SCREENING OF LOCALLY ISOLATED ACTINOMYCETES AND ENDOPHYTIC FUNGI FOR PRODUCTION OF BIOACTIVE COMPOUNDS

By

JEFFREY LIM SENG HENG

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

April 2006

To My
Dad, Mom, Brother,
Sister, Late Grandmother and
my Wife Christine

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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Chairman: Professor Son Radu, PhD

Faculty: Food Science and Technology

Isolation of actinomycetes were done using Humic acid B-vitamins Agar (HVA) while endophytic fungi were from Potato Dextrose Agar (PDA). Isolated strains were then subjected to enzymatic and also antimicrobial testing. Positive strains for antimicrobial testing were then subjected to carbon source utilization testing and viewed under microscope to determine their spores morphology. From the enzymatic test conducted for actinomycetes, 110 isolates showed positive result for cellulase activity, 107 for xylanase activity and 22 for mannanase activity. Fifteen isolates of endophytic fungi have the ability to degrade cellulose, 28 of the isolates were able to degrade xylan and 12 isolates have the potential to degrade mannan. Thirteen isolates of actinomycetes showed positive result towards the 5 strains of pathogenic microorganisms with the highest on *Yersinia enterocolitica*. While test done using endophytic fungi showed only 1 isolate with antimicrobial property toward *Xanthomonas campestris*. Biolog test was done to determine the metabolite diversity of each actinomycetes. Twelve of the 13 isolates of actinomycetes were identified to be from the genus of *Streptomyces* by observing their

spore arrangement and 1 of the isolate could not be identified. Biolog test could not be done on the endophytes fungi strain 13 because this strain does not produce spores. Through microscopic imaging the identity of the endophytes fungi isolate also could not be determined. Actinomycetes isolate number 200 was further identified by targeting its 16S rRNA gene. By using this technique Actinomycetes isolate number 200 was confirmed to be from the genus *Streptomyces*.

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

PENYARINGAN ISOLAT AKTINOMISET DAN ENDOFIT FUNGI TEMPATAN UNTUK PENGHASILAN KOMPOUN BIOAKTIF

Oleh

JEFFREY LIM SENG HENG

April 2006

Pengerusi: Professor Son Radu, PhD

Fakulti: Sains dan Teknologi Makanan

Pemencilan actinomiset dilakukan dengan menggunakan Agar Asid Humik vitamin B (HVA) dan endofit fungi menggunakan Agar Potato Dextros (PDA). Setiap strain kemudiannya diuji dengan ujian enzim dan juga ujian antimikrob. Strain yang memberikan keputusan positif dalam ujian antimikrob akan diuji kebolehannya menggunakan sumber karbon (Ujian Biolog) dan morfologi sporanya akan diperhatikan dibawah mikroskop. Daripada ujian enzim yang dijalankan terhadap actinomiset, 110 isolate memberikan keputusan positif terhadap selulos, 107 isolate dengan xilan dan 22 isolate dengan mannan. Bagi endofit fungi pula, 15 isolate memberikan keputusan positif untuk ujian selulose, 28 dengan xilan dan 12 dengan mannan. Tiga belas isolate daripada actinomiset memberikan keputusan positif terhadap 5 strain mikrob patogenik dan di dapati kebanyakannya mempunyai ringtangan terhadap *Yersinia enterocolitica*. Hanya satu isolate endofit fungi saja yang memberikan keputusan positif terhadap *Xanthomonas campestris*. Dengan menggunakan Ujian Biolog, kepelbagaian metabolic actinomiset dapat diketahui. Dengan menggunakan mikroskop, 12 daripada 13 isolate actinomiset yang didapati, adalah berasal daripada genus *Streptomyces* berpandukan pada susunan

spora manakala satu lagi isolate tidak dapat dikenalpasti. Ujian biolog tidak dapat dijalankan bagi isolate endofit fungi kerana ianya tidak berspora dan tidak dapat dikenalpasti dengan menggunakan mikroskop. Aktinomiset isolate 200, seterusnya di identifikasikan dengan mensasarkan gen 16S rRNAnya. Melalui teknik ini actinomiset isolate 200 ini di sahkan berasal dari genus *Streptomyces*.

ACKNOWLEDGEMENTS

I would like to take this opportunity to express my deepest gratitude to my main supervisor Professor Dr. Son Radu for his willingness to let me to do my Master's project under his supervision. I would also like to thank him for his support, patience, invaluable advice and guidance through out the time of my study.

Also my deepest thanks to my two co-supervisors, Dr. Sahilah Abd. Mutalib from Malaysia Agricultural Research and Development Institute and also Dr. Clemente Michael Wong Vui Ling for their help and also advices through out my years of studies. My special thanks to Dr. Sahilah for financially supporting me by hiring me as her Research Assistant for the past years. I would also like to thank Malaysia Agricultural of Research and Development Institute for allowing me to work and use every available resource in the laboratory for all these years.

I would also like to thank Pn. Tosiah Sadi from Malaysia Agricultural Research and Development Institute for helping me with all the microscopic work. Without her guidance the microscopic part would not be available.

I would also like to extend my thanks to all those helpful people of the Faculty of Food Science and Technology who has assisted me either directly or indirectly through out the duration of my project. I would also like to take this opportunity to express my gratitude

to Kak Rosnah, Kak Napsiah, Sam and Jacintha for their guidances and assistance in the lab.

Last but not less important is my thanks to my family members for their understanding and also their morale support from the beginning of my project to the end of it and who are still doing so now. May god bless all of you.

I certify that an Examination Committee has met on 4th April 2006 to conduct the final examination of Jeffrey Lim Seng Heng on his Master of Science thesis entitled "Screening of Locally Isolated Actinomycetes and Endophytic Fungi for Production of Bioactive Compounds" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The member of the Supervisory Committee are as follow:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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JEFFREY LIM SENG HENG

Date:

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