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论著

牛带绦虫亚洲亚种乳酸脱氢酶基因的克隆表达及免疫原性分析

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摘要
目的 对牛带绦虫亚洲亚种成虫乳酸脱氢酶基因（LDH）进行克隆、表达和免疫原性分析。 方法 将牛带绦虫亚洲亚种成虫TaLDH克隆到原核表达质粒pET-30a（+）中，在大肠埃希菌BL-21/DE3中用异丙基-β-D-硫代半乳糖苷（IPTG）诱导表达，表达产物通过十二烷基硫酸钠-聚丙烯酰胺凝胶电泳（SDS-PAGE）进行鉴定，用镍离子金属螯合剂亲和层析柱进行纯化，纯化的重组蛋白pET-30a（+）-TaLDH用蛋白质印迹（Western blotting）分析其免疫原性。 结果 PCR、双酶切及DNA测序结果均显示重组质粒pET-30a（+）-TaLDH构建成功。SDS-PAGE结果表明，目的基因在大肠埃希菌BL-21/DE3中获得高效表达，经亲和层析获得了高纯度蛋白，浓度为0.9 mg/ml。Western blotting分析结果显示，重组蛋白pET-30a（+）-TaLDH能识别感染牛带绦虫亚洲亚种的猪血清和患者血清，在相对分子质量(Mr) 35 000处有一清晰条带，表明其具有免疫反应性。 结论 牛带绦虫亚洲亚种成虫乳酸脱氢酶基因可在原核表达系统中获得具有免疫学活性的高效表达。

关键词 牛带绦虫亚洲亚种 乳酸脱氢酶基因 克隆 原核表达

分类号

Cloning and Prokaryotic Expression of Malate Dehydrogenase Gene of Taenia saginata asiatica and Immunogenicity Analysis of the Recombinant Protein

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Abstract
Objective To clone and express the lactate dehydrogenase （LDH） gene of Taenia saginata asiatica and analyze the immunogenicity of the recombinant protein. Methods By screening the full length cDNA plasmid library，the coding region of LDH was amplified with PCR，and cloned into the prokaryotic expression vector pET-30a （+），then expressed in E. coli BL21 with IPTG induction. The recombinant protein was detected by SDS-PAGE and purified by Ni-IDA affinity chromatography, and its immunogenicity was analyzed by Western blotting. Results PCR, double enzyme digestion and DNA sequencing confirmed that the recombinant expression plasmid was constructed. The expression products were obtained and purified by Ni-IDA affinity chromatography. Western blotting analysis of LDH recombinant protein testified that the recombinant protein could be recognized by sera of the Taenia saginata asiatica infected swine and the patient. Conclusions The LDH gene of Taenia saginata asiatica has been cloned and expressed，and the purified protein has been confirmed with immunogenicity.

Key words *Taenia saginata asiatica* Lactate dehydrogenas Cloning Prokaryotic expression

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