Diseases Caused by Bacteria and Phytoplasmas

First Report of Bleeding Canker of Pear Tree Trunks Caused by *Dickeya fangzhongdai* in Korea

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Funding: This work was supported by a grant from the National Institute of Horticultural and Herbal Science (NIHHS), funded by the Rural Development Administration (RDA) of the Republic of Korea (PJ015095052020). Plant Dis. 105:2238, 2021; published online as <https://doi.org/10.1094/PDIS-09-20-1948-PDN>. Accepted for publication 9 February 2021.

Pears (*Pyrus pyrifolia* L.) are cultivated nationwide as one of the most economically important fruit trees in Korea. At the end of October 2019, bleeding canker was observed in a pear orchard located in Naju, Jeonnam Province (34°53'50.54" N, 126°39'00.32" E). The canker was observed on trunks and branches of two 25-year-old trees, and the diseased trunks and branches displayed partial die-back or complete death. When the bark was peeled off from the diseased trunks or branches, brown spots or red streaks were found in the trees. Bacterial ooze showed a rusty color, and the lesion was sap-filled with a yeasty smell. Trunks displaying bleeding symptoms were collected from two trees. Infected bark tissues (3 × 3 mm) from the samples were immersed in 70% ethanol for 1 min, rinsed three times in sterilized water, ground to fine powder using a mortar and pestle, and suspended in sterilized water. After streaking each suspension on Luria-Bertani agar, the plates were incubated at 25°C without light for 2 days. Small yellow-white bacterial colonies with irregular margins were predominantly obtained from all the samples. Three representative isolates (ECM-1, ECM-2, and ECM-3) were subjected to further characterization. These isolates were cultivated at 39°C and utilized (–)-D-arabinose, (+)-melibiose, (+)-raffinose, mannitol, and myo-inositol but not 5-keto-D-gluconate, β-gentiobiose, or casein. These isolates were identified as *Dickeya* sp. based on the sequence of 16S rRNA (MT820458 to MT820460) gene amplified using primers 27f and 1492r (Heuer et al. 1997). The 16S rRNA sequences matched *Dickeya fangzhongdai* strain ND14b (99.93%; CP009460.1) and *D. fangzhongdai* strain PA1 (99.86%; CP020872.1). The *recA*, *fusA*, *gapA*, *purA*, *rplB*, and *dnaX* genes

and the intergenic spacer (IGS) regions were also sequenced as described in van der Wolf et al. (2014). The *recA* (MT820437 to MT820439), *fusA* (MT820440 to MT820442), *gapA* (MT820443 to MT820445), *purA* (MT820446 to MT820448), *rplB* (MT820449 to MT820451), *dnaX* (MT820452 to MT820454), and IGS (MT820455 to MT820457) sequences matched *D. fangzhongdai* strains JS5, LN1, and QZH3 (KT992693 to KT992695, KT992697 to KT992699, KT992701 to KT992703, KT992705 to KT992707, KT992709 to KT992711, KT992713 to KT992715, and KT992717 to KT992719, respectively). A neighbor-joining phylogenetic analysis based on the concatenated *recA*, *fusA*, *gapA*, *purA*, *rplB*, *dnaX*, and IGS sequences placed the representative isolates within a clade comprising *D. fangzhongdai*. ECM-1 to 3 were grouped into a clade with one strain isolated from a waterfall, *D. fangzhongdai* ND14b from Malaysia. A pathogenicity test was performed using isolate ECM-1. Three 2-year-old branches and flower buds on a 10-year-old pear tree (cv. Nittaka), grown at the National Institute of Horticultural and Herbal Science Pear Research Institute (Naju, Jeonnam Province in Korea), were inoculated with 10 and 2 µl of a bacterial suspension (10⁸ CFU/ml), respectively, after wounding the inoculation site with a sterile scalpel (for branches) or injecting with a syringe (for flower buds). Control plants were inoculated with water. Inoculated branches and buds in a plastic bag were placed in a 30°C incubator without light for 2 days (Chen et al. 2020). Both colorless and transparent bacterial ooze and typical bleeding canker were observed on both branches and buds at 3 and 2 weeks postinoculation, respectively. No symptoms were observed on control branches and buds. This pathogenicity assay was conducted three times. We reisolated three colonies from samples displaying the typical symptoms and checked the identity of one by sequencing the *dnaX* locus. *D. fangzhongdai* has been reported to cause bleeding canker on pears in China (Chen et al. 2020; Tian et al. 2016). This study will contribute to facilitate identification and control strategies of this disease in Korea. This is the first report of *D. fangzhongdai* causing bleeding canker on pears in Korea.

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The author(s) declare no conflict of interest.

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Keywords: bleeding canker, *Dickeya fangzhongdai*, *Pyrus pyrifolia*

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