

荒漠昆虫小胸鳖甲抗菌肽Attacin基因的克隆及低温表达模式分析

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Cloning and expression profiling of an attacin gene in response to cold stress in the desert beetle *Microdera punctipennis* (Coleoptera: Tenebrionidae)

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摘要 昆虫在低温冷驯化过程中会发生很多基因表达的改变, 从转录组层面上开展广泛的研究有助于全面认识昆虫对冷响应的分子机制。为了对荒漠昆虫小胸鳖甲*Microdera punctipennis* 的 4℃低温转录组数据中一个上调表达的Attacin基因 (*MpAttacin1*) 进行深入了解并分析其对低温诱导的响应情况, 本研究通过生物信息学分析对该基因进行了鉴定, 并利用实时荧光定量PCR对其在低温下的mRNA水平进行了检测。结果表明: 获得的*MpAttacin1* cDNA长度为523 bp, 包含一个456 bp的开放阅读框和67 bp的5'端非翻译区。*MpAttacin1*编码含有151个氨基酸残基的多肽, N端含有17个氨基酸的信号肽序列。同源性分析表明, 其氨基酸序列与其他鳞翅目、双翅目和鞘翅目昆虫的抗菌肽Attacin具有30%~40%的一致性。以邻接法 (neighbor-joining, NJ) 构建的系统进化树表明, 小胸鳖甲Attacin1与其他鞘翅目昆虫的Attacin起源于共同的祖先, 属于Attacin_C超家族。实时荧光定量PCR分析结果显示, 在4℃与-4℃低温胁迫时, *MpAttacin1*基因的转录都呈现先升高后降低的应激反应趋势, 但在对低温的响应时间和强度上有所不同。在4℃处理5 h和9 h后, *MpAttacin1*的表达量分别为对照组的2.3和3.8倍, 而在-4℃处理7 h和9 h后, 分别为对照组的2.4和1.5倍。这些研究表明, 除已知的抗菌功能外, Attacin在昆虫的低温适应过程中可能也发挥着重要的作用。

关键词: 荒漠昆虫 小胸鳖甲 冷响应基因 攻击素 低温 抗菌肽 表达谱

Abstract: Cold acclimation usually alters gene expression in insects. Extensive studies from the genomic level will help to fully understand the molecular mechanism of insects in response to cold. In order to get the further information about the up-regulated attacin gene (*MpAttacin1*) obtained from the transcriptomic data generated at 4℃ from the desert beetle *Microdera punctipennis*, and to analyze the responsive expression of this gene induced by low temperature, *MpAttacin1* was characterized by bioinformatic analysis. Real-time quantitative PCR was performed to detect the mRNA level of *MpAttacin1* at low temperatures. The results showed that the obtained *MpAttacin1* cDNA is 523 bp with an open reading frame of 456 bp and the 5' -untranslated region of 66 bp. It encodes a polypeptide of 151 amino acid residues containing a putative signal polypeptide of 17 amino acids at the N terminal end. Homology analysis showed that the encoded product of this gene shares 30%-40% identity at the amino acid level with attacins from other insects of Lepidoptera, Diptera, and Coleoptera. The phylogenetic tree generated by Neighbor-Joining method indicated that *MpAttacin1* and attacin proteins from other coleopterans are descended from a common ancestor, and they belong to Attacin_C superfamily. Analysis of real time quantitative PCR showed that the expression of *MpAttacin1* presented a stress-response tendency when stressed at both 4℃ and -4℃, increasing first and then decreasing. However, there were differences in their responsive time and strength between these two treatments. The mRNA level of *MpAttacin1* at 4℃ for 5 h and 9 h was 2.3- and 3.8-fold as high as that of the control at room temperature, respectively, while that at -4℃ for 7 h and 9 h was 2.4- and 1.5-fold as high as that of the control, respectively. The results suggest that in addition to the typical function as an anti-microbial peptide, attacin may also be involved in cold adaptation in insects.

Key words: Desert insect *Microdera punctipennis* cold-responsive genes attacin low temperature
antibacterial peptide expression profiling

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