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研究报告

短小芽孢杆菌碱性蛋白酶基因启动子的克隆、鉴定及其应用

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摘要

利用TAIL-PCR(Thermal asymmetric interlaced PCR)从短小芽孢杆菌基因组中扩增到碱性蛋白酶基因编码区上游的启动子片段。对该片段的序列测定和分析表明, 此片段长797 bp, 但与基因表达有关的序列长约390 bp。对启动子片段进行不同长度的缺失突变, 以获得最小的基因启动子片段, 结果表明, 该基因起始密码子上游约160 bp的DNA片段就可以启动基因的表达。将含有该片段的碱性蛋白酶基因WApQ3插入大肠杆菌-芽孢杆菌穿梭质粒载体pSUGV4中, 构建了碱性蛋白酶基因表达质粒pSUBpWApQ3。将该质粒分别转入枯草芽孢杆菌和短小芽孢杆菌中表达, 可在胞外检测到碱性蛋白酶活性, 最高酶活分别为466.5 U/mL和3060 U/mL。

关键词 短小芽孢杆菌 枯草芽孢杆菌 碱性蛋白酶 基因启动子 基因表达

分类号

Cloning, characterization and application of the promoter of alkaline protease gene in *Bacillus pumilus*

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Abstract

<P>A 797 bp promoter fragment of alkaline protease gene was cloned from the Bacillus pumilus genome by employing TAIL-PCR strategy. The sequence analysis of this promoter fragment showed that the sequence accounting for gene expression was approximately 390bp. Deletion analysis of the fragment defined the minimal required sequence of promoter for initiating transcription lies on a 160 bp region upstream of the start codon. The alkaline protease gene WApQ3 containing the cloned promoter fragment was inserted into the shuttle vector pSUGV4 and the constructed expression plasmid pSUBpWApQ3 was transformed into Bacillus subtilis and B. pumilus. Active alkaline protease was successfully expressed in both host strains. The peak value of extracellular alkaline protease activities of B. subtilis and B. pumilus recombinants reached to 465.5 U/mL and 3060 U/mL, respectively. </P>

Key words Bacillus pumilus Bacillus subtilis alkaline protease promoter gene expression

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