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研究报告

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基于CTC-流式细胞仪活性细菌总数的快速检测技术研究

Rapid detection of viable bacteria by integrated CTC (5-Cyano-2, 3-ditoyl tetrazolium chloride) dying and flow cytometry assay (CTC-FCM)

关键词：CTC(5-cyano-2 3-ditolyl tetrazolium chloride)染料;流式细胞仪;活性细菌总数;实际环境水样;快速检测

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摘要：以大肠杆菌作为研究对象,建立一种 5-cyano-2,3-ditolyl tetrazolium chloride(CTC)染色结合流式细胞仪(CTC-FCM)的方法,以选择性检测水环境中具有代谢活性的细菌总数.该方法的原理是细菌与具有氧化还原性的染料CTC发生反应,形成红色荧光物质,被流式细胞仪特异性识别进而可选择性检测活性菌.研究结果表明,CTC染色的最佳反应条件为:CTC浓度为2 mmol·L^{-1}、37 ℃避光孵育3 h.该方法最低检测限为10^3 个·mL^{-1}.通过比较培养法和CTC-FCM方法检测热灭活后的大肠杆菌,结果表明CTC-FCM方法可准确区分活性菌和灭活菌,且与培养法之间具有较好的线性关系(R^2=0.9465).应用CTC-FCM方法检测实际样品,结果显示该方法与培养法之间有较好的线性关系(R^2 = 0.8121).本研究建立的CTC-FCM方法可满足饮用水水质标准需求,且检测时间比平板培养法缩短20~40 h,可以用于环境水样中活性细菌总数检测.

Abstract: An integrated tetrazolium redox CTC (5-Cyano-2,3-ditoyl tetrazolium chloride) dying and flow cytometry assay (CTC-FCM) was developed by using *Escherichia coli* as a representative organism. This method can selectively detect and quantify bacteria with metabolic activity, based on the principle that only active bacteria can react with CTC and form a fluorescent red intracellular CTC-formazan (CTF) easily detected and counted by flow cytometry. The results showed that the optimized detection parameters were 2 mmol·L^{-1} CTC at 37 ℃ for 3-hour incubation. The detection limit of CTC-FCM method was 10^3 CFU·mL^{-1}. Compared with culture-based method for detection of heat-treated bacteria, CTC-FCM method can effectively distinguish viable bacteria from non-viable bacteria, and a good correlation was observed between these two methods (R^2 = 0.9465). This method was also applied to detect viable bacteria in environmental water samples, including tap water and reclaimed water. Results showed that the correlation coefficient (R^2) between CTC-FCM and culture-based method was 0.8121. The CTC-FCM method meets the needs of drinking water quality standards and the detection time was reduced by 20~40 hours, therefore is an effective and quantitative tool for detecting viable bacteria in environmental waters.

Key words: CTC stains flow cytometry viable bacteria environmental water sample fast detection