ISOLATION OF BETA-KETOACYL-ACP SYNTHASE II GENE FROM MARINE MICROALGAE (*Chlorella* sp.)

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Isolation of beta-ketoacyl-acp synthase in gene from marine microalgae (chlorella sp.) / Wan Ratmaazila Wan Makhtar.

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By

Wan Ratmaazila binti Wan Makhtar

Research Report submitted in partial fulfillment 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 Beta Ketoacyl-ACP Synthase II Gene from Marine Microalgae (*Chlorella* sp.) oleh **Wan Ratmaazila binti Wan Makhtar**, No. Matrik **UK 6637**
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

bp	Base pair
$CaCl_2$	Calcium Chloride
cDNA	complementary Deoxyribonucleic Acid
dNTP	Deoxynucleotide triphosphate
DNA	Deoxyribonucleic Acid
E.coli	*Escherichia.coli*
EDTA	Ethylene Diamide Tetra-Acetate
G+C	Guanocine+Cytosine
kb	Kilo base
KCl	Potassium Chloride
LB	Luria-Bertani
$MgCl_2$	Magnesium Chloride
nt	Nucloetide
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
OD	Optical density
TAE	Tris-Acetate-EDTA
~	Approximately

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ABSTRACT

The synthesis of plant fatty acids occurs in a complex pathway. Beta-Ketoacyl-ACP Synthase (KAS II) is the enzyme that converts 16:0-ACP to an 18:0-ACP in fatty acid biosynthesis pathway. The Polymerase Chain Reaction (PCR) method was applied to isolate the KAS II gene from *Chlorella* sp.. The total genomic DNA from *Chlorella* sp. sample was used. Four combinations of different primers: KAS II-F1, KAS II-F2, KAS II-R1 and KAS II-R2 were used during the PCR. The expected KAS II band (650 bp) was successfully amplified and was excised for purification. The KAS II fragment was managed to purify and was cloned into pGEM-T vector through ligation. Plasmid was isolated from putative clone and was successfully digested by using *Eco*R1 enzymes. The digested plasmid was successfully confirmed to contain the DNA insert of interest. This study can be carried out further by sending the extracted plasmid from the putative recombinant clone for sequencing and further analysis.

PEMENCILAN GEN BETA KETOASIL-ACP SINTASE II DARI MIKROALGA MARIN (*Chlorella* sp.)

ABSTRAK

Sintesis asid lemak pada tumbuhan berlaku dalam tapak jalan yang kompleks. Beta-Ketoasil-ACP Sintase (KAS II) adalah enzim yang memangkinkan tindakbalas penukaran 16:0-ACP kepada 18:0-ACP di dalam tapak jalan biosintesis asid lemak. Kaedah Tindakbalas Berantai Polimerase (PCR) telah digunakan untuk memencilkan gen KAS II daripada *Chlorella* sp.. Genomik DNA keseluruhan bagi sampel *Chlorella* sp. telah digunakan. Empat set kombinasi pencetus iaitu: KAS II-F1, KAS II-F2, KAS II-R1 dan KAS II-R2 telah digunakan semasa PCR. Jalur KAS II (650 bp) yang dijangka telah berjaya diamplifikasi dan dipotong keluar untuk ditulenkan. Fragmen KAS II telah berjaya ditulenkan dan telah diklonkan ke dalam vektor pGEM-T secara ligasi. Plasmid telah dipencil daripada klon putatif dan telah berjaya dicerna dengan menggunakan enzim *Eco*R1. Plasmid yang dicerna telah disahkan mengandungi selitan DNA yang dikehendaki. Kajian ini boleh diteruskan dengan menghantar plasmid yang diekstrak daripada klon putatif rekombinan untuk dilakukan penjujukan DNA dan analisis seterusnya.