ISOLATION OF PHYTOENE SYNTHASE GENE FROM MARINE MICROALGAE (*Chlorella sp.*)

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ISOLATION OF PHYTOENE SYNTHASE GENE FROM MARINE MICROALGAE (*Chlorella* sp.)

By

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LIST OF ABBREVIATIONS

at	*Arabidopsis thaliana*
β- carotene	Beta-carotene
bp	Base pair
cDNA	Complementary DNA
cp	*Citrus x paradise*
dc	*Daucus Carota*
DNA	Deoxyribonucleic acid
GGPP	Geranyl-geranyl pyrophosphate
ha	*Helianthus annuus*
KCl	Potassium chloride
LB	Lurie Bertani
le	*Lycopersicon esculentum*
$MgCl_2$	Magnesium chloride
NaCl	Sodium chloride
NADH	Reduced Nicotinamide Adenine Dinucleotide
NADPH	Reduced Nicotinamide Adenine Dinucleotide Phosphate
NaOH	Sodium hydroxide
nt.	Nucleotide
OD	Optical density

PCR	Polymerase chain reaction
PS	Putative phytoene syntase purified product
pPS	Putative plasmid of phytoene synthase
PDS	Phytoene desaturase
TAE	Tris-acetate-EDTA
Tm	Melting temperature
ZDS	Zeta-carotene desaturase

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ABSTRACT

Pyhtoene synthase gene converts geranylgeranyl pyrophosphate to phytoene in the carotenoid biosynthesis pathway in plants. The cDNA clones of this enzyme have been isolated and sequenced in several carotenoid producing species such as carrot and tomato. Nowadays, microalgae that include *Chlorella* sp. are known as one of the source of beta carotene supplement. There is not much information about the carotenoid biosynthesis process in this species. As an initial step towards the understanding the role of phytoene synthase gene in *Chlorella* sp., we have designed two heterologous forward primers (PS-F1 and PS-F2) and two heterologous reverse primers (PS-R1 and PS-R2) based on the conserved region of phytoene synthase gene from five different plant species. Putative phytoene synthase gene was isolated by using PCR-based method with PS-F2/PS-R2 primer combination, which successfully produced a clear 600 bp band. Methods for the purification and cloning of PCR product from *Chlorella* sp. are presented. *Eco*R1 digestion confirmed the successful cloning of the putative phytoene synthase gene from *Chlorella* sp.. This is the first communication to document on the isolation and cloning of putative phytoene synthase gene in *Chlorella* sp..

PEMENCILAN GEN PHYTOENE SYNTHASE DARI MIKROALGA MARIN (*Chlorella* sp.)

ABSTRAK

Gen phytoene synthase merupakan enzim yang menukarkan dua sebatian geranilgeranil pirofosfat kepada phytoene dalam tindakbalas biosintesis carotenoid pada tumbuhan. Urutan cDNA bagi enzim ini telah dipencil dan di sirikan daripada beberapa spesis yang menghasilkan karotenoid seperti lobak dan tomato. Kini, mikroalga seperti *Chlorella* sp. dikenali sebagai sumber makanan tambahan bagi beta-karotin. Tiada banyak maklumat tentang proses biosintesis karotenoid dalam spesis ini. Sebagai permulaan untuk memahami peranan gen phytoene synthase ini dalam *Chlorella* sp., kami telah mereka bentuk empat pencetus yang heterologus berdasarkan kawasan terpelihara bagi lima spesis tumbuhan yang berbeza. Gen jangkaan phytoene synthase dipencilkan dengan kaedah PCR iaitu dengan kombinasi pencetus PS-F2/PS-R2 yang menghasilkan jalur 600 bp yang jelas. Kaedah untuk menulen dan mengklon gen jangkaan ini turut dinyatakan. Proses pencernaan oleh *Eco*R1 membuktikan kehadiran gen jangkaan phytoene synthase ini. Dokumen ini merupakan dokumen pertama yang menerangkan tentang proses pemencilan dan pengklonan gen jangkaan phytoene synthase dalam *Chlorella* sp..