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蜜蜂表皮蛋白*apidermin*（*apd*）基因家族3个新成员的特性鉴定及昆虫APD家族序列特征的分析

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Characterization of three new members of the *apidermin* (*apd*) gene family from honeybees and sequence analysis of the insect APD family

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摘要 Apidermin (APD)蛋白家族是一个新的昆虫结构性表皮蛋白家族。本研究结合生物信息学和RT-PCR扩增， 对意大利蜜蜂*Apis mellifera ligustica*（简称“意蜂”）的apd-1-like, apd-3-like和中华蜜蜂*Apis cerena cerena*（简称“中蜂”）的apd-2 等3个新的apd基因的结构特征和表达进行了分析， 并分析了昆虫APD蛋白家族的序列特征。结果显示， 在西方蜜蜂*Apis mellifera*（简称“西蜂”）中， apd基因家族的6个成员串联排列在基因组序列第4号连锁群上， 它们在*A. m. ligustica*雄蜂头部中的转录水平差异明显， 且其启动子序列所含顺式元件也不同。中蜂apd-2和意蜂apd-1-like都含有3个外显子和2个内含子， 而意蜂apd-3-like则由4个外显子和3个内含子组成。蛋白序列分析结果显示， 目前已知的10条APD蛋白序列N末端均具有相似的信号肽序列，其成熟蛋白分子量为6.0~37.0 kD， pI为6.2~10.8。其中西蜂的APD1-3、APD-like和东方蜜蜂*Apis cerena*的APD-2等5条较短的多肽中疏水氨基酸残基达52%~67%， 且Ala含量最为丰富（占25%~34%）； 而丽蝇蛹集金小蜂*Nasonia vitripennis*的APD 1-3和西蜂APD-1-like， APD-3-like等另外5条APD多肽富含Gly（21%~30%）， 其序列中疏水氨基酸残基含量为35%~41%。多肽序列多重比对和系统进化分析结果显示， APD家族可划分为2个亚家族。亚家族Ⅰ含有西蜂APD 1-3和东方蜜蜂APD-2等4条较短的多肽序列， 其N末端为一个长33 aa的保守基序； 亚家族Ⅱ由另外6条相对较长的多肽序列组成， 其N末端保守基序长50 aa, C末端保守基序长16 aa。本文所描述的APD蛋白家族序列特征有助于以后从其他昆虫中鉴定新的apd基因。

关键词： 西方蜜蜂 东方蜜蜂 表皮蛋白 *apidermin*基因 APD家族 序列分析 生物信息学分析

Abstract： The apidermin (APD) protein family is a novel structural cuticular protein family of insects. To gain a better understanding of this protein family, by using bioinformatics and RT-PCR amplification, we identified three novel *apd* genes (apd-1-like, apd-3-like from *Apis mellifera ligustica*, and apd-2 from *Apis cerana cerana*) and investigated their structural features, and then we revealed the characteristic motifs of the APD family. The results showed that a cluster of six apd genes were tandemly arranged on chromosome 4 of *Apis mellifera*. These apd genes were differentially expressed in drone head of *A. m. ligustica*, and their expression pattern was consistent with that of the *cis*-acting elements in their promoter sequences. The genomic DNA of apd-2 from *A .c. cerana* and apd-1-like from *A. m. ligustica* contain three exons and two introns, while that of apd-3-like from *A. m. ligustica* contain four exons and three introns. As the deduced proteins of the apd genes were analyzed, it was found that the presently available 10 APDs possess similar N-terminal signal peptide sequences. The mature APD proteins ranged in size from 6.0 to 37.0 kD, and their pI ranged from 6.2 to 10.8. Intriguingly, five small APD proteins, including APD-2 from *A. cerana*, and APD 1-3 and APD-like from *A. mellifera*, were rich in hydrophobic amino acid residues (52%-67%) with Ala being the most abundant (25%-34%). However, the other five larger APDs, including APD 1-3 from Nasonia vitripennis, and APD-1-like and APD-3-like from *A. mellifera*, were Gly-rich (21%-30%) proteins with hydrophobic amino acid residues constituting 35%-41% of their amino acid sequences. Multiple alignment and phylogenetic analysis revealed that the APD protein family could be classified into two subfamilies. Subfamily Ⅰ, which contains four low complexity sequences (APD 1-3 from A. *mellifera* and APD-2 from *A. cerana*), was characterized by a 33-aa N-terminal conserved motif. The other six larger APD proteins were classified as subfamilyⅡ, which was featured by a 50-aa N-terminal motif and a 16-aa C-terminal motif. The diagnostic features of APD protein family described here will be very helpful for identifying novel apd genes from arthropods.

Key words: *Apis mellifera* *Apis cerana* cuticular protein *apidermin* gene APD family sequence analysis bioinformatical analysis

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