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论著

肝片吸虫组织蛋白酶L基因的克隆、表达和免疫原性分析

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摘要

目的 克隆和表达肝片吸虫组织蛋白酶 L 基因（FhCL），分析其免疫原性。 方法 根据GenBank公布的FhCL基因序列设计引物，以肝片吸虫总RNA为模板，通过RT-PCR扩增FhCL基因编码序列，PCR产物经TA克隆，通过*Eco*RⅠ、*Hin*dⅢ双酶切和测序鉴定获得重组质粒pMD18-T/FhCL，并将其亚克隆入原核表达载体pET30a（+），经PCR，以及*Bam*HⅠ、*Hin*dⅢ双酶切和测序鉴定，构建原核表达质粒pET30a（+）-FhCL，转化大肠埃希菌(*E. coli*)BL21（DE3）pLysS，异丙基-β-D-硫代半乳糖苷（IPTG）诱导表达并获得纯化的重组蛋白FhCL，用十二烷基硫酸钠-聚丙烯酰胺凝胶电泳（SDS-PAGE）鉴定，以及蛋白质印迹（Western blotting）分析该重组蛋白对感染肝片吸虫的山羊血清及免疫的SD大鼠血清的免疫反应性。结果 PCR和*Bam*HⅠ、*Hin*dⅢ双酶切均可见约1 000 bp的条带，测序结果显示重组质粒pET30a（+）-FhCL构建成功。SDS-PAGE结果表明，重组蛋白相对分子质量约为 *Mr* 42 000（含6个组氨酸标签），与目的蛋白相符，以包涵体形式表达。Western blotting分析结果显示，纯化的重组蛋白FhCL可被感染肝片吸虫的山羊血清和免疫的SD大鼠血清识别，在目的条带Mr 42 000处见单一特异性条带，而阴性对照血清则无反应带。 结论 克隆及表达了肝片吸虫组织蛋白酶 L 编码基因，重组蛋白具有良好的免疫原性。

关键词 肝片吸虫；组织蛋白酶L基因；克隆；原核表达

分类号

Cloning，Expression and Immunogenicity Analysis of Cathepsin L-like Protease of *Fasciola hepatica*

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Abstract

Objective To clone and express the cathepsin L-like protease gene of *Fasciola hepatica*（FhCL）and investigate the immunogenicity of the recombinant FhCL protein. Methods Specific primers were designed according to the reported FhCL gene in GenBank. Using total RNA from adult worms of *F. hepatica*，FhCL gene was amplified by RT-PCR. The PCR product was cloned into pMD18-T vector and then subcloned into pET30a（+） vector. The recombinant plasmid was transformed into *E. coli* BL21（DE3） and followed by expression of the protein induced by IPTG. The expression situation of recombinant FhCL was analyzed by SDS-PAGE. Its immunoresponse to the sera of infected goat and the antisera of SD rats against FhCL was examined by Western blotting analysis. Results PCR and double enzyme digestion showed that the FhCL gene fragment was about 1 000 bp in length. The constructed recombinant plasmid pET30a（+）-FhCL was identified by sequencing. The recombinant protein（*Mr* 42 000）was expressed in the form of inclusion body. The protein was recognized respectively by the sera of infected goat and the sera from rat immunized with FhCL. Conclusion The recombinant plasmid pET30a（+）-FhCL has been constructed，which shows high antigenicity.

Key words *Fasciola hepatica*；Cathepsin L-like protease；Cloning；Prokaryotic expression

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