

Overexpression of *celB* Gene Coding for β -Glucosidase from *Pyrococcus furiosus* Using a Baculovirus Expression Vector System in Silkworm, *Bombyx mori*

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β -Glucosidase is a member of the glycosyl hydrolases that specifically catalyze the hydrolysis of terminal nonreducing β -D-glucose residues from the end of various oligosaccharides with the release of β -D-glucose. *CelB* gene, encoding the thermostable β -glucosidase, was amplified from the *Pyrococcus furiosus* genome and then cloned into the baculoviral transfer vector under the control of the *polyhedrin* gene promoter. After co-transfection with the genetically modified parental *Bombyx mori* nucleopolyhedrovirus (BmNPV), the recombinant virus containing *celB* gene was used to express β -glucosidase in silkworm. The recombinant β -glucosidase was purified to about 81% homogeneity in a single heat-treatment step. The optimal activity of the expressed β -glucosidase was obtained at pH 5.0 and about 105 °C; divalent cations and high ionic strength did not affect the activity remarkably. This suggested that the enzymatic characteristics of recombinant β -glucosidase were similar to the native counterpart. The expressed β -glucosidase accounted for more than 10% of silkworm total haemolymph proteins according to the protein quantification and densimeter scanning. The expression level reached 10,199.5 U per ml haemolymph and 19,797.4 U per silkworm larva, and the specific activity of the one-step purified crude enzyme was 885 U per mg. It was demonstrated to be an attractive approach for mass production of thermostable β -glucosidase using this system.

Key words: β -Glucosidase, *Pyrococcus furiosus*, Baculovirus Expression Vector System