Diseases Caused by Bacteria and Phytoplasmas

First Report of *Erwinia persicina* Causing Stalk Rot of Celery in China

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Species in the genus *Erwinia* cause diseases in many economically important plants. 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 stalks, but at the same time the leaves and roots were asymptomatic. Infected plants rapidly developed brownish rotten stalks. The disease incidence in two greenhouses (0.15 ha in size) was more than 50%. Affected stalk pieces ~0.5 cm in length were surface sterilized by dipping them in 75% ethanol for one min and then rinsed three times with sterile distilled water. The tissues were immersed in 200 µl of 0.9% saline for 15 min. Aliquots (20 µl) of two 10-fold dilutions of the tissue specimen soaking solution were plated onto Luria-Bertani (LB) medium 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, Beijing, China). The 16S rDNA region was amplified by PCR using the universal primers 27F/1492R and sequenced. BLASTn analysis of the 16S rDNA amplicons (MZ614654 and MZ614655) indicated that the bacterial isolates (BY21211 and BY21212) belonged to the genus *Erwinia*. Housekeeping genes including *mdh*, *gapA*, *icdA*, *rpoS*, *acnA*, and *proA* were also amplified using a set of PCR primers (Ma et al. 2007) followed by sequencing (MZ643221 to MZ643232). No sequence variation was observed at any multi-locus sequence analysis (MLSA) locus between BY21211 and BY21212. To

determine the species of BY21211 and BY21212, MLSA was performed with six housekeeping genes, and a phylogenetic tree was reconstructed using RAxML v8.2.12 (<https://github.com/stamatak/standard-RAxML>), which showed that BY21211 and BY21212 were clustered with *E. persicina* type strain NBRC102418^T (Hao et al. 1990). When celery plants (Queen of France) had eight to nine true leaves, they were inoculated with BY21211 by injecting 20 µl of bacterial suspension (10⁷ CFU/ml) into the stalks, and negative controls were injected with 20 µl of 0.9% saline. The seedlings were grown at 25°C and 50% relative humidity. Three days after inoculation, only the bacterial-inoculated 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. Therefore, BY21211 fulfills Koch’s postulates for stalk rot of celery. BY21211 and BY21212 produced a water-soluble pink pigment on sucrose-peptone agar. They were gram-negative and rod-shaped, and negative for oxidase, urease, and indole production, and gelatin liquefaction and acid production from xylose and glycerol. They were positive for catalase, citrate utilization, acid production from sorbitol, raffinose, glucose, arabinose, cellobiose, rhamnose, maltose, saccharose, inositol, lactose, and esculin (hydrolysis). *E. persicina* has been reported to cause pink seed, crown and stem rot, soft rot, or leaf spot on many plant hosts including pea (*Pisum sativum*), soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), lucerne (*Medicago sativa*), barley (*Hordeum vulgare*), onion (*Allium cepa*), garlic (*Allium sativum*), tomato (*Lycopersicon esculentum*), and cucumber (*Cucumis sativus*) (Cho et al. 2019; Gálvez et al. 2015; González et al. 2005, 2007; Hao et al. 1990; Kawaguchi et al. 2021; Zhang and Nan 2014). To our knowledge, this is the first report of *E. persicina* causing stalk rot in celery. Stalk rot of celery has increased in recent years in the Baoding region; it can cause significant yield loss and no cultivar has been found to be resistant to this disease. 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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The author(s) declare no conflict of interest.

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