Bacterial Blight of Sweet Onion Caused by *Pseudomonas viridiflava* in Vidalia, Georgia

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ABSTRACT

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A bacterial blight of sweet spanish onions (*Allium cepa*) was observed for the first time in the winter and spring of 1990 and again in 1991 in the Vidalia region of Georgia. A fluorescent bacterium, the colonies of which initially were white but turned yellow with age on King's medium B agar, was isolated consistently from necrotic streaks and oval, water-soaked lesions on upper portions of leaves, rotted areas at the base of leaves, and from one or more discolored inner scales of bulbs. Many strains of the onion pathogen were copper-tolerant. The bacterium was negative for oxidase and arginine dihydrolase, was variable for hypersensitive reaction in tobacco, slowly produced acid from sucrose (7–20 days), rotted carrot and potato, incited rust-colored symptoms on snap bean pods, and was ice nucleation active. It degraded sodium polypectate gel at pH 8.5 but not at pH 5.0. The onion pathogen had a relatively simple fatty acid profile. The key features were the presence of delta-cis-9,10,-methylene hexadecanoic acid and that the ratio of alpha-hydroxy lauric acid to lauric acid was greater than 1. Based on these characteristics, the onion pathogen was identified as *Pseudomonas viridiflava*. Koch's postulates were fulfilled using greenhouse-grown onion plants. Onion strains of the pathogen were also pathogenic on greenhouse-grown snap bean, soybean, and tomato.

In the winter of 1990, a bacterial disease of onion (*Allium cepa* L.) was observed for the first time in the Vidalia region of Georgia. Later in the season and again in 1991, the disease was observed in most onion-growing areas of Georgia. In some locations, the disease was so severe that onions were not harvested. A fluorescent pseudomonad was isolated consistently from all diseased plant parts. Based on differential physiological tests, the characteristics of the onion pathogen were most similar to those of *Pseudomonas viridiflava* (Burkholder) Dowson (1,3,5,9,16). In addition, onion growers and extension personnel indicated that copper compounds did not control the disease. Therefore, strains of *P. viridiflava* were collected from onions from several locations and tested for their sensitivity to copper. The purpose of this paper is to describe the first occurrence of this disease of onion in the field (onion has been reported previously as a host when artificially inoculated) (2) and to report the occurrence of copper-tolerant strains of *P. viridiflava*.

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MATERIALS AND METHODS

Isolation and identification. Portions of diseased onion leaves and bulbs were triturated in 0.1 ml of sterile PBS (0.05 M KH_2PO_4·K_2HPO_4, 0.85% NaCl, pH 7.4), and loopfuls of the suspension were streaked on plates of King's medium B (KMB) agar (14). Plates were incubated for 48 hr at 30 C. Colonies were picked from the plates, transferred to KMB agar, checked for purity, and stored in 15% glycerol at −72 C (6). Representative strains were compared physiologically and biochemically with two strains of *Pseudomonas syringae* pv. *syringae* van Hall (PSS 84-16 and PSS 84-34) from tomato (*Lycopersicon esculentum* Mill.) transplants from Tifton, GA; three strains of *P. viridiflava* (5777-4 from tomato, 5785-2 from tomato, and G 9A from parsnip [*Pastinaca sativa* L.] from J. B. Jones, Bradenton, FL); and one strain of *P. viridiflava* (PV 13222) from the American Type Culture Collection (ATCC), Rockville, MD.

The following tests were used to characterize the strains: Gram reaction (21); production of levan, cytochrome oxidase, soft rot in potato and carrot, arginine dihydrolase, and a hypersensitive reaction in tobacco (6,15,16); degradation of sodium polypectate at pH 5.0 and 8.5 (9); ice nucleation activity (12); utilization of gelatin, D(-)tartrate, DL-lactate, and erythritol (6); response on excised snap bean (*Phaseolus vulgaris* L. 'Bush Blue Lake 274') pods (4); analysis of cellular fatty acids (7,8,19); and production of acid from sucrose (10). All tests were replicated three times.

Additional tests for the production of acid in sucrose (10) were conducted with new glassware, syringes, tubing, and fresh chemicals to eliminate the possibility of chemical residues and/or chemical contamination. In addition, the sucrose medium was prepared with Baker Analyzed HPLC reagent water (J. T. Baker, Inc., Phillipsburg, NJ) and SeaKem genetic technology grade agarose (FMC Marine Colloids Division, Springfield, NJ). Data were recorded every 36–48 hr for 40 days. Tests prepared with the HPLC water and agarose were replicated three times and repeated twice.

Pathogenicity tests. Representative strains originally isolated from diseased onions from Vidalia, GA, were transferred from KMB agar to nutrient broth. Bacteria were harvested from 24-hr-old broth cultures and adjusted to approximately 1×10^8 cfu/ml as previously described (8). A suspension of each strain was atomized with a chromatography sprayer onto leaf surfaces of 6- to 8-wk-old greenhouse-grown onion plants (cv. Granex 33). Controls were treated with sterile deionized water instead of the inoculum suspension. Plants were either predisposed by placing them in a mist chamber for 24 hr before inoculation or left on the greenhouse bench. After inoculation, all plants were maintained in the mist chamber for an additional 24 hr, after which they were moved to greenhouse benches. Temperatures in the greenhouse were 20–25 C.

Plants were also inoculated with sterile toothpicks, the tips of which had been moved through a colony of *P. viridiflava* on KMB agar. The contaminated toothpick was stabbed into the base of the onion leaves immediately above the neck of the bulb. Controls were prepared with sterile toothpicks. With both inoculation methods, inoculated plants were examined after 10–14 days for disease development.

Inoculation tests were performed on leaves of 4- to 6-wk-old tomato cv. FM

6203; snap bean cv. Bush Blue Lake 274; and soybean (*Glycine max* L.) cv. Twiggs dusted with Carborundum. Leaves of dusted plants were rubbed gently with a cotton-tipped applicator that had been soaked in the inoculum suspension prepared as described earlier. Sterile water inoculations were used as controls. Plants were placed in a mist chamber for 24 hr, after which they were removed to greenhouse benches as described earlier.

Copper sensitivity tests. Modified methods of Marco and Stall (18) were used to determine sensitivity of onion strains DD-1, DD-3, R-2, and CT-1 to copper. The diluent was 0.125 M sucrose in deionized water, and the source of copper was cupric sulfate pentahydrate. Cupric sulfate pentahydrate was also added to nutrient agar (50, 100, and 200 $\mu g\ ml^{-1}$) alone or in combination with mancozeb (12.5, 25, and 50 $\mu g\ ml^{-1}$). The control media contained nutrient agar or nutrient agar with 50 $\mu g\ ml^{-1}$ of mancozeb. A suspension of bacteria was prepared in PBS and adjusted to approximately 1×10^8 cfu/ml as previously described (8). The suspensions were diluted serially (1:9) and 0.1-ml aliquots of selected dilutions were spread onto nutrient agar media that contained the various combinations of copper and mancozeb. Strains DD-1, DD-3, R-2, and CT-1 of the onion pathogen were compared with known copper-tolerant (XCV 23 and XCV 96) and copper-sensitive (XCV 13 and XCV 25) strains of *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye. Plates were incubated at 30 C for 48 hr. Colonies were counted and estimates of the original population were made. The test was replicated three times.

RESULTS

Symptoms. Disease symptoms included water-soaked, oval lesions and streaks on leaves and a soft rot of basal areas of leaves above the neck of the bulb. Streaks darkened with age and became nearly black as leaves withered and collapsed. Inner scales of bulbs were discolored (reddish brown) and decayed, however, the rot typically was firmer than that associated with sour skin, slippery skin, or soft rots caused by *Erwinia* spp. The rot tended to be restricted by the scales and develop in a ringlike pattern.

Characterization of the pathogen. In all cases, a white, gram-negative, aerobic, rod-shaped bacterium that produced a fluorescent, yellow-green, water-soluble pigment on KMB was isolated from diseased tissues. Colonies were initially white to cream on KMB but turned yellow after 5–7 days. The onion strains were negative for oxidase and arginine dihydrolase activity. A variable hypersensitive reaction was obtained in tobacco, whereas *P. s. syringae*, used as a positive control, consistently induced a positive reaction. Strains of the onion pathogen and known strains of *P. viridiflava* produced pectinolytic enzymes as demonstrated by their ability to rot potato and carrot slices and to cause pits in a sodium-polypectate gel at pH 8.5 but not at pH 5.0. Strains of *P. s. syringae* failed to pit the sodium-polypectate gels at either pH. The onion strains either did not produce levan or produced levan so weakly that it was questionable. All onion strains tested positive for ice nucleation activity, hydrolyzed gelatin, and utilized D-tartrate, DL-lactate, and erythritol. The onion strains and known strains of *P. viridiflava* caused rust-colored lesions within 48 hr on excised pods of Bush Blue Lake 274, whereas strains of *P. s. syringae* caused brown lesions. None of the onion strains or known strains of *P. viridiflava* produced acid in sucrose within 7 days at 30 C, whereas *P. s. syringae* produced acid in sucrose within 3 days. However, all strains of *P. viridiflava* produced acid in sucrose within 20 days (Table 1). Fatty acid profiles of onion strains were characterized by the presence of delta-cis-9,10,-methylene hexadecanoic acid (C-9,10 17:0) and the ratio of alpha-hydroxy lauric acid (2-OH 12:0) to lauric acid (12:0) was greater than 1 (Table 2). In contrast, the ratio of 2-OH 12:0 to 12:0 was less than 1 in fatty acid profiles of *P. s. syringae*.

Pathogenicity tests. Plants predisposed by mist treatments or inoculated by the toothpick method displayed foliar leaf spots, streaks, and soft rot of basal areas similar to those observed in the field. Plants not subjected to a preinoculation mist treatment had less severe symptoms or no symptoms. Less than 10% of the bulbs had internal symptoms after 4 wk with onion plants inoculated in the greenhouse. Leaf spot symptoms also occurred on greenhouse-grown snap bean, soybean, and tomato.

Copper-sensitivity tests. Cells of strains DD-1 and DD-3 of the onion pathogen were not killed after a 3-hr exposure to sucrose solutions containing copper at 32 $\mu g\ ml^{-1}$, whereas strains R-2 and CT-1 died after an exposure to a solution containing copper at 0.06 $\mu g\ ml^{-1}$. Similar results were obtained from assays conducted on agar media containing either copper or copper with mancozeb (Table 3). Strains DD-1 and DD-3 grew on nutrient agar in the

Table 1. Production of acid from sucrose[a] by strains of *Pseudomonas syringae* pv. *syringae*, *P. viridiflava*, and strains isolated from Vidalia onions

	Days of incubation at 30 C							
Strain	**3**	**7**	**10**	**14**	**17**	**20**	**24**	**40**
P. s. syringae[b]	+[c]	+	+	+	+	+	+	+
P. viridiflava								
ATCC 13222	–	–	–	(+)[d]	+	+	+	+
PV G 9A	–	–	–	+	+	+	+	+
PV 5777-4	–	–	–	(+)	+	+	+	+
PV 5785-2	–	–	–	+	+	+	+	+
Onion strains								
R-2	–	–	(+)	+	+	+	+	+
CT-1	–	–	–	(+)	+	+	+	+
DD-1	–	–	–	–	–	(+)	+	+
DD-3	–	–	–	–	–	(+)	+	+
Control	–	–	–	–	–	–	–	–

[a] According to Hugh and Leifson (9), with HPLC water, SeaKem agarose, newly opened sucrose, peptone, sodium chloride, and dibasic potassium phosphate; the medium was prepared in new glassware.
[b] Data from *P. s. syringae* represents four strains.
[c] All values are representative of three replications.
[d] (+) = Weak reaction.

Table 2. Values (percentages of total fatty acid methyl esters [FAMES]) for strains isolated from onions in Georgia (PV-Onion) contrasted with values for *Pseudomonas syringae* pv. *syringae* (PSS), known strains of *P. viridiflava* (PV), and *Xanthomonas campestris* pv. *vesicatoria* (XCV)[a]

	Relative % FAMES present			
FAMES	**PSS[b]**	**PV**	**PV-O**	**XCV**
12:0	1.19	3.79	3.80	0.00
2OH-12:0	1.55	0.69	0.85	0.00
14:0	0.88	0.54	0.37	0.80
c16:1^9	41.41	41.11	40.43	18.60
16:0	37.49	35.18	31.82	1.90
c-9,10 17:0	4.88	3.36	2.80	0.00
t18:1^9	12.07	14.80	19.21	0.00
18:0	0.53	0.54	0.74	0.00

[a] A nonfluorescent bacterium used for comparison.
[b] Values represent the mean of four strains each of PSS, PV, and PV-O and of a single strain of XCV.

Table 3. Growth of strains (cfu/ml) of *Pseudomonas viridiflava* from onion in Georgia on nutrient agar supplemented with copper compared with known copper-tolerant and copper-sensitive strains of *Xanthomonas campestris* pv. *vesicatoria*

	Growth on agar							
	Copper/mancozeb ($\mu g\ ml^{-1}$)							
Strain	**0:0**	**12:0**	**25:0**	**50:0**	**12:12**	**25:25**	**50:50**	**0:50**
P. viridiflava								
DD-1	8.4[a]	8.3	8.2	8.1	0.0	0.0	0.0	7.9
DD-3	8.4	8.3	8.3	8.2	0.0	0.0	0.0	8.3
R-2	8.5	8.4	7.9	0.0	0.0	0.0	0.0	7.8
CT-1	7.8	7.3	7.0	0.0	0.0	0.0	0.0	6.8
X. c. vesicatoria								
XCV 23	8.3	8.6	8.5	8.4	0.0	0.0	0.0	8.0
XCV 96	8.5	8.6	8.5	8.4	0.0	0.0	0.0	8.2
XCV 25	8.7	6.9	0.0	0.0	0.0	0.0	0.0	8.1
XCV 13	8.7	0.0	0.0	0.0	0.0	0.0	0.0	8.6

[a] Values are log values and represent the mean of three replications.

presence of copper at 50 $\mu g\ ml^{-1}$ but failed to grow in the presence of copper and mancozeb when each were at 12.5 $\mu g\ ml^{-1}$.

DISCUSSION

Only two fluorescent pseudomonads exhibit the characteristics of being negative for both oxidase and arginine dihydrolase, i.e., *P. s. syringae* and *P. viridiflava*. The bacterial strains from onion were most similar to known strains of *P. viridiflava* on the basis of their ability to rot potato and carrot slices (1), to pit a sodium-polypectate gel at pH 8.5 (9), utilize D-tartrate, produce rust-colored lesions on excised snap bean pods (4), and develop a yellow color upon prolonged growth on KMB (3). In addition, the fatty acid profiles of the onion strains were more similar to *P. viridiflava* than to *P. s. syringae*.

In contrast with most reports (1,2,3,11,13,17), in our tests, the onion strains and known strains of *P. viridiflava* slowly produced acid from sucrose (within 10–17 days). Known strains of *P. s. syringae* produced acid from sucrose within 3 days. Clara (5), Suslow and McCain (20), and Wilkie et al (22) isolated strains of *P. viridiflava* that use sucrose. In our tests, stringent conditions, including the use of HPLC water, new glassware and chemicals, and agarose were employed to determine that the slow production of acid was not attributable to a chemical contaminant. Furthermore, recovery of bacteria from the sucrose tubes once acid was indicated always resulted in a pure culture of *P. viridiflava*, thus reducing the possibility of a microbial contaminant.

Previous reports (1,11,13,17,20) referred to *P. viridiflava* as an opportunistic or weak pathogen. Results from our greenhouse inoculations support the view that *P. viridiflava* is opportunistic because preinoculation mists and wounds were required as predisposing factors to obtain typical symptoms. But, the disease was severe and widespread in onion-growing areas of Georgia, evidence that onions in the field were predisposed by environmental conditions, wounds, or other unknown factors.

Results from lab tests confirmed the presence of copper-tolerant strains and provide an explanation of anecdotal reports that copper bactericides do not control the disease in the field. Results from lab tests also confirmed that copper-tolerant strains of *P. viridiflava* are sensitive to mixtures of copper and mancozeb as previously reported for *X. c. vesicatoria* (18). The source of inoculum for this disease in Georgia is still unknown. Numerous hosts (1,2,22) including parsnip (11), tomato (13), alfalfa (17), poinsettia (20), and soybean (3,5), have been reported susceptible to *P. viridiflava*. Although soybean is commonly used in rotation with onion in Georgia, the role that soybean or any other potential host plays in bacterial disease epidemics in onion remains to be determined.

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