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DISEASE NOTES

First Report of *Pectobacterium carotovorum* subsp. *brasiliense* Causing Soft Rot on Squash and Watermelon in Serbia

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Watermelon (*Citrullus lanatus*) and squash (*Cucurbita pepo*) are traditionally grown in northern Serbia, in the province of Vojvodina. Most of the cucurbit cultivars grown in Serbia originate from imported seed. In July 2013, round, water-soaked lesions were observed on the fruits on sporadic squash plants grown in two fields of the breeder variety trials in Bački Petrovac. Affected tissue was light brown, slightly sunken, soft, and macerated. From symptomatic plants (cultivar GL Maxima), showing 30% symptom intensity, a uniform population of bacterial colonies was isolated, and one strain was chosen for the collection. In August of the following season, infection of the vine was observed on watermelon plants (cultivar unknown) grown in a commercial field in Čelarevo. Soft rot brownish lesions developed on infected stems, resulting in collapse and wilting of entire vines. The number of plants affected with symptoms was less than

10%. From the colonies isolated from the diseased tissue, two bacterial strains were purified and stored in the collection. Being pectolytic and isolated from cucurbits, the squash strain and two watermelon strains were subjected to further characterization. The strains developed white-gray, nonmucoid, round colonies with irregular margins on nutrient agar medium. They were gram and oxidase negative, tobacco HR and catalase positive, facultative anaerobes, nonfluorescent, caused soft rot on potato tuber slices, grew at 37°C, and reduced nitrate. They neither utilized arginine nor produced acids from sorbitol and inositol, showing characteristics of *Pectobacterium* species (De Boer and Kelman 2001). The PCR assay was carried out using primers BR1f/L1r (Duarte et al. 2004), specific for *Pectobacterium carotovorum* subsp. *brasiliense* (*Pcb*). A specific fragment of 322 bp was amplified for all three strains tested. The 16S rDNA sequence analysis (GenBank nos. MH031784, MH031785, and MH031786) showed 99% identity to the sequences of *Pcb* isolated from cucumber (CP020350), pepper (KX377597), and cabbage (KY021040) in China and potato in Japan (LC146476). The *recA* (MK911735, MK911736, and MK911737) and *gapA* (MK911738, MK911739, and MK911740) sequence analysis of three strains showed the highest nucleotide identity (100 to 98.35% and 99.42 to 98.08%, respectively) with *Pcb* previously deposited in NCBI GenBank database. Pathogenicity of the strains was tested on 2-week-old watermelon (cv. Rosa) and melon (cv. Sezam) seedlings, grown in a commercial potting mix in a greenhouse, as well as on mature zucchini fruits. Three seedlings per strain were inoculated by spraying with bacterial suspension (approx. 1×10^8 CFU/ml) using a handheld sprayer. Inoculated plants were incubated under plastic bags for 24 h and afterward maintained in a greenhouse at $28 \pm 2^\circ\text{C}$. Two days after inoculation, water-soaked lesions developed on cotyledons of the watermelon and melon plants. No symptoms were observed on control plants sprayed with water. Three zucchini fruits were inoculated by pricking with a syringe and hypodermic needle and leaving a droplet of bacterial suspension (approx. 1×10^7 CFU/ml) at the point of inoculation. Inoculated fruits were placed on wet filter paper in a sealed plastic container at $28 \pm 2^\circ\text{C}$. Treatment with sterile distilled water was used as a negative control. Discoloration of the tissue and soft rot spreading from the inoculation point developed within 48 h from inoculation. No symptoms developed on the control fruits. To complete Koch's postulates, bacteria were reisolated from the inoculated plant and fruit tissue and were identified as *Pcb* by PCR assay (Duarte et al. 2004). This is the first report of *Pcb* causing soft rot of squash and watermelon in Serbia. The origin of the pathogen remained unknown. It might have been present in Serbia for some time but misidentified in previous studies that could not discriminate this subspecies or recently

introduced by infected seed.

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