中国农学通报 2011, 27(第21期8月) 221-224 DOI: ISSN: 1000-6850 CN: 11-1984/S

生物技术—研究报告

实时荧光定量RT-PCR法对志贺菌外排泵基因emrE的表达分析

穆瑞瑞[1],朱凤荣[2],段广才[2],吴健[2]

1. 新乡学院

2.

摘要：

测定临床耐多药志贺菌株H24的外排泵emrE基因的表达情况，尝试建立一个快速检测外排泵基因表达的方法。提取多耐药志贺菌株H24的RNA，用实时荧光定量RT-PCR方法验证H24的外排泵emrE基因mRNA表达水平和耐药的相关性。实时荧光定量RT-PCR获得H24的外排泵基因emrE的mRNA相对表达量，emrE基因的相对表达量为内参基因gapA的2万多倍；同一标本重复扩增，emrE和gapA基因相对拷贝数的平均变异系数分别为1.4%和1.3%，提示本方法可准确检测二者的相对拷贝数。由emrE基因的高表达可以推断它与志贺菌的多耐药有正相关性，这个推断结果与以前通过克隆emrE基因的方法得到的结果一致。所建立的emrE基因荧光定量RT-PCR诊断方法特异性好、敏感性高，SYBR Green Ⅰ荧光定量RT-PCR可进一步应用于志贺菌多耐药株其他外排泵的临床诊断和科学研究。

关键词： 荧光定量RT-PCR

The Expression Analysis of Drug Efflux Gene emrE mRNA in Shigella spp. by Real-time RT-PCR

2, 2, 2

Abstract:

The aim was to establish a rapid detection method of expression of the efflux pump genes via determination expression of emrE efflux pump gene from clinical isolated multidrug resistance Shigella spp. strain H24. RNA of multidrug-resistance Shigella H24 was extracted, and real-time fluorescent quantitative RT-PCR was performed to investigate the correlation between emrE gene mRNA expression level and the drug resistance ability. The relative expression content of the efflux pump gene emrE mRNA from Shigella H24 was obtained by real-time fluorescent quantitative RT-PCR, and the relative expression of emrE gene was 20000 times more than house gene gapA. The same sample repeat amplification, and average coefficient of variation of emrE and gapA relative copy numbers was 1.4%和1.3%, it revealed this method detected both relative copy numbers exactly. The high expression of emrE gene could infer that it was related with multidrug resistance of Shigella spp.. This result was consistent with the findings by cloning emrE gene formerly. The SYBR Green Ⅰ FQ RT-PCR method had a highly sensitivity and specificity, and could be further applied to other efflux drugs, clinical diagnosis and research from multidrug-resistance.

Keywords: fluorescence quantitative RT-PCR (FQ RT-PCR)

收稿日期 2011-04-20 **修回日期** 2011-06-16 **网络版发布日期** 2011-09-06

DOI:

基金项目:

通讯作者: 穆瑞瑞

作者简介:

作者Email: ruirui5200@163.com

参考文献：

本刊中的类似文章