Diseases Caused by Bacteria and Phytoplasmas

First Report of Bacterial Streak and Rot of Onion Caused by *Pseudomonas viridiflava* in Japan

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In March 2020, a bacterial streak and rot symptom was observed on onion (*Allium cepa* L.) leaves in Akita Prefecture of Japan. On the beginning, oval and dark-greenish water-soaked lesions with grayish-white necrotic center, 2 to 3 mm in diameter, appeared on the middle or the tip of upper leaves. Lesions, frequently surrounded by a light yellow halo, expanded along veins and overlapped together. As lesions grew, the center of the lesions turned to light brown necrosis. The basal areas of diseased leaves often rotted, causing the withering of a whole leaf at the end. From the water-soaked tissues of young lesions, a bacterium forming cream white colonies and producing fluorescent pigment on King's medium B was consistently isolated and was suggested to be a member of genus *Pseudomonas*. The isolates were positive for potato soft rot and tobacco hypersensitive reaction, and negative for levan production, oxidase, and arginine dihydrolase activity, indicating that they belonged to LOPAT group II, *Pseudomonas viridiflava*, defined by Lelliott et al. (1966). *P. viridiflava* is known as a pathogen of bacterial streak and bulb rot of onion in the United States and Uruguay, although it has not been reported in Japan. Four isolates were chosen for further examination. Pathogenicity tests on onion leaves stabbed with selected isolates reproduced similar symptoms as observed in field samples. After 2 days of inoculation, isolates produced water-soaking around the stabbed holes, which developed

into grayish-white necrosis on 4 days after inoculation. After 10 days of inoculation, lesions grew to necrotic streaks with light brown center surrounded by water-soaking and a light yellow halo. Additionally, on onion scales, isolates caused water-soaked rot, yellow to brown colored, in 1 to 2 days after inoculation. From water-soaked areas of both leaves and scales, inoculum was reisolated, fulfilling Koch's postulates. In phenotypic properties, isolates showed consistent results with *P. viridiflava* strains identified in former studies. They were gram negative and aerobic, positive for hydrolysis of esculin, casein, and gelatin, and negative for nitrate reduction and indole production. Regarding the utilization of carbohydrates, they were positive for 2-keto-gluconate, glucose, fructose, D-galactose, mannose, L(+)-arabinose, glycerol, mannitol, sorbitol, myo-inositol, meso-erythritol, meso-tartrate, D(-)-tartrate, gluconate, n-caprate, DL-malate, citrate, L-arginine, L-aspartate, and L-glutamate and negative for D-arabinose, maltose, sucrose, raffinose, adonitol, trehalose, L-tartrate, L-rhamnose, and acetate. The 16S rRNA gene sequences (Frank et al. 2008) of four isolates (1,377 bp) showed 100% similarity with that of *P. viridiflava* type strain LMG 2352^T (GenBank accession no. Z76671) in BLASTN search. In phylogenetic analysis using *gyrB* (910 bp) and *rpoD* (801 bp) genes (Maeda et al. 2006), isolates formed a cluster with *P. viridiflava* strains deposited in public databases, independent from other closely related *Pseudomonas* species. Sequences of 16S rRNA (GenBank accession nos. LC597475 to LC597478), *gyrB* (LC597479 to LC597482), and *rpoD* (LC597483 to LC597486) genes were deposited in the DNA Data Bank of Japan. According to these results, the isolates were identified as *P. viridiflava* (Burkholder 1930) Dowson 1939. This is the first report of the occurrence of bacterial streak and rot of onion caused by *P. viridiflava* in Japan, causing severe damage on onion growth.

References:

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