

利用实时荧光PCR方法检测香蕉软腐细菌

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Utilization of a Real-time PCR-based Approach for Rapid Quantitative Detection of Bacterial Soft Rot of Banana

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摘要 由 *Dickeya* spp. 引起的香蕉细菌性软腐病是近年来在中国广东发生的一种严重危害香蕉的病, 检测方法的建立和应用是防止病害传播和实时防治的重要手段。依据报道的 *Dickeya* spp. 通用引物, 建立了用于香蕉细菌性软腐病发病植株和带菌土壤检测的实时荧光PCR方法。优化后的检测体系对香蕉软腐细菌 (XJ8-3-3) 靶片段克隆质粒DNA的检测灵敏度可达到 $2.4 \times 10^{-5} \text{ ng} \cdot \mu\text{L}^{-1}$, 对菌悬液的检测灵敏度可达到 $4.0 \times 10^2 \text{ cfu} \cdot \text{mL}^{-1}$, 而常规PCR对其检测的灵敏度为 $2.4 \times 10^{-3} \text{ ng} \cdot \mu\text{L}^{-1}$ 和 $4.0 \times 10^4 \text{ cfu} \cdot \text{mL}^{-1}$, 实时荧光PCR的灵敏度比常规PCR高100倍。利用实时荧光PCR能够快速的检出香蕉细菌性软腐病发病植株和带菌土壤中的病原菌量, 对梯度稀释的菌悬液接种的带菌土壤检测结果表明, 可检测到病菌DNA最低含量为 $0.35 \text{ pg} \cdot \text{L}^{-1}$ 。该方法适用于对香蕉软腐病菌的检测和监控。

关键词: 香蕉 软腐病菌 实时荧光定量PCR 检测

Abstract: Bacterial soft rot of banana, caused by *Dickeya* spp., is a destructive disease on banana discovered recently in Guangdong, China. Establishment and application of detection method is an important means to prevent the disease from spreading and occurring. A real-time fluorescent PCR assay was developed based on the universal primers for detecting *Dickeya* spp. from infectious banana plants and soil. The results showed that the lowest detection limits for the Plasmid DNA and bacterial suspensions of bacterial isolate (XJ8-3-3) was $2.4 \times 10^{-5} \text{ ng} \cdot \mu\text{L}^{-1}$, and $4.0 \times 10^2 \text{ cfu} \cdot \text{mL}^{-1}$, respectively, by the optimized real-time fluorescent PCR system, which indicated that detection sensitivity with real-time fluorescent PCR was at least hundred times higher than that with conventional PCR. The bacterial DNA of XJ8-3-3 in the infected banana plant tissues and soil could be accurately and rapidly identified by the developed detection system. The detection limit of bacterial DNA from the soil inoculated with different concentrations of XJ8-3-3 suspensions was $0.35 \text{ pg} \cdot \mu\text{L}^{-1}$ per reaction in real-time quantitative PCR. In conclusion, this real-time PCR method could be utilized to assist in the implementation of quarantine measures for prevention and control of the bacterial soft rot of banana caused by *Dickeya* spp.

Keywords: banana, bacterial soft rot, real-time fluorescent PCR, detection

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