


Diseases Caused by Bacteria and Phytoplasmas

First Report of *Pseudomonas mediterranea* Causing Tomato Pith Necrosis in Croatia

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In May 2021, a tomato producer reported an occurrence of a previously unknown disease in a greenhouse near Split, Croatia. About 30% of plants (cultivar Signora) were affected. Symptoms resembled tomato pith necrosis, a bacterial disease caused by *Pseudomonas corrugata*, known to occur sporadically in tomato greenhouse production in Croatia. Leaves on plants developed interveinal chlorosis, followed by necrosis and leaf collapse. When main stems were longitudinally cut, a brown, disintegrated, and water-soaked partly hollow pith was evident. Severely affected plants wilted. With the suspected presence of *P. corrugata*, bacteria were isolated from surface-sterilized pith tissue of two tomato plants by plating onto sucrose peptone agar (SPA) and King's B medium (KB). Colonies recovered were cream colored on SPA and nonfluorescent on KB. Two isolates, 1-KB and 3A, were first identified by amplification of the internal transcribed spacer (ITS1) between 16S rRNA and 23S rRNA using primers D21 and D22 (Manceau and Horvais 1997). The 550-bp PCR products obtained were purified and sequenced. A subsequent BLAST search showed the sequences to have 100% identity with the strain DSM 16733 isolated from tomato in Italy (accession no. LT629790.1) and 99.77% identity with the strain SM664-12 isolated from tomato in the United States (acc. no. KC405207.1) of

Pseudomonas mediterranea from NCBI. The ITS sequence for isolate 3A was deposited in GenBank under accession no. OP765279. Further identification was performed using species-specific primers PC1/1-PC1/2 for *P. mediterranea* (Catara et al. 2000, 2002). Amplification of a 600-bp DNA fragment confirmed the identity of isolates 1-KB and 3A as *P. mediterranea*. For this region, the sequence of isolate 3A was deposited in GenBank under acc. no. OP068273. Pathogenicity was assessed on tomato plants (cultivar Moneymaker) grown in pots in a bio-chamber. Plants were grown at 25/20°C 12/12h dark/light regime until the eight-leaf stage (BBCH 18). *Pseudomonas mediterranea* isolate 3A was used for the inoculation. Inoculum was prepared from the isolate grown on KB medium for 48 h and suspended in sterile distilled water (concentration of 10⁹ CFU ml⁻¹) by dilution plate counts. Ten plants were inoculated with 10 µl of bacterial suspension injected into the stem with a syringe. Five control plants were inoculated with sterile distilled water. After 40 days of plant growth, symptoms were visible on all plants inoculated with *P. mediterranea* isolate 3A. Although no wilting was observed and all plants were alive, chlorosis was observed on upper leaves and chlorosis and necrosis on middle leaves, while basal leaves wilted. Longitudinal cross-sections of stems revealed brownish pith tissue with longitudinal watery pits spreading from inoculation points. Symptoms were not observed on control plants. Bacteria reisolated from three plants showing the most severe symptoms proved to be identical to the original using species-specific primer pair PC1/1-PC1/2. To our knowledge, this is the first confirmation of *P. mediterranea* causing tomato pith necrosis in Croatia. Tomato pith necrosis caused by *P. mediterranea* may become a significant bacterial disease of greenhouse tomato in Croatia.

References:

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