STUDY ON THE CHITIN-DEGRADING ENZYMES FROM EXOPALAEMON CARINICAUDA

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Degradation and synthesis of chitin in crustacean plays an important role in growth and resistance of pathogen infection. Chitinase, an indispensable enzyme in crustacean, can degrade chitin to make the process be performed. By sequencing and analyzing the transcriptome of the ridgetail white prawn Exopalaemon carinicauda, 17 sequences were annotated to be chitinase. By analyzing the data further and confirmation by amplification the partial sequence using PCR, 6 sequences belonged to chitinase. Based on the above results, we attempt to isolate the native chitinase proteins in E. carinicauda using ion-exchange column chromatography (IEC), and molecular sieve chromatography. At least two kinds of chitinase (EcchiA and EcchiB) were purified from hepatopancreas of this species finally. They were showed an apparent molecular mass of 63kDa and 40kDa by SDS-PAGE, respectively. Based on the results of LC-ESI-MS-MS and the data of transcriptome, the nucleotide sequences and open reading frame (ORF) of these two kinds of chitinase were obtained. The mature peptide of the two ORFs were amplified by PCR and cloned into the expression vector pCT7-CHISP6H. After induced with IPTG, high levels of chitinolytic activity were detected in the cell-free culture supernatant of E. coli BL21(DE3) cells harboring the recombinant plasmid. The secreted recombinant proteins were purified by His-Tag affinity chromatography. The difference of the enzymatic characteristics and the mechanism of substrate hydrolysis between the native chitinases and recombinant proteins were analyzed. After that, the differential expression of the chitinases in mRNA level was analyzed during the embryonic and larval development process, in different organs and different molting period. And then, the dsRNA for special chitinase was synthesized and injected into shrimp. The results of the interference were valued by the observation of the survival, ecdysis, etc.

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