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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

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

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中文摘要:

建立克罗诺杆菌的特异、灵敏的TaqMan-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TaqMan-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r^2=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、△Rn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；△Rn: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 (TaqMan-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 (△Rn) 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 △Rn: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.ConclusionThe 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.

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