遗传 2003 25 (6): 685-690 ISSN: 0379-4172 CN: 11-5450/R

牛肠激酶轻链基因的克隆及其在大肠杆菌中的融合表达 Cloning and Fusion Expression of Bovine Enterokinase Light Chain Gene in Escherichia Coli

黄鹤, 甘一如, 孙彦 HUANG He, GAN Yi-Ru, SUN Yan

天津大学生物工程系,天津 300072 Department of Biochemical Engineering,Tianjin University,Tianjin 300072,China

收稿日期 修回日期 网络版发布日期 接受日期

摘要 为克隆表达牛肠激酶（enterokinase）轻链(EKL)编码基因，以期应用于融合蛋白的切割与纯化，从市售北方黄牛十二指肠组织中提取总RNA,以RT-PCR方法扩增其cDNA片段，将此片段克隆于pUCmT载体中，经过特异性限制性内切酶酶切分析片段正确后，进行全序列分析。结果表明,克隆的cDNA与GenBank上的序列相比完全一致，得到了编码正确的牛肠激酶轻链基因全序列。随后，将目的基因片段插入pET39b中，构建了融合型表达载体pET39b-EKL，转化大肠杆菌BL21(DE3)，用IPTG诱导表达。所获得的pET39b-EKL经过酶切鉴定和测序，证实其插入方向、读码框架正确，所表达重组蛋白经SDS-PAGE分析,相对分子量为65 kDa,表达量达28%，通过镍亲和层析纯化得到融合蛋白DsbA-rEKL的单一条带。该粗酶经脱盐后在适宜的缓冲体系中21℃温育过夜，显示出较高的自催化切割活性，为进一步进行重组牛肠激酶活性的研究及应用奠定了基础。

Abstract:The objective of the study was to obtain the gene of bovine enterokinase light chain, which would be used in the cleavage and purification of fusion proteins. The fragment of bovine enterokinase light chain cDNA was obtained by RT-PCR from a sold bovine′s duodenal mucosa, then cloned into the pUCmT cloning vector and sequenced.Compared with the sequence deposited in GenBank, the cloned gene sequence is correct.Then the interested gene fragment was inserted into the pET39b expression plasmid.The recombinant vector pET39b-EKL was transformed into E.coli BL21 (DE3) and induced by IPTG.It was confirmed that the nucleotide sequence was correct on the conjunction site between the recombinant DNA 5′ terminal multi-cloning site and recombinant fragment after the analysis of the nucleotide sequence.SDS-PAGE analysis indicated that target product was about 65 kDa which occupied 28% of the total protein.A pure fusion protein was obtained by nickel chelating chromatogram using His*Binding Resin.After desalting and changing buffer,the crude kinase was incubated at 21℃ overnight and demonstrated a high autocatalytic cleavage activity.This investigation would be able to lay a foundation for enterokinase activity research and farther application of expression products on a large scale.

关键词 牛肠激酶轻链 克隆 表达 自催化切割 Key words bovine enterokinase light chain cloning expression autocatalytic cleavage

分类号

Abstract

Key words

DOI:

通讯作者