

ISOLATION OF PHENOTOLE SYNTHASE GENE FROM
MARINE MICROBIAL (Chloroflexus sp.)

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

By

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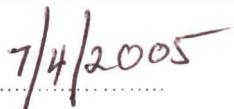
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LIST OF ABBREVIATIONS

| | |
|--------------------|---|
| at | <i>Arabidopsis thaliana</i> |
| β - carotene | Beta-carotene |
| bp | Base pair |
| cDNA | Complementary DNA |
| cp | <i>Citrus x paradise</i> |
| dc | <i>Daucus Carota</i> |
| DNA | Deoxyribonucleic acid |
| GGPP | Geranyl-geranyl pyrophosphate |
| ha | <i>Helianthus annuus</i> |
| KCl | Potassium chloride |
| LB | Lurie Bertani |
| le | <i>Lycopersicon esculentum</i> |
| 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 menukar 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 dipenculkan 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 penceraaan oleh *EcoR*1 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..