山东大学学报(理学版) 2008, 43(7) 78-82 DOI: ISSN: 1671-9352 CN: 37-1389/N



论文

# 乳酸乳球菌nisin抗性基因的克隆及作为筛选标记的研究

郭婷婷，孔文涛，孔 健*，季明杰

山东大学微生物技术国家重点实验室， 山东 济南 250100

摘要：

在添加500U/mL nisin和溴甲酚紫的GM17选择性培养基上，分离到5株nisin抗性菌株。根据形态观察，生理生化特性和16S rDNA基因序列比对被鉴定为乳酸乳球菌。根据已报道nisin抗性基因设计引物，分别以这5株菌的染色体和质粒DNA为模板进行PCR扩增，结果从1株抗性菌株的染色体上得到目的基因产物。通过序列测定和同源性比对，证明为nisin抗性基因（nsr）。将nsr基因克隆到乳酸菌-大肠杆菌穿梭质粒pTRKH2上，重组质粒命名为pT-nsr。pT-nsr电转化乳酸乳球菌MG1614，获得的重组菌MG1614/pT-nsr 在含有500U/mL nisin的培养基中生长情况良好。这表明nsr基因 赋予宿主菌抗nisin特性，并且与红霉素抗性功能相同，因此nsr基因可以作为筛选标记用于食品级载体的构建。

关键词： 乳酸乳球菌 nisin抗性基因（nsr） 食品级筛选标记

# Cloning of a nisin resistance gene from Lactococcus lactis and its application in food-grade selection marker

GUO Ting-ting, KONG Wen-tao, KONG Jian*, JI Ming-jie

State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100, Shandong, China

Abstract:

Five nisin-resistant strains were isolated from fresh milk on GM17 plates supplemented with bromocresol purple and nisin at a final concentration of 500U/mL. By morphological, physiological， biochemical and 16S rDNA
sequence analysis, all of the isolates were identified as Lactococcus lactis. A pair of primers was designed on the basis of the DNA sequences of a reported nisin resistance gene. PCR amplification was carried out with chromosome and plasmid DNA as templates from five strains, respectively. An expected PCR product amplified from one of the five strains was obtained. After being sequenced, the amplicon was
confirmed as nsr by BLAST analysis. The nsr gene was cloned into the E.coli-L.lactis shuttle vector pTRKH2, resulting in the plasmid pT-nsr. The construct was obtained when the plasmid pT-nsr was electroporated into L.lactis MG1614 competent cells. When the medium contained a maximum of 500U/mL nisin, the construct carrying pT-nsr showed the same growth curve as L.lactis MG1614, which suggests that the nsr gene could be used as a marker for constructing a food-grade vector.

Keywords:

Lactococcus lactis nisin resistance gene (nsr) food-grade selection marker

收稿日期 1900-01-01 修回日期 1900-01-01 网络版发布日期 2006-10-24

DOI:

基金项目:

通讯作者: 孔 健

作者简介:

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孙芝兰,孔文涛,孔　健*

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