

Title Electrochemical biosensor for a simple and rapid detection of canker (*Xanthomonas axonopodis* pv. *Citri*) in pomelo for export

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Abstract

Xanthomonas axonopodis pv. *citri* (Xac) is a bacterial pathogen that causes citrus canker, a serious disease of most citrus species. For pomelo, this disease has great economic impact since it is considered a quarantine disease. Fruit with suspicious symptoms can not be exported despite disinfestation treatments. Therefore, it is important to confirm if such symptoms are produced by Xac. Accordingly a simple and rapid detection method, based on loop-mediated isothermal DNA amplification with an electrochemical DNA sensor, was developed to enhance efficiency of the fruit analysis program and to assure quality on asymptomatic fruit. DNA signals was detected based on a phenomenon of DNA aggregation induced by Hoechst 33258 (2'-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi(1*H*-benzimidazole) in conjunction with changes in anodic current peaks measured via a carbon screen printed electrode on linear sweep voltammetry, in a designed DNA stick tube without fluorescence detection. Signals of anodic current peaks of the loop-mediated isothermal amplification products from positive specimens were below 1.08 μ A while those of negative specimens were more than 1.80 μ A. Detection was accomplished within 60 min under isothermal condition at 63°C, without the need for costly devices, by employing a rapid DNA extraction method and amplification targeting of the *rpf* gene of Xac. The assay was highly sensitive, with a detection limit of five copies per test. No cross-reactivity was observed from samples of other related *Xanthomonas* species. This assay provides a rapid yet simple test for detection of Xac that is suitable for field application because of its ease of operation requiring only a regular water bath or heat block and small sensor unit for the detection process.