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1031

Molecular detection of the *Xanthomonas* species complex causing tomato bacterial spot

(Detecção molecular do complexo de espécies de *Xanthomonas* causadoras da mancha bacteriana do tomateiro)

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PCR-based methods for diagnosis of tomato bacterial spot have been developed. However, only up to recently, specific primers for the four *Xanthomonas* species associated with the disease have been reported. The objectives of this work were to validate these primers with Brazilian isolates, verify their specificity and sensitivity, and also to establish a multiplex PCR protocol for simultaneous detection of all species associated with bacterial spot for routine diagnostic use. Thirty strains of *Xanthomonas euvesicatoria*, 30 of *X. vesicatoria*, 50 of *X. perforans*, and 50 of *X. gardneri*, and 32 strains of other fungal and bacterial plant pathogens were tested. The primers were considered highly specific, amplifying only the target DNA. Sensitivity varied from 10^2 to 10^4 CFU/mL for bacterial suspensions and up to 50 $\mu\text{g}/\mu\text{L}$ of purified DNA. Using the multiplex PCR protocol different combinations of DNA from the four species were detected. Specific detection was also accomplished directly from symptomatic tomato leaves. This protocol is rapid, specific and may be an efficient tool for bacterial spot diagnosis, allowing simultaneous detection and identification of all associated species.

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