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论著

安氏隐孢子虫热休克蛋白编码基因的克隆、表达和分析

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摘要

目的 克隆、表达和分析安氏隐孢子虫Mr 70 000热休克蛋白（CaHSP70） 的部分编码基因。 方法 依据公布的CaHSP70基因序列设计引物，以江苏徐州安氏隐孢子虫（XZ-BOV）总RNA为模板，反转录PCR（RT-PCR）扩增目的编码基因。PCR产物经TA克隆后，亚克隆入pET28a原核表达载体，构建重组质粒pET28a-CaHSP70，转化感受态大肠埃希菌BL21（DE3），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（简称为rCaHSP70）。用十二烷基磺酸钠-聚丙烯酰胺凝胶电泳（SDS-PAGE）、蛋白质印迹（Western blotting）和ELISA对该重组蛋白进行分析和鉴定。采用相关生物信息学软件对序列进行分析。 结果 根据克隆的目的基因序列推导的氨基酸序列与GenBank登录的CaHSP70一致。SDS-PAGE和Western blotting分析显示，重组蛋白（rCaHSP70） Mr约为43 000（含6个组氨酸），以包涵体的形式存在，可被辣根过氧化物酶标记的抗组氨酸抗体、安氏隐孢子虫感染的小鼠血清、微小隐孢子虫感染的儿童血清和rCaHSP70免疫小鼠血清识别。rCaHSP70存在多个功能位点和潜在的抗原决定簇。种系发生分析表明XZ-BOV与安氏隐孢子虫进化关系最近。ELISA检测结果表明，rCaHSP70免疫的C57BL/6小鼠与BALB/c小鼠血清特异性抗体滴度均显著高于免疫前。 结论 XZ-BOV HSP70部分编码基因的克隆获得成功，研究获得的重组蛋白具有一定的免疫原性和免疫反应性。

关键词 安氏隐孢子虫 热休克蛋白 重组抗原 表位分析

分类号

Cloning， Expression and Analysis of the Heat Shock Protein of Cryptosporidium andersoni

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Abstract

Objective To clone and express the partial encoding sequence of *Mr* 70 000 heat shock protein of *Cryptosporidium andersoni* （CaHSP70） in Escherichia coli and identify the recombinant protein. Methods Total RNA was extracted from oocysts of *C.andersoni* isolated from Xuzhou， Jiangsu （XZ-BOV）. The CaHSP70 gene was amplified by RT-PCR. The PCR product was cloned and then subcloned into pET28a vector， and the recombinant plasmids were transformed into E.coli BL21（DE3） subsequently. The expressed protein induced by IPTG was purified and identified by SDS-PAGE and western blotting， and was further analyzed by relevant bioinformatics softwares. The specific IgG antibodies in mice immunized by rCaHSP70 were detected by western blotting and ELISA respectively. Results The deduced amino acid sequence showed to be identical with that of *C. andersoni Mr* 70 000 heat shock protein （HSP70）. The recombinant protein expressed in the form of inclusion body was about *Mr* 43 000. It could be recognized by anti-His G labeled HRP antibodies and all the sera from mice infected with C. andersoni and children infected with *C. parvum* as well as sera from mice immunized with rCaHSP70 respectively. The rCaHSP70 possibly had multiple domains and potential antigenic determinants. Phylogenetic analysis showed that XZ-BOV and C. andersoni were in the same clade. ELISA showed that the level of specific antibodies against rCaHSP70 in immunized BALB/c and C57BL/6 mice was significantly higher than that of mice before immunization. Conclusion The recombinant plasmid pET28a-CaHSP70 has been constructed. The purified rCaHSP70 exhibits high antigenicity and seems a potential candidate antigen for immunodiagnosis of cryptosporidiosis.

Key words *Cryptosporidium andersoni* Heat shock protein Recombinant antigen Epitope analysis

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