Diseases Caused by Bacteria and Phytoplasmas

First Report of *Dickeya fangzhongdai* Causing Soft Rot in Orchid in Canada

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Dickeya fangzhongdai was originally described as the causal agent of bleeding canker of pear tree in China. Recently, *D. fangzhongdai* was isolated and identified as the causal agent of soft rot in an orchid plant purchased in a local supermarket in Prince Edward Island, Canada. A water-soaked dark green spot on the leaf surface was observed and later became larger soft rot symptom. The origin of the orchid plants was traced back to a producer in Ontario, Canada, who propagated them with cuttings originally imported from the Netherlands and Taiwan. Bacterial isolations were made from a soft rot lesion on an orchid leaf by surface sterilization of small pieces of marginal tissue of the diseased leaf in 70% alcohol. The small pieces of leaf tissue were then washed three times using sterile water and immersed in drops of sterile water. Bacterial streaming was observed under the microscope and nonfluorescing bacterial colonies were isolated on King's B and casamino acid-peptone-glucose agar plates and purified as isolates 908, 909, 910, and 911. The DNA samples were extracted from the four isolates, as well as the diseased leaf tissue, and tested with a qPCR assay with the specific primer/probe set (DfF/DfR/DfP) for *D. fangzhongdai* (Tian et al. 2020). The assay yielded PCR amplicons of 135 bp with a melting temperature of $86.5 \pm 0.6^\circ\text{C}$ as did two control reactions using genomic DNA from *D. fangzhongdai* strains JS5^T and QZH3 originally isolated in China, providing presumptive identification of the orchid isolates as *D. fangzhongdai*. To fulfill Koch's postulates, freshly purchased healthy orchid plants ($n = 4$) were inoculated by leaf injection with the bacterial isolates obtained in this study and strains JS5^T and QZH3 at $\sim 10^7$ CFU/ml. Three leaves of the same side of the plants were inoculated with the same strains in triplicate. Sterile water was used as the negative control. Inoculated plants were incubated in a growth chamber

with a 16 h photoperiod at 23°C . Water soaked lesions developed 3 to 5 days after inoculation followed by soft rotting in leaves inoculated with the new bacterial strains from orchid plants, while strain QZH3 caused soft rot 10 days after inoculation. The nonfluorescing bacteria on King's B plates with colony morphology similar to those inoculated were reisolated from the inoculated leaves and confirmed to be *D. fangzhongdai* by qPCR. Phylogenetic analysis of the assembled 16S rRNA sequence of isolate 908 (GenBank accession no. MT984340), together with GenBank data of all *Dickeya* spp. and some *Pectobacterium* spp., using the neighbor-joining (NJ) method inferred with MEGA X software (Kumar et al. 2018) showed that isolate 908 clustered with strains JS5^T and QZH3 at a phylogenetic distance of 0.0007. This clearly indicated that isolate 908 and JS5^T and QZH3 belong to the same genus. Species-level identification of isolate 908 was achieved by genome sequencing and analysis based on average nucleotide identity (ANI). Genomic DNA of isolate 908 was sequenced with Illumina MiSeq to provide approximately 180x genome coverage. After quality checking using FastQC (Andrews 2010), de-novo assembly was performed with VelvetOptimizer v2.2.6 (Zerbino and Birney 2008). The draft genome size of strain 908 was 4,938,027 bp consisting of 76 contigs with 56.8% G+C content and 63,801 bp as N50. The draft genome was checked for misassembled fragments using QUAST v5.0.2 (Gurevich et al. 2013) and found to be of good quality. The draft genome sequence is deposited in GenBank under accession JADCNJ000000000. The draft genome sequence of strain 908 was compared with that of *D. fangzhongdai* JS5^T type strain genome using FastANI v1.2 (Jain et al. 2018) resulting in an ANI value of 98.9%, which is above the 95% cutoff for the same species. Previously, it was reported that *D. fangzhongdai* caused soft rot in orchid in Europe (Alič et al. 2018) and in onions in New York (Ma et al. 2020). The difference in virulence among *D. fangzhongdai* strains warrants further investigation and their pathogenicity on potato is being investigated to evaluate any threat to the potato industry. To our knowledge, this is the first report of *D. fangzhongdai* causing soft rot disease on orchids in Canada and North America.

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