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导出

肺炎链球菌SpxB蛋白的细胞表达定位、保守性分析及其生物学功能初探

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《第三军医大学学报》[ISSN:1000-5404/CN:51-1095/R] 卷: 36 期数: 2014年第14期 页码: 1486-1491 栏目: 论著 出版日期: 2014-07-30

Title: Protein expression localization, conservation analysis and biological functions of SpxB in *Streptococcus pneumoniae*

作者: 王哲; 王建敏; 马峰; 张帅; 黄远帅; 张雪梅
重庆医科大学检验医学院临床检验诊断学教育部重点实验室

Author(s): Wang Zhe; Wang Jianmin; Ma Feng; Zhang Shuai; Huang Yuanshuai; Zhang Xuemei
Key Laboratory of Laboratory Medical Diagnostics of Ministry of Educaticn, College of Laboratory Medicine, Chongqing Medical University, Chongqing, 400016, China

关键词: 肺炎链球菌; 丙酮酸氧化酶; 细胞表达定位; 保守性

Keywords: *Streptococcus pneumoniae*; pyruvate oxidase; protein expression localization; conservation

分类号: R372; R378.12; R394-33

文献标志码: A

摘要: 目的 鉴定肺炎链球菌中 spxB 基因编码蛋白的丙酮酸氧化酶活性、细胞表达定位和保守性表达,并初步研究该基因对细菌毒力影响的部分机制。 方法 利用PCR方法扩增肺炎链球菌D39菌株的*spxB*基因全长序列,并将其整合至表达载体pET-28a(+),经测序鉴定后,将重组质粒转化至大肠埃希菌BL21(DE3),以IPTG诱导表达含有His标签的rSpxB蛋白,经Ni-NTA亲和层析柱纯化后,使用SDS-PAGE鉴定蛋白纯度,采用一种商品丙酮酸氧化酶活性质控方法鉴定其酶活性。以rSpxB蛋白免疫昆明小鼠获得其多克隆抗体。采用ELISA检测多克隆抗体效价,采用Western blot鉴定抗体的特异性和蛋白的保守性表达。采用流式细胞术鉴定*spxB*的细胞表达定位。构建*spxB*缺陷菌,通过与流感嗜血杆菌进行共同培养初步探索其毒力机制。 结果 实现了具有丙酮酸氧化酶活性SpxB蛋白的可溶性表达,该蛋白免疫小鼠后获得高效价特异的抗SpxB蛋白抗血清,Western blot鉴定显示SpxB蛋白在1、2、4、6B、14、19F和23F等血清型肺炎链球菌均有表达,流式细胞术检测显示SpxB蛋白的荧光信号较阴性对照有右移,但较阳

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更新日期/Last Update: 2014-07-18

性对照的荧光信号弱很多。野生菌株D39产生的过氧化氢显著高于*spxB*缺陷菌，D39菌株对流感嗜血杆菌的生长抑制作用显著强于*spxB*缺陷菌。　结论　肺炎链球菌*spxB*基因编码的蛋白具有丙酮酸氧化酶活性，在肺炎链球菌中有保守表达，且主要表达于细胞内。该蛋白有助于肺炎链球菌与流感嗜血杆菌共同生长时的优势生长。

Abstract: Objective To investigate the enzyme activity and protein expression localization of pyruvate oxidase encoded by *spxB* gene from *Streptococcus* pneumoniae (*S.pn*), its conservation in different serotypes of *S.pn*, and its virulence mechanisms. Methods The full-length *spxB* gene of *S.pn* D39 was amplified by PCR with specific primers, and then inserted into pET-28a(+). Recombinant plasmid pET-28a(+)-spxB was transferred to *E. coli* BL21(DE3) and then the positive clones were induced with IPTG to express His-tagged rSpxB recombinant protein. The recombinant protein was purified by affinity chromatography column, and the protein purity was checked by SDS-PAGE. The enzyme activity assay was performed in a commercial pyruvate oxidase quality control method. The KM mice were immunized with the purified rSpxB protein to obtain polyclonal antibody. The antibody titers were determined by ELISA, and the antibody specificity and protein conservation were determined by Western blotting. The antibody was used for protein expression localization of SpxB in *S.pn* by flow cytometry. The virulence mechanism was studied preliminarily through the construction of *spxB*-deficient mutant strain and co-culture with *Haemophilus influenzae*. Results Soluble expression of SpxB with pyruvate oxidase activity was realized. Antiserum of high titer against SpxB protein was obtained from KM mice. Western blotting showed high antiserum specificity to SpxB and the conservation of spxB in the 1, 2, 4, 6B, 14, 19F, and 23F serotypes of *S.pn*. Flow cytometry results showed that the fluorescence signal of SpxB shifted to the right compared to the negative control, but much weaker than that of the positive control. The wild D39 strain which produced hydrogen peroxide inhibited the growth of Haemophilus influenzae, while the SpxB-deficient strains showed much weaker activity. Conclusion Pyruvate oxidase encoded by SpxB is highly conserved in 7 different serotypes of *S.pn* and has mostly intracellular expression. SpxB helps *S.pn* hold advantages to Haemophilus influenza in co-culture.

参考文献/References:

王哲，王建敏，马峰，等. 肺炎链球菌SpxB蛋白的细胞表达定位、保守性分析及其生物学功能初探[J].第三军医大学学报,2014,36(14):1486-1491.

相似文献/References:

[1]张巧,马千里,李琦,等.肺炎链球菌黏附素A基因疫苗的构建及对小鼠鼻咽部肺炎链球菌携带的防御作用[J].第三军医大学学报,2008,30(08):739.
ZHANG Qiao,MA Qian-li,LI Qi,et al.Construction of DNA vaccine of pcDNA3.1-PsaA and its effects against nasopharyngeal colonization of Streptococcus pneumoniae[J].J Third Mil Med Univ,2008,30(14):739.

[2]马千里,张巧,姚伟,等.肺炎链球菌毒力蛋白DNA疫苗优势抗原组合筛选及鉴定[J].第三军医大学学报,2009,31(10):922.
MA Qian-li,ZHANG Qiao,YAO Wei,et al.Screening of dominant antigen formats of DNA vaccine against Streptococcus pneumoniae[J].J Third Mil Med Univ,2009,31(14):922.

[3]崔瑾,张群,董杰,等.肺炎链球菌dnaJ基因缺陷菌株的构建及毒力变化的初步研究[J].第三军医大学学报,2011,33(19):2000.
Cui Jin,Zhang Qun,Dong Jie,et al.Construction of dnaJ-defecient mutant strain of Streptococcus pneumoniae and preliminary study of its virulence[J].J Third Mil Med Univ,2011,33(14):2000.

[4]张巧,林科雄,姚伟,等.肺炎链球菌表面蛋白A多价DNA疫苗交叉免疫保护作用评价[J].第三军医大学学报,2011,33(21):2230.
Zhang Qiao,Lin Kexiong,Yao Wei,et al.Evaluation of cross-immune protection of PspA multivalent DNA vaccine against Streptococcus pneumoniae[J].J Third Mil Med Univ,2011,33(14):2230.

[5]贺潇,袁军,王虹,等.肺炎链球菌溶血素的体外表达及活性鉴定[J].第三军医大学学报,2011,33(03):237.
He Xiao,Yuan Jun,Wang Hong,et al.In vitro expression of pneumolysin and identification of its biological activity[J].J Third Mil Med Univ,2011,33(14):237.

[6]王强,王跃,刘明方.红霉素体外诱导肺炎链球菌耐药的研究[J].第三军医大学学报,2009,31(12):1185.
WANG Qiang,WANG Yue,LIU Ming-fang.Study on the Streptococcus pneumoniae resistance induced by erythromycin in vitro[J].J Third Mil Med Univ,2009,31(14):1185.

[7]姚润,姜学美,闫文娟,等.热灭活疫苗HID39免疫小鼠可抵抗肺炎链球菌感染[J].第三军医大学学报,2011,33(05):485.
Yao Run,Jiang Xuemei,Yan Wenjuan,et al.Heat-inactivated vaccine HID39 against Streptococcus pneumoniae infections in mice[J].J Third Mil Med Univ,2011,33(14):485.

[8]张群,胥文春,尹一兵,等.clpP基因缺陷对肺炎链球菌毒力表达降低的研究[J].第三军医大学学报,2009,31(04):307.
ZHANG Qun,XU Wen-chun,YIN Yi-bing,et al.clpP gene deletion attenuates virulence of Streptococcus pneumoniae [J].J Third Mil Med Univ,2009,31(14):307.

[9]袁军,陈曜,曹炬,等.肺炎链球菌毒力因子ClpP蛋白酶的体外表达及纯化[J].第三军医大学学报,2008,30(18):1753.
YUAN Jun,CHEN Yao,CAO Ju,et al.Expression and purification of ClpP of Streptococcus pneumoniae in vitro[J].J Third Mil Med Univ,2008,30(14):1753.

[10]闵迅,黄健,黄美容,等.肺炎链球菌蛋白PotD的表达纯化及免疫活性分析[J].第三军医大学学报,2014,36(16):1660.
Min Xun,Huang Jia,Huang Meirong,et al.Expression, purification and immunological activity analysis of PotD protein