DISEASE NOTES

First Report of Bacterial Leaf Spot of Chard (*Beta vulgaris* subsp. *cicla*) Caused by *Pseudomonas syringae* pv. *syringae* in Serbia

M. Ignjatov, J. Gvozdanović-Varga, D. Milošević, Z. Nikolić, Ž. Ivanović, and T. Popović

Affiliations

Authors and Affiliations
M. Ignjatov
J. Gvozdanović-Varga
D. Milošević
Z. Nikolić , Institute for Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia
Ž. Ivanović
T. Popović , Institute for Plant Protection and Environment, Teodora Drajzera 9, 11000 Belgrade, Serbia.

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During May and June 2014, previously unreported symptoms of a bacterial leaf spot were observed on commercial chard (*Beta vulgaris* subsp. *cicla*) in the Bačka region of Serbia. Up to 6,000 ha chard was grown in 2014. Disease symptoms developed after periods of cool and rainy weather and incidence ranged from 2 to 20%. Symptoms included brown angular leaf spots ~5 to 15 mm in diameter that were often surrounded by yellow margins mostly on the edges of leaves. Ten symptomatic plants were sampled and from each, one symptomatic leaf was taken for isolations. Leaves were rinsed with water, dried at room temperature, and sections (2 × 2 mm) taken from the margins of necrotic tissue were macerated in sterile phosphate buffer and streaked onto nutrient agar with 5% (w/v) sucrose (NAS). After incubation, round, shiny, white colonies 2 to 3 mm in diameter were consistently obtained. A total of 20 bacterial strains found to be gram-negative, aerobic, and according to the results of LOPAT tests, all belonged to *Pseudomonas* Group Ia, which includes *Pseudomonas syringae* pathovars (Lelliott and Stead 1987). All tested strains showed oxidative metabolism of glucose, formed catalase, hydrolyzed esculin and gelatin,

but were unable to hydrolyze starch, reduce nitrates, or produce indole. Strains produced acid from mannitol, inositol, sorbitol, erythritol, and sucrose, but not from trehalose and D-tartrate. Reactions were identical to those for reference strain *P. syringae* pv. *syringae* GSPB 1142, which was included for comparison. Repetitive extragenic palindromic sequence (rep)-PCR was used for genetic fingerprinting of the 20 strains from chard, using the REP, ERIC, and BOX primers (Louws et al. 1994). All strains yielded identical banding patterns. The *gyrB* housekeeping gene was sequenced from three representative strains with primers GyrB-F and GyrB-R (Ferrente and Scortichini 2010). Sequences were deposited in GenBank under accession nos. KP027950, KP027951, and KP027952. BLAST showed that the partial g*yr*B gene sequences had 100% homology with *P. syringae* pv. *syringae* (KC852129). Pathogenicity tests were performed with three representative strains on cotyledons of 6-day-old chard cv. Srebrnolisna seedlings (five seedlings/strain) by pricking with a hypodermic needle dipped in each bacterial suspension of ~10^6 CFU ml^{-1}. Using the same method, reference strain *P. syringae* pv. *syringae* GSPB 1142 and sterile distilled water served as positive and negative controls, respectively. Following inoculation, seedlings were incubated in a growth chamber at 22 to 24°C with 80% relative humidity and a 12-h photoperiod. Symptoms observed after 6 to 7 days on seedlings were dark green, water-soaked spots from which the pathogen could be reisolated. Negative control plants were symptomless. Reisolates were confirmed to be the same bacterium using LOPAT tests and rep-PCR analysis. Pathogenicity was also confirmed using conventional tests described for *P. syringae* pv. *syringae* (Lelliott and Stead 1987). All strains caused deep black, necrotic lesions on lemon fruits, dark sunken spots on immature pear fruits, and water soaked lesions on bean pods. The phenotypic data, genetic analyses, and pathogenicity indicated that strains obtained from chard were *P. syringae* pv. *syringae*. To our knowledge, this is the first report of *P. syringae* pv. *syringae* causing bacterial leaf spot of chard in Serbia. The economic importance of this pathogen could be reflected in the loss of chard leaf quality required for the market. Also, infected chard may serve as an inoculum source for many other cultivated crops since *P. syringae* pv. *syringae* has a wide host range.

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