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研究论文

卡尼鄂拉蜂锌转运蛋白-7相似蛋白（ZnT-7-like）的基因克隆、 抗体制备及差异表达

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Gene cloning, polyclonal antibody preparation and differential expression of zinc transporter 7-like (ZnT-7-l in *Apis mellifera carnica* (Hymenoptera: Apidae)

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摘要 【目的】本研究旨在克隆卡尼鄂拉蜂*Apis mellifera carnica*锌转运蛋白 7相似蛋白（zinc transporter 7-like, ZnT-7-like）基因， 制备ZnT-7-like多克隆抗体， 了解该基因在卡尼鄂拉蜂王浆腺中不同时期的差异表达情况。【方法】运用RT PCR法从卡尼鄂拉蜂王浆腺总RNA中扩增ZnT-7-like基因， 进行生物信息学分析， 为避开跨膜结构的干扰， 选择克隆其部分序列（273 bp）作为多肽免疫序列。将其亚克隆入原核表达载体pGEX-4T-1， 转入大肠杆菌*Escherichia coli* BL21 （DE3）中诱导表达获得融合蛋白， 然后将融合蛋白纯化后免疫新西兰大白兔制备多克隆抗体， 分别用间接ELISA和Western blot检测抗体的效价和特异性， 最后采用实时荧光定量RT-PCR以及Western blot技术检测该基因在不同日龄成虫王浆腺中的相对表达量。【结果】克隆得到卡尼鄂拉蜂ZnT-7-like基因， 大小为1 065 bp。SDS-PAGE电泳结果显示融合蛋白成功表达； 制备的多克隆抗体效价高达1∶64 000， 且具有很高的特异性。ZnT-7-like在卡尼鄂拉蜂5个日龄成虫中的转录情况存在较大差异， 表现为3日龄成虫中的表达量极显著高于其他日龄（$P<0.01$）， 12日龄表达量最低， 其他日龄间表达量两两差异显著($P<0.05$)或极显著（$P<0.01$）；ZnT-7-like蛋白表达与转录水平基本一致。【结论】成功克隆了卡尼鄂拉蜂ZnT-7-like基因， 制备了兔抗蜂ZnT-7-like多克隆抗体， 并在转录和翻译两个水平上测定了ZnT-7-like在卡尼鄂拉蜂王浆腺中不同时期的相对表达量。这些结果为深入研究卡尼鄂拉蜂ZnT-7-like基因的功能奠定了基础。

关键词： 卡尼鄂拉蜂 王浆腺 锌转运蛋白 7相似蛋白 基因克隆 抗体制备 差异表达

Abstract： 【Aim】 In this study, we cloned a zinc transporter 7-like (ZnT-7-like) gene in *Apis mellifera carnica* in order to prepare the polyclonal antibody against recombinant ZnT-7-like, and to understand ZnT-7-like gene in hypopharyngeal glands of *A. m. carnica* differentially expressed in different periods. 【Methods】 The ZnT-7-like gene in *A. m. carnica* was amplified by RT-PCR method from the total RNA of hypopharyngeal glands. To avoid the interference of transmembrane domains, the partial sequence (273 bp) was chosen as peptide sequence through bioinformatics analysis, then sub-cloned into prokaryotic expression vector pGEX-4T-1 and expressed in *E. coli* BL21 (DE3) host cells. Fusion protein was purified and used to immunize New Zealand white rabbits so as to prepare polyclonal antibody. We also detected the sensitivity and specificity of the polyclonal antibody prepared through indirect ELISA and Western blot methods, respectively. Finally, the expression levels of this gene in different day-old adults were detected by real-rime quantitative RT-PCR and Western blot methods. 【Results】 ZnT-7-like gene was cloned from *A. m. carnica* , and the obtained fragment was 1 065 bp in length. SDS-PAGE electrophoresis showed that the fusion protein was well expressed. The anti-ZnT-7-like polyclonal antibody showed high sensitivity (1∶64 000) and specificity. There was a big difference in the transcriptional level of ZnT-7-like gene in different day-old *A. m. carnica* adults. The expression level of 3-day-old bees was extremely significantly higher than that of other ages ($P<0.01$), the 12-day-old bees showed the minimum expression level, and significant ($P<0.05$)or extremely significant differences ($P<0.01$) existed among 6-day-old, 9-day-old and 12-day-old bees. The protein expression level of ZnT-7-like was generally consistent with the transcription level. 【Conclusion】 The ZnT-7-like gene was successfully cloned, the rabbit anti-*A. m. carnica* ZnT-7-like polyclonal antibody was prepared, and the relative expression of ZnT-7-like in hypopharyngeal glands of different day-old *A. m. carnica* adults were measured at the transcription and translation levels. These results lay the foundation for further studies on functions of *A. m. carnica* ZnT-7-like gene.

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Key words: *Apis mellifera carnica* hypopharyngeal gland zinc transporter 7-like (ZnT-7-like) gene cloning antibody preparation differential expression

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