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论著

粉尘螨6类变应原（Der f6）的克隆表达、纯化及免疫学特性鉴定

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摘要

目的 构建粉尘螨6类变应原（*Dermatophagoides farinae*，Der f6）基因的高效原核表达载体，并进行表达、纯化及生物学功能分析。 方法 根据Der f6基因已知序列，设计1对引物，通过对纯培养的粉尘螨提取总RNA，采用RT-PCR方法扩增出Der f6基因片段，PCR产物克隆入pMD18-T载体，转化大肠埃希菌（*E.coli* Top10），经PCR和酶切鉴定并测序。将上述所得阳性克隆菌株扩大培养，碱裂解法提取质粒，所得重组质粒pMD18-T-Der f6和空质粒pET-24a同时用限制性内切酶*Eco*RⅠ和*Xho*Ⅰ双酶切，经纯化后连接并转化至*E.coli* Top10。构建的重组质粒pET24a-Der f6经PCR、酶切和测序鉴定后，再转化至*E.coli* BL21（DE3）， 异丙基-β-D-硫代半乳糖苷（IPTG）诱导表达。用十二烷基磺酸钠-聚丙烯酰胺凝胶电泳（SDS-PAGE）和蛋白质印迹法（Western blotting）鉴定其表达效果，用Ni+离子亲和层析柱纯化重组质粒pET24a-Der f6表达产生的组氨酸重组蛋白。 结果 构建了重组质粒pMD18-T-Der f6和pET24a-Der f6。SDS-PAGE 结果表明Der f6基因在*E.coli* Bl21(DE3）中获得良好的表达，所得重组蛋白相对分子质量(*Mr*) 为31 000, 与理论值一致, 经亲和层析纯化后，SDS-PAGE 结果显示单一条带。该蛋白以尘螨过敏患者血清进行Western blotting, 结果表明具有良好的IgE结合活性。 结论 克隆、表达并纯化了具有良好尘螨致敏患者IgE结合活性的Der f6。

关键词 粉尘螨 Der f6 基因表达 纯化 蛋白质印迹

分类号

Cloning， Expression， Purification and Identification of Der f6 Gene and its Immunological Characteristics from the Dust House Mite

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Abstract

Objective To construct, purify and characterize a recombinant expression plasmid containing Der f6 gene of *Dermatophagoides farinae*. Methods A pair of primers was designed according to the known sequence of Der f6 gene. The live mites identified and cultured locally were picked and the total RNA was extracted. The Der f6 gene fragment was amplified by RT-PCR，and cloned into pMD18-T vector， and then transferred into *E.coli* Top10. The target gene obtained from the recombinant plasmid by digestion with *Eco*RⅠ and *Xho*Ⅰ was connected to the prokaryotic expression vector pET-24a. The expressed recombinant plasmid containing Der f6 gene was constructed by cloning target gene into pET-24a and first transferred into *E.coli* Top10，then into *E.coli* Bl21（DE3）. The expressed recombinant protein was analyzed by SDS-PAGE and Western blotting，and was purified by immobilized metal ion affinity chromatography（IMAC）. Results The two recombinant plasmids，pMD18-T-Der f6 and pET24a-Der f6，were constructed. SDS-PAGE showed a correct molecular weight of the recombinant Der f6 protein. After purification by affinity chromatography，the protein showed only one strip on SDS-PAGE gel and appropriate combination ability with IgE in sera of allergic patients. Conclusion The Der f6 gene has been cloned into plasmid pMD18-T vector and sub-cloned into the expression vector pET-24a，the recombinant plasmid pET24a-Der f6 has been expressed in *E.coli* BL21（DE3），purified by IMAC，and showed appropriate IgE-combined ability.

Key words *Dermatophagoides farinae* Der f6 Expression Purification Western blotting

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