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论著

mRNA差异显示技术分离猬迭宫绦虫幼虫特异表达基因

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摘要

目的 查找猬迭宫绦虫幼虫裂头蚴阶段特异性表达基因。 方法 裂头蚴和成虫组织用异硫氢酸胍一步法提取总 RNA,用 DNA酶去除总 RNA中污染的 DNA。使用 T1 2 MA、T1 2 MC、T1 2 MG和 T1 2 MT4种锚定引物反转录合成 c DNA,再用 1种随机引物与上述 4种锚定引物在含同位素的反应液中进行 PCR反应。将 PCR产物用变性聚丙烯酰胺凝胶电泳分离 ,经放射自显影后 ,从凝胶上选出裂头蚴与成虫不同的差异带 ,PCR扩增后经杂交试验鉴定出不同种类的基因片段 ;以筛选出的差异带作探针 ,分别与裂头蚴和成虫 RNA进行 Northern杂交证实裂头蚴阶段表达基因 ;将差异带测序后与 Gen Bank中的序列进行同源性比较。 结果 从凝胶中共选出 11条差异带。将回收的 11条带再经PCR扩增和杂交试验 ,从中选出 3种不同的基因片段。经 Northern杂交证实片段 1和片段 2为裂头蚴特异表达基因 ,而片段 3为裂头蚴和成虫共同表达的基因。3个基因片段测序后与 Gen Bank中的基因进行同源性分析 ,基因片断 1和 2无同源序列 ;基因片段 3与多种生物的 2 8S r RNA同源。 结论 通过 m RNA差异显示技术查找出 2个裂头蚴阶段特异性表达基因片段

关键词 mRNA差异显示 猬迭宫绦虫 PCR 基因 阶段表达

分类号

Screening of Stage-Specific Expression Genes of Spirometra erinacei-europaei Plerocercoid by mRNA Differential Display Technique

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Abstract

Objective To screen stage-specific expression genes of plerocercoid of Spirometra erinacei-europaei. Methods RNA was extracted by the acid guanidinium thiocyanate-phenol-chloroform from plerocercoid and adult worm of Spirometra erinacei-europaei. Contaminated DNA in the RNA was digested by RNase-free DNase. cDNA was synthesized by using T_{12}MA, T_{12}MC, T_{12}MG and T_{12}MT primers, and PCR was then done using the same T_{12}MN and one random primers. The PCR products were fractionated on 8% denatured polyacrylamide gel, differential bands of plerocercoid found in the gel were cut out, amplified by PCR and sequenced after the gel was dried out and autoradiographed. Northern hybridization was conducted to identify the stage-specific expression genes. Results Eleven differential bands were selected from the gel and classified into 3 kinds of gene fragments by hybridization after they were amplified by PCR. The fragments 1 and 2 were confirmed to express specifically in plerocercoid by Northern hybridization, but the fragment 3 was expressed in both plerocercoid and adult worm. When the 3 gene fragments were homologically analyzed in GenBank, the sequence which was homologous with the fragments 1 and 2 was not found, but the fragment 3 had high homology with many kinds of 28S rRNA. Conclusion The gene expression of plerocercoid was different from that of adult worm probably because they live in different hosts. Two kinds of different gene fragments in plerocercoid were identified by mRNA differential display technique.

Key words mRNA differential display Spirometra erinacei europaei PCR gene stage specific expression

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