Diseases Caused by Bacteria and Phytoplasmas

First Report of Leaf Blight of *Adenophora triphylla* var. *japonica* Caused by *Pseudomonas viridiflava* in Korea

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Plant Dis. 105:3738, 2021; published online as <https://doi.org/10.1094/PDIS-11-20-2506-PDN>. Accepted for publication 28 April 2021.

A severe disease with leaf spots and necrotic symptoms was observed in *Adenophora triphylla* var. *japonica* (Regel) Hara (*A. triphylla*) during a survey in July 2020 on a field in Andong, Gyeongbuk province, Korea. It is a highly valued medicinal plant used to treat various diseases, including cough, cancer, and obesity. The infected plants initially showed spots with a halo lesion; at later stages, spots enlarged and spread over the leaves, with the lesions becoming yellow and chlorotic. In some areas, disease incidence was up to 15% of the plants. Symptomatic samples were collected from *A. triphylla* and cut into 4- to 5-mm squares, surface sterilized in 1% sodium hypochlorite for 1 min, rinsed three times, and macerated in sterile distilled water (SDW). They were spread onto nutrient agar plates and incubated at 28°C for 3 days. The representative bacterial strains selected for identification showed fluorescent colonies on King's medium B (KB). Fifteen isolates from independent samples were subjected to biochemical and pathogenicity tests. The isolates induced a hypersensitive reaction in tobacco leaves, gave a reaction in the anaerobe respiratory test, and were negative for levan, oxidase,

arginine dihydrolase, gelatin hydrolysis, aesculin hydrolysis, and starch hydrolysis. The isolated strains presented the following LOPAT profile: -- + - +. The Biolog GN2 microplate and the Release 4.20 system putatively found the isolate to exhibit 93% similarity with the bacterium *Pseudomonas viridiflava*. Likewise, analysis of fatty acid methyl ester profiles using the microbial identification system (Sherlock version 3.1) also characterized the representative bacterial strain as *P. viridiflava* with 87% similarity. The genomic DNA of the isolate was extracted, and the 16S rDNA sequence was amplified with a universal bacterial primer set (27F and 1492R). The sequence was submitted to GenBank under the accession number MT975233. BLASTn analysis yielded 99.79% identity with *P. viridiflava* strain RT228.1b (accession no. AY604846.1) and 99.72% similarity with *P. viridiflava* KNOX249.1b strain (accession no. AY604848.1). A phylogenetic dendrogram was constructed from the comparative analysis of 16S rDNA gene sequences showing the relationship between *P. viridiflava* GYUN274 and related *Pseudomonas* species. Pathogenicity tests were conducted three times on seedlings of *A. triphylla* by spraying 50 ml of bacterial suspensions of a 24-h culture in KB medium (10⁸ CFU/ml). The leaves inoculated with SDW alone did not develop symptoms; however, the plants treated with isolated bacterial suspensions developed halo and blight symptoms similar to those observed in the field 7 days postinoculation. Finally, Koch's postulates were verified by reisolating *P. viridiflava* from all symptomatic tissues and determined to be morphologically identical to the original isolates. To our knowledge, this is the first report of leaf blight disease of *A. triphylla* caused by *P. viridiflava* in Korea. Based on the observed symptoms, and identification by morphological characteristics, molecular data, and pathogenicity against the host plant, the proper control measures can be identified in the future studies.

The author(s) declare no conflict of interest.

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Keywords: *Pseudomonas viridiflava*, *Adenophora triphylla* var. *japonica* (Regel) Hara, 16S rDNA, sequence, pathogenicity

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