PARTIAL PURIFICATION AND CHARACTERIZATION OF A MONOTERPENE SYNTHASE EXTRACTED FROM YOUNG LEAVES OF *MICHELLIA ALBA*

By

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

February 2006

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

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Chairman : Associate Professor Radzali Muse, PhD

Faculty : Biotechnology and Biomolecular Sciences

This study was conducted to partially purify linalool synthase from the young leaves of *Michellia alba* (Cempaka Putih) from the Magnoliaceae family. The technique used to determine the amount of linalool produced from enzyme activity was the combination of solid-phase microextraction (SPME) and gas chromatography with flame-ionization detecter (GCFID) technique. The substrate used for this enzyme activity assay was geranylpyrophosphate (GPP). Optimal conditions such as temperature and incubation time for SPME technique were also determined. The linalool synthase exhibited a strict requirement for a divalent metal cofactor with a preference for Mg^{2+}, Mn^{2+} and K^{+} ions. The optimal pH and temperature of the enzyme was 6.0 and 30^{o}C respectively. The enzyme was inhibited by 1,2-Di(2-aminoethoxy)ethane-N,N,N'N'-tetra-acetic acids (EGTA). Three steps of partial purification of enzyme were carried out, including the crude extraction of young

leaves, ultra centrifugation and Mono-Q anion exchange chromatography. The partially purified linalool synthase was characterized and studied for its enzyme kinetic properties. The linalool synthase has a K_m of 83μM for substrate GPP. The SPME-GCFID technique was later proved to be reliable and sensitive in determination of monoterpene products.

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

PENULENAN SEPARA DAN PENGKAJIAN SIFAT SATU MONOTERPENE SINTASE DARIPADA DAUN MUDA *MICHELLIA ALBA*

Oleh

LEE YUAN CHERN

Februari 2006

Pengerusi : Profesor Madya Radzali Muse, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Kerja penyelidikan ini telah dijalankan bagi penulenan separa satu enzim , iaitu Linalool Sintase daripada daun muda *Michellia alba* (cempaka putih) daripada famili Magnoliaceae. Satu teknik hasil gabungan antara teknik 'Solid-phase microextraction' (SPME) dan 'gas chromatography with flame ionization detecter' (GCFID) telah digunakan untuk mengkaji jumlah linalool yang dihasilkan daripada aktiviti enzim tertentu. Substrat yang telah digunakan untuk aktiviti enzim ini adalah geranil pirofosfat (GPP). Keadaan optimum suhu dan masa pengeraman untuk kaedah SPME juga telah dikaji. Linalool sintase memerlukan ion Mg^{2+}, Mn^{2+} dan K^{+} sebagai kofaktor kation dwivalent. pH dan suhu optimum bagi aktiviti enzim adalah 6.0 dan 30^{o}C masing-masing. Enzim ini juga didapati direncat oleh 1,2-Di(2-aminoethoxy)ethane-N,N,N'N'-tetra-acetic acids (EGTA). Tiga langkah penulenan telah dijalankan, iaitu ekstrak kasar, pengemparan-ultra dan kromatografi pertukaran anion 'Mono-Q' anion. Sifat-sifat dan kajian kinetik enzim yang separa tulen itu juga

telah dikaji. Enzim ini mempunyai nilai K_m 83μM untuk substrat GPP. Teknik SPME-GCFID kemudiannya telah dibukti sensitif dan berguna di dalam menentukan produk monoterpene yang dihasilkan.

ACKNOWLEDGEMENTS

I am greatly indebted to my supervisor, Assoc. Prof. Dr Radzali Muse as well as my co-supervisors, Prof. Dr Mohd. Arif Syed, Assoc. Prof. Dr Mohd. Aspollah Sukari and Dr Mohd. Yunus Shukor for their invaluable guidance, encouragement, help and patience that lead to the completion of this project.

I wish to thank Ministry of Science, Environment and Innovation (MOSTI) for the IRPA grant awarded and Universiti Putra Malaysia for the laboratory and library facilities provided throughout the whole project.

I also wish to express my sincere appreciation and gratitude to the staff of department of Biochemistry for their kind assistance and guidance.

Special thanks are also extended to my colleagues in the Laboratory of Secondary Product Research for their invaluable helps and co-operation during this interesting study.

Last but not least, sincere gratitude to my family for their love, supports and helps throughout the project.

I certify that an Examination Committee has met on 6th February 2006 to conduct the final examination of Lee Yuan Chern on his Master of Science thesis entitled " Partial Purification and Characterization of a Monoterpene Synthase Extracted from Young Leaves of *Michellia alba* " in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the 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 fulfilment of the requirement for the degree of Master of Science. The members 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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