Diseases Caused by Bacteria and Phytoplasmas

First Report of Root Mat Disease in a Hydroponic Tomato Production System Caused by Rhizogenic *Agrobacterium* Biovar 1 in South Korea

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In June 2019, root mat disease was observed in hydroponically cultivated tomatoes in Jinju, South Korea, which occurred in at least 30% of the plants in the greenhouse. To isolate the causal bacteria, 10 g of infested tomato root mat sample was ground with 50 ml of sterile water. A 100- μ l aliquot of the homogenate was serially diluted and spread on mannitol-glutamate medium amended with 0.1% yeast extract (MGY) and incubated at 28°C for 48 h. Fifteen dominant colonies that formed on the MGY medium were purified and subjected to diagnostic polymerase chain reaction (PCR) based on the *virD2-ipt* gene loci (Weller et al. 2000). Because Ti-plasmid harbors both *virD2* and *ipt* genes, Ri-plasmid-borne *Agrobacterium* species with only *virD2* can be differentiated using *virD2-ipt* PCR. To amplify *virD2*, the primers 5'-ATGCCCGATC-GAGCTCAAGT-3' and 5'-TCGTCTGGCTGACTTTCGTCATAA-3' were used; for *ipt* amplification, the primers 5'-GATCG(G/C)GTCCAATG(C/T)TGT-3' and 5'-GATATCCATCGATC(T/C)CTT-3' were used. Amplification involved an initial 94°C for 1 min and then 40 cycles at 94, 50, and 72°C for

1 min at each temperature, with a final 5-min extension at 72°C. For three strains (GNIY2, J10, and J11), *virD2* PCR products, but no *ipt* PCR products, were identified, indicating that three strains are Ri-plasmid-borne *Agrobacterium* species. A pathogenicity test was performed on 2-week-old tomato plants. Bacteria isolates (GNIY2, J10, and J11) cultured overnight in lysogeny broth were made into a bacterial suspension (10^7 CFU/ml) in 50 mM phosphate buffer. Five tomato seedling roots were cut with sterilized scissors and soaked in each bacterial suspension for 1 h. Phosphate buffer was used as a negative control. The inoculated tomato seedlings were transplanted in new pots and placed in a greenhouse with 25°C day and 20°C night temperature set points in natural light. After 9 weeks, all inoculated tomato plants produced overgrown roots, whereas the negative control plants had no symptoms. To satisfy Koch's postulates, reisolation was performed from the diseased tomato, and the reisolated bacteria were subject to partial 16S rDNA sequencing. Biovar tests performed as previously described (Bosmans et al. 2015) revealed that all three isolates were biovar 1. A representative strain (GNIY2) was deposited in the Korean Agricultural Culture Collection (KACC 21759). To confirm the identity, four housekeeping genes of KACC 21759 were sequenced (16S rRNA, *trpE*, *rpoB*, and *recA*) (Bosmans et al. 2015) and deposited in GenBank (accession nos. MT071560, MT444428, MT444429, and MT444430). Multilocus sequence analysis performed as previously described (Bosmans et al. 2015; Costechareyre et al. 2010; Vargas et al. 2020) showed that the KACC 21759 strain was grouped in *Agrobacterium* genomospecies 4. This is the first report on mat root disease caused by *Agrobacterium* biovar 1 in hydroponic tomatoes in South Korea.

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