daripada 'internal transcribed spacer-1' (ITS 1) region' daripada ribosomal DNA.

Variasi elektroforetik bagi sembilan sistem isozim telah dikaji pada 47 isolat daripada 3 spesies *Trichoderma,* iaitu *T. harzianum, T. aureoviride* dan *T. longibrachiatum*. Melalui penggunaan analisis kluster UPGMA, populasi *T. harzianum* dan *T. longibrachiatum* secara keseluruhannya dipisahkan antara satu sama lain, manakala *T. aureoviride* menunjukkan persamaan genetik yang rapat dengan populasi *T. harzianum*. Tiada kesimpulan yang tepat dapat dilakarkan daripada dendrogram kerana aras pemisahan mungkin tidak menunjukkan perbezaan spesies. Pendekatan molekular kedua, dengan ekstrak DNA dan analisis menggunakan 'Random Amplified Microsatelite DNA' (RAMS). Dendrogram dengan generat RAMS telah menunjukkan kumpulan *T. harzianum* dan luar kumpulannya adalah jelas terpisah antara satu sama lain tetapi tiada pemisahan yang jelas didapati melalui 'neighbor joining' (NJ).

Pendekatan ketiga pula menggunakan 'internal transcribed spacer 1' (ITS 1) region' daripada rDNA. Turutan nukleotida telah disusun secara rawak dan dibandingkan dengan susunan strain sebelumnya yang diperolehi daripada pangkalan data NCBI dan *Tricho*BLAST Genbank. Keputusan menunjukkan 25 daripada 26 sampel dikenalpasti putatif sebagai *T. harzianum* kerana mempunyai genom yang sama dengan strain *T.*

harzianum yang sebelumnya, manakala satu sampel lagi adalah *T. virens*. Sembilan sampel telah salah dikenalpastikan sebagai *T. aureoviride* kerana 7 daripadanya ialah *T. harzianum* dan 2 lagi ialah *T. virens* berdasarkan pangkalan data Genbank. Stren tunggal *T. longibrachiatum* (IMI: 375055) adalah sama dengan stren sebelumnya. Kajian ini menunjukkan bahawa walaupun cara tradisional mengenalpasti *T. harzianum* telah dilakukan dalam keadaan yang rapi, ia masih boleh membawa kepada pengenalpastian spesis yang salah. Menerusi kajian ini didapati teknik molekular seperti isozim tidak depat megnhasilkan pengasingan yang sempurna pada peringkat spesies. Dendrogram yang dihasilkan menerusi UPGMA bagi teknik analisis RAMS telah menyokong analisis turutan gen ITS 1 , tetapi ia tidak dapat memberikan kepastian mengenai peringkat spesifik spesis.

Kajian ini menunjukkan pencirian gen sampel *Trichoderma* pada ‘non-coding ITS 1 region’ adalah teknik yang tepat untuk pengenalpastian dengan kestabilan ‘bootstrap’ pada 100% dan homologi sebanyak 98-100%.

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I certified that an Examination Committee met on 9th November, 2007 to conduct the final examination of **Md. Shafiquzzaman Siddiquee** on his **Master's degree** entitle "**Molecular Evaluation of Selected *Trichoderma harzianum* Rifai Isolates and its Inhibitory Effects**" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the student be awarded the Master's degree.

Members of the Examination Committee were as follows:

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Date: 17 December 2007

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:

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Date: 22 January 2008

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledge. 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.

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MD. SHAFIQUZZAMAN SIDDIQUEE

Date: 9 November 2007

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