Diseases Caused by Bacteria and Phytoplasmas

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

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Cabbage (*Brassica oleracea* var. *capitata*) is one of the important vegetables in Japan. In the summer of 2019, some cabbages with soft rot were found in commercial fields in Hokkaido, the northern island in Japan. All diseased plants showed gray to brown discoloration and expanding water-soaked lesions on leaves. We obtained two independent strains (NACAB191 and NACAB192) from diseased leaves. DNA from these strains yielded an expected single-size amplicon with the primer set of PhF/PhR for *Pectobacterium wasabiae* (De Boer et al. 2012) by PCR but 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. These two strains grew at 37°C and had the ability to utilize raffinose and lactose. These bacterial strains were gram-negative and rod-shaped. 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 strains exhibited pectolytic activity on potato slices. The sequence analysis of 16S rDNA (LC597897 and LC597898) showed more than 98% identities to *P. wasabiae* strain (e.g., HAFL01 in Switzerland) by BLAST analysis. In addition, multilocus sequence analysis (Ma et al. 2007) was performed by MEGA10 (Kumar et al. 2018) using

concatenated DNA sequences of seven housekeeping genes (aconitate hydratase [*acnA*, LC597923 and LC597924], glyceraldehyde-3-phosphate dehydrogenase A [*gapA*, LC597970 and LC597971], isocitrate dehydrogenase [*icdA*, LC597996 and LC597997], malate dehydrogenase [*mdh*, LC598022 and LC598023], mannitol-1-phosphate dehydrogenase [*mitD*, LC598048 and LC598049], glucose-6-phosphate isomerase [*pgi*, LC598074 and LC598075], and gamma-glutamyl phosphate reductase [*proA*, LC598079 and LC598080]), and all clustered NACAB191 and NACAB192 into a clade containing other confirmed strains of *P. wasabiae*. As a result, these two strains shared high identity with each other (>98%, E-values showed 0). The clade containing these two strains was consistently placed in a larger clade with the other *P. wasabiae* and 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 cabbage (cv. Rakuen) was confirmed by the field experiments with 5-weeks-growth plants sprayed with bacterial suspension (1×10^7 CFU/ml). Thirty cabbages per strain were used in this study (12 plants treated the suspension of NACAB191 and 16 plants treated the suspension of NACAB192), which died with the same soft rot symptoms about 4 weeks after inoculation, whereas water-inoculated plants remained symptomless. Strains reisolated 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 on the cultural, molecular, and pathological features of the strains, we conclude that the soft rot symptoms of cabbage in Hokkaido in 2019 were caused by *P. wasabiae*. To our knowledge, this is the first report of *P. wasabiae* as a soft rot disease agent of cabbage in Japan.

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