

ORIGINAL ARTICLE

Occurrence of *Pseudomonas viridiflava* in tomato plants in Uberlândia, Minas Gerais

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Data de recebimento: 16/09/2020. Aceito para publicação em: 24/06/2023

10.1590/0100-5405/243634

The bacterium *Pseudomonas viridiflava* (Burkholder) Dowson is the causal agent of leaf bacterial speck (8) and pith necrosis (2, 10) in tomato plants (*Solanum lycopersicum* L.). In 1973, this species was reported by Wilkie et al. (22) causing pith necrosis and leaf speck symptoms and was subsequently associated with symptoms in the fruits (6). *Pseudomonas viridiflava* was described causing leaf bacterial speck on tomato in Greece (5) and the United States (8), as well as pith necrosis in Greece (2, 12), Argentina (1), Portugal (18), Turkey (3, 21), Macedonia (15) and Serbia (17). In Brazil, symptoms of bacterial speck were reported in Bahia and São Paulo States (13), while pith necrosis was found in Santa Catarina State (16). In 2013, in a commercial staked tomato field in Uberlândia, Minas Gerais State, plants were observed showing leaf bacterial speck symptoms (Figure 1A), lower leaf wilt, leaf chlorosis, and severe pith necrosis (Figure 1B) including dark brown lesions and premature death. The pathogen was isolated by indirect method (19) from infected leaves and from pith necrosis on 523 culture medium (9). The isolates (Figure 1C) were biochemically characterized as Gram-negative, aerobic growth, fluorescence in King B medium, levan production-negative, oxidase-negative, pectolytic activity-positive, arginine dihydrolase-negative, tobacco hypersensitivity reaction-positive (LOPAT/- - + - +), and identified as *P. viridiflava* (10, 20). The pathogenicity of isolates was confirmed by spraying of tomato leaves until runoff and stem injection with the bacterial suspension at 1×10^8 CFU.mL⁻¹. The plants were kept in a moist chamber 24 h before and after inoculation. Twenty days after inoculation, symptoms of leaf bacterial speck were observed on the leaf adaxial surface and petioles (Figure 1D), including small circular and water-soaked lesions, while pith necrosis symptoms were found in the stem (Figure 1E). The bacteria were then reisolated to complete Koch's postulates. No symptoms were observed for control plants inoculated with sterile distilled water. The disease was less severe for inoculated leaves than for tomato plants in the field. According Monteiro et al. (16), leaf symptoms were not observed for plants inoculated with the strain obtained from the stem which, however, was reported to be capable of infecting various plant parts. Thus, *P. viridiflava* is less aggressive on leaves, while tomato production losses are more related to pith necrosis. The genomic DNA of bacterial strains was not amplified with the primer pair Primer 1/Primer

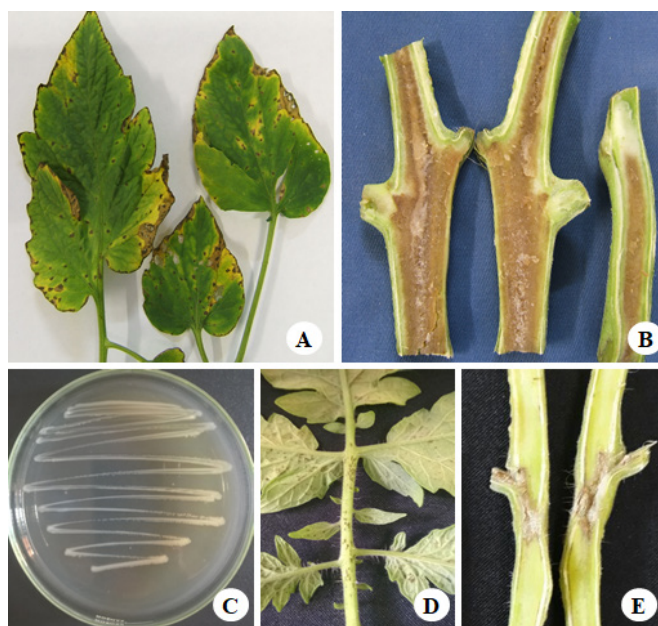


Figure 1. Symptoms of leaf bacterial speck (A) and tomato pith necrosis (B), caused by *Pseudomonas viridiflava*. Bacterial growth on culture medium (C). Symptoms of leaf bacterial speck (D) and tomato pith necrosis (E), 20 days after inoculation.

2, specific for *P. syringae* pv. *tomato* (Okabe) Young, Dye & Wilkie (4), or with the primer pair PF/PR, specific for *P. syringae* pv. *syringae* van Hall (7). Considering BOX-PCR (11), the isolates had the same pattern and number of bands. Sequencing of the 16S rRNA gene region using the universal pair of primers 27F/1492R (14) was compared with sequences deposited in the GenBank and aligned closely (99.02% similarity) with *P. viridiflava* (GenBank No. NR_114482.1), query cover of 100% sequence. Correct characterization and identification of this bacterium can improve the disease management in the field,

especially because pith necrosis symptoms can be confused with other fluorescent *Pseudomonas* species in tomato plants. This is the first occurrence of *P. viridiflava* causing tomato pith necrosis and leaf bacterial speck in Uberlândia, Minas Gerais, Brazil. The strains are maintained in the phytopathogenic bacteria collection of the Institute of Agrarian Sciences at Federal University of Uberlândia and were codified as UFU E36 and UFU E37.

FUNDING

University Support Foundation (FAU) of the Federal University of Uberlândia (UFU) and CNPq.

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Editor associado para este artigo: Luadir Gasparotto