New Disease Reports

First report of *Ralstonia solanacearum* causing tomato bacterial wilt in Mexico

J. Hernández-Romano [1]*, S. Ramírez-Rojas [2] and C.J. Ydrac-Morales [1]

[1] *Universidad Politécnica del Estado de Morelos. Boulevard Cuauhnáhuac #566, Col. Lomas del Texcal, Jiutepec, Morelos, CP 62550, Mexico*; [2] *Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experimental Zacatepec, Km. 0.5 Carretera Zacatepec-Galena, Galeana, Zacatepec, Morelos, Mexico*

*E-mail: jhernandez@upemor.edu.mx

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Worldwide, Mexico ranks first in the export of fresh tomato (*Solanum lycopersicum*) (SAGARPA, 2010; USDA, 2009), and Morelos is in the top ten producing states in the country (SIAP, 2010). Since June 2010, several greenhouse-based tomato crops from at least five districts of Morelos have been affected by wilt without foliar yellowing. When cut stems of affected plants were submerged in water, whitish ooze was evident and longitudinal sections showed a brown discolouration (Fig. 1). Mucous, opaque, pleomorphic and convex colonies with red centres and whitish periphery were isolated from affected plants on nutrient broth and yeast extract (NBY) agar with 2.5% glucose alone or supplemented with triphenyl tetrazolium chloride (TZC, 0.005%) (Fig. 2).

For pathogenicity tests, tomato plants cv. Ramses at four-true-leaf phenological stage were infected using a needle dipped in a pure liquid bacterial culture with A600nm=0.100 (approximately $2x10^8$ cfu/ml), with which the stem was pierced at the height of the first true leaf. Five plants were inoculated in this way, while five plants were pierced without bacteria and five were untreated controls. All plants were kept at 28°C and in a 16h/8h light/dark cycle. Plants inoculated with bacteria wilted 13 to 15 days after inoculation. Infected plants showed oozing brown lesions of variable size that never recovered at the point of inoculation together with the emergence of adventitious roots. Dissection showed bacterial dissemination through the plant xylem in these plants (Fig. 3). *R. solanacearum* was recovered on TZC medium from the five symptom-bearing plants that had been inoculated. In contrast, lesions made with needle without bacteria healed spontaneously and no adventitious roots were seen on the stem. Control plants remained healthy.

Identification of the pathogen was done by PCR using two primers pairs: 759/760 F(5'-GTCGCCGTCAACTCACTTTCC-3', 5'-GTCGCCGTCAGCAATGCGGAATCG-3'; Opina, *et al*, 1997), Nmult21:2F/Nmult22:RR (5'-AAGTTATGGACGGTGGAAGTC-3', 5'-TCGCTTGACCCTATAACGAGTA-3'; Fegan & Prior, 2005). Each amplification reaction generated 282 bp and 372 bp amplicons, respectively, the first corresponding to the upstream region of *lpxC* gene and used to identify *Ralstonia solanacearum* at the species level; the second corresponding to the 16S-23S ITS region specific to phylotype II of *Ralstonia solanacearum*. These results confirmed *Ralstonia solanacearum* phylotype II as the wilt causal agent (Fig. 4). This is the first report of *Ralstonia solanacearum* causing bacterial wilt in tomato crops in Mexico. Symptoms share characteristics with previous reports (Loreti *et al.*, 2007). Features shared by greenhouses affected by this pathogen are high temperatures (35°-50°C) and high relative humidity (above 80%). Tomato crops in the open air do not show signs of infection. Tomato is the main vegetable cultivated in Mexico, with many people depending on this crop for their livelihood. This underlines, on the one hand, the importance of understanding the factors associated with the emergence of bacterial wilt and, on the other hand, the need of timely environment- and human-friendly control measures.

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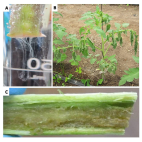

Figure 1

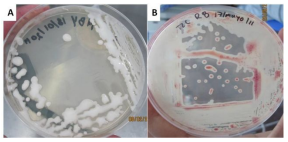

Figure 2

Figure 3

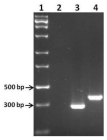

Figure 4

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