Occurrence, Isolation, and Identification of *Acidovorax citrulli* from Melon in Turkey

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Abstract

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During February and August of 2010 and 2011, disease symptoms were detected in melon (*Cucumis melo* cv. Surmeli) fields and commercial nurseries in Adana and Mersin provinces (Eastern Mediterranean Region, Turkey). Lesions on leaves and fruits were observed in nearly 75 and 85 acres production areas of melon in 2010 and 2011, respectively. Initial symptoms were water-soaked irregular spots, light brown to reddish spots advancing through veins on leaves, small water-soaked lesions, greasy, dark olive green colour areas on the surfaces of melon fruit followed by brown lesions, softening, and cracks. Thirty-five non-fluorescent, slow-growing, round, cream and Gram-negative bacterial isolates were isolated from symptomatic plant materials. The pathogenicity of the isolates was proved using melon seedlings and fruits. According to classical, serological, and molecular assays, the causal organism was identified as *Acidovorax citrulli*. To the best of our knowledge, this is the first report and occurrence of bacterial fruit blotch disease on melon as a new host in Turkey.

Keywords: *Acidovorax*; *Cucumis melo*; disease; fruit blotch; ELISA; PCR

Nearly 10% of the melon (*Cucumis melo*) production is conducted in Cukurova region located at the Eastern Mediterranean Region of Turkey. In February 2010, water-soaked foliar disease symptom was observed in commercial melon (*Cucumis melo* cv. Surmeli) seedling production companies in Adana and Mersin provinces. Subsequently, in May–August of 2010 and 2011, foliar and fruit disease symptoms were also detected in melon fields in Adana province, particularly within overhead irrigated areas. Typical lesions on leaves and fruits were observed in almost 75 and 85 acres melon fields in the summer of 2010 and 2011, respectively. Several complaints were also reported from commercial nurseries where many seedlings were infected by the disease agent. In commercial nurseries, seedlings showing disease symptoms were eradicated immediately. Initial symptoms of the disease consist in water-soaked angular spots following light brown to reddish spots delimited by the leaf veins. In the Cukurova region of Turkey, small, brown, water-soaked lesions were observed on melon fruit surfaces, followed by cracks, softening and rotting. At the late stage of the infection, the fruit was contaminated with secondary metabolites like saprophytic bacteria and fungi. Up to 80% of marketable fruit were destroyed in some melon fields.

Bacterial fruit blotch caused by the bacterium *Acidovorax citrulli* is a devastating disease of cucurbit hosts in many producing countries (Latin & Rane 1990; Evans & Mulrooney 1991; Somodi *et al.* 1991; Jacobs *et al.* 1992; Black *et al.* 1994; Babadoost & Pataky 2002). Furthermore, the pathogenic bacterium developed disease symptoms on other cucurbit hosts including musk melon (Sowell 1981), honeydew (Isakeit *et al.* 1997), citron melon (Isakeit *et al.* 1998), melon (Assis *et al.* 1999), rock

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melon (O'Brien & Martin 1999), cucumber (Martin *et al*. 1999), pumpkin (Langston *et. al*. 1999), and Hami melon (Zhao *et al*. 2001). Burdman *et al*. (2005) characterised the *Acidovorax citrulli* strains isolated from different cucurbit hosts according to DNA fingerprinting profiles and their study indicated the two differentiated groups within *Acidovorax citrulli*: watermelon group and non-watermelon cucurbits group.

In Turkey, the disease agent was first reported on watermelon in Edirne province (Demir 1996). After the eradication of the contaminated fields in Edirne in the summer of 2005, sudden disease symptoms appeared in watermelon fields in Cukurova region (Mirik *et al*. 2006). Following the first disease report in the region, a significant outbreak occurred in 2009 in watermelon fields. A year later, similar symptoms were observed in both watermelon and melon fields and commercial nurseries in the studied Turkish provinces.

The purposes of the research were to isolate and identify the causal agent of melon bacterial fruit blotch in the Cukurova region of Turkey by using morphological, physiological, biochemical, serological, and molecular studies. This is the first detailed study on bacterial fruit blotch disease of melon in Turkey.

MATERIAL AND METHODS

Collection of bacterial strains. Melon seedlings or leaves showing necrotic lesions were collected during February and August of 2010 and 2011 from commercial nurseries and fields in Adana and Mersin provinces of Turkey. Some fruit samples were submitted by the growers.

Isolation and identification of the pathogen. Diseased samples were processed at the day of sampling. Leaves were first washed under tap water until removing the plant debris or soil particles. Small pieces of necrotic leaf tissue with healthy part and fruit lesion were cut into small pieces, and then all tissues were disinfected by immersion in the solution of 70% ethanol for few seconds. Each necrotic piece was macerated in a sterile mortar with approximately 2 ml of sterile saline buffer (0.85% NaCl). A 20 µl sample was streaked onto King's Medium B (KB) (King *et al*. 1954) plates. The plates were incubated at 28°C for 2–3 days. Each bacterial colony from plates was purified and stored at –80°C in 20% aqueous glycerol and also kept on yeast dextrose calcium carbonate (Lelliott & Stead 1987) at 5°C. For all bacterial isolates, gram reactions were determined by the lysis of bacteria in 3% potassium hydroxide (KOH) (Gregersen 1978). Biochemical and physiological tests including LOPAT (levan production, oxidase reaction, potato soft rot, arginine dihydrolase, and hypersensitivity on tobacco leaves) characters were used for identification of the bacterial isolates.

Pathogenicity tests. All representative isolates were grown on KB for 48 hours. Three-week-old healthy melon seedlings (*Cucumis melo* cv. Balkovan) at 3–5 true leave stage and mature melon fruits (*Cucumis melo* cv. Balkovan) obtained from healthy fields were used for the pathogenicity tests. Both tests were conducted with 2 replicates. Bacterial suspensions were adjusted to 10^7 CFU/ml using a spectrophotometer, and young melon seedlings were sprayed with the pathogen. The same bacterial suspensions were injected into the healthy mature melon fruit, no deeper than 1 cm with hypodermic needles. Sterile water was used as negative control. A watermelon isolate of *Acidovorax citrulli*, which was previously identified (Mirik *et al*. 2006), was used as positive control. Inoculated plants and fruits were placed in a growth chamber at 28°C with 85% relative humidity for 2–7 days. Disease development on leaves and fruits was evaluated at 2–7 days after inoculations. Re-isolations were made from symptomatic plant materials.

Serological test by ELISA. The serological identification of the bacterial isolates was performed as three replications according to the manufacturer's instructions with DAS-ELISA kit, Agdia SRA 14800 (Agdia Inc., Belkart, USA). A watermelon isolate of *Acidovorax citrulli* and healthy melon leaves were used as positive and negative controls, respectively. Absorbance values were read using ELISA micro plate reader (Medispec ESR 200; Medispec Ltd., Gaithersburg, USA) at 405 nm after 45 minutes.

Molecular test by species-specific PCR. The molecular identification of *Acidovorax citrulli* isolates was confirmed using the WFB1 (5'-GACCAGCCACACTGGGAC-3') and WFB2 (5'-CTGCCGTACTCCAGCGAT-3') primer pair. All representative bacterial isolates were grown on KB at 28°C for 48 hours. The DNA isolation step was performed by modifying the method used by Nejat *et al*. (2009). Purified DNA was stored in a new 1.5 ml centrifuge tube at –20°C until use. PCR assays were performed in 25 µl of PCR master mix K0171 (FermentasThermo Fisher Scientific, Vilnius, Lithuania) containing reaction buffer, 4mM $MgCl_2$, 0.4mM of each dNTP, 0.05 u/µl of *Taq* DNA polymerase, 2 µl of each primer (10 pmol), and 2 µl of each genomic DNA per reaction. DNA amplification was carried out in a thermal cycler

Techne TC-4000 (Bibby Scientific Ltd., Staffordshire, UK). PCR products were analyzed after 2-h electrophoresis at 40 V in a 1.5% agarose gel stained with ethidium bromide at 0.1 µg/ml. A 100 bp ladder SM0241 (Thermo Fisher Scientific, Vilnius, Lithuania) was used as a size marker.

RESULTS

Collection of bacterial strains. Samples of melon (*C. melo* cv. Surmeli) seedlings or true leaves and fruits showing bacterial symptoms were collected from commercial fields and nurseries in Adana and Mersin provinces in Cukurova region (Turkey) during two growing periods in February–August 2010 and 2011, respectively. In commercial nurseries, initial symptoms were observed in February: water-soaked areas on cotyledons (Figure 1a), angular spots following light brown to dark brown necrotic lesions on true leaves limited by the veins (Figure 1b). However, symptoms on true leaves did not appear so often owing to the epiphytic survival of the pathogen, but some symptoms observed in the fields were irregular, brown to reddish brown coloured necrotic lesions on leaves (Figure 1c), additionally, initial water-soaked, sunken, browning in fruit flesh following cracks, softening and rotting (Figures 1d–f). The affected fruits became unmarketable due to disease damage. Disease symptoms in fields and nurseries were observed only on a local melon cultivar (*C. melo* cv. Surmeli).

Isolation and identification of the pathogen. Isolations from necrotic lesions of melon cotyledons, true leaves, and necrotic rind of fruit consistently yielded a single type of bacterial colony from the lesions both on leaves and fruits on King's Medium B. The purified thirty-five bacterial isolates were non-fluorescent, slow-growing, round, cream, and Gram-negative (Table 1). These bacterial strains were negative for levan production, pectolytic activity on potato slices, and arginine dihydrolase, positive for oxidase test and hypersensitivity response (HR) on tobacco.

Table 1. *Acidovorax citrulli* strains isolated from melon cv. Surmeli seedlings and fruits

Number of strains	Year of isolation	Isolationpart
6	2010	seedling
15	2010	fruit
14	2011	fruit

Pathogenicity tests. All representative isolates, which were artificially inoculated to the healthy melon (*Cucumis melo* cv. Balkovan) seedlings, caused water soaked lesions, which expanded rapidly followed by dark brown spots on infected leaf tissues seven days after spraying. Initial disease symptoms on mature melon fruits were observed three days after inoculations, starting with water soaked lesions followed by sunken, brown necrosis, cracks, and finally rotting on fruit rind within seven days. By positive control seedlings or fruits, typical disease symptoms such as water-soaked irregular spots, dark brown lesions on cotyledons and true leaves, water-soaked, dark olive green lesions, sunken, brown necrosis and rotting on fruits were produced. Control plants and fruits were negative for disease symptoms. Re-isolations were

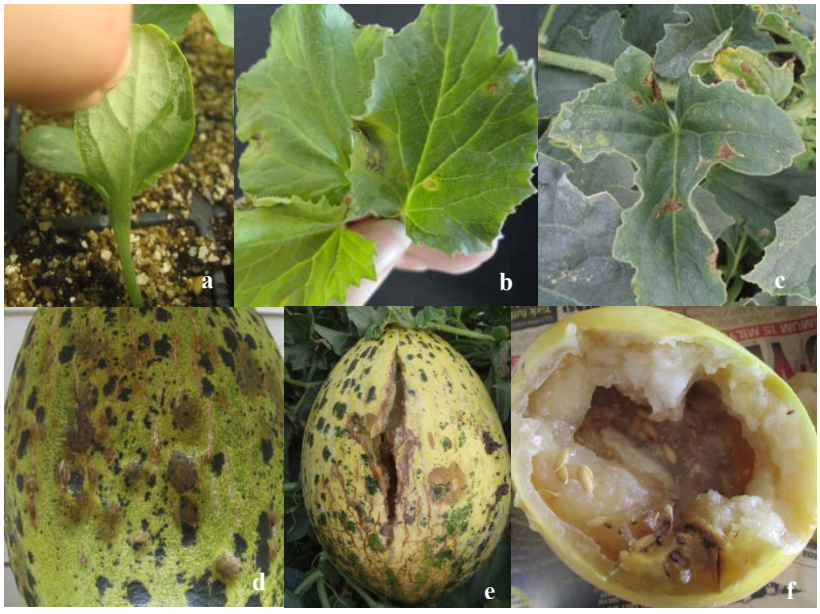

Figure 1. Natural symptoms of melon bacterial fruit blotch disease caused by *Acidovorax citrulli*, (a) water soaked lesions on cotyledons, (b) angular spots following light brown to dark brown necrotic lesions on true leaves limited by the veins, (c) irregular, brown to reddish brown coloured necrotic lesions on leaves, (d, e) water-soaked, sunken browning in fruit flesh followed by cracks on melon cv. Surmeli, (f) softening and rotting on melon cv. Balkovan (pathogenicity test)

made from symptomatic plant materials yielded by pure bacterial colonies of *Acidovorax citrulli*.

Serological test by ELISA. In DAS-ELISA tests, all melon isolates were positive and mean absorbance values of three replications measured using Medispec ESR 200 ELISA plate reader were 1.880–2.020 at 405 nm wavelength. Positive and negative controls exhibited absorbance values 2.512 and 0.356 nm, respectively.

Molecular test by species-specific PCR. The identification of *Acidovorax citrulli* strains was confirmed by using *A. citrulli* species-specific primer pair, WFB1/WFB2. All melon strains yielded a 360 bp amplified fragment after a two-hour electrophoresis (40 V, 1.5% agarose gel stained with ethidium bromide at 0.1 µg/ml). On the basis of their classical, serological, and molecular characteristics, the 35 melon isolates were identified as *Acidovorax citrulli*.

DISCUSSION

To our knowledge, this is the first detailed study on the occurrence of *Acidovorax citrulli* on melon in the commercial nurseries and fields in Cukurova region as well as in whole Turkey. Surmeli, as a local melon cultivar, is one of the most common cucurbit crop grown in the region, and its seeds are the main source of transferring the pathogen isolated from melon field and nurseries. Bacterial blotch disease on melon was also reported in Brazil (Assis *et al*. 1999). The disease symptoms have still persisted in fields and the spread of the disease to other commercial fields can be prevented by implementing suitable phytosanitary measures. Mengulluoglu and Soylu (2012) showed that thyme and origanum essential oils inhibited *Acidovorax citrulli* population in *in vitro* petri assays. Fessehaie and Walcott (2005), Oliveira *et al.* (2006), Medeiros *et al*. (2009), and Wang *et al.* (2009) indicated that using antagonistic bacteria or yeasts is promising to manage bacterial fruit blotch disease. Our ongoing studies will be focused on the determination of *Acidovorax citrulli* isolates from infected watermelon and melon in the nurseries or fields in order to reveal the genetic relationships, epidemiology of the disease in the region, and to propose the disease management strategies.

References

Assis S.M.P., Mariano R.L.R., Silva-Hanlin D.M.W., Duart V. (1999): Bacterial fruit blotch caused by *Acidovorax avenae* subsp. *citrulli* in melon, in the State of Rio Grande do Norte, Brazil. Fitopatologia Brasileira, **24**: 191.

Babadoost M., Pataky N. (2002): First report of bacterial fruit blotch of watermelon caused by *Acidovorax avenae* subsp. *citrulli* in Illinois. Plant Disease, **86**: 443.

Black M.C., Isakeit T., Barnes L.W., Kucharek T.A., Hoover R.J., Hodge N.C. (1994). First report of bacterial fruit blotch of watermelon in Texas. Plant Disease, **78**: 831.

Burdman S., Kots N., Kritzman G., Kopelowitz J. (2005): Molecular, physiological and host-range characterization of *Acidovorax avenae* subsp. *citrulli* isolates from watermelon and melon in Israel. Plant Disease, **89**: 1339–1347.

Demir G. (1996): A new bacterial disease of watermelon in Turkiye: Bacterial fruit blotch of watermelon (*Acidovorax avenae* subsp. *citrulli* (Schaad et al.) Williams et al.). Journal of Turkish Phytopathology, **25**: 43–49.

Evans T.A., Mulrooney R.P. (1991): First report of watermelon fruit blotch in Delaware. Plant Disease, **75**: 1074.

Fessehaie A., Walcott R.R. (2005): Biological control to protect watermelon blossoms and seed from infection by *Acidovorax avenae* subsp. *citrulli*. Phytopathology, **95**: 413–419.

Gregersen T. (1978): Rapid method for distinction of gram-negative from gram-positive bacteria. European Journal of Applied Microbiology and Biotechnology. **5**: 123–127.

Isakeit T., Black M.C., Barnes L.W., Jones J.B. (1997): First report of infection of honeydew with *Acidovorax avenae* subsp. *citrulli*. Plant Disease, **81**: 694–694.

Isakeit T., Black M.C., Barnes L.W., Jones J.B. (1998): Natural infection of citronmelon with *Acidovorax avenae* subsp. *citrulli*. Plant Disease, **82**: 351–351.

Jacobs J.L., Damicone J.P., Mccraw B.D. (1992): First report of bacterial fruit blotch of watermelon in Oklahoma. Plant Disease, **76**: 1185.

King E.O., Ward M. K., Raney D.E. (1954): To simple media for the demonstration of pyocyanin and flueorescein. Journal of Clinic Medicine, **44**: 301–307.

Langston Jr. D.B., Walcott R.R., Gitaitis R.D., Sanders F.H. (1999): First report of a fruit rot of pumpkin caused by *Acidovorax avenae* subsp. *citrulli* in Georgia. Plant Disease, **83**: 199.

Latin R.X., Rane K.K. (1990): Bacterial fruit blotch of watermelon in Indiana. Plant Disease, **74**: 331.

Lelliot R.A., Stead D.E. (1987): Methods for the Diagnosis of Bacterial Diseases Plants. Blackwell Scientific Publications, Oxford.

Martin H.L., O'Brien R.G., Abbott D.V. (1999): First report of *Acidovorax avenae* subsp. *citrulli* as a pathogen of cucumber. Plant Disease, **83**: 965.

Medeiros F.H.V., Moraes Iale S.F., Silva Neto E.B.D., Silveira E.B., Mariano Rosa D.L.R. (2009): Manage-

ment of melon bacterial blotch by plant beneficial bacteria. Phytoparasitica, **37**: 453–460.

Mengulluoglu M., Soylu S. (2012): Antibacterial activities of essential oils extracted from medicinal plants against seed-borne bacterial disease agent, *Acidovorax avenae* subsp. *citrulli*. Research in Crop. **13**: 641–646.

Mirik M., Aysan Y., Sahin F. (2006): Occurrence of bacterial fruit blotch of watermelon caused by *Acidovorax avenae* subsp. *citrulli* in the Eastern Mediterranean Region of Turkey. Plant Disease, **90**: 829.

Nejat N., Sijam K., Abdullah S.N.A., Vadamalai G., Dickinson M. (2009): Molecular characterization of a phytoplasma associated with Coconut Yellow Decline (CYD) in Malaysia. American Journal of Applied Sciences, **6**: 1331–1340.

O'Brien R.G., Martin H.L. (1999): Bacterial blotch of melons caused by strains of *Acidovorax avenae* subsp. *citrulli*. Australian Journal of Experimental Agriculture, **39**: 479–485.

Oliveira A. de., Santos M.H.M. dos, Silveira E.B. da., Gomes A.M.A., Mariano R. de L.R. (2006): Biocontrol of bacterial blotch of melon by seed treatment with epiphytic and endophytic bacteria. Horticultura Brasileira, **24**: 373–377.

Somodi G.C., Jones J.B., Hopkins D.L., Stall R.E., Kucharek T.A., Hodge N.C., Watterson J.C. (1991): Occurrence of a bacterial watermelon fruit blotch in Florida. Plant Disease, **75**: 1053–1056.

Sowell G. Jr. (1981): A bacterial disease causing severe damage to susceptible plant introductions of muskmelon. Plant Disease, **65**: 609–610.

Wang X., Li G., Jiang D., Huang H.-Ch. (2009): Screening of plant epiphytic yeasts for biocontrol of bacterial fruit blotch (*Acidovorax avenae* subsp. *citrulli*) of hami melon. Biological Control, **50**: 164–171.

Zhao T.C., Sun F. Z., Wang B.W., Hui W.G. (2001): Pathogen identification of Hami melon bacterial fruit blotch. Acta Phytopathologica Sinica, **31**: 357–364.

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