Quantitative Real-time PCR for diagnosis and identification of *Xanthomomas citri* pv. citri pathotypes, the causal agent of Asiatic Citrus Canker

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Asiatic Citrus Canker disease is induced by *Xanthomonas citri* pv. *citri* (*Xcc)* and threatens most of *Citrus* species and cultivars in many citrus-producing countries. Two pathogenic variants with different host range were described within *Xcc*. The pathotype *Xcc*-A infects a wide range of citrus and some related genera, has a worldwide distribution and is a permanent threat for citriculture. In contrast, *Xcc*-A* is naturally restricted to two *Citrus* species, limes (*C. aurantifolia*) and alemow (*C. macrophylla*) in limited areas. A rapid and reliable test using molecular methods is useful for accurate identification at pathotype level of this bacterium, classified as quarantine organism. Several PCR-based diagnostic tools have been developed for the identification of *Xcc*. In a preliminary study, we showed a lack of specificity for most of them when assayed on a large collection of *Xcc* strains and non-target strains. The aim of this study was to propose a new diagnostic tool for detection and identification of pathotypes of *X. citri* pv. *citri*. A multiplex quantitative real-time PCR assay (qPCR) was developed using hydrolysis probes targeting two markers of the bacterium.

PCR primers targeting genes involved in pathogenicity and/or plant interactions were designed from the complete sequence of the *Xcc*-pathotype A strain IAPAR 306. Their specificity was assayed on a collection of *Xcc*-A strains (n=21), *Xcc*-A* (n=14) and other genetically related *Xanthomonas* (n=23) (1). A specific *Xcc* sequence encoding a conserved hypothetical protein related to chemotaxism and a specific *Xcc*-A sequence in a housekeeping gene were retained for the qPCR assay.

The specificity of the qPCR assay was verified on the same strains collection of *Xcc* strains and non-target *Xanthomonas*. The qPCR assay detected all the *Xcc* strains and identified the pathotypes from cultures and citrus plant samples artificially infected with the bacterium: a sample is tested positive for the presence of A strains if both markers are amplified. No amplification or amplification of the housekeeping gene marker alone led to a negative result for *Xcc.* Amplification of the *Xcc*-specific marker alone is the signature for A* strains. Standard curves with high correlation coefficients (R^2>0,99) were obtained from 10-fold bacterial suspensions dilutions. The qPCR assay allowed the detection of 10^7 to 10^3 CFU per ml of bacterial suspension or per g of lime leaves (*Citrus aurantifolia*) with calculated amplification efficiencies around 90% and 80% respectively.

This qPCR assay is a powerful tool that allows distinguishing pathotypes with different economic incidence. It would be useful for indexing propagation material in nurseries and for surveillance of international movement of *Xanthomonas citri* pv. *citri*

References:

1. Rademaker *et al.* 2005. *Phytopathology*. 95: 1098-1111