Diseases Caused by Bacteria and Phytoplasmas

First Report of Bacterial Leaf Spot Disease on Sunflower (*Helianthus annuus*) Caused by *Pseudomonas viridiflava* in South Korea

H. Luo, Y. J. Lee, and J. M. Yu[†]

Department of Applied Biology, Chungnam National University, Daejeon, South Korea

Funding: This project was supported in part by the Rural Development Administration <http://dx.doi.org/10.13039/501100003627>, Republic of Korea, “Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ014507022020)” and the Chungnam National University research fund. Plant Dis. 105:2235, 2021; published online as <https://doi.org/10.1094/PDIS-01-21-0083-PDN>. Accepted for publication 24 March 2021.

In September 2019, bacterial leaf spot symptoms were observed on sunflowers in an experimental field in Eumseong, South Korea. The leaves of infected plants initially showed irregular brown spots surrounded by haloes; as the disease progressed, the spots became enlarged and darkened. At the flowering stage, leaves became dry and showed signs of blight including defoliation; dark brown spots were also observed on sunflower stems and petioles but not on floral discs. Disease incidence ranged from 5 to 30% in three surveyed plots of the field. Symptomatic leaf tissue was surface sterilized, macerated with sterile distilled water, and cultured on nutrient agar plates at 28°C for 48 h. After incubation, nine bacterial isolates, representing individually collected samples from each field, were selected for further study. All nine isolates were gram-negative and fluorescent pigments produced under ultraviolet light on King’s medium B. With the LOPAT test, the isolates were levan negative, oxidase negative, positive for pectinolytic activity, arginine dihydrolase negative, and positive for tobacco hypersensitivity. Based on 16S rRNA sequences, all isolates shared 100% identity with *Pseudomonas viridiflava* strain KNOX209.1 (GenBank accession no. AY604847). The 16S rRNA sequences of nine isolates were deposited in GenBank (accession nos. MT393747, MW446479, to MW446486). Based on the phylogenetic analysis of the 16S rRNA, all isolates were grouped with *P. viridiflava* strains isolated from globe artichoke, Chinese

cabbage, and rape (Liu et al. 2019; Myung et al. 2010; Sanver et al. 2019). The isolates CPB 19362, CPB 19366, and CPB 19372, which represent each plot, were selected for further phylogenetic analysis and pathogenicity assays. The identity of these isolates was confirmed by sequences of housekeeping genes of the gyrase B subunit (*gyrB*) and RNA polymerase σ^{70} factor (*rpoD*) (Yamamoto et al. 2000) (GenBank accession nos. MT409400, MW446487, and MW446494 for *gyrB* and MT409401, MW446495, and MW446502 for *rpoD*). Based on the phylogenetic analyses of *gyrB* and *rpoD*, the three isolates belong to the same clade as the *P. viridiflava* pathotypes and were distinguished from *P. syringae* complex. These results indicated that the bacteria isolated from the spots on the sunflower plants were *P. viridiflava* strains. To confirm pathogenicity, bacterial suspensions (approximately 10^8 CFU/ml) of three representative isolates sprayed onto 4-week-old sunflower (cv. Common) seedlings separately until runoff occurred. Sterile distilled water was used as a control and inoculated in the same manner. After inoculation, plants were covered with transparent plastic bags at room temperature for 24 h. Plastic bags were then removed, and plants were grown on a plant growth shelf at 25°C in 50% relative humidity. The leaves of plants inoculated with the bacterial suspensions developed small brown spots after 24 h. After 3 days, brown spots surrounded by chlorotic or necrotic areas were observed on infected leaves. These spots gradually increased in size and formed brown lesions with haloes similar to those of infected field-grown plants, but not on the controls treated with sterile water. The pathogenicity test was repeated three times. Isolates recovered from infected leaves showed the same morphological, biochemical, and molecular characteristics as the original isolates from field samples. To our knowledge, this is the first report of bacterial leaf spot on sunflower caused by *P. viridiflava* in South Korea.

References:

- Liu, Y., et al. 2019. Plant Dis. 103:1764.
- Myung, I. S., et al. 2010. Plant Dis. 94:1164.
- Sanver, U., et al. 2019. Plant Dis. 103:2123.
- Yamamoto, S., et al. 2000. Microbiology 146:2385.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: *Pseudomonas viridiflava*, sunflower, leaf spot

[†]Indicates the corresponding author.
J. M. Yu; junyu@cnu.ac.kr