Pseudomonas viridiflava: Causal Agent of Bacterial Leaf Blight of Tomato

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ABSTRACT

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A leaf spot disease of tomato was observed in several fields near Bradenton, FL, in the late winter and early spring of 1983. A fluorescent bacterium identified as *Pseudomonas viridiflava* was isolated consistently. In controlled-environment chambers, the disease developed only when plants were water-soaked by misting before and after inoculation or where wounds were inoculated. The bacterium appears to be an opportunistic parasite that attacks plants stressed by unfavorable environmental conditions.

Historically, bacterial spot of tomato incited by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye (XCV) has been the most important bacterial foliar disease of tomato in the southeastern United States. Recently, bacterial speck caused by *Pseudomonas syringae* pv. *tomato* (Okabe) Young et al (PST) and a leaf spot caused by *P. syringae* pv. *syringae* van Hall (PSS) have occurred (7,14). In March 1983, a leaf spot of tomato affecting nearly 100% of the plants was observed in several fields in southwestern Florida. The disease caused extensive tissue necrosis, resulting in losses of as much as 50% of the foliage. On the basis of symptoms, the disease was diagnosed as bacterial spot. The consistent isolation of a fluorescent, oxidase-negative pseudomonad that induced a hypersensitive reaction in tobacco indicated that either PST or PSS was responsible. Results of differential laboratory tests, however, indicated that the physiological and biochemical characteristics of the organism were almost identical to those described for *P. viridiflava* (Burkholder) Dowson (PV) (1,5).

The purpose of this paper is to report the first occurrence of *P. viridiflava* as a foliar pathogen of tomato in the United States.

MATERIALS AND METHODS

Isolation and identification. Lesions from leaves were triturated in drops of sterile distilled water. Loopfuls of the suspension were streaked onto plates of nutrient yeast-dextrose agar (7) and medium B of King et al (KMB) (8); the plates were incubated at 28 C for 48 hr. Representative colonies were transferred to plates of KMB for later characterization.

Isolated cultures were compared physiologically and biochemically with two strains each of PST and PSS from our laboratory and with one strain of PV from J. E. Hunter, New York State Agricultural Experiment Station, Geneva. The following tests were used for characterizing all the strains: Gram reaction (15); production of cytochrome oxidase (7); production of a fluorescent pigment (8); arginine dihydrolase activity (20); tobacco hypersensitivity reaction (9); levan production (10); degradation of sodium polypectate at pH 5.0 and 8.3–8.5 (5); utilization of erythritol, D(−)-tartrate, DL-lactate, sucrose, mannitol, mesotartrate, and sorbitol (13); and ability to cause soft rot of potato (10). Two of the unknown strains and one each of PSS and PST were also compared in the following tests: oxygen relationship, motility in stabs of semisolid agar, starch hydrolysis, nitrate reduction, citrate utilization, casein hydrolysis, gelatin liquefaction, catalase production, ammonia production, urease activity, ice-nucleating ability, aesculin hydrolysis, growth in 5% NaCl, and reaction of litmus milk. Standard methods of testing were used (3,4,7,16,17).

Pathogenicity tests. Cultures for inoculum were grown for 48 hr on KMB at 25 C, and bacterial suspensions were prepared with deionized water and adjusted to 10^8 colony-forming units per milliliter. Four inoculation methods were used for testing pathogenicity: 1) plants sprayed to runoff with inoculum, then enclosed in clear polyethylene bags for 48 hr; 2) plants rubbed with a cotton swab soaked in inoculum, then treated like the sprayed plants; 3) water-congested plants (produced by placing plants in a mist chamber for 6 hr before inoculation) sprayed with inoculum and held in a mist chamber for 36 hr; and 4) water-congested plants (kept in a mist chamber for 12 hr) rubbed with sterile sand bags to provide injury, then sprayed to runoff with inoculum and held for 36 hr in mist. Controls were prepared using distilled water in each test. Plants were held at 20–21 C in controlled-environment chambers after the moisture treatments. They were observed for symptoms daily for 7–10 days after inoculation. Tomato cultivars Walter or Libby 8990 were used in all experiments; they were grown in the greenhouse until they were 15–18 cm tall, at which time they were inoculated. In some trials, a strain of PST was used for comparative purposes.

RESULTS

Characterization of the pathogen. A fluorescent bacterium was isolated consistently from leaf and stem lesions. These isolates induced a weak, hypersensitive reaction in tobacco. The bacterium was aerobic, gram-negative, and negative for oxidase and arginine dihydrolase activity. Results of key determinative tests made with 17 isolates agreed closely with those obtained with a known strain of *P. viridiflava*, except the unknown tomato isolates degraded pectate gel at pH 5.0 (Table 1). These isolates were different from PSS and PST in their ability to rot potatoes and degrade pectate gel at pH 8.3 and in their inability to utilize sucrose. They also differed from PST in their ability to utilize erythritol. When two of the tomato strains and one each of PST and PSS were compared in a battery of tests, all were positive for citrate utilization, aesculin hydrolysis, and catalase. All were negative for nitrate reduction, urease activity, starch hydrolysis, and lipolysis. The two unknown isolates from tomato were weakly positive or negative for motility, whereas the PST and PSS were positive. PST and PSS were negative and weakly positive, respectively, for ammonia production, but the two tomato isolates were positive. The two tomato isolates were positive for growth in 5% sodium chloride, casein hydrolysis, and peptonization of litmus milk, whereas PST and PSS were negative. All strains except PSS liquefied gelatin. PST

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Table 1. Comparison of the tomato isolates with isolates of *Pseudomonas viridiflava, P. syringae* pv. *syringae*, and *P. syringae* pv. *tomato* in differential tests

Tests	Tomato isolates (17)[a]	*P. viridiflava* (1)	*P. syringae* pv. *syringae* (2)	*P. syringae* pv. *tomato* (2)
Potato soft rot	17[b]	1	0	0
Levan	0	0	2	2
Pectate degradation at pH 5.0	17	0	0	2
Pectate degradation at pH 8.3	17	1	0	0
DL-Lactate	17	1	2	2
D(−)-Tartrate	17	1	2	2
Mannitol	17	1	2	2
Mesotartrate	17	ND[c]	2	2
Sucrose	0	0	2	2
Sorbitol	13	1	2	2
Erythritol	17	1	2	0

[a] Number in parentheses is number of isolates tested.
[b] Number of isolates positive for a given test.
[c] Not determined.

was the only strain negative for ice-nucleating ability.

Pathogenicity tests. Results of inoculation tests differed greatly, depending on the method used. Disease was least severe when inocula of the unidentified isolates were applied by spraying leaves or rubbing them with a cotton swab without a preinoculation mist treatment. One isolate produced some foliage blight when the spray or rub treatments were used, but most isolates produced either small, necrotic spots or no reaction. Plants inoculated similarly with PST developed numerous leaf spots with halos. Plants given preinoculation and postinoculation mist treatments to induce and maintain water-soaking showed leaf spotting and necrosis similar to that observed in the field. Plants wounded by rubbing with a sand bag had more severe symptoms than uninjured plants.

DISCUSSION

The bacterium associated with the leaf blight that occurred on tomato in southwestern Florida during early spring of 1983 appears to be *P. viridiflava* on the basis of the determinative schemes of Billing (1) and Hildebrand and Schroth (5). This apparently is the first report of *P. viridiflava* as a foliar pathogen of tomato in the United States. The pathogen was reported to cause a stem rot of tomato in Pennsylvania (12) and it has been associated with foliar lesions on tomatoes grown in other countries (2,21). It is also a foliage, fruit, stem, or root pathogen of several other crops in the United States and elsewhere (6,11, 18,19,21).

Our inoculation results indicate that *P. viridiflava* is a weak parasite of tomato. Water congestion of plant tissues favors disease development. The outbreak of the disease in Florida during 1983 was associated with excessive rainfall during February and March. Rainfall for these months was 25.8 and 21.9 cm, respectively, compared with a previous 29-yr average of 7.8 and 7.5 cm for these months. Other stress factors before and during the epidemic included subnormal temperatures and high winds, which resulted in blowing sand. The disease diminished rapidly with the onset of more favorable growing conditions.

The role attributed to *P. viridiflava* as a plant pathogen has varied with the investigator (1,21). Billing (1) considered the bacterium a weak parasite or a secondary invader that follows entrance by another pathogen. Billing believed that PV is primarily an epiphyte that, under conditions conducive for lesion production, may have pathogenic capability. Wilkie et al (21), however, considered the bacterium to be a pathogen in its own right and listed several hosts. In their test, water-soaking leaves of young tomato plants with PV inoculum followed by a 48-hr mist period at 20 C (conditions somewhat similar to those in our experiments) resulted in disease. Most workers (1,6,11,18) have considered *P. viridiflava* to be an opportunistic pathogen that attacks plants that have been wounded or otherwise stressed. On the basis of our research, *P. viridiflava* should be considered an opportunistic pathogen that incites leaf spot on tomatoes. We believe that if the pathogen were not opportunistic, the disease would have continued to progress, although at a slower rate, once the weather conditions became less favorable (low precipitation).

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