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变形链球菌表面蛋白抗原(PAc)的A区和P区融合共表达、纯化及抗原性研究

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Research on the fusion,co expression,purification and antigenicity to fuctional A and P region of surface protein antigen(PAc) of Streptococcus mutans

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摘要

变形链球菌（Streptococcus mutans）是引起人类龋齿的主要致病菌,其表面蛋白(PAc)是龋齿疫苗研究的主要对象.在该研究中,变形链球菌 pac 基因的功能A区和P区经PCR扩增后,以连接链Gly 4SerGly 4连接,并克隆至pET32a质粒中构建成pET32a-A-P重组表达载体,经IPTG诱导表达后,表达产物A-P重组蛋白(rA-P蛋白)经饱和硫酸铵沉淀、镍亲和层析、疏水层析、离子交换层析纯化,其纯度明显提高,可达96%以上.rA-P蛋白再经过肠激酶切除质粒融合标签和经镍亲和层析纯化后,获得rsA-P目的蛋白,其分子质量为80?ku左右,纯度可达98%以上.Western blot结果表明,目的蛋白rsA-P与鼠抗 S.mutans MT8148菌血清以及鼠抗rsA-P蛋白血清发生较好的免疫反应.因此,rsA-P目的蛋白具有PAc的A区和P区抗原性的完整性,为变形链球菌龋齿基因工程亚单位疫苗的研制奠定基础.

关键词： 变形链球菌 PAc 重组蛋白 龋齿

Abstract:

Streptococcus mutans is the major pathogen causing denta

l caries for hunman,which its surface protein antigen(PAc) is widely researched

as vaccine for dental caries.The fuctional A & P region of pac gen

e of Streptococcus mutans were amplified by PCR and linked by 3′ ter?minal

of A region and 5′ terminal of P region with a linker encoding a flexible pep

tide Gly 4SerGly 4,and the recombinant expression vector pET32a A P was cons

tructed by inserting the A P fuction gene into pET32a vector.After induced with

IPTG,the products,recombinant A P protein (rA P),were purified through four s

teps,including saturated ammonium sulfate(SAS),Nickel affinity chromatography,hy

drophobic chromatography and ion exchange chromatography,and its purity was up t

o 96%.Futhermore,the target rsA P protein,which its Mr was about 80?ku and its

purity was up to 98%,was prepared by digesting rA P with enterokinase to cleav

aged the fusion tag and being purifed by nickel affinity chromatography.And rsA

P protein reacted with high strong specificity to two antiseruns against Str

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eptococcus mutans MT8148 and rsA P protein,which were derived from mice.There fore,the target rsA P protein,showed the antigenic integrity for the fuctional A & P region of PAc,and was provided to research on genetic subunit vaccine of dental caries to Streptococcus mutans .

Key words:

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