

ISOLATION OF GAMMA TOCOPHEROL METHYLTRANSFERASE
GENE FROM MARINE MICROALGAE (*Chlorella* sp.)

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ISOLATION OF GAMMA TOCOPHEROL METHYLTRANSFERASE GENE FROM
MARINE MICROALGAE (*Chlorella* sp.)

By

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**JABATAN SAINS BIOLOGI
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**PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: ISOLATION OF GAMMA TOCOPHEROL METHYLTRANSFERASE GENE FROM MARINE MICROALGAE (*Chlorella* sp.) oleh MALINNA BINTI JUSOH, No. Matrik UK6587 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperoleh 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
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Triphosphate
EDTA	Ethylene Diamide Tetra-Acetate
G+C	Guanine and Cytosine Content
Kb	Kilo Base
KCl	Potassium Chloride
LB	Lurie Bertani
MgCl ₂	Magnesium Chloride
NaCl	Sodium Chloride
NADH	Reduced Nicotinamide Adenine Dinucleotide
NADPH	Reduced Nicotinamide Adenine Dinucleotide Phosphate
NaOH	Sodium Hydroxide
nt	Nucleotide
OD	Optical Density
TAE	Tris-Acetate-EDTA
γ-TMT	Gamma Tocopherol Methyltransferase

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ABSTRACT

Tocopherols are essential components of the human diet and are synthesized exclusively by photosynthetic organisms. Tocopherols are micronutrients with antioxidant properties that play important roles in animal and human nutrition. Due to these health benefits, there is considerable interest in identifying the genes involved in tocopherol biosynthesis to allow transgenic alteration of both tocopherol levels and composition in agricultural crops. The Polymerase Chain Reaction (PCR) method was utilized to isolate gamma tocopherol methyltransferase (γ -TMT) gene from *Chlorella* sp. culture. The extracted genomic DNA (1 μ g) was used for each amplification reaction of γ -TMT gene. Four sets of primer combinations (TMTF1+TMTR1, TMTF2+TMTR1, TMTF1+TMTR2 and TMTF2+TMTR2) were used to amplify the γ -TMT gene. Three putative bands were obtained from PCR products. These bands were named as TMT-1, TMT-2 and TMT-3 and have a size of 300 bp, 400 bp and ~500 bp respectively. These putative bands were excised from the gel and the DNA was recovered for DNA cloning. The putative recombinant colonies were selected with ampicillin and the present of the inserted DNA was determined by colony-PCR method. The putative plasmids of pTMT-1, pTMT-2 and pTMT-3 were successfully isolated and the digestion of EcoR1 restriction enzymes further confirmed the present of the DNA inserted.

Pemencilan Gen Gamma Tocopherol Metiltransferase dari Mikroalga Marin (*Chlorella* sp.)

ABSTRAK

Tocopherol merupakan komponen perlu dalam diet manusia dan sebatian ini disintesis secara eksklusif oleh organisma berfotosintesis. Tocopherol adalah mikronutrien bersifat anti-oksida yang memainkan peranan penting dalam nutrisi haiwan dan juga manusia. Oleh yang demikian, kajian tentang gen yang terlibat dalam biosintesis tocopherol adalah sesuatu yang bermanfaat untuk mengubah tahap dan komposisi tocopherol di dalam tanaman pertanian. Kaedah Tindakbalas Berantai Polimerase (PCR) telah digunakan untuk memencilkan serpihan gen gamma tocopherol metiltransferase (γ -TMT) dari kultur *Chlorella* sp.. Genomik DNA (1 μ g) yang berjaya diekstrak digunakan untuk setiap tindakbalas amplifikasi gene γ -TMT. Empat kombinasi pencetus (TMTF1+TMTR1, TMTF2+TMTR1, TMTF1+TMTR2 dan TMTF2+TMTR2) digunakan untuk tujuan amplifikasi, dan tiga jalur putatif telah berjaya didapati dari produk PCR. Jalur-jalur ini dinamakan TMT-1, TMT-2 dan TMT-3 yang masing-masing mempunyai saiz serpihan 300 bp, 400 bp dan ~500 bp. DNA dari ketiga-tiga jalur putatif berjaya dituliskan untuk pengklonan. Koloni rekombinan yang putatif dipencilkan melalui ampicillin dan kehadiran DNA selitan disahkan melalui kaedah Koloni-PCR. Plasmid putatif bagi pTMT-1, pTMT-2 dan pTMT-3 berjaya dipencilkan dan kehadiran DNA selitan sekali lagi disahkan dengan pencernaan enzim *EcoR*1.