景明.逆转录定量PCR选择性检测环境样品中的活性分枝杆菌[J].环境科学学报,2015,35(2):484-490

逆转录定量PCR选择性检测环境样品中的活性分枝杆菌

Selective detection of viable *Mycobacterium* spp. in environmental samples using reverse transcription quantitative PCR

关键词：RT-qPCR 分枝杆菌 活性菌 环境样品

基金项目：国家水体污染控制与治理科技重大专项(No.2011ZX07506-003-005)

作 者 单位

景 明 1. 苏州环境监测中心, 苏州 215000;2. 同济大学环境科学与工程学院, 上海 200092

摘要：以分枝杆菌(*Mycobacterium* spp.)为研究对象,建立了一种逆转录定量PCR(Reverse transcription quantitative PCR,RT-qPCR)方法,选择性检测环境样品中的活性分枝杆菌.研究结果表明,稳定期(48~144 h)分枝杆菌细胞内*hsp*65基因的RNA转录稳定,单位细胞内*hsp*65的cDNA含量约为1拷贝,以此作为活性分枝杆菌的定量依据,达到快速确定环境样品中活性分枝杆菌浓度的目的.相比qPCR,RT-qPCR方法能够区分3个数量级的非活性分枝杆菌;样品的底物基质对RT-qPCR方法的影响较小;对于实际样品,与qPCR方法相比,RT-qPCR方法检测实际样品中活性分枝杆菌的结果与培养法更接近,线性关系更加显著(R^2=0.9390).本研究表明,作为一种新的检测技术,RT-qPCR可以快速、准确地检测环境样品中的活性分枝杆菌.

Abstract：A detection method based on reverse transcription quantitative PCR (RT-qPCR) was developed to quantify the concentration of viable *Mycobacterium* spp. in environmental samples. The results showed that the transcription of *hsp*65 gene of *Mycobacterium* spp. was stable during the stationary grow phase (48~144 h), and the concentration of cDNA targeting on *hsp*65 was about 1 copy per cell. Based on the above results, the established method can quantify viable *Mycobacterium* spp. in environmental samples. Compared to qPCR, RT-qPCR could distinguish inactivated *Mycobacterium* spp. only with 3 lg level. Besides, the substrate of environmental sample had non-significant effect on RT-qPCR. Finally, RT-qPCR results were similar and highly linearly correlated to those obtained by culture assay for environmental samples, and the linear correlation (R^2) between RT-qPCR and culture-based method was 0.9390. In summary, RT-qPCR shows to be a promising method that can detect viable *Mycobacterium* spp. in environmental samples rapidly and accurately.

Key words：reverse transcription quantitative PCR(RT-qPCR) *Mycobacterium* spp. viable bacteria environmental sample