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Sensitive and specific detection of *Xanthomonas axonopodis Pv. vesicatoria* by direct colony polymerase chain reaction (PCR)

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The present study describes the colony PCR assay to detect the bacterial spot disease caused by *Xanthomonas axonopodis* pv. vesicatoria that affects tomato. The direct method of diagnosis of spot disease has been so far by culture isolation and traditional methods by biochemical characterization which is also time consuming and fails to identify the infecting pathovars. The rhs family gene is well characterized and sequenced completely in *Escherichia coli* K-12 due to high degree of sequence conservation maintained among its members. However, BLAST search using the nucleotide sequences of this rhs family gene, which are deposited in GenBank database, found sequence divergence at pathovar level. One set of PCR primer was custom synthesized to amplify gene required for an *rhs* family gene homologous to *rhsA*, cell envelop. We have developed direct colony PCR assay for the detection of Xanthomonas axonopodis pv. vesicatoria from infected samples recovered on selective media without DNA extraction. Single colony of *Xanthomonas axonopodis* pv. *vesicatoria* was picked up with a sterile pipette tip and added directly to the PCR mix as a template for DNA amplification. Successful amplification was achieved at 517 bp in over 95% of the colonies recovered from infected plant material/seed samples with species - specific primers. Application of this direct colony PCR technique for early disease diagnosis and the source of pathogen can be explored. This method can be a quantitative tool for pathogen detection that may provide more information. The use of colony PCR for specific and sensitive detection of *Xanthomonas axonopodis* pv. *vesicatoria* is discussed in the present study.