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Cloning and Expression of Zeaxanthin Glucosylase (CrtX) from *Paracoccus* sp. BC7417

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Zeaxanthin [(3R,3′R)-β,β-carotene-3,3′diol] is one of the two carotenoids contained within the retina. It is the pigment that gives corn, saffron, and many other plants their characteristic color. Zeaxanthin breaks down to form picrocrocin and safranal, which are responsible for the taste and aroma of saffron. The product of the zeaxanthin glucosylase gene (*crtX*) mediate the formation of zeaxanthin to zeaxanthin diglucoside. Zeaxanthin glucosylase (CrtX) was isolated from the marine bacterium *Paracoccus* sp. BC7417. The *crtX* gene consisted of 1248 bp encoding 415 amino acid residues. The nucleotide sequences of *crtX* was analyzed with that of other species and it turned out to be well conserved during evolution, which was confirmed from a phylogenetic tree. It has been expressed that the CrtX followed by subcloning into the *E. coli* expression vector, pET-44(a)+. The plasmid containing *crtX* gene produced the recombinant protein of 46.9 kDa. The protein was purified to homogeneity and the purified proteins are enzymatically active. The enzymatic properties of gene product of *crtX* gene from *Paracoccus* sp. BC7417 was analyzed by chromatographic and spectroscopic methods.

Key words: Zeaxanthin glucosylase, *Paracoccus*, CrtX, zeaxanthin diglucoside

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Overexpression and Purification of Human Procarboxypeptidase B from Yeast

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Carboxypeptidase B (EC 3.4.17.2, CPB) is a digestive proteolytic enzyme capable of hydrolyzing peptide linkages that involve carboxyl terminal amino acid residues of arginine and lysine in the peptide chains. Also, CPB is a zinc-dependent metallocarboxypeptidase and naturally synthesized in form of zymogen with an 11 kDa N-terminal pro domain that covers the catalytic pocket of the enzyme. A cDNA containing the human pancreatic pro-CPB was cloned and fused to *Saccharomyces cerevisiae* alpha factor-1 secretion signal (MFα1), in which the transcription of MFα 1-pro-CPB was under the control of *GAL10* promoter. The constructed plasmids were pYα-hproCPB (7.72 kb) and pYα-hproCPBI(8.38 kb), which was fused to a polyhistidine tag, and transformed into *S. cerevisiae* cell. The recombinant human pro-CPB (hproCPB) was successfully expressed in *S. cerevisiae* after induction of galactose, and could be secreted into the culture medium. The secreted hproCPB was purified by nickel-nitrotriacetic acid-agarose column. By analyses of SDS-PAGE and western blotting of the purified hproCPB, the molecular weight was estimated to be 45 kDa. The activity of extracellular hCPB after removal of pro-region by trypsin treatment reached about 2.59 unit/ml at the flask culture for 60 hr.

Key words: Carboxypeptidase B, *GAL10* promoter, *Saccharomyces cerevisiae,* alpha factor