中国病理生理杂志 2007 23 (11): 2262-2264 ISSN: 1000-4718 CN: 44-1187/R

论著

淋病奈瑟菌临床菌株外膜蛋白PIA基因序列分析及原核表达系统的构建

孙爱华[1],宋春涵[2],吴森林[1],毛亚飞[3],周海鸥[1],严杰[3△]

1 浙江医学高等专科学校,浙江 杭州 310053;2 金华职业技术学院医学院，浙江 金华 321027;3 浙江大学医学院微生物和寄生虫学教研室,浙江 杭州 310031

收稿日期 2006-4-6 修回日期 2006-10-16 网络版发布日期 2008-8-28 接受日期 2006-10-16

摘要 目的： 分析本地区淋病奈瑟菌临床菌株外膜蛋白PIA基因核苷酸及氨基酸序列，构建PIA基因的原核表达系统。方法：采用高保真PCR扩增9株淋病奈瑟菌全长PIA基因序列，T-A克隆测序后与GenBank公布的序列进行同源性比较。构建PIA原核表达系统。采用不同浓度IPTG诱导重组目的蛋白rPIA表达，10%SDS-PAGE和Bio-Rad凝胶图像分析系统检测rPIA表达情况。采用Ni-NTA亲和层析法提纯rPIA，SDS-PAGE检测提纯效果。结果： 与报道的PIA基因序列（GenBank No: L19962）比较，9株淋病奈瑟菌核苷酸和氨基酸序列相似性分别高达99.6%-100%和99.1%-100%，均属于IA6血清型。rPIA表达量可占细菌总蛋白量的50.1%，提纯后仅显示单一的目的蛋白条带。结论： IA6为本地区淋病奈瑟菌优势血清型，该基因序列相当保守。所构建的PIA基因原核表达系统能高效表达rPIA，为今后研制淋病奈瑟菌血清学检测试剂盒及疫苗研制奠定了基础。

关键词 奈瑟球菌,淋病 基因,PIA 序列分析 原核表达

分类号 R363

Sequence analysis and prokaryotic expression system construction of PIA genes isolated from Neisseria gonorrhoeae

SUN Ai-hua[1],SONG Chun-han[2],WU Sen-lin[1],MAO Ya-fei[3],ZHOU Hai-ou[1],YAN Jie[3]

1 Zhejiang Medical College,Hangzhou 310053,China;2 Medical School of The Professional Technique College of Jinhua,Jinhua 321027,China;3 Department of Medical Microbiology and Parasitology,Zhejiang University School of Medicine,Hangzhou 310031,China.E-mail: Med_bp@zju.edu.cn

Abstract

<FONT face=Verdana>AIM: To analyze the nucleotide and putative amino acid sequences of PIA genes isolated from N.gonorrhoeae and to construct the prokaryotic expression system of PIA gene.
METHODS: The entire PIA genes from 9 strains of N.gonorrhoeae were amplified by using high fidelity PCR.The target amplification fragments were sequenced after T-A cloning.Homology comparison of the nucleotide and putative amino acid sequences of PIA genes from the isolates with the reported sequences in GenBank was then performed.A prokaryotic expression system of PIA gene was constructed.Different dosages of IPTG were applied to induce the expression of the target recombinant protein (rPIA) and 10% SDS-PAGE plus Bio-Rad Agarose Image Analysor was used to determine the expression level of rPIA.rPIA was extracted using Ni-NTA affinity chromatography and the purified effect was detected by SDS-PAGE.
RESULTS: In comparison with the reported PIA gene sequences (GenBank No: L19962),the homologies of nucleotide and putative amino acid sequences of PIA genes from the isolates were 99.6%-100% and 99.1%-100%,respectively,which indicated that all the isolates were belonging to serovars IA6.Output of rPIA was as high as 50.1% of the total bacterial proteins.The purified rPIA only showed a single target protein fragment in gel.
CONCLUSION: Serovar IA6 is dominant in the local N.gonorrhoeae isolates and sequences of the encoding gene are relatively conserved.The constructed prokaryotic expression system is able to express rPIA with high efficiency,which may lay a foundation for further development of serological detection kit and vaccine of N.gonorrhoeae.</FONT>

Key words Neisseria gonorrhoeae Genes PIA Sequence analysis Prokaryotic expression

DOI: 1000-4718

通讯作者 严杰 Med_bp@zju.edu.cn