DEVELOPMENT AND ANALYSES OF EXPRESSED SEQUENCE TAGS FROM *Gracilaria changii* FOR FUNCTIONAL GENOMIC STUDIES

By

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October 2004

Chairman : Ho Chai Ling, Ph.D.

Faculty : Biotechnology and Biomolecular Sciences

Macroalgae from the genus *Gracilaria* is the most common agarophytic genus in Malaysia (Phang *et al.*, 1996). This wild population of seaweed has been identified as an important source of raw material for the agar industry. Despite its potential to produce good gel strength agar, *Gracilaria* sp. was genetically less studied. The aims of this study are to generate and sequence a thousand Expressed Sequence Tag (EST) sequences from *G. changii* for further cDNA microarray to facilitate functional genomic research. RNA extraction from *G. changii* is difficult due to poor yield, polysaccharide contamination and gel formation. To circumvent these problems the RNA isolation procedure was modified and repeated more than 150 times (more than 10 kg of fresh samples were used) to obtain high quality RNA for further studies. From the three modified RNA extraction methods, the modified method of Kim *et al.* (1997) was chosen for rapid RNA isolation from *G. changii*. This method can be completed within 1 day and many samples can be processed at the same

time. The yield was increased from 0.018 μg/g to 1.14 μg/g of tissue with an average purity measured as $A_{(260/280)}$ of 1.90. After the modification, the mRNA was recovered from the total RNA of *G. changii* at a ratio of 0.5 – 1.0%. Starting from 5 μg of mRNA, a primary cDNA library of 1.14 x 10^6 clones was constructed and 1.375 x 10^{10} *pfu*/mL plaques were established for the amplified library. A total of 1854 cDNA clones were successfully sequenced. The database consists of ESTs with putative functions in protein synthesis (6%), energy (4%), protein destination and storage (3%), metabolism (3%), transportation (2%), transcription (2%), signal transduction (1%), cell structure/maintenance (1%), disease and defence (1%), cell growth and division (1%), intracellular traffic (1%) and other miscellaneous functions (2%). Putative proteins with unknown functions (67%), and novel sequences (6%) that do not show significant matches to the existing sequence databases are also present. Among the ESTs, 1342 sequences (72.38%) were clustered as singleton, and the remaining 512 were clustered into 168 contigs.

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

PEMBANGUNAN DAN ANALISIS EXPRESSED SEQUENCE TAGS BAGI *Gracilaria changii* UNTUK KAJIAN GENOMIK FUNGSIAN

Oleh

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Genus *Gracilaria* merupakan makroalga penghasil agar yang paling umum di Malaysia (Phang *et al.*, 1996). Populasi liar rumpai laut ini telah pasti dikenal sebagai sumber bahan mentah penting untuk industri agar. Walaupun ia berpotensi dalam penghasilan agar dengan kekentalan gel yang baik, *Gracilaria* sp. kurang dikaji dari segi genetiknya. Tujuan kajian ini adalah untuk menghasilkan dan menjujukan seribu jujukan EST (Expressed Sequence Tag) dari *G. changii* untuk pengaturan mikro cDNA bagi memudahkan pengajian genomik fungsian. Ekstrasi RNA dari *G. changii* adalah sukar disebabkan penghasilan yang tidak memuaskan, pencemaran polisakarida dan pembentukan gel. Untuk mengelakan masalah-masalah ini, kaedah pemencilan RNA telah diubahsuaikan dan diulang lebih daripada 150 kali (lebih daripada 10 kg sampel segar telah digunakan) untuk memperolehi RNA yang berkualiti tinggi bagi pengajian seterusnya. Daripada tiga kaedah ekstraksi RNA yang telah diubahsuaikan, kaedah Kim *et al.* (1997) yang telah

diubahsuai telah dipilih sebagai kaedah pemencilan RNA daripada *G. changii*. Kaedah ini membolehkan banyak sampel diproses pada masa yang sama dan dapat ditamatkan dalam masa sehari sahaja. Penghasilan RNA telah ditingkatkan daripada 0.018 μg/g kepada 1.14 μg/g tisu dengan ketulenannya ($A_{(260/280)}$) 1.90. Setelah pengubahsuaian, nisbah mRNA yang diperolehi daripada jumlah RNA *G. changii* adalah 0.5 – 1.0%. Bermula dengan 0.5 μg mRNA, suatu khazanah cDNA yang mengandungi 1.14 x 10^6 klon telah dihasilkan. Khazanah cDNA yang telah diamplifikasi mempunyai 1.375 x 10^{10} *pfu*/mL Sejumlah 1854 EST telah berjaya dijujukan. Pengkalan data yang terdiri daripada EST mempunyai fungsi putatif dalam sintesis protein (6%), tenaga (4%), penyimpanan dan destinasi protein (3%), metabolisma (3%), pengangkutan (2%), transkripsi (2%), transduksi isyarat (1%), struktur dan penyelenggaraan sel (1%), penyakit dan pertahanan (1%), pertumbuhan dan pembahagian sel (1%), intraselular trafik (1%) dan lain-lain kepelbagaian fungsi (2%). Protein putatif dengan fungsi yang tidak diketahui (67%) dan jujukan baru (6%) yang tidak menunjukkan persamaan yang signifikan kepada jujukan yang sedia ada pada pengkalan data juga dikesan. Di kalangan EST yang diperolehi, 1342 jujukan (72.38%) dikelompokkan sebagai ‘singleton’ dan yang selebihnya, 512 jujukan, dikelompokkan kepada 168 ‘contigs’.

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Frank Llyod Wright once wrote; “The thing always happen that you believe in and the belief in a thing makes it happen.” This verse had accompanied me throughout my life since I knew it. Making it the principle of my life, I had encountered through many obstacle and thorns along the path of my life but this special verse kept me going on and strong. Most important of all, are those people around me that always lend a hand when I needed them most. Life as a postgraduate student is not as easy as one thought it is. Without the help, guidance, advise and support given by those dear to me, I believe I will never make it this far. Here, I would like to take the opportunity to express my gratitude and appreciation towards their contribution for my current success and well being.

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I certify that an Examination Committee met on date of viva to conduct the final examination of Teo Swee Sen on her degree in Master of Science thesis entitled "Functional Genomic Studies of *Gracilaria changii* (Gracilariales, Rhodophyta) ~ An Expressed Sequence Tag (EST) Approach" 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 requirements for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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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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TEO SWEE SEN

Date:

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