2018/11/19 《中国食品卫生杂志》

並文

下试试看

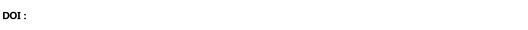
首页 | 期刊介绍 | 投稿指南 | 排行榜 | 光荣榜 | 編委会 | 期刊订阅 | 留言板 | 联系我们 | 自荐編委/审稿人 | 广告合作

谭翰清、蔡建生、谭海芳、林凤、程洁萍、TagMan-MGB探针实时荧光定量PCR检测克罗诺杆菌MMS基因方法的建立[J].中国食品卫生杂 志,2014,26(1):40-44.

TaqMan-MGB探针实时荧光定量PCR检测克罗诺杆菌MMS基因方法的建 立

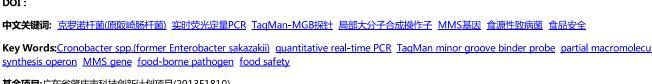
Establishment of quantitative real-time PCR targeting the MMS gene of Cronobacter spp. based on TaqMan-MGB probe

投稿时间: 2013-09-22



Key Words: Cronobacter spp. (former Enterobacter sakazakii) guantitative real-time PCR TagMan minor groove binder probe partial macromolecular synthesis operon MMS gene food-borne pathogen food safety

基金项目:广东省肇庆市科技创新计划项目(2013E1810)



11	潜	单位	E-mail
這	輸責	广东省肇庆市疾病预防控制中心,广东肇庆526060	tanhanqing2000@163.com
玄	建生	广东省肇庆市疾病预防控制中心,广东肇庆526060	
遣	<u> </u>	广东省肇庆市疾病预防控制中心,广东 肇庆 526060	
乜	凤	广东省肇庆市疾病预防控制中心,广东肇庆526060	
種	<u> </u>	广东省肇庆市疾病预防控制中心,广东肇庆526060	

摘要点击次数: 602 全文下载次数: 644

中文摘要:

建立克罗诺杆菌的特异、灵敏的TagMan-MGB探针实时荧光定量PCR检测方法。方法 根据GenBank公布的克罗诺杆菌MMS基因高保守 序列,设计特异引物和TagMan-MGB探针,建立和优化反应体系,用25种其他常见致病菌评价反应体系的特异性,用克罗诺杆菌MMS基因重组质 粒构建实时荧光定量PCR标准曲线,对重组质粒、纯菌和人工模拟污染样本进行灵敏度试验,并与FDA推荐的TaqMan探针实时荧光PCR比较配 对t检验分析两种方法对Ct值和荧光强度的差异。结果采用TaqMan-MGB探针实时荧光定量PCR检测克罗诺杆菌MMS基因仅需40min,与25 种非目标菌无交叉反应,仅对克罗诺杆菌有特异性扩增;所构建方法线性关系良好,相关系数r²=0.999,扩增效率为99.972%,对重组质粒、纯 菌、人工模拟污染样品标本的灵敏度分别达10拷贝/反应、3.8和38cfu/ml;与FDA推荐的TaqMan探针实时荧光PCR相比、TaqMan-MGB探 针实时荧光定量PCR的Ct值更小、ARn值更高,灵敏度和分辨率差异均有统计学意义(Ct.t=-14.406,P < 0.01; ARn:t=14.230,P < 0.01)。结论 本研究建立的TaqMan-MGB探针实时荧光定量PCR反应体系能够快速、特异、灵敏地检测克罗诺杆菌MMS基因,可用于婴幼儿奶粉中克罗诺 杆菌的快速筛查和鉴定,具有较大的的应用价值和推广价值。

Abstract:

To establish a specific and sensitive TaqMan-MGB quantitative real-time PCR assay for the rapid detection of Cronobacter spp..MethodsBased on the conservative sequence of partial macromolecular synthesis operon gene of Cronobacter spp. published on GenBank, specific primers and TaqMan Minor groove binder (TagMan-MGB) probes were designed, and the rapid real-time PCR assay was estabilished and optimized. The specificity was evaluated with 25strains of other Enterobacteriaceae and some common pathogens. The quantitative standard curve was established with the recombinant plasmids and the sensitivity for the assay was evaluated for recombinant plasmids, pure cultures and contaminated food samples. Comparing with the TaqMan real-time PCR recommended by U.S. FDA, paired-samples t-test for the variables of cycle threshold (Ct) and relative fluorescence intensity (ARn) was done between the two methods. ResultsThe TaqMan-MGB quantitative real-time PCR assay could be finished detection in 40minutes. It was specific enough to discriminate Cronobacter spp. from all other Enterobacter and non-Enterobacter strains tested. The relative coefficient of the quantitative standard curve was 0.999, and the amplification efficiency of the quantitative standard curve was 99.972%. The sensitivity for the assay was 10copies per reaction for recombinant, 3.8cfu/ml for pure culture, and 38cfu/ml for contaminated food samples, respectively. There were statistical differences between two real-time PCR methods by paired-samples t-test (Ct:t=-14.406, P<0.01and An:t=14.230, P<0.01). The TaqMan-MGB real-time PCR was better than the TaqMan real-time PCR recommended by U.S. FDA in sensitivity and resolution. Conclusion The TaqMan-MGB quantitative real-time PCR assay targeted the partial macromolecular synthesis operon gene of Cronobacter spp. is rapid, specific and sensitive. It would had a good value in the screening and identification of Cronobacter spp. from infant milk powder for food safety and risk monitor.

查看全文 查看/发表评论 下载PDF阅读器

您是第27822371位访问者 今日一共访问162次

版权所有:《中国食品卫生杂志》编辑部 京ICP备12013786号-3 地址:北京市朝阳区广渠路37号院2号楼501室 邮编:100022

E-mail:spws462@163.com 电话/传真:010-52165456/5441 (编辑室) 010-52165556 (主编室)

未经授权禁止复制或建立镜像

技术支持:北京勤云科技有限公司

