Diseases Caused by Bacteria and Phytoplasmas

First Report of *Rhodococcus fascians* Causing Fasciation of Lilies (*Lilium longiflorum*) in South Korea

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Rhodococcus fascians is a bacterium that causes growth abnormalities such as leafy galls, fasciation, and shoot proliferation in many plants, including ornamental plants. In February 2020, the Animal and Plant Quarantine Agency of South Korea detected 492,000 contaminated lily bulbs (*Lilium longiflorum* Thunb.) using an in-house PCR test based on the *R. fascians* *fasD* gene, and subsequently 1.3 million imported bulbs were destroyed. Because no pathogen isolation was associated with this diagnosis, there has been great cultivator demand for bacterial isolation evidence of lily bulb infection with pathogenic *R. fascians*. To isolate the causal bacterium of the PCR tests, we sampled leaf, stem, and bulb tissues from 130 lilies with growth abnormality symptoms, collected from 24 South Korean mass production lily farms from June to August 2020. Supernatants of the homogenized samples were spread on mD2 medium (Kado and Heskett 1970) and incubated at 28°C for 10 days. Yellow to orange colonies were isolated into pure culture on mD2. Total DNA was extracted from cultures grown in yeast extract broth at 28°C for 24 h with a Wizard DNA prep kit (Promega, Madison, WI). PCR was performed to test for pathogenicity genes *fas* (*A*, *D*, and *R*) and *att* (*A* and *R*) (Putnam and Miller 2007). Colonies that produced at least one amplicon from these pathogenicity genes were analyzed by partial 16S rRNA gene sequencing to determine the corresponding species. Three strains that were isolated

from the bulbs of fasciated lilies from Wanju (35°56'22.1"N; 127°08'52.0"E), Gwacheon (37°26'51.6"N; 127°00'11.8"E), and Yeongwol (37°18'45.8"N; 128°11'05.6"E), or W1, G3, and Y5 strains, yielded PCR products of the expected size for *fas* and *att* genes with the primer sets published in Serdani et al. (2013) and developed in this study (*attAF*, 5'-CCCGGCTACACGCATTTCGC-3'; *attAR*, 5'-CGAACGCGGTGTG-CAGGT-3'; and *attRF*, 5'-AGTGTCCCGTCGCGAG-3'; *attRR*, 5'-CGCGGCAGATCGAAGTCCT-3'). Sequences of the three strains were deposited in GenBank for *fasA* (accessions MW122940 to 942), *fasD* (G3: MW122935 and 936), and *fasR* (MW122937 to 939); all shared 98.3 to 100% nucleotide identity to corresponding sequences from phytopathogenic *R. fascians* A25f (CP049745.1 Protein_ID *fasA*:QII09280.1, *fasD*: QII09282.1, and *fasR*:QII09277.1). The *attA* and *attR* products were only present in G3 (*attA*, MW122943; *attR*, MW122944) and resulted in 100% identity to those of A25f (CP049745.1 Protein_ID *attA*:QII09269.1, *attR*: QII09267.1). Partial 16S rRNA gene sequences were obtained (MW064131 to 133) and clustered with phytopathogenic *R. fascians* strains D188, A21d2, and A25f. Thus, we concluded that the strains (W1, G3, and Y5) corresponded to *R. fascians*. To test the pathogenicity of these three strains, 10 seeds of garden peas for each strain were inoculated at 10⁸ CFU/ml according to Nikolaeva et al. (2009), and the length of the main stem of each seedling was calculated 22 days postinoculation. Seedlings inoculated with G3 and Y5 resulted in a stunted phenotype with up to 40% height reduction ($P \leq 0.001$) compared with noninoculated seedlings. As for the seedlings inoculated with W1, they exhibited as much as 15% height reduction ($P \leq 0.001$). Colonies were recovered from the inoculated seedlings, and identity was confirmed through colony PCR for *fas* and *att* genes. To our knowledge, this is the first report of phytopathogenic *R. fascians* in lilies cultivated in South Korea.

References:

- Kado, C., and Heskett, M. G. 1970. Phytopathology 60:969.
- Nikolaeva, E. B., et al. 2009. Plant Dis. 93:499.
- Putnam, M. L., and Miller, M. L. 2007. Plant Dis. 91:1064.
- Serdani, M., et al. 2013. Plant Dis. 97:517.

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