

ISOLATION OF DELTA-9-STEARONL-ACP DESATURASE
GENE FROM MARINE MICROALGAE (*Chlorella* sp.)

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2005

ISOLATION OF DELTA-9-STEAROYL-ACP DESATURASE GENE FROM
MARINE MICROALGAE (*Chlorella* sp.)

By

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Research Report submitted in partial fulfillment of
the requirements for the degree of
Bachelor of Applied Science (Biodiversity Conservation and Management)

Department of Biological Sciences
Faculty of Science and Technology
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
2005

This project should be cited as:

Rajakumar, P.D. 2005. Isolation of delta-9-stearoyl-ACP desaturase gene from marine microalgae (*Chlorella* sp.). Undergraduate thesis, Bachelor of Applied Science in Biodiversity Conservation and Management, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu. 65p.

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 PROJEK PENYELIDIKAN I DAN II**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk:
 Isolation of Delta-9-Stearoyl-ACP Desaturase Gene from Marine Microalgae
 (*Chlorella* sp.)

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ACKNOWLEDGEMENTS

A number of important people have made substantial contribution to the completion of this project and deserve special recognition. First and foremost, I would like to thank my project supervisor, Dr. Cha Thye San, for guiding and supporting me throughout this research. I thank him also for unsparingly enriching my knowledge in the field of molecular biology.

My utmost gratitude also goes out to my parents and brother for their constant moral support, encouragement and prayers.

I thank all my friends, lab assistants and research assistants that have helped me when I encountered problems during the project.

Above all, I thank my God for granting me wisdom, knowledge and understanding, so that this project could be accomplished successfully.

Thank You Everyone!

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LIST OF ABBREVIATIONS

ATP	Adenosine Triphosphoshate
bp	Basepair
CaCl ₂	Calcium Chloride
cDNA	Complimentary DNA
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Trihosphates
<i>E. coli</i>	<i>Eschericia coli</i>
EDTA	Ethelene Dwiamine Tetra-Acetate
G+C	Guanine and Cytosine Content
Kb	Kilobase
KCl	Potassium Chloride
LB	Luria-Bertanni
MgCl ₂	Magnesium Chloride
NaCl ₂	Sodium Chloride
NaOH	Sodium Hydroxide
nt.	Nucleotide
OD	Optical Density
U	Unit
TAE	Tris-bes EDTA

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ABSTRACT

Delta-9-stearoyl-ACP desaturase enzyme catalyzes the first double-bond formation in the fatty acid biosynthesis to convert saturated stearoyl-ACP to monounsaturated oleoyl-ACP. This enzyme plays an important role in the synthesis of unsaturated fatty acids in plant storage oil. Therefore, this gene was isolated using PCR-based method. The primers involved in the PCR amplification are the heterologous gene specific primers, designated SAD-F1, SAD-F2, SAD-R1 and SAD-R2, that were designed based on the conserve region of delta-9-stearoyl-ACP desaturase gene from six different plant species. Two putative bands, designated SAD1 (400 bp) and SAD2 (300 bp) were obtained from PCR amplification and were cloned into the pGEM-T vector. The presence of the DNA insert in the recombinant colonies were confirmed by using Colony-PCR method. Four out of ten colonies screened for both SAD1 and SAD2 showed positive signal. Only one colony from each clone, designated pSAD1 and pSAD2 were selected for plasmid extraction. The *Eco*RI digestion verification further confirmed that pSAD1 and pSAD2 recombinant clones contains the DNA insert of interest. This study could be carried out further by sending the extracted plasmid of the putative recombinant clones for sequence analysis and homology search in the Gene Bank Database.

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

ABSTRAK

Enzim delta-9-stearoil-ACP desaturase memungkinkan proses pembentukan ikatan ganda dua pertama untuk menukarkan stearoil-ACP ke oleoil-ACP dalam biosintesis asid lemak. Enzim ini memainkan peranan penting dalam pembentukan asid lemak tak tepu dalam simpanan minyak tumbuhan. Oleh sebab itu, gen delta-9-stearoil-ACP desaturase telah dipencilkan dari *Chlorella* sp. dengan menggunakan teknik PCR. Pencetus-pencetus yang terlibat dalam amplifikasi PCR ini adalah pencetus gen spesifik heterologous, SAD-F1, SAD-F2, SAD-R1 dan SAD-R2, yang direka berpandukan kawasan terabadi delta-9-stearoil-ACP desaturase daripada enam jenis spesies tumbuhan. Dua produk putatif PCR, SAD1 (400 bp) dan SAD2 (300 bp) yang diperolehi telah diklonkan ke dalam vektor pGEM-T. Saiz DNA selitan koloni rekombinan putatif yang diperolehi dipastikan dengan teknik PCR-Koloni. Empat daripada sepuluh koloni yang dipastikan menunjukkan petunjuk positif. Hanya satu koloni daripada setiap klon dipilih untuk ekstraksi plasmid. Ujian dengan pemotongan oleh enzim *EcoRI*, telah membuktikan bahawa koloni rekombinan, pSAD1 dan pSAD2 mengandungi DNA selitan yang diinginkan. Kajian ini boleh diteruskan dengan menghantar ekstraksi plasmid koloni rekombinan positif yang diperolehi untuk kajian analisis jujukan dan pencarian homologi di Data Gen Bank.