ISOLATION OF BACTERIAL FOR THERMOSTABLE CELLU FROM MARINE HYDROZOAN (*Obelia* sp.)

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

By

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PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk:
Isolation of Palmitoyl-ACP thioesterase gene from marine microalgae (*Chlorella* sp.) oleh **Siti Mahirah Yusuf**, No. Matrik **UK6726** 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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LIST OF ABBREVIATIONS

ATP	Adenosine Tri-phosphate
$CaCl_2$	Calcium Chloride
cDNA	Complementary Deoxyribonucleic Acid
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide triphosphate
EDTA	Ethylene Diamine Tetra-Acetate
G+C	Guanocine + Cytocine Content
KCl	Pottasium Chloride
LB	Luria-Bertani
$MgCl_2$	Magnesium Chloride
$NaCl_2$	Sodium Chloride
NaOH	Sodium Hydroxide
nt.	Nucleotide
OD	Optical density
TAE	Tris-Acetate-EDTA
Tris-HCL	Tris [Hydroxymethyl] aminomethane hydrochloride
~	Approximately

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ABSTRACT

Palmitate (C16) is the predominant saturated fatty acid that found in plant membrane lipid. The Polymerase Chain Reaction (PCR) method was used to isolate the PAT gene from *Chlorella* sp. culture. The extracted genomic DNA (1 μg) was used for every amplification reaction of PAT gene. Five putative bands were successfully amplified from four different primer combinations such as PAT-F1+PAT-R1, PAT-F1+PAT-R2, PAT-F2+PAT-R1 and PAT-F2+PAT-R2. Two putative bands, PAT1 and PAT2 were selected for further analysis. The size of the PAT1 band is ~550 bp, while the PAT2 is ~350 bp. The DNA of these two bands was successfully recovered from the gel slice and were managed to clone into pGEM-T vector. The putative plasmids of pPAT1 and pPAT2 were successfully isolated and the present of the DNA of interest was further confirmed by using the Colony-PCR method and digestion of plasmids with *Eco*R1enzymes.

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

ABSTRAK

Palmitoil-ACP thioesterase menghidrolisiskan Palmitoil-ACP dan membebaskan palmitat dari plastid ke sitosol sel tumbuhan. Palmitat (C16) adalah asid lemak tepu yang lebih dominan dijumpai dalam membran tumbuhan. Tindakbalas Berantai Polimerase (PCR) telah digunakan untuk memencilkan serpihan gen Palmitoyl-ACP thioesterase (PAT) dari kultur *Chlorella* sp. Genomik DNA yang telah diekstrak (1μg) telah digunakan bagi setiap tindakbalas amplifikasi gen PAT. Lima jalur putatif telah berjaya diamplifikasi daripada empat kombinasi pencetus yang berlainan iaitu PAT-F1+PAT-R1, PAT-F1+PAT-R2, PAT-F2+PAT-R1 dan PAT-F2+PAT-R2. Dua jalur putatif PAT1 dan PAT2 telah dipilih untuk analisis seterusnya. Saiz serpihan bagi PAT1 ialah ~500 bp manakala saiz serpihan bagi PAT2 ialah ~350 bp. DNA bagi kedua-dua jalur ini telah ditulenkan dan diklonkan dalam vektor pGEM-T. Plasmid putatif bagi klon pPAT1 dan pPAT2 telah berjaya dipencilkan dan kehadiran DNA selitan disahkan dengan menggunakan Kaedah Koloni-PCR dan pencernaan dengan menggunakan enzim *Eco*R1.