Pectolytic Xanthomonads in Mixed Infections with *Pseudomonas syringae* pv. *syringae*, *P. syringae* pv. *tomato*, and *Xanthomonas campestris* pv. *vesicatoria* in Tomato and Pepper Transplants

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ABSTRACT

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Gram-negative, yellow, aerobic, cellulolytic, rod-shaped bacteria were originally isolated in mixed culture along with other phytopathogenic bacteria from tomato and pepper transplants, or in pure culture from pecan and weeds. Based on fatty acid composition, presence of xanthomonadin pigment, and standard bacteriological characteristics of the bacteria, the unknown strains were identified as xanthomonads. However, all strains differed from endemic strains of *Xanthomonas campestris* pv. *vesicatoria* because they typically displayed strong starch hydrolysis, pectolytic activity on crystal violet pectate medium and did not elicit a hypersensitive response in tobacco. When atomized onto tomato and pepper foliage, the pectolytic xanthomonads failed to induce disease; however, they produced restricted necrotic areas in association with wounds produced by the Carborundum-rub method of inoculation. In addition, soft-rot symptoms were induced in 82% of tomato fruits and 38% of pepper fruits when 20 μl of a dilute inoculum suspension (5×10^3 cfu per milliliter) was placed in wounds. Approximately 20% of the test strains were lysed by bacteriophages that were originally developed for *X. campestris* pv. *campestris*. Fatty acid profiles of the pectolytic xanthomonads had a high similarity to known profiles of *X. campestris* pv. *raphani*. Except for two strains from pecan that caused black rot, the test strains only produced localized necrosis in association with wounds when inoculated onto cabbage and radish.

Southern transplants account for a major proportion of the tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicum annuum* L.) plants grown for the processing industry in the northeastern United States and Canada. Because southern-grown plants may introduce pathogens that may cause disease problems in northern production areas, a stringent certification program is conducted to minimize the shipment of diseased plants. Accurate diagnosis of disease problems is required because a disease such as syringae leaf spot, caused by *Pseudomonas syringae* pv. *syringae* van Hall, is economically unimportant and is not regulated, whereas plants with bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye or bacterial speck, caused by *P. s.* pv. *tomato* (Okabe) Young, Dye, and Wilkie, are placed under quarantine (8,10,11).

Approximately 10% of the isolations from foliar spots of tomato and pepper during 1981–1985 yielded a mixture of one of the above tomato pathogens with a xanthomonad (8). In greenhouse pathogenicity tests, none of the xanthomonad strains produced typical bacterial spot symptoms on susceptible tomato plants.

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Furthermore, unlike most of the strains of *X. c.* pv. *vesicatoria* endemic to Georgia and Florida, the avirulent xanthomonads were positive for starch hydrolysis. Considering the economic importance of accurate identification of bacterial flora on transplants, this study was initiated to characterize xanthomonad strains from tomato and pepper transplants. In addition, several previously unidentified xanthomonads recovered as epiphytes from various symptomless weeds were included in this study for analysis. The importance of these xanthomonad strains as potential disease-inciting agents and the role that they play in the Georgia and Florida plant-certification programs were evaluated.

MATERIALS AND METHODS

Bacterial strain. Seventeen test strains isolated from tomato and pepper (T1-T16 and F1), 17 strains of *X. c.* pv. *vesicatoria* (XCV1-XCV17), 16 strains of *X. c.* pv. *campestris* (Pammel) Dowson (XCC1-XCC16), nine test strains from weeds (*Erigeron canadensis* L., *Linaria vulgaris* Hill, *Physalis heterophylla* Nees, and *Solanum nigrum* L.) (T17-T22 and F2-F4), eight strains of *X. c.* pv. *vignicola* (Burkholder) Dye (XVG1-XVG8), three strains of *X. c.* pv. *raphani* (White) Dye (XCRl-XCR3), two strains of *X. c.* pv. *malvacearum* (Smith) Dye (XCM1-XCM2), two test strains from pecan (*Carya illinoensis*) (Wang.) K. Koch (P1-P2), one strain of *X. c.* pv. *pruni* (Smith) Dye (XPR1), and one strain of *Clavibacter michiganense* (Smith) Davis (CM1) for a total of 76 bacterial strains were used in various tests. All strains except CM1 were collected from their respective hosts in Georgia and Florida and were maintained in 2 ml of sterile tap water in screw cap vials at room temperature and in 15% glycerol at −73 C. Strain CM1 of *C. michiganense* was provided by D. Emmatty (Heinz USA, Bowling Green, OH) and stored in a similar manner.

Morphological characters. All strains were tested for Gram reaction by the nonstaining (KOH) method (20). Flagellation was determined by the method of Mayfield and Inniss (15). Cell morphology was determined from cultures grown on nutrient agar. Culture and colony comparisons were made on nutrient agar.

Physiological and biochemical reactions. Selected tests specific for *Xanthomonas* spp. (4,5) and routine bacteriological tests (6) were used to characterize the bacterial strains. Cellulolytic activity was determined on a carboxymethylcellulose medium (CMC) (8). Pectolytic activity was determined on crystal violet pectate medium (CVP) (3). Aesculin, casein, gelatin, and starch hydrolysis; catalase, oxidase, and urease reactions; nitrate reduction; methyl red test and production of acetoin; production of indole; and litmus milk reactions were performed by standard bacteriological techniques (6).

Tobacco hypersensitivity. Intercostal areas of mature leaves of greenhouse grown tobacco (*Nicotiana tabacum* L. 'Samsun') were infiltrated with an aqueous suspension ($\cong 10^8$ cfu per milliliter) of each bacterial strain by injection with a hypodermic needle (27 gauge) and syringe. Plants were incubated for 24 hr at 25–30 C and results were recorded at that time. All tests had three replications and were repeated twice.

Xanthomonadin pigment production. Methanolic extraction of bacterial pigments was done according to the methods of Irey and Stall (9). Xanthomonadin pigments were detected by thin-layer chromatography and identified by Rf value.

Fatty acid composition. Previously reported methods for analysis of cellular fatty acids were used (14,16,17). Bacteria were grown on trypticase soy agar at 28 C for 48 hr. A loopful of bacteria was added to 1 ml of 1.2 N NaOH in 50% aqueous methanol in a screw cap tube and saponified for 30 min at 100 C. After cooling, samples were acidified with 0.5 ml of 6 M HCl (final pH 2). Samples were methylated with 1 ml of 12% BCl_3 reagent and incubated in a water bath at 85 C for 5 min. After cooling, fatty acid methyl esters were extracted with 1 ml of a hexane and diethyl ether mixture (1:1). After gentle mixing (3 min), the lower aqueous phase was removed. Samples were washed with 3 ml of 0.3 M NaOH and agitated (end-over-end five times). The upper organic phase was removed for analysis. Fatty acid methyl esters were analyzed by gas chromatography with the Microbial Identification System (HP5898A) (Hewlett-Packard, Palo Alto, CA). Eighteen of the test strains, five strains of *X. c.* pv. *vesicatoria* and one strain each of *X. c.* pv. *campestris*, pv. *pruni*, pv. *vignicola* and *C. michiganense* were compared with the HP5898A library.

Bacteriophage typing. Bacteriophages P1 and P8 were isolated in our laboratory from diseased cabbage leaves infected with *X. c.* pv. *campestris*. Known strains of *X. c.* pv. *campestris* were used in a nutrient broth-$CaCO_3$-cabbage leaf enrichment (1). Enrichments were incubated at room temperature for 48 hr. After removal of cabbage leaves, broth suspensions were centrifuged 20 min at 2,000 *g* to remove bacterial cells. Supernatants were then filter-sterilized (0.22 μm). The supernatants were spotted against the original strains used in the enrichment. Bacteriophages were recovered from resulting plaques and were purified by three successive single-plaque isolations. Bacteriophages HXX, OH_2, HT_1 were provided by A. Alvarez, University of Hawaii, Honolulu. All bacteriophages were stored in 5 ml of tap water at room temperature and in 15% glycerol at −73 C.

Bacteriophage typing was performed on test strains seeded onto nutrient agar by the standard double-agar layer method. A 10-μl drop of bacteriophage suspension (titres of 10^{10}–10^{12} plaque-forming units per milliliter) was spotted onto the resulting bacterial lawns. Lytic responses were determined after a 24-hr incubation at 28 C. Purity of bacteriophage suspensions was periodically checked on the appropriate propagating hosts.

Pathogenicity tests. All test strains and representative strains of *X. c.* pv. *vesicatoria*, *X. c.* pv. *campestris*, and *X. c.* pv. *raphani* were tested for pathogenicity on tomato (FM6203), pepper (Early Calwonder), cabbage (*Brassica oleracea* var. *capitata* L. 'Rio Verde'), and radish (*Raphanus sativus* L. 'Champion'). Suspensions containing approximately 10^8 cfu per milliliter were used for all inoculations except where indicated otherwise. A suspension of each strain was atomized with a chromatography sprayer onto leaf surfaces of 6–8-wk-old plants. Plants were predisposed by placing them in a mist chamber for 24 hr before inoculation. After inoculation, plants were replaced in the mist chamber for 24 hr, after which they were moved to greenhouse benches. All inoculation tests were replicated three times.

A second inoculation test was performed on plants that were dusted with Carborundum. Leaves of dusted plants were rubbed with a cotton-tipped applicator that had been soaked in the inoculum suspension. In both the atomized and Carborundum-rub tests, inoculated plants were examined after 10–14 days for disease development. All tests were replicated three times.

Tomato and pepper fruit were obtained from a retail grocery and used for soft-rot pathogenicity tests. Three wounds were made in each fruit with a sterile dissecting needle. Bacterial suspensions of three selected test strains at 5×10^3 cfu per milliliter were spotted onto the wound sites (20 μl per wound). Fruit were incubated at 30 C for 72 hr. There were five replicates and three wound sites for each bacterial strain that was tested. Sterile water inoculations were used as controls. The test was repeated a second time.

Populations of selected test strains after inoculation into tomato and cabbage leaves were determined by the dilution plate method. Inoculum was prepared from 24-hr nutrient broth cultures. Bacteria were harvested by centrifugation and pellets were resuspended in sterile water. Inoculum density was adjusted photometrically until populations were approximately 5×10^7 cfu per milliliter. Bacterial suspensions were infiltrated into the intercellular spaces of tomato and cabbage leaves with a hypodermic needle and syringe. Plants were maintained in the greenhouse for 5 days, at which time 1.0-cm disks were cut from inoculated areas. Disks were macerated in 1.0 ml of buffered saline (0.85% NaCl and 0.01 M K_2HPO_4-KH_2PO_4, pH 7.0) and serially diluted (1:9). Aliquots of 0.1 ml were spotted and spread on nutrient agar plates. Characteristic xanthomonad colonies were counted after a 3-day incubation at 28 C. All tests were replicated three times.

RESULTS

Morphological characters. All test strains were gram-negative based on the KOH method, and all were motile by means of monotrichous flagellation. Cell morphology was rod-shaped and no endospores were observed microscopically under phase-contrast. All strains produced yellowish growth and copious slime when grown on nutrient agar supplemented with 1% glucose.

Physiological and biochemical reactions. All test strains could not be differentiated from *X. campestris* by standard physiological and biochemical tests (Table 1). Unlike *X. c.* pv. *vesicatoria*, all test strains were pectolytic when grown on CVP. In addition, all *X. c.* pv. *vesicatoria* strains were negative for starch hydrolysis.

Tobacco hypersensitivity. Strains of *X. c.* pv. *campestris* were variable in their ability to induce a hypersensitive response in tobacco. All strains of *X. c.* pv. *vesicatoria*, pv. *vignicola*, pv. *malvacearum*, and pv. *pruni* induced a hypersensitive response in tobacco after 24 hr. Twenty-two out of 28 test strains did not elicit a hypersensitive response in tobacco. Strains T1, T6, T10, T12, P1, and P2 induced a hypersensitive response.

Xanthomonadin pigment production. All test strains produced a yellow pigment with an Rf value of 0.36–0.49. All but two strains (T4 and T12) had a second spot on the thin-layer silica gel plates with an Rf value of 0.59–0.75. Reference strains of *X. c.* pv. *vesicatoria* had pigments with Rf values of 0.41–0.49 and 0.68–0.70.

Fatty acid composition. Fatty acid composition for the pectolytic test strains were similar to known pathovars of *X. campestris* (Table 2). The test strains were identified as *Xanthomonas* based on the presence and percentage of C11:0 *iso*, C11:0 *iso* 30H, C15:0 *iso* and C15:0 *anteiso*. The ratio of C15:0 *iso* to C15:0 *anteiso* in all test strains was greater than one but less than 10, as for known strains of *X. campestris*. Identification to pathovar was more tentative when ratios of selected peaks were

TABLE 1. Physiological and biochemical characteristics of strains of opportunistic xanthomonads from tomato and pepper compared with strains of *Xanthomonas campestris* pv. *vesicatoria*

Characteristic	Reaction	
	Test Strains	*X. c.* pv. *vesicatoria*
Number of strains tested	28	17
Yellow mucoid colonies on nutrient agar with glucose	+[a]	+
Water-insoluble yellow pigment	+	+
Gram-negative rods	+	+
Monotrichous flagellation	+	+
Utilization of glucose:		
oxidative	+	+
fermentative	–	–
Utilization of asparagine	–	–
Catalase reaction	+	+
Oxidase reaction	–	–
Starch hydrolysis	+	–
Aesculin hydrolysis	+	+
Gelatin hydrolysis	+	+
Casein hydrolysis	+	+
Methyl red reaction	–	–
Acetoin production	–	–
Nitrate to Nitrite	–	–
Urease reaction	–	–
Indole production	–	–
Litmus milk reaction	Proteolytic[b]	Proteolytic
Cellulolytic (CMC)	+	+
Pectolytic (CVP)	+	–

[a] + = positive reaction and – = negative reaction within 7 days incubation at 28 C.

[b] After 14 days, incubation at 28 C.

TABLE 2. Fatty acid composition of unknown pectolytic xanthomonads from tomato and pepper transplants and weeds compared with fatty acid composition of *Clavibacter michiganense* and several known pathovars of *Xanthomonas campestris*

Strain	No. tested	C11:2 *iso*	C11:0 *iso* 30H	C12:0 30H	C13:0 *iso* 30H	C14:0	C15:0 *iso*	C15:0 *anteiso*	C15:0	C16:0 *iso*	C16:1 B&C	C16:0	C17:1 C9	C17:0 *iso*	C17:0 *anteiso*
Clavibacter michiganense	(1)	0	0	0	0	0	0.9	65.8	0.6	5.1	0	7.4	0	0	16.7
Xanthomonas campestris															
pv. *campestris*	(1)	4.5	3.1	2.5	4.6	0.7	29.8	16.5	1.2	3.8	11.7	3.5	0.8	6.1	0.7
pv. *vesicatoria*	(5)	4.6[a]	2.1	2.5	4.6	0.8	24.3	15.1	0.8	1.9	18.6	3.5	0.7	7.1	1.2
pv. *pruni* (1)		3.8	1.9	3.3	2.4	1.5	27.1	20.9	4.5	2.1	15.5	4.8	1.8	3.8	1.0
pv. *vignicola*	(1)	3.4	1.5	2.5	3.2	1.5	20.8	16.1	1.2	3.5	20.3	7.3	1.0	6.2	1.0
Pectolytic test strains from:															
tomato	(9)	4.3[a]	2.3	3.0	3.4	1.1	28.6	14.7	2.3	2.5	14.5	5.2	2.1	5.8	0.6
pepper	(3)	4.2[a]	2.7	3.3	3.6	1.3	32.2	15.5	2.0	2.5	13.5	4.8	1.4	5.8	0.6
weeds	(6)	5.3[a]	3.9	3.6	5.4	1.1	28.5	15.6	1.7	2.3	12.3	4.0	1.6	4.9	0.5

Fatty acids (%) for all columns after "No. tested".

[a] Mean of number of strains tested.

TABLE 3. Similarity of fatty acid composition of pectolytic xanthomonads to known bacterial strains in the Hewlett-Packard HP5898A Microbial Identification System library

Test strains	No. tested	Similarity to known *Xanthomonas campestris* pathovars as most likely matches						
		pv. *campestris*	pv. *citri*	pv. *manihotis*	pv. *pruni*	pv. *raphani*	pv. *vesicatoria*	pv. *vignicola*
X. campestris								
pv. *campestris*	(1)	0.57[a]	0.00	0.00	0.00	0.52	0.00	0.00
pv. *vesicatoria*	(5)	0.07[b]	0.00	0.49	0.00	0.00	0.38	0.00
pv. *pruni*	(1)	0.00	0.00	0.00	0.19	0.00	0.00	0.00
pv. *vignicola*	(1)	0.00	0.00	0.53	0.00	0.00	0.00	0.40
Pectolytic strains from:								
tomato	(9)	0.14[b]	0.08	0.00	0.19	0.56	0.00	0.00
pepper	(3)	0.44[b]	0.00	0.00	0.00	0.64	0.00	0.00
weeds	(6)	0.04[b]	0.09	0.00	0.00	0.26	0.00	0.00

[a] Similarity values 0.00 = no match, 1.0 = 100% match.

[b] Mean of number of strains tested.

compared to a library of known profiles in the HP5898A Microbial Identification System. Pectolytic test strains displayed a similarity to *X. c.* pv. *campestris* and *X. c.* pv. *raphani* and showed no relationship to *X. c.* pv. *vesicatoria* despite having their origin from tomato and pepper (Table 3). Known strains of *X. c.* pv. *vesicatoria* were similar to the library strains of *X. c.* pv. *vesicatoria* as well as to the library strains of *X. c.* pv. *manihotis*. All of the pectolytic test strains were unrelated to *X. c.* pv. *manihotis*. The known strains of *X. c.* pv. *campestris*, pv. *pruni*, and pv. *vignicola* were identified to most likely pathovar level by their fatty acid profiles (Table 3).

Bacteriophage typing. The five bacteriophages were fairly specific for *X. c.* pv. *campestris* (Table 4). However, no bacteriophage was capable of lysing all *X. c.* pv. *campestris* stains. Also, no bacterial strain was sensitive to all bacteriophages. Several of the pectolytic test strains (P1, P2, T1, T6, T10, and F1) were sensitive to bacteriophages isolated from a *X. c.* pv. *campestris* enrichment (Table 4).

Pathogenicity tests. All reference strains of *X. c.* pv. *vesicatoria* produced typical bacterial spot symptoms on FM6203 tomato plants by both atomized and Carborundum-rub methods of inoculation. Three reference strains of *X. c.* pv. *raphani* produced typical leaf spot symptoms when atomized onto cabbage and radish. All but two of the 28 test strains produced no symptoms on tomato, pepper, cabbage, or radish when plants were inoculated by the atomization method. The two test strains, P1 and P2, that did produce symptoms caused typical black rot symptoms on cabbage and radish. The remaining 26 test strains produced mild necrotic flecks in all test plants but only in association with wounds produced by the Carborundum-rub method of inoculation. These areas remained restricted and were apparently devoid of further colonization. After incubation for 5 days, populations of selected test strains that were infiltrated into intercellular spaces of leaf tissues were approximately a 100-fold and significantly ($P = 0.01$) lower than populations of *X. c.* pv. *vesicatoria* and *X. c.* pv. *campestris* in tomato and cabbage, respectively (Table 5).

Thirty-seven of 45 inoculation sites on tomato fruit developed soft-rot symptoms when spotted with 20 μl of a suspension of three selected test strains that were at a concentration of 5×10^3 cfu per milliliter. Only 10 out of 45 sites on pepper fruit developed soft rot when spotted with the same strains. Strain T10, although capable of rotting tomato fruit, did not affect pepper fruit other than accelerating senescence. All sterile water control inoculations remained negative for soft rot development.

TABLE 4. Lytic reactions of various pathovars of *Xanthomonas campestris* and unknown opportunistic xanthomonads from weeds and tomato and pepper transplants when incubated with bacteriophages

Bacterial strain	Strains (no.)	Bacteriophages 1	OH2	HT7	HXX	8
X. c. pv. *campestris*:						
Type I	(2)	+	+	+	−	−
Type II	(1)	+	+	−	−	−
Type III	(4)	+	−	−	−	−
Type IV	(2)	−	−	+	−	−
Type V	(3)	−	−	−	−	+
Type VI	(2)	−	−	−	+	−
Type VII	(2)	−	−	−	−	−
X. c. pv. *pruni*	(1)	−	−	−	−	−
X. c. pv. *vignicola*	(8)	−	−	−	−	−
X. c. pv. *malvacearum*	(2)	−	−	−	−	−
X. c. pv. *vesicatoria*	(17)	−	−	−	−	−
Pectolytic Test Strains:[a]						
P1	(1)	−	−	−	−	+
P2	(1)	−	−	−	−	+
T1	(1)	+	−	−	−	−
T6	(1)	−	−	−	+	−
T10	(1)	+	−	−	−	−
F1	(1)	−	−	−	+	−
Remainder	(22)	−	−	−	−	−

[a] Twenty-eight strains tested, 22 of which were not lysed by any bacteriophage with the six positive strains listed.

DISCUSSION

Strains of *Xanthomonas* isolated from tomato and pepper transplants and several weeds were differentiated from *X. c.* pv. *vesicatoria* by their pectolytic activity, hydrolysis of starch, general inability to elicit a hypersensitive response, and fatty acid profiles. Further evidence that the pectolytic xanthomonads were different from *X. c.* pv. *vesicatoria* was their lack of virulence when atomized on tomato and pepper foliage. In addition, multiplication patterns of the test strains differed from those of *X. c.* pv. *vesicatoria* when infiltrated into tomato leaves. In each instance, populations of bacteria increased after inoculation irrespective of the strain. However, the concentration of bacteria did not increase as much for the pectolytic strains as for reference strains of *X. c.* pv. *vesicatoria* (Table 5). This disparity was similar to differences in population trends documented for the infiltration of *X. c.* pv. *vesicatoria* into resistant and susceptible tissues (18). Consequently, the pectolytic strains multiplied in tomato tissues in a pattern normally associated with resistant varieties or a nonhost. Two of the strains (P1 and P2) were able to induce black rot and were lysed by bacteriophages developed for *X. c.* pv. *campestris*. The remaining test strains could not be easily separated from *X. c.* pv. *campestris* but they lacked virulence on cabbage. Population trends of test strains in cabbage compared with multiplication patterns of *X. c.* pv. *campestris* were similar to what occurred in tomato. Highest populations were attained by the homologous pathogen, whereas populations of the pectolytic strains were significantly lower (Table 5).

Fatty acid composition was most useful for the identification of the strains to the species level and was of some use for identification to pathovar. The HP5898A library contains data on numerous strains of many organisms. Consequently, when a similarity index is calculated, it is based on the comparison of the test strain to a composite of profiles in the library. Therefore, it would be unlikely that a theoretical match of a similarity index value of 1.0 would actually be obtained. The data presented here were the three highest similarity index values matched to the particular strains tested. In general, there was an excellent match of the reference strains that were tested. Fatty acid profiles developed for the pectolytic strains, regardless of origin (tomato, pepper, symptomless weeds), had a greater similarity to *X. c.* pv. *raphani* than any other xanthomonad. In all cases, the similarity of test strains with *X. c.* pv. *vesicatoria* was less than for *X. c.* pv. *raphani*

TABLE 5. Populations of *Xanthomonas campestris* pv. *campestris* and pv. *vesicatoria* compared with populations of pectolytic and opportunistic xanthomonads in cabbage and tomato leaves

Bacterial strain[x]	Populations Log 10 cfu/ml/1.0-cm-diam. disk[y] Tomato	Cabbage
X. c. pv. *vesicatoria*:		
XCV 1	9.42 a[yz]	7.09 b
XCV 2	8.87 b	6.49 c
XCV 3	8.85 b	5.78 d
X. c. pv. *campestris*:		
XCC 1	8.08 c	8.20 a
XCC 2	8.45 bc	8.31 a
Pectolytic test strains:		
T1	7.16 d	7.19 b
T4	7.13 d	6.51 c
T6	7.15 d	6.36 c
T7	7.21 d	6.45 c
T8	7.10 d	6.46 c
T10	7.37 d	7.29 b
F1	7.08 d	6.36 c

[x] Initial bacterial suspension of 5×10^7 cfu per milliliter was infiltrated into the intercellular spaces of intercostal areas, which gave on the average 5×10^5 cfu 1.0-cm disk on day one.

[y] Mean population of three replicates after incubation for 5 days.

[z] Values within a column followed by the same letter are not significantly different by Duncan/Waller k-ratio test.

or *X. c.* pv. *campestris*.

Bacteriophage typing schemes using highly specific bacteriophages have been used to detect and identify bacterial pathogens (2,7,13). Although developed for other reasons, the bacteriophages used in this test were of some value. All were highly specific for *X. c.* pv. *campestris* and none of the 17 strains of *X. c.* pv. *vesicatoria* or other reference strains of *Xanthomonas* were sensitive to the bacteriophages. Lysis of approximately 20% of the pectolytic test strains would indicate that they are more closely related to *X. c.* pv. *campestris* than to *X. c.* pv. *vesicatoria*.

These data present a problem in classifying these pectolytic xanthomonads under the current concept of the genus *Xanthomonas* (19). On the basis of physiological, biochemical, and fatty acid tests, the pectolytic strains should be classified as *X. campestris*. Beyond that, the pathovar system classifies organisms based on host specificity (5). The pectolytic xanthomonads are opportunistic; they are found in nature in association with other pathogens such as *P. syringae* or as epiphytes on weeds. Based on fatty acid analysis, the pectolytic test strains may be more related to *X. c.* pv. *campestris* and *X. c.* pv. *raphani* than to *X. c.* pv. *vesicatoria*. *X. c.* pv. *raphani* has an extensive host range that includes tomato and pepper (21). However, the test strains, unlike *X. c.* pv. *raphani*, were unable to infect tomato, pepper, or radish except for a localized necrosis associated with wounds. It is possible that these strains are a nonparasitic and an atypical form of *X. c.* pv. *raphani* that survives epiphytically or as an opportunistic pathogen. Other atypical xanthomonads have been reported to be saprophytes in nature in apple buds, causing damage only to explants in tissue culture (14). Xanthomonads have also been isolated from soft rots of vegetables (12). It is possible that the pectolytic strains survive epiphytically without causing foliar disease problems and then colonize and rot the fruit.

The association of pectolytic, opportunistic xanthomonads with tomato and pepper transplants creates a dilemma for the Georgia and Florida plant certification programs. In the past, these xanthomonads isolated from tomato and pepper were probably confused with *X. c.* pv. *vesicatoria* and resulted in loss of certification. The inoculation studies reported in this paper demonstrate their relative lack of pathogenicity on tomato and pepper. The avirulent xanthomonads capacity for inducing soft rot on the tomato and pepper fruits may affect fresh market produce (12), but this probably is of less importance to the processing industry. Until additional studies demonstrate otherwise, it is our belief that the presence of pectolytic xanthomonads found on tomato and pepper transplants should not be considered as reasons for loss of plant certification.

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