Disease Notes

First Report of Broccoli Soft Rot Caused by *Pectobacterium carotovorum* subsp. *carotovorum* in Serbia

K. Gašic, V. Gavrilović, Ž. Ivanović, and A. Obradović

Affiliations

Authors and Affiliations

K. Gašic

V. Gavrilović

Ž. Ivanović , Institute for Plant Protection and Environment, Plant Pathology Dept., Teodora Drajzera 9, 11040 Belgrade, Serbia

A. Obradović , University of Belgrade, Faculty of Agriculture, Plant Pathology Dept., Nemanjina 6, 11080 Belgrade, Serbia. This research was supported by projects III46008 and TR31018, Ministry of Education, Science and Technological Development, Serbia

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Abstract

In September 2012, soft rot symptoms on broccoli (*Brassica oleracea* L. var. *italica* Plenck) were observed in several commercial fields in the western part of Serbia. Following the first harvest, water-soaked areas developed on broccoli stem tissue and progressed into soft rot decay of entire plants. The incidence of disease was approximately 30%. In Serbia, broccoli is grown on smaller fields compared to other vegetables, but its production and consumption increased significantly in recent years. From the diseased tissue, shiny, grayish white, round colonies were isolated on nutrient agar. Six non-fluorescent, gram-negative, facultative anaerobic, oxidase-negative, and catalase-positive bacterial strains were chosen for further identification. All strains caused soft rot on potato and carrot slices and did not induce hypersensitive reaction on tobacco leaves. They grew at 37°C and in yeast

salts broth medium containing 5% NaCl (2), did not produce acid from α-methyl glucoside, but utilized lactose and trehalose, and did not produce indole or lecitinase. Investigated strains formed light red, 1.5-mm-diameter colonies on Logan's medium (2), and did not produce blue pigmented indigoidine on glucose yeast calcium carbonate agar (2) nor "fried egg" colonies on potato dextrose agar. Based on biochemical and physiological characteristics (1) and ITS-PCR and ITS-RFLP analy (4), the strains were identified as *Pectobacterium carotovorum* subsp. *carotovorun* The 16S rRNA gene sequence from two strains (GenBank KC527051 and KC527052) showed 100% identity with sequences of *P. carotovorum* subsp. *carotovorum* previously deposited in GenBank (3). Pathogenicity of the strains was confirmed by inoculation of broccoli head tissue fragments. Three florets per strain were inoculated by pricking the petals with a syringe and hypodermic needle and depositing a droplet of bacterial suspension (approx. 1×10^8 CFU/ml) at the point of inoculation. Sterile distilled water was used as a negative control. Inoculated florets were placed in a sealed plastic container and incubated in high humidity conditions at 28°C. Tissue discoloration and soft rot developed around the inoculation point within 48 to 72 h. No symptoms developed on control florets. Identity of bacterial strains reisolated from inoculated plant tissues was confirmed by ITS-PCR using G1/L1 primers followed by digestion of PCR products with *Rsa* I restriction enzyme (4). In Serbia, *P. carotovorum* subsp. *carotovorum* has been isolated from potato, some vegetable crops, and ornamentals, but not from broccoli until now.

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The American Phytopathological Society (APS)

3340 Pilot Knob Road, St. Paul, MN 55121

USA

+1.651.454.7250

FAX +1.651.454.0766

APS