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MOLECULAR CHARACTERIZATION OF GDPMANNOSE PYROPHOSPHORYLASE, GDPMANNOSE-3', 5'-EPIMERASE AND GALACTOSE-1-PHOSPHATE URIDYLYLTRANSFERASE RECOMBINANT PROTEINS FROM GRACILARIA CHANGII I. A. ABBOTT

SIOW ROUH SAN

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By

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

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SIOW ROUH SAN

MASTER OF SCIENCE

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Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

MOLECULAR CHARACTERIZATION OF GDP-MANNOSE PYROPHOSPHORYLASE, GDP-MANNOSE-3', 5'-EPIMERASE AND GALACTOSE-1-PHOSPHATE URIDYLYLTRANSFERASE RECOMBINANT PROTEINS FROM *GRACILARIA CHANGII* I.A. ABBOTT

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2012

Chairman : Assoc. Prof. Dr. Ho Chai Ling, PhD

Faculty : Faculty of Biotechnology and Biomolecular Sciences

Gracilaria changii is a red seaweed which grows in the muddy and silted mangroves fringing the west coast of Peninsular Malaysia such as Morib, Selangor. *Gracilaria* plays an important role in the phycocolloid industry for agar production. GDP-mannose pyrophosphorylase (GMP), GDP-mannose-3', 5'-epimerase (GME) and galactose-1-phosphate uridylyltransferase (GALT) are the enzymes involved in the biosynthesis of D- and L-galactose (basic unit of agar). Although the complete biosynthetic pathway of agar and agarose biosynthesis is not known, the regulating steps of agar and agarose biosynthesis is believed to lie in the intermediate pathways involving the biosynthesis of UDP-D and GDP-L-galactose. The objectives of this study were to express the cDNAs encoding GcGALT, GcGME and GcGMP as recombinant protein in *Escherichia coli* for biochemical assays and to isolate the 5' flanking regions of these three enzymes from *G. changii*. The recombinant proteins of GcGALT and GcGME were successfully expressed as soluble proteins in *E. coli* strain BL21 (DE3) pLysS. The enzyme activity of recombinant GcGALT was

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determined in a coupled assay by monitoring the reduction of NAD and NADP. For the forward reaction, the K_m (UDP-glucose) and K_m(galactose-1-phosphate) were 0.134 mM and 0.116 mM, respectively. For the reverse reaction, K_m(glucose-1-phosphate) and K_m(UDP-galactose) were 0.092 mM and 0.051 mM, respectively. The analysis of high performance liquid chromatography (HPLC) showed that the purified recombinant GcGME formed two products, most probably GDP-L-galactose and GDP-L-gulose. The recombinant protein of GcGMP was expressed as inclusion bodies in *E. coli* strain Origami (DE3) pLysS. The inclusion bodies were solubilized and refolded for enzyme assay using HPLC. However, the refolded recombinant GcGMP did not show any activity. The structural gene sequences of GcGALT, GcGME and GcGMP isolated from the genomic DNA of *G. changii* were devoid of introns. Cis-acting regulatory element related to light, methyl jasmonate responses and meristem specific activation/expression were found at the 5' flanking regions of *GcGALT*, *GcGME* and *GcGMP*. The cis-acting regulatory element involved in light response showed the highest frequency in the 5' flanking regions of *GcGALT*, *GcGME* and *GcGMP*. The molecular and biochemical characterization of recombinant GcGALT, GcGME and GcGMP may facilitate the understanding of agar production in *G. changii*.

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

PENCIRIAN MOLEKUL GDP-MANNOSE PYROPHOSPHORYLASE, GDP-MANNOSE-3', 5'-EPIMERASE DAN GALACTOSE-1-PHOSPHATE URIDYLYLTRANSFERASE PROTEIN REKOMBINAN DARIPADA *GRACILARIA CHANGII* I.A. ABBOTT

Oleh

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Pengerusi : Prof. Madya Dr. Ho Chai Ling

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Gracilaria changii merupakan sejenis rumpai laut merah yang tumbuh di paya bakau berlumpur di sekitar pantai barat Semenanjung Malaysia, seperti Morib di Selangor dan Port Dickson di Negeri Sembilan. *Gracilaria* memainkan peranan penting dalam industri fikokoloid untuk penghasilan agar. GDP-mannose pyrophosphorylase (GMP), GDP-mannose-3', 5'-epimerase (GME) dan galactose-1-phosphate uridylyltransferase (GALT) merupakan enzim yang terlibat dalam biosintesis D- dan L-galaktosa iaitu unit asas untuk pembinaan agar. Walaupun pengetahuan mengenai tapak jalan biosintesis agar dan agaros yang lengkap tidak diketahui, biosintesis agar dan agarosa dipercayai terletak di laluan perantaraan yang melibatkan biosintesis UDP-D dan GDP-L-galaktosa. Objektif kajian ini adalah untuk menghasilkan protein rekombinan dengan mengekspres cDNA yang mengekodkan GcGALT, GcGME dan GcGMP di dalam *Escherichia coli* untuk asai biokimia dan untuk mengasingkan bahagian terapit 5' *GcGALT*, *GcGME* dan

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GcGMP daripada *G. changii*. Protein rekombinan GcGALT dan GcGME dihasilkan sebagai protein yang larut di dalam *E. coli* BL21 (DE3) pLysS. Aktiviti enzim bagi rekombinan protein GcGALT ditentukan oleh asai berpasang dengan memantau reduksi NADP dan NAD. Bagi tindak balas ke depan, K_m (UDP-glucose) dan K_m (galactose-1-phosphate) adalah 0.134 mM dan 0.116 mM masing-masing. Bagi tindak balas ke belakang, K_m (glucose-1-phosphate) dan K_m (UDP-galactose) adalah 0.092 mM and 0.051 mM masing-masing. Analisis kromatografi cecair berprestasi tinggi menunjukkan bahawa protein rekombinan GcGME yang tulen membentuk dua produk, yang berkemungkinan iaitu GDP-L-galactose dan GDP-L-gulose. Protein rekombinan GcGMP dihasilkan sebagai protein yang tidak larut di dalam *E. coli* Origami (DE3) pLysS. Protein rekombinan GcGMP yang tidak larut telah dilarutkan untuk penglipatan semula dan menjalani asai enzim dengan menggunakan kromatografi cecair berprestasi tinggi. Namun, protein rekombinan GcGMP tidak menunjukkan sebarang aktiviti. Jujukan gen struktur *GcGALT*, *GcGME* dan *GcGMP* daripada DNA genomik *G. changii* adalah tanpa intron. Elemen pengawalaturan cis yang berkaitan dengan tindak balas cahaya, metil jasmonat dan pengaktifan/ekspresi meristem dijumpai di bahagian terapit 5’ *GcGALT*, *GcGME* dan *GcGMP*. Elemen pengawalaturan cis yang berkaitan dengan tindak balas cahaya menunjukkan kekerapan tertinggi dalam bahagian terapit 5’ *GcGALT*, *GcGME* dan *GcGMP*. Pencirian molekul dan biokimia *GcGALT*, *GcGME* and *GcGMP* dapat meningkatkan pemahaman tentang penghasilan agar daripada *G. changii*.

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Approval Sheet 1

I certify that a Thesis Examination Committee has met on (4th September 2012) to conduct the final examination of Siow Rouh San on her thesis entitled "Molecular characterization of GDP-mannose pyrophosphorylase, GDP-mannose-3', 5'-epimerase and galactose-1-phosphate uridylyltransferase recombinant proteins from *Gracilaria changii* I.A. Abbott" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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Approval Sheet 2

This thesis was 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 members of the Supervisory Committee were as follows:

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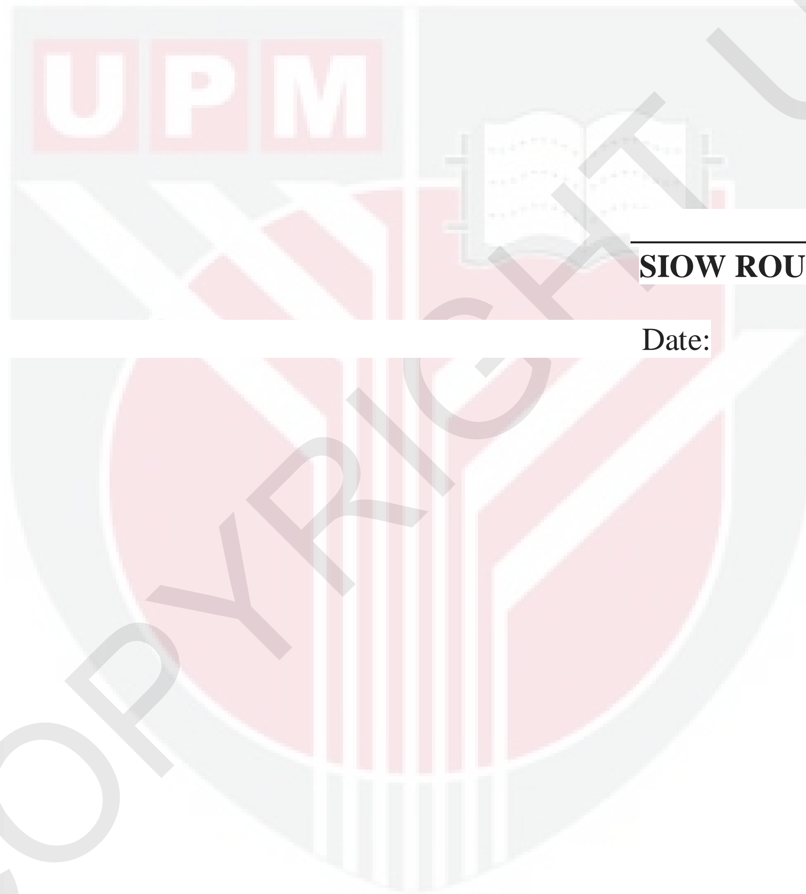
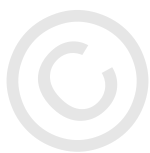
Declaration Form

DECLARATION

I declare that the thesis is my original work except for the 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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SIOW ROUH SAN

Date:

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