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生物技术 生命科学

大肠杆菌外膜蛋白酶基因OmpT的克隆及表达

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摘要:

OmpT(Outer-membrane proteases T)是革兰氏阴性细菌分泌到细胞表面具有丝氨酸蛋白酶活性的一种重要蛋白。利用大肠杆菌OmpT降解抗菌肽特性来筛选和改造新型抗菌肽,可以为开发抗OmpT蛋白酶水解新抗菌肽序列提供新的思路和创造条件。根据大肠杆菌外膜蛋白酶OmpT的基因序列设计一对引物,应用聚合酶链式反应(PCR)方法,从大肠杆菌K12基因组中扩增获得一段为954 bp的序列,测序结果显示此序列与已公布序列同源性达99.99%。将其序列定向克隆到原核表达载体pET28a上构建重组表达质粒pET28a-OmpT。经IPTG诱导后,在表达宿主菌中特异性的表达出分子量约为36 kDa且具有生物活性的OmpT蛋白。生长曲线试验显示,抗菌肽LL37对带重组表达质粒pET28a-OmpT的大肠杆菌生长没有影响,而对照组的生长明显受到抗菌肽的抑制作用。

关键词: OmpT;克隆与表达;外膜蛋白酶;抗菌肽

Cloning and Expression of Escherichia coli Outer Membrane Proteases Gene OmpT

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Abstract:

The outer membrane proteases T (OmpT) of Escherichia coli is a surface membrane protein with serine protease activity. When OmpT could degrade antimicrobial peptide, it was used to screen and reconstruct novel antimicrobial peptide, which provided new idea for developing antimicrobial peptides of OmpT. In this paper, a pair of primers was designed according to OmpT sequence of E.coli, and a 954 bp sequence was obtained by PCR from E.coli K12 genome, which had 99.99% similarity with the opened sequence. Then the gene was cloned into the prokaryotic expression vector pET28a to construct the recombinant expression plasmid pET28a-OmpT. A specific molecular weight of about 36 kDa of OmpT protein that has biological activity was expressed in E.coli BL21 after induced by IPTG. With the present of antimicrobial peptide (AMP) LL37, growth of E.coli pET28a was inhibited, while the growth of E.coli pET28a-OmpT was not affected after OmpT protein was induced. Current results indicated that OmpT could increase the resistance of E.coli to the AMP.

Keywords: OmpT cloning and expression outer membrane proteases antimicrobial peptide

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