DISEASE NOTES

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First Report of *Pseudomonas syringae* pv. *syringae* on Pea (*Pisum sativum*) in Serbia

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In May 2014, during a cool, humid spring period, bacterial spot symptoms were observed for the first time on pea (*Pisum sativum* L.) in a home garden in the Bačka region of Vojvodina Province in Serbia. Commercial pea is grown on 11,533 ha, as well as in small vegetable farms in Serbia, with average yields of 4 t/ha. Observed spots on leaves were necrotic and progressed inward from the margins. Disease incidence approached 10 to 30%. Bacteria consistently isolated from margins of leaf lesions macerated in sterile phosphate buffered saline (PBS, pH 7.4) formed round, shiny, white, 2- to 3-mm in diameter colonies on nutrient agar with 5% (w/v) sucrose (NAS) and a green fluorescent pigment on King's medium B (KB). A collection of 20 strains selected for further study was strictly aerobic, gram-negative; positive for levan and tobacco hypersensitive response and negative for oxidase, arginine dihydrolase, and potato soft rot (LOPAT group Ia). Strains were characterized by repetitive (rep)-PCR, sequence typing, and pathogenicity. To determine genetic heterogenicity among the tested strains, rep-PCR genetic fingerprinting was conducted using the REP, ERIC, and BOX primers (Louws et al. 1994). Profile analyses yielded identical banding patterns for all tested strains. To identify three representative strains by sequence typing, the housekeeping genes *gyrB* and *gltA* were amplified (Ferrante

and Scortichini 2010). Sequences were deposited in GenBank under accession numbers KP188575 to 77 for *gyrB* and KP188578 to 80 for *gltA*. BLAST analysis showed that the regions were 100% homologous for the partial *gltA* gene of the reference strain *P. syringae* pv. *syringae* CFBP 4702 (KF937504) and 99% homologous for the partial *gyrB* gene of the reference strain *P. syringae* pv. *syringae* PD 2021 (KJ158894). Pathogenicity of the three tested strains was tested by using lesion tests and stem inoculation (Mazarei and Kerr 1990). For all tests, a reference strain *P. syringae* pv. *syringae* GSPB 1142 and PBS served as a positive and negative controls, respectively. In lesion tests, young bean pods and pale yellowish-green lemon fruits were inoculated by placing drops of bacterial suspension (with 10^8 CFU/ml) on the surface and pricking lightly through the drops with a sterile needle. Fruits and pods were incubated at 25°C for 7 days. Tested strains produced small reddish-brown depressed lesions on bean pods and dark brown depressed lesions on lemon fruits. For stem inoculation, pea cv. Mali provansalac plantlets were grown from seed for 10 to 14 days. Bacteria grown on KB for 24 h were scraped from the surface with a sterile needle and stabbed into the main stem at the youngest two nodes. Inoculation resulted in extensive necrosis and collapse with stunting on the plants 10 days after inoculation. Control strain GSPB 1142 developed similar symptoms to those of the strains tested. No symptoms were observed on plants inoculated with PBS. The bacterium was reisolated from lesions of test plants for the tree tested strains and confirmed to be the same bacterium using LOPAT tests and rep-PCR genetic fingerprinting. To our knowledge, this is the first report of *P. syringae* pv. *syringae* causing bacterial brown spot of pea in Serbia. The information of this species as a pea pathogen is still limited but is considered to be one of the limiting factor in pea cropping (Martín-Sanz et al. 2011).

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