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简单异尖线虫D-天冬氨酸蛋白酶基因的克隆和表达

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Cloning and Expression of D-like Aspartic Protease of *Anisakis simplex*

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摘要 目的 克隆简单异尖线虫Ⅲ期幼虫（L3）的D-天冬氨酸蛋白酶基因（AsAP）全长，研究其表达蛋白的特性。 方法 根据GenBank中简单异尖线虫D-天冬氨酸蛋白酶基因表达序列标签的部分信息，设计特异引物并用cDNA末端快速扩增技术得到AsAP全长序列，分析推导的蛋白序列特征，并预测其三级结构。用RT-PCR扩增简单异尖线虫L3的AsAP基因编码序列，产物用*Eco*RⅠ和*Sal*Ⅰ双酶切，连入表达载体pET32a（+），转化大肠埃希菌(*E. coli*）BL21（DE3），以异丙基-β-*D*-硫代半乳糖苷（IPTG）诱导表达，表达产物经十二烷基硫酸钠-聚丙烯酰胺凝胶电泳（SDS-PAGE）检测。 结果 简单异尖线虫L3的AsAP基因全长1 753 bp，编码453个氨基酸，与锡兰钩虫（*Ancylostoma ceylanicum*）的D-天冬氨酸蛋白酶相似性达65%。该蛋白具有两个保守的催化域，1个活性中心翼环，S2和S3亚位点各1个；具有由20个氨基酸组成的N端信号肽，构成疏水性强的跨膜域。不同浓度的IPTG（0.2～1.6 mmol/L）诱导对AsAP表达的影响较小，1.0 mmol/L IPTG诱导2 h后表达量达到最高水平。 结论 克隆并表达了简单异尖线虫的D-天冬氨酸蛋白酶。

关键词： 简单异尖线虫 D-天冬氨酸蛋白酶 克隆 原核表达

Abstract: Objective To clone and express the full length of D-like aspartic protease gene (AsAP) of the third stage larvae of *Anisakis simplex*. Methods According to the partial information of D-like aspartic protease encoding gene of *A. simplex* from GenBank, specific primers were designed to amplify 3′ end and 5′ end of AsAP gene using rapid amplification of cDNA ends (RACE), and the full length of the D-like aspartic protease gene was obtained. Using total RNA of the third-stage larvae of *A. simplex*, coding sequence of the AsAP gene was amplified by reverse transcription-PCR (RT-PCR). The PCR product was digested by *Eco*RⅠ and *Sal*Ⅰ, and cloned into pET32 vector. The recombinant plasmid was checked by double enzyme digestion and sequencing, and the positive recombinant plasmid was transformed into *E. coli* BL21 (DE3). Expression of the protein induced by IPTG under gradient concentration and different time was conducted. Result A 1 753 bp full length of AsAP was obtained, which contained 30 bp 5′ UTR, 361 bp 3′ UTR and a 1 362 bp open reading frame (ORF) encoding 453 amino acids with a predicted molecular mass of M_r 50 726. It showed 65% identity with the D-like aspartic protease of *Ancylostoma ceylanicum*. The predicted amino acid sequence contains two conserved catalytic motif, an active site flap, an S2 subsite and an S3 subsite. A 20 amino acids signal peptide was found in the N-terminus, with significant hydrophobic property. Different concentration of the IPTG (0.2~1.6 mmol/L) showed little effect on the expression, and the production of the protein was up to maximum after 2 hours induction. Conclusion The AsAP gene has been cloned and expressed.

Keywords: *Anisakis simplex* D-like aspartic protease Cloning Prokaryotic expression

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