Diseases Caused by Bacteria and Phytoplasmas

First Report of Soft Rot of Onion Caused by *Pectobacterium wasabiae* in Japan

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Onion (*Allium cepa* L.) is one of the important vegetables in Japan. In the summer of 2019, onions with soft rot were found in commercial fields in Hokkaido, the northern island in Japan. Diseased onion showed chlorosis, maceration of leaves, and rotted bulbs. We sampled some diseased onions and isolated three independent isolations (NAONI191, NAOI192, and NAOI193) from infected bulbs on LB medium. These strains were identified as *Pectobacterium wasabiae* based on their inability to grow at 37°C and their ability to utilize raffinose and lactose. These bacterial strains were gram-negative, rod-shaped, *N*-acetylglucosaminyl transferase, gelatin liquefaction. The bacterium was positive for *O*-nitrophenyl-beta-D-galactopyranoside, *N*-acetylglucosaminyl transferase, gelatin liquefaction, and acid production from D-galactose, lactose, melibiose, raffinose, citrate, and trehalose. The bacterium was negative for indole production and acid production from maltose, α -methyl-D-glucoside, sorbitol, D-arabitol, inositol, inulin, and melezitose. All the strains exhibited pectolytic activity on potato slices. DNA from these strains yielded a single size amplicon with the primer set of PhF/PhR for *P. wasabiae* (De Boer et al. 2012) by PCR. However, DNA from these strains did not yield the expected amplicon with the primer set of BR1f/L1r for *P. carotovorum* subsp. *brasiliense* (Duarte et al. 2004) and Eca1f/Eca2r for *P. atrosepticum* (De Boer and Ward 1995) by PCR. The sequence analysis of 16S rDNA (LC597917 to

LC597919) showed more than 98% identities to *P. wasabiae* strains (e.g., HAFL01 in Switzerland) by BLAST analysis. In addition, multilocus sequence analysis (Ma et al. 2007) was performed by MEGA6.06 using concatenated DNA sequences of seven housekeeping genes (aconitate hydratase [*acnA*, LC597925 to LC597927], glyceraldehyde-3-phosphate dehydrogenase A [*gapA*, LC597972 to LC597974], isocitrate dehydrogenase [*icdA*, LC597998 to LC598000], malate dehydrogenase [*mdh*, LC598024 to LC598026], mannitol-1-phosphate dehydrogenase [*mtlD*, LC598050 to LC598052], glucose-6-phosphate isomerase [*pgi*, LC598076 to LC598078], and gamma-glutamyl phosphate reductase [*proA*, LC598099 to LC598101]), and all clustered into a clade containing other confirmed strains of *P. wasabiae*. As a result, these three strains shared high identity with each other (>98%, E-values showed 0). The clade containing these three strains was consistently placed in a larger clade with the other *P. wasabiae* with 100% bootstrap support for its separation from other *Pectobacterium* species available in GenBank when the consensus tree was constructed using the maximum likelihood method. Pathogenicity of these strains against onion (cv. 'Hayate') was confirmed by the field experiments with 5-weeks-growth plants sprayed with bacterial suspension (1×10^7 CFU/ml), resulting in soft rot on the plants about 4 weeks after inoculation, whereas water-inoculated plants remained symptomless. Strains re-isolated from the artificially diseased stems were confirmed as *P. wasabiae* using the methods as biochemical characterization and multiple genetic analyses. Based on the disease symptoms and the cultural, molecular, and pathological features of the strains, we conclude that the soft rot symptoms of onion in Hokkaido in 2019 were caused by *P. wasabiae*. To our knowledge, this is the first report of *P. wasabiae* as the soft rot disease agent of onion in Japan.

References:

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