

研究论文

企鹅珍珠贝组织蛋白酶D的cDNA克隆、序列特征分析和应激表达研究

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摘要:

笔者初步研究了企鹅珍珠贝 (*Pteria penguin*) 组织蛋白酶D (cathepsin D, CTSD) 的基因克隆和功能, 通过同源克隆方法和cDNA末端快速扩增技术 (RACE) 获得了企鹅珍珠贝组织蛋白酶D基因 (命名为 *pgCTSD*)。该基因cDNA全长1 767 bp, 其中5' UTR为38 bp, 3' UTR为553 bp, ORF为 1 176 bp, 编码392个氨基酸, 包括信号肽 (Met₁-Ala₁₈)、前体域 (Leu₁₉-Lys₄₇) 和成熟域 (Tyr₄₈-Ser₃₉₂) 三部分, 分子量为42.3 kDa, 等电点为8.04。pgCTSD氨基酸序列与大珠母贝 (*Pinctada maxima*) pmCTSD的相似性最高 (79%), 与其他物种的相似性为59%~75%。荧光定量分析表明, 空白对照组中 *pgCTSD* mRNA在闭壳肌、性腺、肝胰脏、外套膜和鳃组织中都有表达, 且在闭壳肌中表达量最少, 肝胰脏中最高。与试验对照组相比, 脂多糖(LPS)刺激6 h后性腺和肝胰脏显著下降, 闭壳肌的表达量虽不大但增加显著, 外套膜和鳃组织变化不显著; 哈维弧菌 (*Vibrio harveyi*) 刺激6 h后肝胰脏和外套膜显著下降, 闭壳肌和鳃显著上升, 性腺无显著变化。肝胰脏中pgCTSD对LPS和弧菌刺激的应答反应表明pgCTSD可能参与了免疫反应。

关键词: 企鹅珍珠贝 组织蛋白酶D LPS刺激 弧菌刺激 组织表达模式

cDNA cloning, characterization and challenge-based expression profiles of cathepsin D in winged pearl oyster *Pteria penguin*

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Abstract:

We cloned the cathepsin D gene of winged pearl oyster (*Pteria penguin*) and conducted preliminary studies on its expression profiles. The cDNA sequence of cathepsin D was obtained from *P. penguin* (named as *pgCTSD*) by using homology cloning method and RACE approach. The full length of the cDNA is 1 767 bp long, with a 5' UTR of 38 bp, an ORF of 1 176 bp, encoding 392 amino acids and a 3' UTR of 553 bp. The predicted amino acid sequence is consisted of a signal peptide of 18 aa, a pro-sequence of 29 aa and a mature protein of 345 aa, with an estimated isoelectric point of 8.04 and molecular mass of 42.3 kDa. The amino acid sequence of its pgCTSD is highly similar with that of *Pinctada maxima* (79% similarity), and shares 59%~75% similarity with other organisms. The fluorescent quantitative analysis suggests that *pgCTSD* mRNA expresses in all the tissues tested including adductor muscle, gonad, hepatopancreas, mantle and gill, with higher expression level in hepatopancreas and lower level in adductor muscle. Compared with the control, the expression level of pgCTSD in the LPS-challenged test groups decreases in tissues of gonad and hepatopancreas significantly but increases in adductor muscle in 6 h after injection of LPS, yet no obvious change was observed in mantle or gill tissues. In 6 h after injection of *Vibrio harveyi*, the expression profile of pgCTSD decreases in hepatopancreas and mantle but increases in adductor muscle and gill, yet no significant change is found in gonad. The cDNA expression's response to challenges of LPS and *Vibrio* bacteria in hepatopancreas, suggests pgCTSD's participation in immune reaction.

Keywords: *Pteria penguin* cathepsin D LPS challenge *Vibrio* challenge tissue expression profile

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