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Cathepsin L of the sea cucumber *Apostichopus japonicus*- molecular characterization and transcriptional response to *Vibrio splendidus* infection.

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Abstract

Cathepsin L, a lysosomal endopeptidase, has been noted for its involvement in the innate immune response in invertebrates. Here, the cathepsin L cDNA of the sea cucumber *Apostichopus japonicus* (*Aj*CatL) is identified from an EST library and then cloned by the rapid amplification of the cDNA ends (RACE) PCR. The full-length cDNA is 1678 bp long containing an open reading frame (ORF) of 1002 bp, an 80 bp 5’ UTR and a 599 bp 3’ UTR. The cDNA encodes 333 amino acid residues with a predicted molecular mass of 37.07 kDa and a theoretical isoelectric point (pI) of 5.01. The full-length *Aj*CatL contains three active sites of eukaryotic thiol (cysteine) protease at positions 133-144, 278-288 and 295-314. Analysis of the predicted tertiary structure of prepro-CatL (17-333 aa) and mature-CatL (116-333 aa) reveals that the propeptide region (17-115 aa) blocks access to the substrate-binding cleft. Phylogenetic analysis shows that the *Aj*CatL is clustered together with two other CatLs from *Strongylocentrotus purpuratus*. The enzymatic activity of *Aj*CatL was verified using a substrate hydrolyzing assay with recombinant m*Aj*CatL. Further analysis of real time-PCR demonstrates that the expression of *Aj*CatL mRNA is significantly up-regulated in the coelomocytes in cases of infection with the common bacterial pathogen, *Vibrio splendidus*. This suggests that the *Aj*CatL is likely to be involved in the immune response.