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论文

铜绿假单胞菌外膜蛋白OprF原核表达载体的构建及表达

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摘要：

目的 克隆铜绿假单胞菌外膜蛋白OprF基因，构建原核表达载体并鉴定表达。方法 从铜绿假单胞菌中提取基因组DNA，PCR扩增OprF羧基端，克隆至原核表达载体pET28b中，构建重组表达载体pET28b-F。重组质粒经酶切和序列测定后，转化E.coli BL21，异丙基-β-D-硫代半乳糖苷（IPTG）诱导表达，进行SDS-PAGE检测。经Ni-NTA亲和层析柱纯化后OprF蛋白免疫BALB/C小鼠，通过杂交瘤方法制备相应单克隆抗体并用ELISA方法进行鉴定。结果 pET28b-F原核表达载体构建成功。重组表达质粒经IPTG诱导后表达外膜蛋白OprF。获得4株高分泌型特异性单克隆细胞株,其中2株单克隆抗体可用于制备双抗体夹心法ELISA试剂盒,检测铜绿假单胞菌。结论 成功克隆铜绿假单胞菌外膜蛋白OprF羧基端基因片段并在大肠杆菌中进行表达，筛选出特异性抗OprF单克隆抗体。

关键词： 假单胞菌，铜绿；原核细胞；抗体，单克隆；膜蛋白质类

Construction and expression of a prokaryotic vector encoding outer membrane protein OprF of Pseudomonas aeruginosa

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Abstract:

Objective To clone the outer membrane protein OprF of Pseudomonas aeruginosa(PA), and to construct and identify a prokaryotic expression plasmid. Methods The DNA genome was extracted from PA, and the carboxyl terminus of the OprF gene was amplified by primers of PCR. The recombinant plasmid pET28b-F was constructed by cloning the OprF cDNA into the prokaryotic expression vector pET28b. After being identified by restriction endonuclease digestion analysis and DNA sequencing, the pET28b-F was transformed into E. coli BL21 and induced by IPTG. The expressed protein was analyzed by SDS-PAGE and Western blot. Monoclonal antibodies(McAbs) against OprF were prepared by the hybidoma technique and screened by ELISA. Results The construction of the recombinant expression plasmid pET28b-F was correct by restriction enzyme, PCR and DNA sequencing. Then the expression plasmid expressed a corresponding protein OprF after induction of IPTG. Four McAbs could specifically combine with the OprF protein of PA. Conclusion Successful construction of OprF in the prokaryotic expression vector and specific McAbs established with this method can provide the basis for further research into preparing the specific antibody to OprF and into conveniently detecting PA in wound infection.

Keywords: Pseudomonas aeruginosa; Prokaryotic cells; Antibodies, monoclonal; Membrane Proteins

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