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赤拟谷盗HSP70基因克隆和竞争定量PCR检测

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Cloning and quantitative competitive PCR assay of HSP70 gene in Tribolium castaneum (Coleoptera: Tenebrionidae)

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全文: PDF (0 KB) HTML (1 KB) 输出: BibTeX | EndNote (RIS) 背景资料

摘要

为研究赤拟谷盗*Tribolium castaneum*受到热胁迫后高度保守的热激蛋白70（heat shock protein 70，HSP70）基因的表达变化，本研究扩增了681 bp的赤拟谷盗*hsp70*片段，编码227个氨基酸残基，GenBank登录号为HM345948。同源性分析表明：赤拟谷盗*hsp70*核苷酸序列与马铃薯甲虫*Leptinotarsa decemlineata*的*hsp70*（GenBank登录号：AF322911.1）同源性最高，为97%；其推测的蛋白序列与马铃薯甲虫、甘蓝夜蛾*Mamestra brassicae*、黑腹果蝇*Drosophila melanogaster*和美洲斑潜蝇*Liriomyza sativae*的HSP70蛋白均有94%以上的同源性。利用RT-PCR技术得到与赤拟谷盗*hsp70*进行竞争定量的内部竞争物， 以等量的目标cDNA和一系列稀释的竞争模板进行竞争PCR扩增，构建了*hsp70*的竞争定量PCR检测体系， 该体系标准曲线的线性方程为Y=1.032X-1.618 (r^2=0.975)。这些结果为赤拟谷盗的*hsp70*定量检测提供了方便，并为热控技术防治害虫提供了基础资料。

关键词：

Abstract:

To study the expression changes of heat shock protein 70 (a highly conserved protein) in *Tribolium castaneum* after exposure to heat stress, a fragment of 681 bp encoding for HSP70 was amplified and sequenced from *T. castaneum*. The fragment encoded 227 amino acid residues with the GenBank accession no. HM345948. The result of homology analysis showed that this fragment shared 97% identity with hsp70 from *L. decemlineata* (AF322911.1). Comparison of the deduced amino acid sequences of HSP70 in *T. castaneum* with those in *Leptinotarsa decemlineata*, *Mamestra brassicae*, *Drosophila melanogaster* and *Liriomyza sativa*e indicated that they shared more than 94% identity. The internal competitor used in quantitative competitive PCR was obtained by RT-PCR. The detection system of hsp70 was constructed by PCR amplification using the same quantity of target cDNA and a series of diluted internal competitor as template. The linear equation of standard curve was Y=1.032X-1.618 (r^2=0.975). This study provides a very convenient method for the quantitation of the hsp70 expression changes in *T. castaneum* and offers the basic data for the prevention and control of pests using thermal control technology.

Key words:

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