中国寄生虫学与寄生虫病杂志
CHINESE JOURNAL OF PARASITOLOGY AND PARASITIC DISEASES
ISSN 1000-7423
CN 31-1248/R
主管：中华人民共和国国家卫生和计划生育委员会
主办：中华预防医学会
中国疾病预防控制中心寄生虫病预防控制所

中国寄生虫学与寄生虫病杂志 » 2014, Vol. 32 » Issue (1) :38-41 DOI:

淡色库蚊转铁蛋白在毕赤酵母中的分泌表达及其抑菌活性的初步研究

吴松泉1 *，王光丽1，周武1，钟石根2

1 丽水学院医学院，丽水 323000；2 杭州师范大学医学院，杭州310036

Cloning and Expression of Transferrin Protein from Culex pipiens pallens and a Study of its Antimicrobial Activity

WU Song-quan1 *，WANG Guang-li1，ZHOU Wu1，ZHONG Shi-gen2

1 Medical College, Lishui Unversity，Lishui 323000，China; 2 Medical College, Hangzhou Normal University，Hangzhou 310036

摘要 | 参考文献 | 相关文章

Download: PDF (3928KB) HTML 1KB Export: BibTeX or EndNote (RIS) Supporting Info

摘要 【摘要】 目的 利用毕赤酵母（Pichia pastoris）分泌表达具有生物活性的淡色库蚊（Culex pipiens pallens）转铁蛋白（Transferrin，Tsf），并初步研究其抑菌活性。 方法 RT-PCR扩增淡色库蚊转铁蛋白的编码基因序列，并克隆至毕赤酵母组成型表达载体pGAPZα-A的α-factor信号肽序列下游，构建pGAPZα-A-Tsf重组分泌表达载体。重组载体经Xho Ⅰ和Xba Ⅰ双酶切和测序正确后，采用Bln Ⅰ线性化处理，电击转化毕赤酵母GS115感受态细胞，转化子经Zeocin抗性筛选和菌落PCR构建pGAPZα-A-Tsf/GS115工程菌。重组转铁蛋白表达上清采用十二烷基磺酸钠-聚丙烯酰胺凝胶电泳（SDS-PAGE）和蛋白质印迹（Western blotting）分析。表达上清经镍-氨三乙酸亲和层析柱（Ni-NTA）纯化后，磷酸缓冲液（PBS）稀释为1.000、0.500、0.250和0.125 mg/ml，采用琼脂糖扩散抑菌圈法进行抑菌实验。 结果 RT-PCR结果显示，转铁蛋白基因片段大小为2 100 bp，与预期结果一致。pGAPZα-A-Tsf重组载体双酶切获得2 127 bp条带，与预期结果一致，测序结果表明其构建成功。菌落PCR结果显示，pGAPZα-A-Tsf/GS115工程菌构建成功。SDS-PAGE和Western blotting结果显示，重组转铁蛋白表达了相对分子质量（Mr）约为80 200的蛋白产物，与预期大小一致。Ni-NTA纯化后重组转铁蛋白的抑菌实验表明，添加1.000、0.500、0.250 mg/ml重组转铁蛋白的平板上均有明显的抑菌圈，最低抑菌浓度为0.250 mg/ml。 结论 淡色库蚊转铁蛋白可通过基因重组方式从毕赤酵母中分泌表达具有抑菌活性的转铁蛋白。

关键词： 淡色库蚊 转铁蛋白 毕赤酵母 表达 抑菌作用

Abstract： 【Abstract】 Objective To clone and express transferrin（Tsf） from Culex pipiens pallens in Pichia pastoris，and detect its antibacterial activity. Methods The coding region of transferrin from Culex pipiens pallens was amplified by RT-PCR. The product of RT-PCR was inserted into the downstream of gene encoding α-factor signal sequence in a Pichia pastoris secreting expression vector pGAPZα-A. The recombinant pGAPZα-A-Tsf vector was transformed into P. pastoris GS115 by electroporation. Recombinant strains pGAPZα-A-Tsf/GS115 were screened by Zeocin resistance and PCR. Recombinant protein was detected by SDS-PAGE and Western blotting. The recombinant transferrin protein was purified by using Ni-NTA resin. The antibacterial activity of the purified transferrin against Escherichia coli was detected. Results The transferrin gene with 2 100 bp was obtained by RT-PCR. The product of recombinant plasmid pGAPZα-A-Tsf was approximately 2 127 bp by double digestion with restriction enzymes, consistent with the anticipated fragment length. Sequencing results showed that the inserted sequence was correct. PCR result showed that the recombinant plasmid pGAPZα-A-Tsf/GS115 was constructed. The results of SDS-PAGE and Western blotting showed that the relative molecular weight (Mr) of the protein was about 80 200. The recombinant transferrin protein showed antibacterial activity against Escherichia coli, and the minimum concentration was 0.25 mg/ml. Conclusion The recombinant transferrin protein from Culex pipiens pallens has been expressed in P. pastoris, and shows antibacterial activity against E. coli.

Keywords： Culex pipiens pallens Transferrin Pichia pastoris Expression Antibacterial activity

Service
把本文推荐给朋友
加入我的书架
加入引用管理器
Email Alert
RSS
作者相关文章

引用本文:
吴松泉1 *，王光丽1，周武1，钟石根2.淡色库蚊转铁蛋白在毕赤酵母中的分泌表达及其抑菌活性的初步研究[J] 中国寄生虫学与寄生虫病杂志, 2014,V32(1): 38-41

WU Song-quan1 *，WANG Guang-li1，ZHOU Wu1，ZHONG Shi-gen2.Cloning and Expression of Transferrin Protein from Culex pipiens pallens and a Study of its Antimicrobial Activity[J] , 2014,V32(1):38-41