

苹果蠹蛾热激蛋白Hsp90基因的克隆及热胁迫下的表达分析

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Cloning and analysis of the expression characteristics under heat stress of Hsp90 gene in the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae)

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摘要 世界检疫性害虫苹果蠹蛾*Cydia pomonella*是一种温度耐受可塑性很高的物种。本研究针对温度波动可能导致其耐热性增强的科学问题, 采用生测法鉴定了苹果蠹蛾实验种群的高温耐受阈值, 采用同源克隆、RACE和实时荧光定量PCR (RT-qPCR)等方法研究了苹果蠹蛾热激蛋白Hsp90基因的应激表达对耐热性的重要作用。高温耐受阈值研究结果表明, 苹果蠹蛾实验种群的死亡率随温度的升高和时间的延长显著性升高, 1-5龄幼虫分别经50℃和52℃高温处理2, 5和10 min后, 3龄幼虫耐热性最差, 5龄幼虫最强。50℃和52℃分别处理10 min和5 min均可导致1-4龄幼虫全部死亡, 而5龄幼虫在这两种处理下仍有25.0%和11.1%的存活率。以35℃处理的5龄雌幼虫为材料克隆苹果蠹蛾Hsp90基因全长cDNA, 结果显示该基因全长为2 470 bp, 完整开放阅读框为2 148 bp, 共编码716个氨基酸, 预测分子量为82.07 kDa, 命名为Cphsp90 (GenBank登录号JN624775)。该基因编码的氨基酸序列与亚洲玉米螟*Ostrinia furnacalis*和甘蓝夜蛾*Mamestra brassicae*等昆虫的Hsp90的氨基酸序列一致性高达96%, 表明了Hsp90家族的保守特性。Cphsp90 mRNA的相对表达量在32~44℃高温胁迫下随温度的升高而显著增高, 证实Cphsp90是诱导型热激基因, 且mRNA相对表达量与胁迫程度正相关。Cphsp90基因的表达还具有组织特异性, 35℃处理幼虫的表皮中Cphsp90相对表达量显著高于血淋巴、脂肪体和中肠, 应激响应最为活跃。与未经温热预处理的昆虫相比, 35℃温热预处理3 h后的5龄幼虫在40, 45和50℃更高的温度胁迫下, Cphsp90 mRNA达到最高表达量所需要的胁迫温度有所提升, 由未经预处理的40℃处理10 min提高到45℃处理10 min, 这与温热预处理会增强5龄幼虫耐热性的现象相符, 表明Cphsp90基因的响应表达在苹果蠹蛾耐热性及其可塑性过程中发挥重要的作用。

关键词: 苹果蠹蛾 耐热性 热激蛋白 系统发育 热激诱导 相对表达模式

Abstract: The codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), is a worldwide quarantine pest and possesses the plasticity in thermotolerance ability. The temperature fluctuations may enhance its tolerance to heat stress. In the present study, we determined the critical threshold of tolerance to high temperature by bioassay method in *C. pomonella* laboratory population, and applied the techniques of homology-based cloning, RACE and real-time quantitative PCR (RT-qPCR) to elucidate the function of Hsp90 responding to heat stress in *C. pomonella*. The results of bioassay showed that the mortality of *C. pomonella* was significantly increased as temperature was elevated and time prolonged. When the 1st-5th instar larvae were exposed to 50℃ and 52℃ for 2, 5 and 10 min, the 3rd instar larvae were found to be the most sensitive and the 5th instar larvae the most resistant. The 1st-4th instar larvae were killed 100% when exposed to 50℃ for 10 min and 52℃ for 5 min, while the survival rate of 5th instar larvae still maintained at 25.0% and 11.1%, respectively. The full-length cDNA of hsp90 gene was obtained from the 5th instar larvae of *C. pomonella* exposed to 35℃, which is 2 470 bp in length and has an open reading frame (ORF) of 2 148 bp encoding a protein of 716 amino acids with the deduced molecular weight of 82.07 kDa. This gene was registered in GenBank under the accession number JN624775 and designated as *Cphsp90*. Cphsp90 shares 96% amino acid sequence identity with the Hsp90 from *Ostrinia furnacalis* and *Mamestra brassicae*, suggesting that the Hsp90 family is highly conserved. The result of RT-qPCR showed that the *Cphsp90* was heat inducible, and the relative expression level of *Cphsp90* mRNA was positively correlated with the heat stress when the larvae were exposed to 32-44℃ for 1 h. When the larvae of *C. pomonella* were exposed to 35℃, *Cphsp90* was expressed abundantly in cuticle with the expression level significantly higher than that in the hemolymph, fat body and midgut. The result of the mRNA expression pattern of the *Cphsp90* under higher temperature after pretreatment at 35℃ for 3 h indicated that the maximum

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expression level in the larvae preheated at different temperature (45°C for 10 min) compared with the larvae not preheated (40°C for 10 min). This result further proved that preheat treatment enhanced the thermotolerance of 5th instar larvae. It is so concluded that the positive expression of the *Cphsp90* mRNA may play an important role in thermotolerance and the plasticity of *C. pomonella*.

Key words: *Cydia pomonella* heat resistance heat shock protein (Hsp) phylogeny heat induction relative expression pattern

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