



家蝇III龄幼虫消化系统内源性-葡萄糖苷酶的组织定位和表达差异研究

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Tissue Localization and Expression Difference of Endogenous β -glucosidase in Digestive System of *Musca domestica* Third Instar Larvae

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摘要

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摘要 目的 以家蝇III龄幼虫为研究对象,探讨家蝇(*Musca domestica*)消化系统内源性 β -葡萄糖苷酶(β -glucosidase, BG)的组织分布、表达差异和器官功能定位。方法 用原位杂交鉴定家蝇 β -葡萄糖苷酶mRNA的组织定位。免疫组织化学法鉴定纤维素酶的组织定位。分离家蝇III龄幼虫消化系统组织,用3,5-二硝基水杨酸(DNS)法测定 β -葡萄糖苷酶的活性,实时荧光定量PCR法检测家蝇 β -葡萄糖苷酶mRNA在消化系统各器官中的表达差异。结果 原位杂交显示,中肠、前肠和唾液腺上皮细胞是家蝇 β -葡萄糖苷酶mRNA表达的部位。免疫组织化学染色显示,唾液腺、前肠和中肠上皮细胞是家蝇幼虫产生和分泌纤维素酶的主要部位。DNS法测定结果显示,家蝇幼虫唾液腺、前肠、中肠和后肠的 β -葡萄糖苷酶活性分别为(0.80±0.06)、(0.38±0.02)、(1.20±0.05)和(0.26±0.02) IU/mg,各部位间酶活性差异有统计意义(P<0.05);中肠的酶活性较高,占总酶活性的(45.45±1.27)%,后肠的酶活性最低,为(9.85±0.88)%。实时荧光定量PCR显示,家蝇 β -葡萄糖苷酶mRNA只在前肠、中肠和唾液腺中表达,中肠的表达量约为前肠的5倍,唾液腺的表达量约为前肠的3倍,三者表达量差异有统计学意义(P<0.05),而后肠未见该基因的表达。结论 家蝇III龄幼虫前肠、中肠和唾液腺均能分泌内源性 β -葡萄糖苷酶,唾液腺和中肠为主要分泌器官。

关键词: β -葡萄糖苷酶; 消化系统; 家蝇

Abstract: Objective To study the tissue localization and expression difference of endogenous β -glucosidase in digestive system of *Musca domestica* third instar larvae. Methods The digestive system of the 3rd instar larvae of *Musca domestica* was taken for the below tests. Tissue localization of endogenous β -glucosidase mRNA was identified by in situ hybridization. Cellulase was localized by immunohistochemistry. The enzymatic activity of β -glucosidase was measured by 3, 5-dinitrosalicylic acid (DNS) assay. The relative mRNA expression levels of *M. domestica* β -glucosidase gene in these organs were determined by RT-PCR. Results β -glucosidase mRNA, with in situ hybridization, was shown in the epithelial cells of midgut, salivary glands and foregut of the larvae. The immunohistochemical analysis on larvae tissues revealed that cellulase was produced and secreted by the epithelial cells of the midgut, salivary glands and foregut. β -glucosidase activity in salivary glands, foregut, midgut, and hindgut was (0.80±0.06), (0.38±0.02), (1.20±0.05) and (0.26±0.02) IU/mg, respectively. There was significant difference in β -glucosidase activity among these digestive organs (P<0.05). The activity level of β -glucosidase was highest in midgut [(45.45±1.27)%], and lowest in hindgut [(9.85±0.88)%]. However, β -glucosidase gene were only expressed in the salivary gland, foregut and midgut. Significant differences in gene expression level of β -glucosidase was found among these organs (P<0.05). The relative expression quantity of β -glucosidase gene in midgut and salivary glands were 5 and 3 times higher than that in foregut. Conclusion The endogenous β -glucosidase gene is expressed in the foregut, midgut and salivary glands. The midgut and salivary glands of *Musca domestica* 3rd instar larvae are the primary organs of this enzyme secretion.

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