ISOLATION OF DELTA-9 STEAROYL-ACP DESATURASE GENE FROM *Chlorella* sp.

CHIN SHU MEI

FAKULTI SAINS DAN TEKNOLOGI
UNIVERSITI MALAYSIA TERENGGANU

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Isolation of delta-9 stearoyl-acp desaturase gene from Chlorella sp. / Chin Sau Mei.

ISOLATION OF DELTA-9 STEAROYL-ACP DESATURASE GENE FROM *Chlorella* sp.

By

Chin Sau Mei

A thesis submitted in partial fulfillment of
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JABATAN SAINS BIOLOGI
FAKULTI SAINS DAN TEKNOLOGI
UNIVERSITI MALAYSIA TERENGGANU

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Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: Isolation of delta-9 stearoyl-ACP desaturase gene from *Chlorella* sp. Oleh Chin Sau Mei, no. matrik: UK12786 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Sarjana Muda Sains (Sains Biologi) , Fakulti Sains dan Teknologi,Universiti Terengganu Malaysia.

Disahkan oleh: /Verified by:

.....

Penyelia Utama/*Main Supervisor*

Nama: **DR. CHA THYE SAN**

Cop Rasmi: **Pensyarah**
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu.

Tarikh: 30/4/08

.....

Ketua Jabatan Sains Biologi/*Head, Department of Biogical Sciences*

Nama: **PROF. MADYA DR. AZIZ BIN AHMAD**

Cop Rasmi: **Ketua**
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu

Tarikh: 30 APR 2008

DECLARATION

I hereby declare that this thesis entitled Isolation of delta-9 stearoyl-ACP desaturase gene from *Chlorella* sp. is the result of my own research except as cited in the references.

Signature : 

Name : CHIN SAU MEI

Matrix No : UK12786

Date : 30 April 2008

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LIST OF ABBREVIATION

ATP	Adenosine Triphosphate
bp	Basepair
$Cacl_2$	Calcium Chloride
cDNA	Complimentary DNA
DEPC	Diethylpyrocarbonate
DNA	Deoxyribonucleic triphosphate
dNTP	Deoxyribonucleotide triphosphate
E.Coli	*Escherichia Coli*
EDTA	Ethelene Dwiamine tetra-Acetate
G+C	Guanine and Cytosine content
Kb	Kilobase
KCl	Potassium Chloride
LB	Luria-Bertani
MgCl	Magnesium Chloride
mRNA	Messenger ribonucleic acid
NaOH	Sodium Hydroxide
nt	Nucleotide
RNA	Ribonucleic acid

ABSTRACT

Delta-9-stearoyl-ACP desaturase (SACPD) gene is the key gene in converting the saturated stearoyl-ACP (C:18) to monounsaturated oleoyl-ACP (C18:1) in fatty acid biosynthesis pathways. Manipulation of delta-9 stearoyl-ACP desaturase gene is an important step to enhance the accumulation of oleic acid level, so that, more monounsaturated oleic acid can be produced in *Chlorella* sp. The RT-PCR technique was used to isolate the SACPD cDNA clone from *Chlorella* sp. The total RNA was reverse transcribed with KPN-T17 oligo-dT primer by using M-MLV reverse transcriptase. Four forward primers designed from the conserved regions of SACPD gene were used to amplify corresponding 3'-end regions of the gene. PCR amplification were successfully produce three putative DNA fragments with size between 500-1300bp. These three putative fragments were cloned into pGEM-T Vector and the plasmid was extracted from positive recombinant clones. The plasmid were sent for DNA sequencing to determine their nucleotide sequences and the analysis of sequences results showed no homology to the target SACPD gene. Instead, the clones showed homology to coat protein (pD9D-Ch3 and pD9D-Ch4) and 40s ribosomal protein S19 (pD9D-Ch2).

PEMENCILAN GEN DELTA-9 STEAROIL-ACP DESATURASE DARI MIKROALGA MARIN (*Chlorella* sp.)

ABSTRAK

Gen delta-9-stearoil-ACP desaturase (SACPD) adalah gen yang bertanggungjawab untuk menukarkan steroil-ACP (C18:0) tepu kepada oleoil-ACP desaturase tidaktepu (C:18:1) dalam biosintesis asid lemak. Manipulasi gen delta-9 stearoil-ACP adalah satu langkah yang penting untuk meningkatkan tahap pengumpulan asid oleoil dan seterusnya membolehkan *Chlorella* sp. menghasilkan lebih banyak asid oleoil. Kaedah RT-PCR telah digunakan untuk mempencilkan RNA daripada *Chlorella* sp. RNA yang sempurna telah ditranskripsi terbalik dengan menggunakan pencetus KPN-T17 oligo-dT dan M-MLV transkripstase terbalik. Empat pencetus hadapan daripada empat jenis tumbuahan penghasilan lemak telah direka berdasarkan kawasan terabadi delta-9 stearoil-ACP desaturase. Pencetus-pencetus ini digunakan untuk mengamplifikasikan 3' Kawasan penghujung gen. Daripada amplifikasi PCR, telah berjaya mengamplifikasikan tiga produk putative dengan size daripada 500bp ke 1300bp dan diklonkan ke dalam vektor pGEM-T Easy. Plasmid yang berjaya diekstrak telah dihantar untuk penjujukan DNA. Analisis and pencarian homologi di Genbank menunjukan tiada homologi terhadap gene sasaran SACPD. Sebaliknya, ia menunjukan homologi terhadap protein (pD9D-Ch3 and pD9-DCh4) dan 40S ribosomal protein S19 (pD9D-Ch2).