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

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

By

Priscilla Dorothy A/P Rajakumar

Research Report submitted in partial fulfillment of
the requirements for the degree of
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## PENGAKUAN DAN PENGESAHAN LAPORAN 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.)

oleh Priscilla Dorothy A/P Rajakumar UK6671, no. matrik: ..... telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Bacelor Sains Gunaan ( Pemuliharaan & Pengurusan Biodiversiti), Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

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| | |
|---|---|
| ATP | Adenosine Triphosphoshate |
| bp | Basepair |
| $CaCl_2$ | Calcium Chloride |
| cDNA | Complimentary DNA |
| DNA | Deoxyribonucleic Acid |
| dNTP | Deoxynucleotide Trihosphates |
| *E. coli* | *Eschericia coli* |
| 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 |

## LIST OF APPENDICES

## 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 memangkinkan proses pembentukkan ikatan ganda dua pertama untuk menukarkan stearoil-ACP ke oleoil-ACP dalam biosintesis asid lemak. Enzim ini memainkan peranan penting dalam pembentukkan 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 coloni 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 *Eco*RI, telah membuktikan bahawa koloni rekombinan, pSAD1 dan pSAD2 mengandungi DNA selitan yang diingini. Kajian ini boleh diteruskan dengan menghantar ektraksi plasmid koloni rekombinan positif yang diperolehi untuk kajian analisis jujukan dan pencarian homologi di Data Gen Bank.