

论著

## 日本血吸虫信号蛋白14-3-3在毕赤酵母菌中的分泌表达及其抗原性分析

郑美娟<sup>1</sup>,李敏<sup>2</sup>,王志成<sup>2</sup>,罗飞<sup>2</sup>,罗庆礼<sup>2</sup>,储德勇<sup>2</sup>,李丛磊<sup>2</sup>,沈继龙<sup>2</sup><sup>1</sup> 蚌埠医学院生物化学与分子生物学实验室, 蚌埠 233003;<sup>2</sup> 安徽医科大学病原生物学教研室, 教育部与安徽省共建重要遗传病基因资源利用重点实验室(安徽医科大学), 合肥 230032

收稿日期 修回日期 网络版发布日期 接受日期

摘要

目的 在毕赤酵母菌 (*Pichia pastoris*) 表达系统中表达日本血吸虫信号蛋白14-3-3 (Sj14-3-3), 并与原核表达rSj14-3-3比较其抗原性。方法 以重组质粒pET28a-rSj14-3-3为模板, PCR扩增Sj14-3-3基因, 将特异片段连接到pMD18-T载体, DNA序列分析后, 亚克隆目的片段Sj14-3-3至酵母菌分泌表达载体pPICZαB。测序正确后, 重组质粒经电转化转染至毕赤酵母菌X-33菌株, 经抗生素Zeocin筛选得到高拷贝转化子。经甲醇诱导表达, 取诱导上清进行十二烷基磺酸钠-聚丙烯酰胺凝胶电泳(SDS-PAGE) 和蛋白质印迹法(Western blotting) 分析。用间接ELISA法比较毕赤酵母菌表达的rSj14-3-3和原核表达rSj14-3-3检测血吸虫病患者血清抗体的特异性和敏感性。结果 目的基因已在酵母菌基因组中得到整合, PCR扩增得到约1 300 bp的片段。经甲醇诱导, Sj14-3-3表达并分泌到培养上清中。表达产物经SDS-PAGE 测定为 Mr 35 000。Western blotting 结果显示, Mr 35 000 蛋白可被Sj14-3-3单克隆抗体识别, 表明该真核表达产物具有免疫反应性。间接ELISA检测结果表明, 该重组蛋白检测36份急性血吸虫病患者血清rSj14-3-3抗体, 阳性率为81%。与12份华支睾吸虫感染者血清未见交叉反应, 32份健康人血清假阳性反应率为9.3%。以原核表达的rSj14-3-3为抗原, 间接ELISA检测, 36份急性血吸虫病患者血清的 rSj14-3-3 抗体阳性率为88.9%; 与12份华支睾吸虫感染者的交叉反应率为16.7%, 32份健康人血清假阳性反应率为12.5%, 其差异均无统计学意义 ( $P>0.05$ )。结论 在毕赤酵母菌中成功表达了Sj14-3-3, 培养上清中产物丰度较高, 且免疫反应性良好。

关键词 [日本血吸虫](#) [信号蛋白14-3-3](#) [毕赤酵母菌](#) [真核表达](#)

分类号

## Secreted Expression of Signaling Protein 14-3-3 of *Schistosoma japonicum* in *Pichia pastoris* System with Primary Evaluation on its Antigenicity

ZHENG Mei-juan<sup>1</sup>, LI Min<sup>2</sup>, WANG Zhi-cheng<sup>2</sup>, LUO Fei<sup>2</sup>, LUO Qing-li<sup>2</sup>, CHU De-yong<sup>2</sup>, LI Cong-lei<sup>2</sup>, SHEN Ji-long<sup>2</sup><sup>1</sup> Department of Biochemistry and Molecular Biology, Bengbu Medical College, Bengbu 233003, China; <sup>2</sup> Department of Parasitology, Anhui Medical University, Key Laboratory of Gene Resource Utilization for Important Inherited Diseases, Ministry of Education, Hefei 230032, China

Abstract

Objective To express signaling protein Sj14-3-3 in *Pichia pastoris* and compare its antigenicity with prokaryotic expression one. Methods Sj14-3-3 in gene was amplified from pET28aSj14-3-3 in recombinant plasmid, cloned into vector pMD18-T followed by sequencing. The Sj14-3-3 gene was subcloned into the expression vector pPICZα-B and transformed into *Pichia pastoris* X-33 by electroporation. The transformants were identified by sequencing. Three transformants with high copies were obtained when selected under zeocin, and expression was induced with methanol. The culture supernatant was collected and tested by SDS-PAGE and Western blotting. The specificity and sensitivity of eukaryotic expression rSj14-3-3 in *Pichia pastoris* were compared with that from prokaryotic expression by detecting sera of patients with schistosomiasis by indirect ELISA. Results The Sj14-3-3 in gene was integrated into *Pichia pastoris*, and the gene of interest detected by PCR was with 1 300 bp. After induction by methanol, the Sj14-3-3 in gene was expressed and secreted into the medium. The molecular weight of the recombinant protein was determined as about Mr 35 000 by SDS-PAGE. Western blotting showed that the protein has a high specificity against mouse-anti-Sj14-3-3 monoclonal antibody. The recombinant protein had a promising immune reactivity. Indirect ELISA showed that by using eukaryotic expression rSj14-3-3 in *Pichia pastoris*, the positive rate in 36 cases of acute schistosomiasis was 81%, with no cross-reactivity in 12 cases of *Clonorchis sinensis*, 9.3% cross-reactivity in 32

扩展功能

本文信息

▶ [Supporting info](#)▶ [PDF \(309KB\)](#)▶ [\[HTML全文\]\(OKB\)](#)▶ [参考文献\[PDF\]](#)▶ [参考文献](#)

服务与反馈

▶ [把本文推荐给朋友](#)▶ [加入我的书架](#)▶ [加入引用管理器](#)▶ [复制索引](#)▶ [Email Alert](#)▶ [文章反馈](#)▶ [浏览反馈信息](#)

相关信息

▶ [本刊中包含“日本血吸虫”的相关文章](#)

▶ 本文作者相关文章

· [郑美娟](#)· [李敏](#)· [王志成](#)· [罗飞](#)· [罗庆礼](#)· [储德勇](#)· [李丛磊](#)· [沈继龙](#)

cases of normal sera. While using prokaryotic expression rSj14-3-3 in *E. coli*, the positive rate in 36 cases of acute schistosomiasis was 88.9%, with 16.7% cross-reactivity in 12 cases of *Clonorchis sinensis*, 12.5% cross-reactivity in 32 cases of normal sera. There was no statistically significant difference of the results ( $P>0.05$ ).  
Conclusion The recombinant protein Sj14-3-3 of eukaryotic expression in *Pichia pastoris* has been successfully harvested and shows a promising immunological potential.  
Key words [Schistosoma japonicum](#) [14-3-3 protein](#) [Pichia pastoris](#) [Eukaryotic expression](#)

DOI:

---

通讯作者 沈继龙 [jlshen@ahmu.edu.cn](mailto:jlshen@ahmu.edu.cn)

作者个人主页 郑美娟<sup>1</sup>;李敏<sup>2</sup>;王志成<sup>2</sup>;罗飞<sup>2</sup>;罗庆礼<sup>2</sup>;储德勇<sup>2</sup>;李丛磊<sup>2</sup>;沈继龙<sup>2</sup>