

MOLECULAR CHARACTERIZATION OF BETA-KETOACYL-ACP SYNTHASE I (KAS I) GENE AND THE EFFECT OF ABSCISIC ACID (ABA) ON OIL CONTENT, FATTY ACID COMPOSITION AND THE EXPRESSION OF FATTY ACID SYNTHASE GENES IN *CHLORELLA VULGARIS* (STRAIN UMT-M1)

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**Thesis Submitted in Fulfillment of the Requirement for the Degree
of Master of Science in the School of Fundamental Science
Universiti Malaysia Terengganu**

2015

Abstract of thesis presented to Senate of Universiti Malaysia Terengganu in fulfillment of the requirement for the degree of Master Science

Molecular Characterization of Beta-ketoacyl-ACP synthase I (KAS I) gene and the effect of abscisic acid (ABA) on oil content, fatty acid composition and the expression of fatty acid synthase genes in *Chlorella vulgaris* (Strain UMT-1)

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AUGUST 2014

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Beta-ketoacyl-ACP synthase I (KAS I) is the fatty acid synthase (FAS) which catalyze the elongation of growing fatty acid chains from butyryl-ACP (C4:0) to palmitoyl-ACP (C16:0). Thus, KAS I play important role as key gene that involved in fatty acid biosynthesis pathway. In this present study, a full-length KAS I gene was successfully isolated from genomic DNA (4678 bp) and cDNA (1903 bp) of *Chlorella vulgaris* using genome walking approached and RT-PCR, respectively. Sequenced analysis of full-length KAS I cDNA revealed 1,392 bp of open reading frame (ORF), which encodes polypeptide of 464 amino acid residues show high homology (78-82%) to KAS I gene from various plant species such as *Haematococcus pluvialis*, *Arachis hypogaea*, *Helianthus annuus*, *Solanum lycopersicum* and *Glycine max*. The coding sequences of full-length KAS I gene from genomic DNA was interrupted by 10 introns regions. The isolation and characterization of KAS I gene promoter is an important step towards the study of regulatory mechanism of fatty acids (FAs) accumulation at the gene level. Again, the PCR-based genome walking approached was used to isolate the KAS I gene promoter from *C. vulgaris*. PCR amplification from *EcoRV* genomic library produced a putative DNA fragment with approximately 3107 bp. Sequence analysis of the KAS I promoter region using the online PlantCARE and PLACE program demonstrated the presence of potential functional elements in the promoter region such as basic element in eukaryotic gene promoter, several light responsive elements, abscisic acid responsive element (ABRE), gibberallic acid responsive element (GARE) and fungi elicitor responsive element. The present of 2 ABRE indicates possible regulation of the KAS I promoter by ABA. Thus, the effect of exogenously applied abscisic acid (ABA) in the cultures of *C. vulgaris* was studied in term of total oil production, fatty acids accumulation, and expression of KAS I and omega-3-desaturase gene. The results showed that the exogenous ABA (10, 20 and 80 μ M) significantly affects ($p \leq 0.05$) the biomass, total oil content and fatty acids produced by *C. vulgaris* particularly SFAs, PUFAs, C16, C18, C18:2 and C18:3n3. Exogenous ABA at concentration 10, 20 and 80 μ M also significantly down regulated ($p \leq 0.05$) the expression of KAS I and ω -3 desaturase genes. The results of correlation study propose that both KAS I and ω -3 desaturase are the key rate-limiting genes of synthesis of certain fatty acids such as PUFAs, C18:2 and C18:3n3 because their expression showed linear relationships with synthesis of corresponding fatty acids in *C. vulgaris*. Therefore, from this study, it is suggested that *C. vulgaris* culture might be useful for studying the effects of different

exogenously applied phytohormone on lipid metabolic pathway. Further study will also offer precious insight into the regulation of lipid metabolism in this industrially and economically valuable plant system.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Master Sains

Pencirian molekul gen beta-ketoacyl-ACP synthase I (KAS I) dan Kesan Asid Absisik (ABA) pada Kandungan Minyak, Komposisi Asid Lemak dan Pengekspresan Gen Sintase Asid Lemak dalam *Chlorella vulgaris* (Strain UMT-M1)

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Pusat Pengajian: Pusat Pengajian Sains Asas

Beta-ketoacyl-ACP synthase I (KAS I) adalah enzim sintase asid lemak (FAS) yang menjadi pemangkin kepada pemanjangan berkembang rantaian asid lemak dari butyryl-ACP (C4:0) untuk palmitoil-ACP (C16:0). Oleh itu, KAS I memainkan peranan penting sebagai gen utama yang terlibat dalam laluan biosintesis asid lemak. Di dalam kajian ini, panjang penuh gen KAS I telah berjaya dipencilkan daripada DNA genomik (4678 bp) dan cDNA (1903 bp) daripada *Chlorella vulgaris* masing-masing menggunakan genom berjalan mendekati dan RT-PCR, Analisis panjang penuh KAS I cDNA mendedahkan 1,392 bp panjang rangka bacaan terbuka (ORF), yang mengekod polipeptida daripada 464 residu asid amino menunjukkan homologi yang tinggi (78-82%) kepada gen KAS I daripada pelbagai spesies tumbuhan seperti *Haematococcus pluvialis*, *Arachis hypogaea*, *Helianthus annuus*, *Solanum lycopersicum* dan *Glycine max*. Urutan pengekodan panjang penuh gen KAS I daripada genom DNA terganggu oleh 10 kawasan introns. Pemencilan dan pencirian penganjur gen KAS I adalah satu langkah penting ke arah kajian mekanisme pengawalan pengumpulan asid lemak (AL) di peringkat gen. Sekali lagi, genom berasaskan PCR berjalan menghampiri digunakan untuk mengasingkan penganjur gen KAS I dari *C. vulgaris*. Amplifikasi PCR dari perpustakaan genom *EcoRV* menghasilkan serpihan DNA dengan saiz 3107 bp. Analisis kawasan penganjur KAS I menggunakan program PlantCARE dan PLACE dalam talian menunjukkan kehadiran unsur fungsian yang berpotensi di rantau penganjur seperti elemen asas dalam gen penganjur eukariot, beberapa elemen responsif cahaya, elemen responsif asid absisik (ABRE), elemen responsif asid gibberallik (GARE) dan elemen responsif elisitor kulat. 2 elemen ABRE menunjukkan kemungkinan pengawalaturan penganjur KAS I oleh asid absisik (ABA). Oleh itu, kesan eksogenus ABA terhadap pengkulturan *C. vulgaris* telah dikaji dari segi jumlah pengeluaran minyak, pengumpulan asid lemak, dan ekspresi gen KAS I dan omega-3-desaturase (ω -3 desaturase). Keputusan menunjukkan bahawa eksogenus ABA (10, 20 dan 80 μ M) dengan ketara memberi kesan ($p \leq 0.05$) terhadap biomas, jumlah kandungan minyak dan asid lemak yang dihasilkan oleh *C. vulgaris* terutamanya SFA, PUFA, C16, C18, C18: 2 dan C18: 3n3. Eksogenus ABA pada kepekatan 10, 20 dan 80 μ M juga dengan ketara menurunkan ($p \leq 0.05$) ekspresi gen KAS I dan ω -3 desaturase. Keputusan kajian korelasi mencadangkan bahawa kedua-dua KAS I dan ω -3 desaturase adalah kadar-mengehadkan gen utama sintesis asid lemak tertentu seperti PUFA, C18: 2 dan C18: 3n3 kerana ekspresi mereka menunjukkan hubungan linear

dengan sintesis asid lemak yang berkenaan dalam *C. vulgaris*. Oleh itu, daripada kajian ini, adalah dicadangkan bahawa kultur *C. vulgaris* mungkin berguna untuk mengkaji kesan penggunaan eksogenous fitohormon yang berbeza terhadap laluan metabolik lipid. Kajian lanjut juga akan menawarkan pemahaman yang bernilai terhadap pengawalan metabolisma lipid dalam tumbuhan yang berharga dalam perindustrian dan ekonomi ini.