Bacterial Leaf Spot of Watermelon Caused by Pseudomonas lachrymans

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ABSTRACT

A bacterium isolated from a leaf spot of watermelon reproduced the symptoms of the disease when watermelon was inoculated in the greenhouse. In host range and physiological tests, the watermelon leaf spot bacterium most closely resembled *Pseudomonas lachrymans* and was most pathogenic on several plants in the Cucurbitaceae. Isolates of the watermelon pathogen and *P. lachrymans* were more pathogenic on cucumber than on watermelon. With its yearly recurrence, this disease has the potential to adversely affect watermelon production in Florida.

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A bacterial leaf spot disease of watermelon (*Citrullus lanatus* [Thunb.] Mansf.) was first observed in Florida in 1963 (8) when it caused considerable defoliation in many commercial watermelon fields. The symptoms began as small black spots, usually circular in shape and surrounded by yellow halos. The lesions sometimes had white centers. As the disease progressed, the lesions became irregular in shape and enlarged to envelop entire lobes, or more, of the leaf (8).

To our knowledge, the first bacterial leaf spot disease of watermelon to be described in the literature as occurring naturally was reported by Mullin & Schenck (8). An unidentified bacterium which caused leaf spot symptoms was isolated later from naturally infected seedlings of two watermelon plant introductions in Georgia (11). Successful inoculations of watermelon in the greenhouse with *Pseudomonas lachrymans* (E. F. Sm. & Bryan) Carsner have been reported (1, 10).

The bacterial leaf spot disease has occurred in Florida each year since it was initially observed. Preliminary greenhouse tests identified many suitable hosts on which the pathogen could exist throughout most of the year in South Florida (8). The disease appeared to have the potential to adversely affect watermelon production in Florida, and this prompted our attempt to identify the bacterium and to study its host range.

MATERIALS AND METHODS.—Cultures of the watermelon leaf spot bacterium were obtained from naturally infected watermelon leaves in Florida in 1964 through 1970. Isolates of *P. lachrymans* for comparison were obtained from Dr. P. H. Williams, Department of Plant Pathology, University of Wisconsin, Madison, and from the International Collection of Phytopathogenic Bacteria (ICPB) maintained in the Department of Bacteriology, University of California, Davis.

King's Medium B agar (4) was used to isolate the bacterium from infected leaves, to obtain pure cultures by dilution plating, and to grow cultures. The dilution plating procedure was repeated at least twice with watermelon leaf spot isolates before they were considered pure cultures. Stock cultures were maintained in sterile, deionized water at room temperature.

All isolates of the leaf spot bacterium were tested for pathogenicity to watermelon. The inoculum, taken from 2- to 5-day-old cultures on Medium B, was suspended in sterile deionized water. The concentration of the bacterial suspensions was determined with a Bausch & Lomb Spectronic-20 colorimeter. Watermelon plants in the 3- to 6-leaf stage were inoculated with suspensions containing from 10^3 to 10^8 bacterial cells/ml. Inoculum was either sprayed gently on the lower surface of the leaves or injected into the leaves. After inoculation, plants were either placed in a moist chamber for 18 hr or returned immediately to the greenhouse bench. The relative humidity of the greenhouse during these tests was 70-100% for 16-18 hr of the day, and was rarely below 40% the remaining 6-8 hr. The greenhouse temperature was usually 27 ± 1 C, but occasionally was as low as 21 C at night or as high as 32 C at 2 PM. The plants were examined for leaf spot symptoms 6 days after inoculation.

Representative isolates, 64-3 and 67-1, of the watermelon leaf spot bacterium were compared with known *P. lachrymans* isolates in host-range studies. The bacterial isolates were judged to be pathogenic on a host only when lesions developed within 1 week after a suspension of 5×10^7 cells/ml were sprayed gently on the underside of the leaves and the inoculated plants were returned to the greenhouse bench. Care was taken to avoid water-soaking. This inoculation procedure allowed the recognition of susceptible hosts, and obviated the possibility of hypersensitivity being mistaken for pathogenicity.

The biochemical and physiological tests used by Misaghi & Grogan (7) to place most of the plant-pathogenic nomenspecies of *Pseudomonas* into their Group IA were performed on all of the watermelon leaf spot isolates. These tests were: oxidase, arginine dihydrolase, hypersensitivity

TABLE 1. Susceptibility of various plant species to the watermelon bacterial leaf spot pathogen and *Pseudomonas lachrymans*[a]

Host	Cultivar	Leaf spot isolates		*P. lachrymans*	
		64-3	67-1	PL-104	PL-113
Cucurbitaceae					
Citron (*Citrullus lanatus* var. *citroides* [Thunb.] Mansf.)		+[b]	+	+	+
Cucumber (*Cucumis sativus* L.)	Ashley	+	+	+	+
Cucumber (*C. sativus* L.)	Boston Pickling	+	+	+	+
Cucumber (*C. sativus* L.)	Poinsett	+	+	+	+
Muskmelon (*C. melo* L.)	Hales Best	+	+	+	+
Muskmelon (*C. melo* L.)	Planters Jumbo	+	+	+	+
Pumpkin (*Cucurbita pepo* L.)	Conn. Field	+	+	+	+
Pumpkin (*C. maxima* Duch.)	King of Mammoth	+	+	–	+
Squash (*C. pepo* L.)	Early Yellow Summer Crookneck	+	+	+	+
Squash (*C. moschata* Poir.)	Butternut 23	+	+	+	+
Watermelon (*Citrullus lanatus* [Thunb.] Mansf.)	Charleston Gray	+	+	–	+
Watermelon (*C. lanatus* [Thunb.] Mansf.)	Florida Giant	+	+	–	–
Watermelon (*C. lanatus* [Thunb.] Mansf.)	Garrisonian	+	+	+	+
Melothria pendula L.		+	+	–	+
Luffa (spp. unknown)		+	–	–	–
Leguminosae					
Alyceclover (*Alysicarpus vaginalis* [L.] DC.)		+	+	+	–
Common Bean (*Phaseolus vulgaris* L.)	Bountiful	+	–	–	–
Cowpeas (*Vigna sinensis* [Torner] Savi)		+	+	–	+
Solanaceae					
Tomato (*Lycopersicum esculentum* Mill.)	Rutgers	+	+	–	+
Tropaeolaceae					
Nasturtium (*Tropaeolum majus* L.)	Scarlet Gem	+	+	+	+

[a]Host plants were inoculated with a gentle spray of a 5×10^7 bacterial cells/ml suspension and returned directly to the greenhouse bench.

[b]+ = susceptible; – = not susceptible.

reaction in tobacco, growth at 37 C, and pathogenicity.

Standard bacteriological tests, conducted as described elsewhere (3, 5, 6), were performed on six watermelon leaf spot isolates. The carbohydrates and organic acids used in the carbon utilization studies were sterilized by filtration and added aseptically to a sterilized basal medium to make final concentrations of 1.0% and 0.15%, respectively. The basal medium consisted of the following: $Na(NH_4)HPO_4 \cdot 4H_2O$, 1.5 g; $MgSO_4$, 0.5 g; KH_2PO_4, 0.5 g; Bacto peptone, 0.5 g; and distilled water to 1,000 ml. A 2% solution of bromothymol blue in alcohol served to indicate pH changes.

RESULTS.–*Pathogenicity*.–The watermelon leaf spot bacterial isolates were pathogenic when suspensions of 10^5 or more cells/ml were sprayed on the lower surface of watermelon leaves. Suspensions of 10^3 or more cells/ml injected into leaves also reproduced symptoms of the disease. Although symptoms were more severe when plants were placed in a moist chamber for 18 hr after inoculation, lesions also developed when they were not placed in a moist chamber. Several isolates produced symptoms similar to those seen in the field.

This bacterium was arginine dihydrolase-negative and oxidase-negative, induced hypersensitivity in tobacco, did not grow at 37 C, and was pathogenic. By these criteria, it fits rather well into the Group IA defined by Misaghi & Grogan (7) and the Group I of Sands et al. (9), which consist of the fluorescent pseudomonad plant pathogens.

Host range.–The watermelon leaf spot bacterium and *P. lachrymans* were most pathogenic on hosts in the Cucurbitaceae family (Table 1). There was a wide range in the degree of susceptibility within the Cucurbitaceae. Cucumber and muskmelon appeared to be highly susceptible to the watermelon leaf spot isolates and to *P. lachrymans*. Pumpkin, squash, watermelon, citron, *Luffa*, and *Melothria pendula* were moderately susceptible to the watermelon leaf spot isolates and only weakly susceptible to *P. lachrymans*, developing smaller and fewer lesions than cucumber or muskmelon. Individual lesions from infection with the watermelon leaf spot bacterium and *P. lachrymans* were usually indistinguishable on cucurbits.

The bacterial isolates were also weakly pathogenic on some members of the Leguminosae family, as well as on tomato and nasturtium. Only a few small lesions developed on common bean after inoculation, but alyceclover and cowpeas were more susceptible. Small brown lesions developed in 2-3 days on inoculated primary and trifoliate leaves of cowpea. The lesions were water-soaked, and had yellow halos. Five days after inoculation, the small lesions (2-3

TABLE 2. Plant species found to be nonhosts of both the watermelon leaf spot pathogen and *Pseudomonas lachrymans*[a]

Species	Cultivar
Chenopodiaceae	
Beet (*Beta vulgaris* L.)	Detroit Dark Red
Swiss Chard (*B. vulgaris* var. *cicla* L.)	Lucullus
Compositae	
Endive (*Cichorium endivia* L.)	Green Curled
Lettuce (*Lactuca sativa* L.)	Iceburg
Marigold (*Tagetes patula* L.)	Dwarf Double French
Sunflower (*Helianthus giganteus* L.)	Giant Gray Stripe
Zinnia (*Zinnia elegans* Jacq.)	Eldorado
Cruciferae	
Cabbage (*Brassica oleracea* var. *capitata* L.)	Late Flat Dutch
Cauliflower (*B. oleracea* var. *botrytis* L.)	Early Snowball
Chinese Cabbage (*B. pekinensis* [Lour.] Rupr.)	Michihli
Mustard (*B. juncea* [L.] Coss.)	Florida Broad Leaf
Radish (*Raphanus sativus* L.)	Scarlet Globe
Rutabaga (*B. napobrassica* Mill.)	Purple Top
Turnip (*B. rapa* L.)	White Globe
Gramineae	
Corn (*Zea mays* L.)	Golden Bantam
Leguminosae	
Garden Pea (*Pisum sativum* L.)	Early Perfection
Malvaceae	
Okra (*Hibiscus esculentus* L.)	Clemson Spineless
Solanaceae	
Eggplant (*Solanum melongena* var. *esculentum* Nees)	Black Beauty
Pepper (*Capsicum annuum* L.)	California Wonder
Petunia (*Petunia hybrida* Vilm.)	

[a]Plants were inoculated with a gentle spray of a 5×10^7 bacterial cells/ml suspension and returned directly to the greenhouse bench.

mm) were white-centered with brown borders. Lesions on alyceclover and nasturtium often enlarged to envelop the entire leaf. Symptoms on tomato developed first as small chlorotic spots, and after 1 week consisted of very small (1-2 mm) brown lesions with yellow halos. Water-soaking was not observed on tomato.

Plants in several families were inoculated and found to be nonhosts of both *P. lachrymans* and the watermelon leaf spot bacterium (Table 2).

Physiological reactions.—The watermelon leaf spot bacterium is a motile rod, with one or more polar flagella. Growth on potato-dextrose agar was heavy, grayish-white, and butyrous. Bacto-nutrient broth became turbid in 24 hr. A green fluorescent pigment was produced in Clara's medium. Litmus milk became alkaline without reduction. Neither hydrogen sulfide nor indole were produced, and nitrate was not reduced to nitrite. The bacterium was catalase-positive and liquefied gelatin. It was not lipolytic, and starch was not hydrolyzed. In Bacto-nutrient broth supplemented with NaCl, good growth occurred at 3% salt in 48 hr, slight growth at 4% after 4 days, and no growth at 5% salt after 10 days.

The watermelon leaf spot bacterium produced acid but no gas from the following compounds: sucrose, glucose, fructose, arabinose, xylose, mannose, galactose, and mannitol. Rhamnose, lactose, maltose, raffinose, dulcitol, glycerol, and salicin were not utilized. The sodium salts of succinic, malonic, and citric acid were utilized, and gave an alkaline reaction in 1 to 3 weeks.

DISCUSSION.—The bacterial isolates from watermelon leaf spot in Florida exhibited characteristics of the genus *Pseudomonas*, and met all the criteria for inclusion in the group of plant-pathogenic fluorescent pseudomonads (7, 9). On the basis of host range and limited physiological and biochemical tests (2), the watermelon bacterial pathogen closely resembles *P. lachrymans*. The watermelon leaf spot bacterium varied from the ICPB isolates of *P. lachrymans* in that it liquefied gelatin. This difference seemed insignificant, as the literature does not agree on this characteristic of *P. lachrymans* (2, 5, 7). In host range tests, the watermelon leaf spot isolates and *P. lachrymans* produced identical symptoms on the various hosts to which they were both pathogenic. This host range was determined in the greenhouse, and may vary somewhat from the natural host range in the field.

The fact that Florida watermelon leaf spot isolates were more pathogenic than the *P. lachrymans* isolates from ICPB on watermelon may indicate that Florida has a strain of the bacterium that is more pathogenic on this particular host. Both the Florida and ICPB isolates were more pathogenic on cucumber than on watermelon.

The susceptibility of certain legumes and tomatoes to the watermelon strain of *P. lachrymans* is unusual. Perhaps this pathogenic reaction on these hosts would only occur in certain environmental conditions. The 16-18 hr of high relative humidity in the greenhouse during this test could have resulted in near optimum conditions for the bacterium. These results indicate that the watermelon strain has the potential to occur on hosts other than Cucurbitaceae.

Further investigation is needed on the epidemiology and control of the bacterial leaf spot disease of watermelon. Its recurrence every year raises the question of whether the bacterium is seed-borne in watermelon or is introduced into watermelon from other wild or cultivated cucurbits each year.

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