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Molecular cloning of a marine bacterial carotenoid biosynthesis gene cluster crt Z

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Introduction

A carotenoid biosynthesis gene cluster crt Z for the production of astaxanthin was isolated from the marine bacterium *Rhodobacter sp.* The crt gene clusters responsible for the biosynthesis of carotenoids such as lycopene, beta-carotene and astaxanthin have been isolated from carotenogenic bacteria such as *Erwinia* species and the marine bacterium *Agrobacterium aurantiacum* (Vinuesa, *et al.* 1998). The crt Z gene products which mediated the oxygenation reactions from beta-carotene to astaxanthin.

Materials and Methods

Isolation and cultivation of bacterial strains: *Rhodobacter sp.* was cultured in LB medium at 25°C.

PCR and sequencing: All primers used for the PCR experiments were synthesized by Bioneer(Korea). The nucleotide sequences of cloned cDNAs were determined by a chain-termination method using BigDye terminator premix kit (Perkin Elmer). Sequencing was carried out using T3 and T7 Universal primers. The fluorescence-labeled nucleotides were analyzed on an ABI PRISMTM 310 automatic sequencer (Perkin Elmer).

Overexpression of CRT Z gene: GST-fusion overexpression system method was used. In order to overproduce the crt z, the gene was using PCR with

oligonucleotide primers, CRT-ZF and CRT-ZR. The PCR product was eluted using Double GeneClean, and ligated into the BamH I/Xho I site of the pGEX-4T2 vector. E.coli BL21(DE3) was transformed with the ligation mixture. Chemical overproduction method was used.

Results and conclusion

The crt Z genomic DNA was 489bp and had encoding 162 amino acids. The sequence has a high degree of identity with other crtZ sequences varying from 97% identity with *Agrobacterium aurantiacum*.

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1  ATGACCAATT TCCTGATCGT CGTCGCCACC GTGCTGGTGA TGGAGTTGAC
51  GGCCTATTCC GTCCACCGTT GGATCATGCA CGGCCCCCTG GGCTGGGGCT
101 GGCACAAGTC CCACCACGAG GAACACGACC ACGCGCTGGA AAAGAACGAC
151 CTGTACGGCC TGGTCTTTGC GGTGATCGCC ACGGTGCTGT TCACGGTGGG
201 CTGGATCTGG GCGCCGGTCC TGTGGTGGAT CGCTTTGGGC ATGACCGTCT
251 ATGGGCTGAT CTATTTCTGC CTGCATGACG GGCTGGTCA TCAGCGCTGG
301 CCGTTCCGCT ATATCCC GCG CAAGGGCTAT GCCCGCCGCC TGTATCAGGC
351 CCACCGCCTG CACCACGCG TCGAGGGACG CGACCATTCG GTCAGCTTCG
401 GCTTCATCTA TCGCGCCCG GTCGACAAGC TGAAGCAGGA CCTGAAGACG
451 TCGGGCGTGC TCGGGGCCGA GGCGCAGGAG CGCACGTGA

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Fig 1. ORF sequences of CRT Z gene

Reference

- Vinuesa, P., Rademaker, Jan L. W., DE Bruijn,, F. J. and Werner, D. 1998. Genotypic Characterization of Bradyrhizobium Strains Nodulating Endemic Woody Legumes of the Canary Islands by PCR- Restriction Fragment Length Polymorphism Analysis of Genes Encoding 16S rRNA (16S) rDNA) and 16S-23S rDNA Intergenic Spacers, Repetitive Extragenic Palindromic PCR Genomic Fingerprinting, and Partial 16S rDNA Sequencing. *App Environl Microbiology*, 64(6), 2096-2104.