



B群脑膜炎球菌*lpxL2*基因敲除突变株的构建及初步鉴定

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Construction and primary identification of meningococcal serogroup B mutant strain that knocked out gene *LpxL2*

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摘要 应用基因敲除技术的方法和原理,通过PCR扩增B群脑膜炎球菌*lpxL2*基因及载体pGBK T7上的Kan抗性片段,*lpxL2*片段与puc-18载体连接得到重组质粒*msb-puc*,以重组质粒*msb-puc*为基础,分别通过反向PCR和酶切2种方法构建*lpxL2*基因中间片段的缺失,并在缺失位点连入Kan抗性表达盒,从而得到重组质粒mpK,mpK转化B群脑膜炎球菌,并用PCR的方法对转化子进行初步筛选鉴定,初步确定突变株1株.本研究通过基因敲除MenB中LPS合成途径相关基因*lpxL2*的方法,降低LPS毒性,为B群脑膜炎球菌OMV疫苗的研发做了铺垫.

关键词: 脑膜炎奈瑟氏菌 外膜囊泡 基因敲除 脂多糖

Abstract: To construct *lpxL2* deletion mutants of meningococcal serogroup B, the *lpxL2* gene and Kan resistance gene was amplified from wild-type N.meningitidis serogroup B strain 29325 and plasmid pGBK T7 respectively. The *lpxL2* gene was cloned into plasmid puc-18 to constitute plasmid *msb-puc*. Based on *msb-puc*, inverse PCR and restriction enzyme digestion were performed respectively to delete some internal fragments, the resulting products were digested and ligated with the Kan resistance gene, yielding recombinant plasmid mpK which was used to transform into wild-type strain 29325. Antibiotic-resistant transformants were screened by using PCR, and a mutant strain was primary identified. The LPS biosynthesis gene *lpxL2* mutants can reduce toxicity by means of knocking out gene *lpxL2*, which established approach for the development of OMV vaccine against Nm serogroup B.

Key words: *Neisseria meningitidis* OMV gene knockout lipopolysaccharide

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