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Disease Notes



First Report of *Pseudomonas syringae* pv. *coriandricola* Causing Bacterial Leaf Spot on Carrot, Parsley, and Parsnip in Serbia

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Abstract

During the spring of 2014, a severe leaf spot disease was observed on carrot (*Daucus carota*), parsley (*Petroselinum crispum*), and parsnip (*Pastinaca sativa*) on a 0.5-ha vegetable farm in Vojvodina Province, Serbia. The disease appeared under wet and cool conditions with 5 to 25% of plants infected for each of the three crops. Symptoms were characterized as brown angular leaf spots, ~2 mm in diameter, often limited by veins. Collected symptomatic leaves were rinsed and dried at room temperature, and leaf sections taken from the margin of necrotic tissue were macerated in sterile phosphate buffer and streaked onto nutrient agar with 5% (w/v) sucrose (NAS). After isolation, whitish, circular, dome-shaped, Levan-positive colonies consistently formed. Five strains from each host (carrot, parsley, and parsnip) were used for further study. Strains were gram-negative, aerobic, and positive for catalase and tobacco hypersensitive reaction but negative for oxidase, rot of potato slices, and arginine dihydrolase. These reactions corresponded to LOPAT group Ia, which includes *Pseudomonas syringae* pathovars (3). Repetitive extragenic palindromic sequence (Rep)-PCR fingerprint profiles using the REP, ERIC, and BOX primers (4) were identical for all strains. Sequence typing of the

housekeeping genes *gyrB* and *rpoD* (1) was performed for three representative strains (one from each host). Sequences were deposited in the NCBI GenBank database as accessions KM979434 to KM979436 (strains from carrot, parsnip, and parsley, respectively) for the *gyrB* gene and KM979437 to KM979439 (strains from parsnip, parsley and carrot, respectively) for the *rpoD* gene. Sequences were compared with pathotype strain *Pseudomonas syringae* pv. *coriandricola* ICMP12471 deposited in the Plant Associated and Environmental Microbes Database (<http://genome.ppws.vt.edu/cgi-bin/MLST/home.pl>). BLAST analysis revealed 100% homology for *gyrB* and 99% homology for *rpoD*. Pathogenicity was tested with five representative strains from each host on four-week-old plants of carrot (cv. Nantes), parsley (cv. NS Molski), and parsnip (cv. Dugi beli glatki) using two methods: spraying the bacterial suspension (10^8 CFU ml⁻¹) on the leaves until runoff (5) and injecting the bacterial suspension into leaves with a hypodermic syringe (2). Four plants were used per strain and method. Sterile distilled water was applied as a negative control treatment for each plant species. All plants were kept in a mist room with 100% humidity for 4 h, then transferred to a greenhouse at 25°C and 80% relative humidity and examined for symptom development over a period of three weeks. For all strains, inoculated leaves first developed water-soaked lesions on the leaves 5 to 7 days after inoculation (DAI); 14 DAI lesions became dark brown, often surrounded by haloes. No symptoms were observed on control plants inoculated with sterile distilled water. For fulfillment of Koch's postulates, re-isolations were done onto NAS. Re-isolated bacteria were obtained from each inoculated host and confirmed to be identical to the original isolates using the LOPAT tests and Rep-PCR fingerprinting profiles. Based on the pathogenicity test accompanied by completion of Koch's postulates, sequence analysis, and bacteriological tests, the strains were identified as *P. s.* pv. *coriandricola*. To our knowledge, this is the first report of bacterial leaf spot of carrot, parsley, and parsnip in Serbia. It may present a threat to production due to quality requirements for fresh market.

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