

***Acidovorax avenae* subsp. *citrulli* Transmitted by Solanaceous Seeds**

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Acidovorax avenae subsp. *citrulli* (*Aac*), the causal agent of watermelon fruit blotch, was intercepted in watermelon seeds imported to Israel from the U.S.A. a few times during 1992–94 (1). The bacterium was isolated and identified again in May 1995 from diseased eggplant seedlings grown from imported seeds; the exact origin of the seed lot and the name of the seed company remain unknown. Koch tests with this isolate against watermelon, cucumber, eggplant and tomato seedlings were strongly positive, as were similar tests done with a watermelon *Aac* isolate (1). A polyclonal antiserum (rabbit anti-*Aac* IgG) was prepared using a 1994 watermelon isolate of *Aac* (I. Assouline & O. Assouline, unpublished).

A shipment of tomato seeds (var. HMX 2867 F1) imported to Israel in January 1997 was tested by two laboratories and found infested with: *A. avenae* subsp. *citrulli*, *Clavibacter michiganensis* subsp. *michiganensis*, a pectolytic *Erwinia* sp. and tobacco mosaic virus. The origin of the seeds was Tropica Seeds, Girinagar, Bangalore, India, produced for and exported to Harris Moran Seed Company, Modesto, California. The Indian Phytosanitary Certificate (no. 3018, issued 04.03.1996) declared the seeds free from a number of bacteria, including *C. michiganensis*. The USDA Phytosanitary Certificate for Reexport (FPC 100900-R, issued 11 Dec. 1996) declared the seeds as treated by “hot water, 50 centigrade for 60 min”, and “The commodity has met the entry requirements of the United States”.

Aac was isolated from this lot of seeds on a semi-selective medium (*AacSM*, I. Assouline, unpublished). Identification was confirmed by morphological and biochemical characters as described (1), by ELISA with the antiserum described above, and by fatty acid profile using the MIS gas chromatography system (4). The ELISA reaction (OD >2.0) was as strong as with a watermelon *Aac* isolate used as positive control. The fatty acids profiles of two separate colonies were analyzed (4) and identified as *A. avenae* subsp. *citrulli* with similarity indices >0.800, by the TSBA library of MIDIs AEROBE 3.90 method. The fatty acid composition found: 10:0 (0.39–0.41%); 10:0 3OH (2.12–2.28%); 12:0 (2.32–2.33%); 12:1 3OH (0.25–0.54%); 14:0 (1.62–1.64%); 15:1 ω 6c (1.30–1.56%); 15:0 (5.50–6.51%); 16:1 ω 7c/15:0 iso 2OH (39.98–40.22%); 16:0 (32.32–33.67%); 17:0 Cyclo (1.54–1.80%); 17:0 (1.82–2.04%); 18:1 ω 7c/ ω 9t/ ω 12t/ 18:1 ω 9c/ ω 12t/ ω 7c (8.90–8.92%), is practically identical to that in the IMI description (5).

Koch's postulates were fulfilled by inoculating watermelon seedlings and obtaining the characteristic symptoms. Cotyledons were dusted lightly with carborundum and rubbed with a cottonwool swab dipped in a bacterial suspension of 10^7 – 10^8 cfu/ml. The seedlings were incubated in humid chambers at 23–26°C. Four days postinoculation water-soaked lesions appeared, identical to those described by Hopkins *et al.* (ref. 2: Fig. 1), which later turned into necrotic spots (ref. 2: Fig. 2; ref. 3: Fig. 1H). Control seedlings were rubbed with sterile water. The causal agent was reisolated from lesions of inoculated seedlings and identified as described before. Inoculations and reisolations were repeated twice. The USDA (APHIS) was notified about the interception of *Aac* in this seed shipment.

Watermelon, eggplant and tomato crops are grown in Israel as Speedlings in glasshouses and transplanted to the field. To date no symptoms of watermelon fruit blotch have been encountered in Israeli fields. The eggplant Speedlings with leaf spot symptoms, which constituted the first (1995) interception of *Aac* in solanaceous seeds, were promptly destroyed and precautions were taken to disinfect the glasshouse thoroughly. This bacterium is listed as a quarantine pest for Israel.

Following two recent *Aac* interceptions in imported solanaceous seeds, we feel it is important to review the host list and geographical distribution of this bacterium in the IMI Descriptions (5) and Distribution Maps. To the best of our knowledge, this is the first documented report on transmission of *Aac* by solanaceous seeds.

REFERENCES

1. Assouline, I. (1996) The watermelon fruit blotch disease and other diseases caused by *Acidovorax avenae*. *Phytoparasitica* 24: 136-137 (abstr.).
2. Hopkins, D., Stall, B., Kucharek, T., Gay, D., Gitaitis, R., Cook, W., Keinath, A. and Latin, R. (1994) Bacterial fruit blotch of watermelon. Joint Techn. Publ., Univ. of Florida, Univ. of Georgia, Clemson Univ., Purdue Univ., USA. 4pp.
3. Latin, R.X. and Hopkins, D.L. (1995) Bacterial fruit blotch of watermelon. The hypothetical exam question becomes reality. *Plant Dis.* 79: 761-765.
4. MIDI (1992) Microbial Identification System – Operating Manual, Version 4. Microbial ID, Inc. (MIDI), Newark, DE, USA.
5. Saddler, G.S. (1994) *Acidovorax avenae* subsp. *citrulli*. IMI Descriptions of Fungi and Bacteria No. 1213. *Mycopathologia* 128: 47-48.