ISOLATION OF CLEAVAGE TYROSTERASE GENE

FROM MARINE MICROALGAE (*Chlorella* sp.)

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ISOLATION OF OLEOYL-ACP THIOESTERASE GENE FROM MARINE MICROALGAE (*Chlorella* sp.)

By

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PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk:....**Isolation of oleoyl-ACP thioesterase gene from marine microalgae (*Chlorella* sp.)**...oleh ...**Norazrin binti Md Nor**..., no. matrik: ...**UK 7480**... 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, Kolej Universiti Sains dan Teknologi Malaysia.

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~	approximately
ATP	adenosine triphosphate
$CaCl_2$	calcium chloride
cDNA	complimentary DNA
DNA	deoxyribonucleic acid
dNTP	deoxynucleotidetriphosphate
E.coli	*Escherichia coli*
EDTA	ethylenediaminetetraacetate
G+C	guanocine+cytosine content
LB	Luria-Bertani
$MgCl_2$	magnesium chloride
nt	number of nucleotide
NaOH	sodium hydroxide
ω-3	omega-3
ω-6	omega-6
OD	optical density
PCR	polymerase chain reaction
TAE	Tris-Acetate EDTA

APPENDICES

ABSTRACT

Oleoyl-ACP thioesterases (OAT) intercept the 'prokaryotic' pathway by hydrolyzing the newly formed oleoyl-ACP into oleic acids and exit the plastids to supply the 'eukaryotic' lipid biosynthesis pathway. The cDNA clone of this enzyme have been isolated and sequenced in several oil producing crops species such as jojoba and avocado. As an initial step toward understanding the role of oleoyl-ACP thioesterase gene, the genomic DNA was successfully extracted from the marine microalgae (*Chlorella* sp.). Total genomic DNA (0.4 µg) was used to amplified the gene. Two heterologous forward primers and reverse primers designated OAT-F1, OAT-F2, OAT-R1 and OAT-R2 were used with four different combinations. Primer combination of OAT-F2+OAT-R1 successfully produced a clear 300 bp band. The amplified DNA fragment was cloned into pGEM-T Easy Vector through ligation. The plasmid was successfully extracted from a putative pOAT1 clone. *Eco*R1 digestion confirmed that the putative pOAT1 clone contained the DNA insert of interest. This research could be carried out further by sending the extracted plasmid of the putative recombinant clone for DNA sequencing and further analysis.

PEMENCILAN GEN OLEOYL-ACP THIOESTERASE DARIPADA MIKROALGA MARIN (*Chlorella* sp.)

ABSTRAK

Oleoyl-ACP thioesterase (OAT) merupakan enzim yang menyekat tapak jalan 'prokariot' dengan menghidrolisis oleoyl-ACP yang baru terbentuk kepada asid oleik dan dibawa keluar daripada plastid untuk memasuki tapak jalan biosintesis lipid 'eukariot'. Klon cDNA bagi gen oleoyl-ACP thioesterase telah dipencilkan dan dijujuk daripada beberapa spesis tanaman minyak seperti jojoba dan zaitun. Langkah utama bagi mengkaji fungsi gen oleoyl-ACP thioesterase, maka DNA genom bagi mikroalga marin (*Chlorella* sp.) telah berjaya diekstrak. DNA genom (0.4 μg) telah digunakan untuk mengamplifikasi gen tersebut. Dua pencetus heterologous hadapan dan pencetus berbalik dibina iaitu OAT-F1, OAT-F2, OAT-R1 dan OAT-R2 telah diguna dalam empat kombinasi yang berbeza. Kombinasi primer bagi OAT-F2+OAT-R1 telah berjaya menghasilkan saiz jalur yang jelas pada 300 bp. Hasil amplifikasi serpihan DNA telah diklon ke dalam vektor pGEM-T secara ligasi. Plasmid telah berjaya diekstrak daripada klon putatif pOAT1. Klon putatif bagi pOAT1 yang mengandungi selitan DNA yang dikehendaki telah berjaya di kenalpasti melalui pencernaan dengan menggunakan enzim *Eco*R1. Plasmid yang telah dipencil daripada klon rekombinan putatif akan dilakukan penjujukan DNA dan analisis seterusnya.