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First Report of Bacterial Fruit Blotch on Watermelon Caused by Acidovorax citrulli in the Republic of North Macedonia

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First Report of Bacterial Fruit Blotch on Watermelon Caused by *Acidovorax citrulli* in the Republic of North Macedonia

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Abstract

In late August, 2019, watermelon fruits (*Citrllus lanatus* L., variety Bibo, 1.5 ha and Olakala, 3.5 ha) collected from a field located in Sopot Kavadarci, N. Macedonia exhibited typical disease symptoms resembling bacterial fruit blotch (BFB) caused by *Acidovorax citrulii*.

Watermelon leaves were not symptomatic. On fruit, first symptoms observed were small cracks with some water soaking, and then cracks extended to the entire fruit and water soaking and rotting areas appeared all around.

Fruit samples were collected from the field, washed with running water, and the bacterium was isolated from the margin of lesions from within the mesocarp. The solution of macerated tissue was plated onto King's B medium and non-fluorescent colonies were visible after a few days. The colonies were gram-negative, cream-colored with smooth margins, and convex, and individual cells were rod-shaped. Isolates were oxidase positive, gram-negative, arginine dihydrolase negative and aerobic.

A collection of 20 isolates were characterized based on physiological, biochemical, and pathogenicity tests, and identified applying PCR with *A. citrulli*-specific primer pairs (Schaad et all. 2001) using PCR conditions described in EPPO standard (PM 7/127 (1) OEPP/EPPO (2016). The strains grew at 41°C, and italicize a strong hypersensitive response on tobacco (*N. tabacum* cv Samsun) 24 h after tissue infiltration. Pathogenicity tests were performed by injecting 0.5 to 1 ml suspensions of the bacteria (10^6 CFU/ml) under the rind of three small watermelon fruits (*C. lanatus*), and into the cotyledons of ten, 10-day-old watermelon seedlings. The fruit and seedlings were incubated in plastic bags at 27°C, and treatments with sterile water

served as negative controls. After 4 days, the inoculated with the putative strain seedlings exhibited dark brown necrotic lesions with yellow halos that later coalesced, causing the cotyledons to collapse. The watermelon fruit had completely collapsed in a watery rot after 7 days. No symptoms were observed on the fruits and seedlings treated with sterile water. To confirm the identity of the pathogen, PCR was conducted with the *A. citrulli* primer set and thermal cycling conditions.

A single unique band of 450 bp was amplified for all isolates tested and compared with two positive controls: KFB 0250 (collection from A. Obradovic, University of Belgrade) and *A. avenae* subsp.*citrulii* No. 08154PC (commercial control from LOEWE).

On the basis of the fruit symptoms, pathogenicity on watermelon young fruit and seedlings, results of biochemical tests and an PCR analyses using known positive controls the pathogen was identified as *Acidovorax citrulli*.

To our knowledge, this is the first occurrence of *A. citrulli* causing bacterial fruit blotch on watermelon in N. Macedonia, with significant economic losses (up to 90%).

Considering that is pathogen in on the EPPO A1 List, the Ministry of Agriculture was informed and eradication measures should be implemented to prevent further spread of this bacterium across the region.

References: (1) Schaad N.W., Jones J.B., Chun W., 2001. Laboratory Guide for Identification of Plant Pathogenic Bacteria. 3nd. Ed. APS Press, St. Paul, MN, USA.

(2) PM 7/127 (1) *Acidovorax citrulii*, Bulletin OEPP/EPPO (2016) 46 (3), 444-462

Acidovorax citrulli in the Republic of N. Macedonia

Acidovorax citrulli in the Republic of N.Macedonia

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