



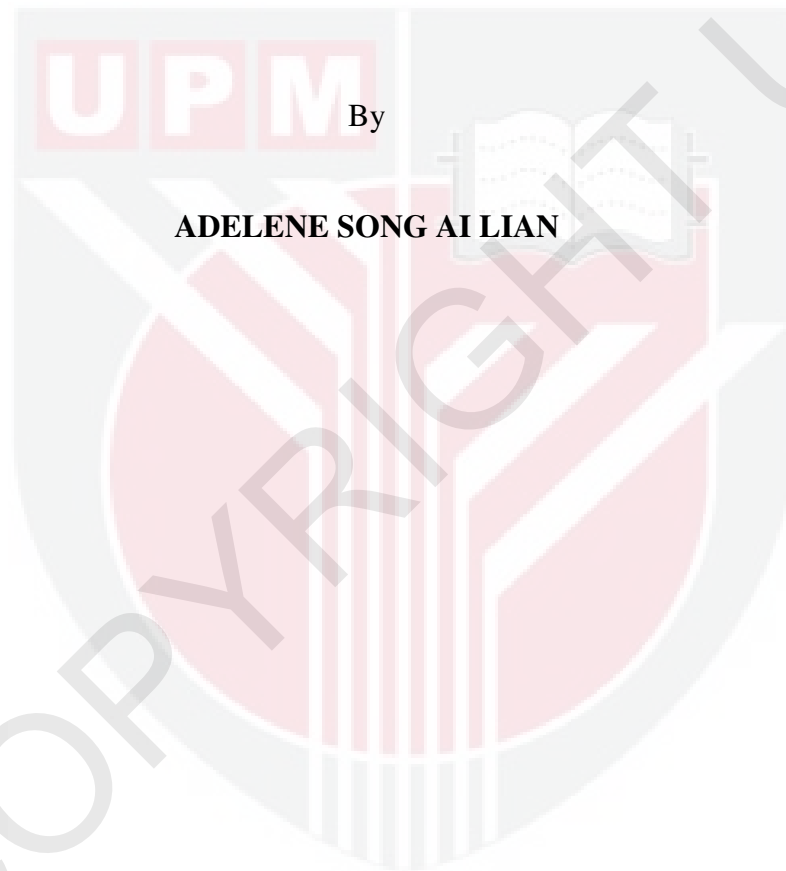
**UNIVERSITI PUTRA MALAYSIA**

***FUNCTIONAL EXPRESSION OF PLANT SESQUITEPENE SYNTHASES  
FOR ISOPRENOID PRODUCTION IN LACTOCOCCUS LACTIS***

**ADELENE SONG AI LIAN**

**FBSB 2012 35**

**FUNCTIONAL EXPRESSION OF PLANT SESQUITEPENE SYNTHASES  
FOR ISOPRENOID PRODUCTION IN *LACTOCOCCUS LACTIS***



By

**ADELENE SONG AI LIAN**

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

**November 2012**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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**Chairman: Prof. Raha binti Haji Abdul Rahim, PhD**

**Faculty: Biotechnology and Biomolecular Sciences**

Plants produce a large variety of isoprenoids as secondary metabolites through the mevalonate (MVA) pathway and the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway. These isoprenoids have appealing characteristics such as flavour, fragrance, and therapeutic properties. However, since plants have many limitations in producing high yields of isoprenoids, systems such as *Escherichia coli* and *Saccharomyces cerevisiae* have been engineered as heterologous hosts to produce plant isoprenoids. *Lactococcus lactis*, a food grade homofermentative bacterium, is another potential heterologous cell factory for plant isoprenoids. Interestingly, *L. lactis* uses the MVA pathway more commonly found in eukaryotes for isoprenoid production. In this study, two different plant sesquiterpene synthases from *Vanda Mimi Palmer* (VMP) and *Polygonum minus*, respectively, were cloned and expressed in *L. lactis*. VMP is a highly scented tropical orchid hybrid of *Vanda tessellata* and *Vanda Tan Chay Yan* while *P. minus* is a highly fragranced local herbaceous plant commonly used for cooking in local delicacies. Four plasmid constructs denoted pNZ:VMPSTS,

pNZ:VMPSTS:mvaA, pNZ:PMSTS and pNZ:PMSTS:mvaA were developed in this study. The sesquiterpene synthases from both plants were expressed optimally at 40 ng/ml nisin, 2 h post-induction. The recombinant proteins were purified and its function identified through *in vitro* enzymatic assays followed by GC-MS analysis. The VMP sesquiterpene synthase was found to produce multiple sesquiterpene products with germacrene D dominating its profile while the sesquiterpene synthase from *P. minus* was identified to be a  $\beta$ -sesquiphellandrene synthase. However, only the recombinant *L. lactis* expressing the *P. minus*  $\beta$ -sesquiphellandrene synthase was able to produce  $\beta$ -sesquiphellandrene *in vivo*. Using this recombinant *L. lactis* strain, an attempt to increase  $\beta$ -sesquiphellandrene production by overexpressing the HMG-CoA reductase (HMGR), an established rate-limiting enzyme in the eukaryotic MVA pathway, was conducted. However, this effort was shown to increase the production of  $\beta$ -sesquiphellandrene only by 1.25-1.60 folds. Therefore, the MVA pathway's transcriptomic profiles of the wild-type *L. lactis* and the various recombinant *L. lactis* constructed in this study were compared in hope of gaining some insights on the prokaryotic MVA pathway and future metabolic engineering strategies which may be feasible for the optimisation of plant isoprenoid production in *L. lactis*. Transcriptomic analysis revealed that HMGR may not be the rate-limiting enzyme in the prokaryotic MVA pathway and instead *mvk* encoding MVA kinase may be a better metabolic engineering gene target for increased isoprenoid production. Also, the genes *mvaD* and *fni* encoding diphosphomevalonate decarboxylase and isopentenyl pyrophosphate isomerase, respectively, may be important genes involved with the ability of the lactococcal host to produce heterologous isoprenoids *in vivo*. In conclusion, apart from demonstrating *L. lactis* as an alternative host for plant isoprenoid production, this study is the first study involving metabolic engineering of

the prokaryotic MVA pathway for increased isoprenoid production and also provides important information on the potential bottle-necks in the lactococcal MVA pathway.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENGEKSPRESAN BERFUNGSI “SESQUITERPENE SYNTHASE” DARI  
TUMBUHAN UNTUK PENGHASILAN ISOPRENOID DI DALAM  
*LACTOCOCCUS LACTIS***

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Tumbuhan menghasilkan pelbagai jenis isoprenoid sebagai metabolit sekunder melalui tapak jalan mevalonate (MVA) dan tapak jalan 2-C-methyl-D-erythritol-4-phosphate (MEP). Isoprenoid ini mempunyai ciri-ciri yang menarik seperti perisa, wangian dan ciri terapeutik. Walau bagaimanapun, oleh kerana tumbuhan mempunyai banyak batasan untuk menghasilkan isoprenoid dalam jumlah yang tinggi, sistem-sistem lain seperti *Escherichia coli* dan *Saccharomyces cerevisiae* telah dijurutera sebagai hos heterologus untuk penghasilan isoprenoid tumbuhan. *Lactococcus lactis*, sejenis bakteria gred makanan yang berciri homofermentasi merupakan kilang sel heterologus untuk penghasilan isoprenoid tumbuhan yang berpotensi. Amat menarik bahawa *L. lactis* menggunakan tapak jalan MVA yang biasanya dijumpai di dalam eukariot untuk penghasilan isoprenoid. Dalam kajian ini, dua “sesquiterpene synthase” dari *Vanda Mimi Palmer* (VMP) dan *Polygonum minus* telah diklon dan diekspreskan di dalam *L. lactis*. VMP merupakan sejenis orkid tropikal yang sangat

beraroma dan merupakan hibrid dari *Vanda tessellata* dan *Vanda Tan Chay Yan*. *P. minus* pula merupakan tumbuhan herba tempatan yang beraroma tinggi yang biasanya digunakan untuk memasak juadah tempatan. Empat plasmid yang dinamakan pNZ:VMPSTS, pNZ:VMPSTS:mvaA, pNZ:PMSTS dan pNZ:PMSTS:mvaA telah dihasilkan di dalam kajian ini. “Sesquiterpene synthase” dari kedua-dua tumbuhan ini telah diekspreskan secara optima dengan 40 ng/ml nisin, 2 jam selepas induksi. Rekombinan protein kemudiannya telah diasingkan dan dikenal pasti melalui pencerakinan enzimatik *in vitro* diikuti dengan analisis GC-MS. “Sesquiterpene synthase” dari VMP didapati menghasilkan pelbagai produk sesquiterpene dengan germacrene D mendominasi profilnya manakala sesquiterpene synthase dari *P. minus* telah dikenal pasti sebagai “ $\beta$ -sesquiphellandrene synthase”. Walau bagaimanapun, hanya *L. lactis* rekombinan yang mengekspreskan “ $\beta$ -sesquiphellandrene synthase” dari *P. minus* berupaya menghasilkan  $\beta$ -sesquiphellandrene di dalam sel. Dengan menggunakan *L. lactis* rekombinan ini, cubaan untuk meninggikan penghasilan  $\beta$ -sesquiphellandrene melalui “overexpression” HMG-CoA reductase (HMGR), enzim kadar-penghad di dalam tapak jalan MVA eukariot telah dilakukan. Walau bagaimanapun, usaha ini telah berjaya meninggikan penghasilan  $\beta$ -sesquiphellandrene sebanyak 1.25-1.60 kali sahaja. Oleh kerana itu, profil transkriptomik tapak jalan MVA untuk *L. lactis* jenis asal berbanding dengan *L. lactis* rekombinan yang telah dihasilkan di dalam kajian ini telah dianalisis dengan harapan untuk mendalami ilmu pengetahuan mengenai tapak jalan MVA di dalam prokariot dan strategi kejuruteraan metabolik yang boleh digunakan untuk meninggikan penghasilan isoprenoid tumbuhan dengan lebih berjaya untuk kajian masa depan. Kajian transkriptomik menunjukkan bahawa HMGR mungkin bukan enzim kadar-penghad di dalam tapak jalan MVA prokariot tetapi *mvk* yang mengekod “MVA

kinase” mungkin merupakan calon kejuruteraan metabolik yang lebih baik. *mvaD* dan *fni* yang mengekod “diphosphomevalonate decarboxylase” dan “isopentenyl pyrophosphate isomerase” juga mungkin memainkan peranan yang penting untuk membolehkan hos *Lactococcus* menghasilkan isoprenoid heterologus di dalam sel. Kesimpulannya, selain dari menunjukkan kebolehan *L. lactis* berfungsi sebagai hos alternatif untuk penghasilan isoprenoid tumbuhan, kajian ini juga merupakan kajian yang pertama yang melibatkan kejuruteraan metabolik ke atas tapak jalan MVA prokariot dan memberi maklumat yang penting mengenai “bottle-neck” yang berpotensi di dalam tapak jalan MVA *Lactococcus*.



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## APPROVAL

I certify that a Thesis Examination Committee has met on 14<sup>th</sup> February 2013 to conduct the final examination of Adelene Song Ai Lian on her thesis entitled “Functional Expression of Plant Sesquiterpene Synthases for Isoprenoid Production in *Lactococcus lactis*” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the University Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

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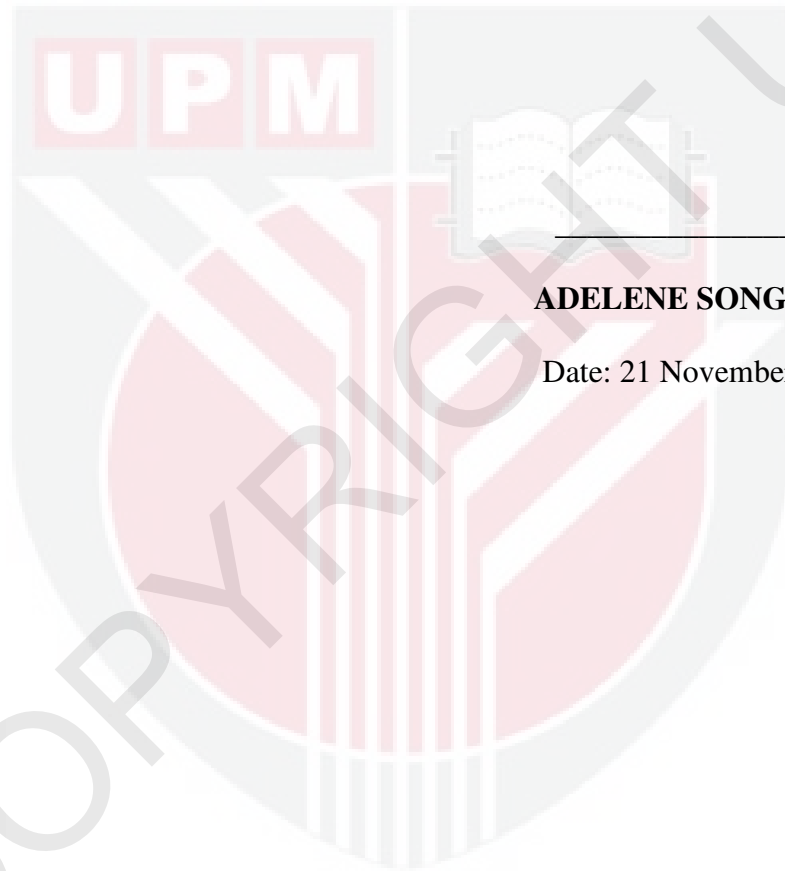
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## DECLARATION

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.



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**ADELENE SONG AI LIAN**

Date: 21 November 2012

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