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研究报告

*Tenebrio molitor*抗冻蛋白基因家族cDNA片段的克隆、序列分析及原核表达

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摘要

利用反转录-多聚酶链式反应(RT-PCR)的方法, 克隆黄粉甲虫(*Tenebrio molitor*)抗冻蛋白基因cDNA片段并进行序列分析和原核表达。同源性分析表明, 获得9条新cDNA片段, 与黄粉甲虫抗冻蛋白基因家族的其他基因序列具有较高的同源性。重组质粒pGEX-4T-1-*tmafp*-XJ430, 转化*E.coli* BL21进行原核表达, SDS-PAGE分析结果表明, 抗冻蛋白基因以可溶性融合蛋白表达, 相对分子量为38 kDa。构建真核表达载体pCDNA3-*tmafp*-XJ430, 免疫小鼠, 获得的抗血清滴度为1:2 000。Western blotting 结果为单一的条带, 证明该抗血清具有针对抗冻蛋白TmAFP-XJ430抗原的专一性。

关键词 黄粉甲虫 抗冻蛋白 cDNA片段 序列分析 原核表达

分类号 Q75

Cloning, Sequencing and Prokaryotic Expression of cDNAs for the Antifreeze Protein Family from the Beetle *Tenebrio molitor*

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Abstract

<P>The partial cDNA sequence coding for the antifreeze proteins in the <EM>Tenebrio molitor</EM> was obtained by RT-PCR. Sequence analysis revealed nine putative cDNAs with a high degree of homology to <EM>Tenebrio molitor</EM>antifreeze proteins. The recombinant pGEX-4T-1-<EM>tmafp</EM>-XJ430 was introduced into E. <EM>coli </EM>BL21 to induce a GST fusion protein by IPTG. SDS-PAGE of the fusion protein demonstrated that the antifreeze protein migrated at a size of 38 kDa. The immunization was performed by intra-muscular injection of pCDNA3- <EM>tmafp</EM>-XJ430, and then antiserum was detected by ELISA.The titer of the antibody was 1:2 000. Western blotting analysis showed the antiserum was specific against the antifreeze protein. This finding could lead to further investigation of the properties and function of antifreeze proteins.</P>

Key words Tenebrio molitor antifreeze proteins cDNA fragment sequence analysis prokaryotic expression

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