

## Molecular cloning and expression of I-type Lysozyme and phage-type like lysozyme gene from freshwater shellfish

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**ABSTRACT** Lysozyme is a widely distributed hydrolase possessing lytic activity against bacterial peptidoglycan, which enables it to protect the host against pathogenic infection. In the present study, A i-type lysozyme, designated as CpPLYZ3, from *Cristaria plicata* and a phage-type lysozyme, designated as HcPLYZ, from *Hyriopsis cumingii* were identified by rapid amplification of cDNA ends and nested PCR. The complete CpPLYZ3 cDNA sequence comprises of 968 bp, and 432 bp of open reading frame (ORF) coded for a 143 amino acids. The multiple sequence alignment analysis indicated that this gene had the two highly conserved i-type lysozyme activity sites (Glu61 and Ser72). The sequence homology analysis found that this gene had 27~87% the high homology with the other i-type lysozyme gene. The full-length cDNA sequence of HcPLYZ was 829 bp with the open reading frame of 468 bp, and encoded a polypeptide of 154 amino acids. The deduced amino sequence of HcPLYZ had no signal peptide and the mature peptide harbored three cysteine residues, and contained two highly conserved phage-type lysozyme activity sites (Glu20 and Asp29). Real-time quantitative-PCR analysis showed that the mRNA expression of CpPLYZ3 was significant high in hepatopaneas, followed by the mantle and gill. The transcripts of HcPLYZ was constitutive expression in all detected tissues with a high level in gills. The expression levels of CpPLYZ3 and HcPLYZ in hemocytes, hepatopaneas and gills of *C. plicata* or *H. cumingii* were increased significantly after the *Aeromonas hydrophila* and PGN challenged. These results indicated the involvement of CpPLYZ3 and HcPLYZ in the innate immunity of freshwater shellfish.

In order to further inquire into the functions of HcPLYZ, PET30-PLYZ prokaryotic expression system was constructed by double digestion, and prokaryotic

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expression plasmid was transformed into *Escherichia coli* (DE3). The recombinant protein was successfully expressed after IPTG induction, and was purified by using the native Ni<sup>2+</sup> affinity chromatography. *Micrococcus luteus* as substrate, the optimum pH of the recombinant HcPLYZ was 5.5, and the optimum temperature was 50 °C.

**Keywords:** *Cristaria plicata*, *Hyriopsis cumingii*, Lysozyme, Gene cloning, Recombinant protein, Bacteriolytic activity