Diseases Caused by Bacteria and Phytoplasmas

First Report of Stalk Rot of Celery Caused by *Erwinia rhapontici* in China

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Species in the genus *Erwinia* cause diseases in many economically important plants (Mansfield et al. 2012). In May 2021, celery plants (*Apium graveolens* var. *dulce* ‘Queen of France’) showing soft rot symptoms were observed in greenhouses in Boye County, Baoding, Hebei Province (North China). Disease symptoms began with pinkish, water-soaked lesions on the midrib of celery stalks, but at the same time the leaves and roots did not show symptoms. The infected celery plants rapidly developed brownish rotten stalks and leaves turned dry and yellow, but roots remained asymptomatic. The disease incidence in two greenhouses (0.15 ha in size) was more than 50%. Affected celery stalk tissues were cut into 0.5-cm pieces, surface sterilized using 75% ethanol for 60 s, and rinsed three times with sterile distilled water. Then, the tissues were immersed in 200 µl of 0.9% saline for 15 min. Aliquots of two 10-fold dilutions of the tissue specimen soaking solution were plated onto Luria-Bertani (LB) agar plates and incubated at 28°C for 24 h. Single colonies were picked and restreaked onto LB agar three times for purity. The bacterial gDNA was extracted using the EasyPure Bacteria Genomic DNA Kit (TransGen Biotech). The 16S rDNA region was amplified by PCR using the universal primers 27F/1492R and sequenced. BLASTn analysis of the 16S rDNA amplicons (MZ489246 and MZ489247) indicated that the bacterial isolates (BY21311 and BY21312) belonged to the genus *Erwinia*. Biolog analysis (GEN III Microplate) identified BY21311 (SIM = 0.668) and BY21312 (SIM = 0.638) as *E. rhapontici*. Housekeeping genes including *acnA*, *gapA*, *icdA*, *mdh*, and *rpoS* were also amplified using a set of PCR primers (Ma et al. 2007; Waleron et al. 2008) followed by sequencing

(MZ463029 to MZ463038). To determine the species of BY21311 and BY21312, multilocus sequence analysis (MLSA) was performed with five housekeeping genes, and the phylogenetic tree was reconstructed using RAxML v8.2.12 (Stamatakis et al. 2005). No sequence variation was observed at any MLSA locus between BY21311 and BY21312. Phylogenetic analysis showed that BY21311 and BY21312 were clustered with *E. rhapontici* isolates. These celery isolates are closely related to the cabbage (*Brassica rapa*) isolate MAFF311153 (AP024329.1) in Japan. When celery plants had eight to nine true leaves, plants (cv. Queen of France) were inoculated with BY21311 by injecting 20 µl of bacterial suspension (10⁶ CFU/ml) into the celery stalks, or injected with 20 µl of 0.9% saline as control. The seedlings were grown at 25°C and 50% relative humidity. Three days after inoculation, only infected seedlings showed disease symptoms resembling those observed in greenhouses. Bacterial colonies were obtained from the infected stalks and were identified using the same PCR primers of housekeeping genes as described above, fulfilling Koch’s postulates. *E. rhapontici* has been reported to cause pink seed, crown and stem rot, soft rot, or leaf spot on many plant hosts including pea (*Pisum sativum*), chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), common bean (*Phaseolus vulgaris*), lucerne (*Medicago sativa*), wheat (*Triticum aestivum*), hyacinth (*Hyacinthus orientalis*), onion (*Allium cepa*), kiwifruit (*Actinidia chinensis*), and peach (*Prunus persica*) (Huang et al. 2003; Kovács et al. 2020; Wang et al. 2017; Zhang et al. 2018). To our knowledge, this is the first report of *E. rhapontici* causing stalk rot in celery. Stalk rot of celery has increased in prevalence over recent years in the Baoding region; it can cause significant yield loss and no cultivar has been found to be resistant to this disease so far. Stalk rot poses a significant threat to local celery production, and further research on epidemiology and disease management options is needed.

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