Two Genetically Diverse Groups of Strains Are Included in *Xanthomonas campestris* pv. *vesicatoria*†

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Two genetically diverse groups of strains were identified among cultures of *Xanthomonas campestris* pv. *vesicatoria* isolated from plants with bacterial spot of pepper and tomato. Group A strains do not pit pectate gels or hydrolyze starch, whereas group B strains are strongly positive for these reactions. Group A strains cause a hypersensitive reaction in plants of tomato breeding line Hawaii 7998, but group B strains do not. Other differences between the two groups of strains were discovered in tests for utilization of carbon compounds, serology, fatty acid profiles, silver-stained protein bands on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels, and DNA restriction enzyme digestion profiles. The levels of DNA homology between strains belonging to the same group were more than 74%, but the levels of DNA homology between strains belonging to different groups were less than 46%. The two groups of strains have different genetic backgrounds, but cause essentially the same disease of tomato and pepper.

The bacterial spot disease of tomato and pepper is caused by the bacterium *Xanthomonas campestris* pv. *vesicatoria*. Necrotic spots on leaves, stems, peduncles, and fruits are distinctive symptoms of the disease (5, 17). Lesions may enlarge or coalesce and cause leaves to become chlorotic and eventually die. When prevalent, lesions on fruits result in loss of marketability. Bacterial spot occurs in all regions of the world where tomatoes and peppers are grown (17).

A large collection of strains of the bacterial spot pathogen of tomato and pepper was accumulated for study (4), and two characteristics, pitting of pectate gels and hydrolysis of starch, were determined for each strain as aids for identifying possible opportunistic xanthomonads in the collection (14). Strains that were pectolytic and amylolytic, characteristics expected for opportunistic xanthomonads, were discovered, but these strains were pathogenic for tomato and/or pepper. One of the pectolytic and amylolytic strains was from Brazil and was designated a new race of *X. campestris* pv. *vesicatoria* on the basis of its ability to cause disease in the resistant tomato breeding line Hawaii 7998 (29). Of 522 strains tested (4), 104 were pectolytic and amylolytic. Most of these 104 strains were from Argentina, but similar strains were received from Australia, Brazil, New Zealand, and Spain. Only 1 of 331 strains from the United States was pectolytic and amylolytic, and no such strain was among the 28 strains from Taiwan tested. The pectolytic and amylolytic strains were not evenly distributed in all regions of the world where bacterial spot of tomato and pepper occurs, but seemed to be most prevalent in countries in the southern hemisphere.

The two groups of strains, which are placed in *X. campestris* pv. *vesicatoria* because of their host specificity, were further characterized to determine the extent of genotypic

and phenotypic diversity of these organisms. Several criteria were used for characterization, including carbon utilization patterns, serology data, fatty acid profiles, protein profiles as determined by sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), DNA restriction enzyme digestion data, and DNA homology data. The purpose of this work was to determine the extent of genetic variation among strains assigned to a pathovar of *X. campestris* and to provide a better understanding of the pathogen of bacterial spot of tomato and pepper.

MATERIALS AND METHODS

Bacterial strains and inoculum preparation. Ten nonpectolytic and nonamylolytic (group A) and 10 pectolytic and amylolytic (group B) strains originally isolated from tomatoes and peppers were selected for characterization. The origins of the strains are shown in Table 1. Many of the strains had been stored as suspensions in screw-cap tubes containing sterile tap water. All were preserved in 15% glycerol at -70°C for long-term storage during this work.

Pathogenicity tests. Single colonies were transferred to nutrient broth and shaken overnight at 30°C , and the bacterial suspensions were centrifuged. The resulting pellets were resuspended in sterile tap water, and the suspensions of cells were standardized to an optical density at 600 nm of 0.3 (concentration equal to 5×10^8 CFU/ml). Seedlings of tomato (cultivar Bonny Best and breeding line Hawaii 7998) and pepper (cultivar Early Calwonder) were transferred to 10-cm pots containing a steamed peat-vermiculite mixture in a greenhouse (23 to 35°C). A soluble 20-20-20 fertilizer (0.4 g per pot; Peters Fertilizer Products, W. R. Grace & Co., Fogelsville, Pa.) was added to the plants biweekly. After 4 weeks the plants were transplanted to 15-cm pots. To observe the disease symptoms produced by the different strains, leaves of both tomato (cultivar Bonny Best) and pepper plants were inoculated by swabbing inoculum onto leaves that were not fully expanded with a cotton applicator saturated with a bacterial suspension amended with carborundum. After inoculation, symptom development was observed periodically for 3 weeks. Fully expanded leaflets of

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TABLE 1. Strains of *X. campestris* pv. *vesicatoria* and *X. campestris* pv. *campestris* used in this study

Strain	Location of isolation	Isolated from:	Source ^a
<i>P. campestris</i> pv. <i>vesicatoria</i>			
Group A strains ^b			
75-3	Florida	Tomato	Stall
87-21	Brazil	Tomato	Poulos
87-47	Taiwan	Tomato	Wang
87-44	Taiwan	Pepper	Wang
86-46	Florida	Pepper	Stall
86-2	Florida	Tomato	Stall
82-4	Florida	Pepper	Stall
85-16	Florida	Tomato	Stall
86-22	Florida	Pepper	Stall
89-10	Australia	Pepper	Hibberd
Group B strains ^b			
56	Brazil	Tomato	Nagai
0226	Australia	Tomato	Hibberd
0350	Australia	Tomato	Hibberd
71-4	New Zealand	Tomato	Starr
BA 27-1	Argentina	Pepper	Alippi
BA 29-1	Argentina	Tomato	Alippi
BV 20-3A	Argentina	Tomato	Canteros
BV 5-3A	Argentina	Tomato	Canteros
BV 5-4A	Argentina	Tomato	Canteros
BV 5-4B	Argentina	Tomato	Canteros
<i>X. campestris</i> pv. <i>campestris</i>			ATCC
ATCC 33913 ^c			

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^b Group A strains are nonpectolytic and nonamylolytic; group B strains are pectolytic and amylolytic.

^c T = type strain.

cultivar Bonny Best and Hawaii 7998 plants were infiltrated with unamended inoculum to test for hypersensitivity. The plants were then transferred to a growth chamber kept at 24°C. Ratings for necrosis were recorded daily for 5 days.

Pectate utilization and starch hydrolysis. A depression in the medium around a bacterial colony on CVP medium (6) prepared without crystal violet or on Hildebrand's B medium (15) was scored as a positive reaction for pectolytic activity. Starch hydrolysis was determined on nutrient agar supplemented with 1.5% soluble starch. A positive reaction consisted of copious growth and a turbid halo around each colony. These reactions were associated with starch hydrolysis previously by flooding the plates with Lugol's iodine solution. Pectolytic and amylolytic activities were scored 48 h after transfer of bacteria to the media.

Carbon source utilization. The 20 strains were tested for utilization of 95 carbon sources with GN MicroPlates (Biology, Inc., Hayward, Calif.). Single colonies were transferred from nutrient agar to Trypticase soy broth agar for 24 h of growth at 27°C. The bacteria were removed from Trypticase soy broth agar with a sterile cotton swab and suspended in sterile saline, and each suspension was adjusted to an optical density at 590 nm of 0.19. Then, 150 µl of the adjusted suspension was added to each well of a GN MicroPlate. After incubation for 24 h at 28°C, the A_{550} of each well was determined with a plate reader (model EAR 400; SLT Lab Instruments USA, Inc., Ronkonkoma, N.Y.).

A data base of the reaction patterns was used to create a dendrogram with the software program accompanying the Biolog system.

Serology. (i) MAb production. Monoclonal antibodies (MAbs) were produced separately in Hawaii and in Florida. In Hawaii, the antibodies were produced as described previously by using formalin-killed whole-cell preparations of strains as immunizing antigens (1). In Florida, BALB/c mice were immunized twice, 12 days apart, by intraperitoneal injection of 10⁸ whole cells mixed 1:1 with Ribi's adjuvant (Ribi Immuno-Chem Research, Inc., Hamilton, Mont.). Each mouse was hyperimmunized 3 days before its spleen was removed. The splenocytes were mixed 7:1 with washed Sp2/0 myeloma cells, centrifuged, and resuspended in 50% polyethylene glycol for 1 min to accomplish fusion. Over the next 5 min, 1 ml of HAT (hypoxanthine, aminopterin, and thymidine) medium was added every 10 s. The mixture was centrifuged, resuspended in HAT medium, and dispensed into 96-well culture plates. The supernatants were screened after 12 days with the homologous antigen (strain 75-3) and a heterologous antigen (strain 56) by performing an enzyme-linked immunosorbent assay (ELISA). Hybridomas producing antibodies that reacted differently with the two strains and hybridomas producing antibodies that reacted equally well with the two strains were selected. These hybridomas were then cloned by limiting dilution.

(ii) ELISA procedure. The antigens consisted of whole cells suspended in 0.01 M carbonate buffer (pH 9.6) and adjusted to an optical density at 600 nm of 0.6. They were spread onto Immulon 2 microtiter plates (Dynatech Laboratories, Inc., Chantilly, Va.) and incubated overnight in a moist environment at 4°C. The plates were washed with phosphate-buffered saline-Tween, and then 1% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) was added to the wells. Appropriate dilutions of MAbs were added directly to the wells, and the preparations were incubated for 1 h at 37°C. The wells were washed again before alkaline phosphatase conjugated to anti-mouse immunoglobulin G was added, and the preparations were incubated as described above for MAbs. The plates were then washed, and 100 µl of an enzyme substrate buffer containing *p*-nitrophenyl phosphate (Sigma) was added to each well (28). The enzyme activity for substrate conversion was quantified for each well by measuring A_{405} with a model EAR 400 plate reader. An A_{405} of 0.12 after 60 min of incubation was considered a positive reaction.

Fatty acid analyses. The fatty acid profiles of 39 group A strains and 39 group B strains were analyzed. The 20 strains listed in Table 1 were included among the strains examined. A single colony of each strain was transferred from a nutrient agar culture to Trypticase soy broth agar, and after 24 h of growth at 28°C approximately 40 mg of cells was collected for analysis. The bacterial fatty acids were derivatized to their methyl esters (20), separated by gas chromatography (23), and identified by using the Microbial Identification System software (version 3.6; MIDI, Newark, Del.). The relationships of the strains based on fatty acid analysis data were determined by analyzing principal components with MIDI software.

Electrophoresis of soluble proteins. The strains listed in Table 1 were characterized by one-dimensional electrophoretic separation of whole-cell proteins, using the method of Laemmli (18) as modified by Jackman (16). Single colonies were transferred to nutrient broth and incubated with shaking for 16 h at 28°C. The cells were harvested by centrifugation and washed twice in sterile deionized water, and then

approximately 20 mg was suspended in 180 μ l of 10% sorbitol. Each cell suspension was mixed with an equal volume of double-strength lysis buffer (125 mM Tris-HCl [pH 6.8], 4% SDS, 10% 2-mercaptoethanol, 20% glycerol) containing 0.01% bromophenol blue. The suspension was heated at 100°C for 10 min and centrifuged at $16,000 \times g$ for 10 min to pellet the cellular debris. The supernatant containing water-soluble proteins was then removed carefully and stored at -20°C until it was needed. Before the protein solution was loaded, the sample was thawed at room temperature and heated at 50°C for 10 min. The proteins were separated with a discontinuous gel consisting of a stacking gel (5% total acrylamide with 2.7% cross-linking *N,N'*-bismethyleneacrylamide, 125 mM Tris-HCl [pH 6.8], 0.1% SDS) layered on top of a separation gel (10% total acrylamide with 2.7% cross-linking *N,N'*-bismethyleneacrylamide, 375 mM Tris-HCl [pH 8.8], 0.1% SDS). Electrophoresis was performed at 4°C by using a Protean II double-slab vertical electrophoresis cell (Bio-Rad Laboratories, Richmond, Calif.) filled with electrophoresis buffer (250 mM Tris [pH 8.3], 192 mM glycine, 0.1% SDS). A constant current of 25 mA per gel was applied until the bromophenol blue reached the separation gel (ca. 45 min). The current was then increased to 35 mA per gel until the tracking dye had migrated about 100 mm through the separation gel (ca. 3 h). The gel was stained for proteins at room temperature with a silver staining kit (Sigma) and finally dried between two sheets of cellophane.

Restriction endonuclease analysis. Genomic DNAs of the 20 group A and B strains listed in Table 1 were prepared and digested with restriction enzymes *Xba*I and *Spe*I as described previously (9). The large DNA fragments were separated by pulsed-field electrophoresis. The gels were stained with 0.5 mg of ethidium bromide per liter and photographed with type 55 Polaroid film. The fragments of all 20 strains were compared on the same gel, and similarity coefficients were determined for strains that shared fragments larger than 100 kb (9). The KITSCH program from the PHYLIP computer package (10) was used to infer a rooted phylogenetic tree by using the Fitch-Margoliash method (11). The input data consisted of a matrix of pairwise genetic distances determined as estimates of the numbers of nucleotide substitutions per site on the basis of similarity coefficients calculated for the combined *Spe*I and *Xba*I digestion data.

DNA-DNA hybridization. The experimental procedure used for DNA reassociation experiments has been described previously (9). The DNA of one strain belonging to each group (strains 75-3 and 56) was labeled by incorporating tritiated dCTP into sheared genomic DNA by the random primer method. Each labeled DNA was hybridized with sheared DNAs of the 20 group A and B strains listed in Table 1. Labeled double-stranded DNA was incorporated onto Whatman GF/C glass fiber filters, and radioactivity was quantified by liquid scintillation counting. The hybridization values for pairs of strains were means of the values from two experiments; in each experiment two hybridization reactions were performed.

RESULTS

Pathogenicity tests. The strains of the two groups caused slightly different symptoms in tomato and pepper leaves. Lesions that were raised above the leaf surface developed when group A strains were inoculated onto tomato and pepper leaves by the swabbing technique. A whitish halo

FIG. 1. Dendrogram of relationships based on patterns of carbon substrate utilization by *X. campestris* pv. *vesicatoria* strains.

surrounded each lesion as it appeared. In contrast, the group B strains caused lesions that were sunken or level with the leaf surface and were not surrounded by whitish halos. The differences were obvious only when lesions first formed after inoculation. Differences in the frequency or diameter of lesions caused by strains of the two groups were not detected. Both types of strains caused chlorosis and epinasty of tomato and pepper leaves.

Necrosis occurred in leaves of tomato line Hawaii 7998 within 24 h after infiltration with high populations of all group A strains. Necrosis occurred after 72 h in the same type of leaves infiltrated with all group B strains. Necrosis in leaves of cultivar Bonny Best plants occurred after 72 h with strains of both groups.

Pectate utilization and starch hydrolysis. The group A strains selected for this research were consistently non-amyolytic and nonpectolytic. The group B strains were amyolytic and pectolytic.

Carbon source utilization. A cluster analysis of the patterns of utilization of 95 carbon sources revealed relative diversity among the 20 strains examined. Four clusters of strains were differentiated at a dendrogram distance of 12 (Fig. 1). One cluster consisted of two group A strains; another cluster contained six group B strains; a third cluster contained six group A strains and two group B strains; and a fourth cluster contained two group A strains and two group

TABLE 2. Serological reactions of MABs with strains of *X. campestris* pv. *vesicatoria*

Strain	Reaction with:			
	MAB 5D12	MAB 3	MAB 4H5	MAB 15
Group A strains ^a				
75-3	+	+	-	-
82-4	+	+	-	-
85-16	+	-	-	-
86-2	+	+	-	-
87-21	+	+	-	-
89-10	+	+	-	-
86-46	+	+	-	-
87-47	+	+	-	-
86-22	-	+	-	-
87-44	-	+	-	-
Group B strains ^a				
56	+	-	+	-
0226	+	-	+	-
0350	+	-	+	-
BV 5-3A	+	-	+	-
BV 5-4B	+	-	+	-
BV 20-3A	+	-	+	-
BA 27-1	-	-	-	+
BA 29-1	-	-	-	+
BV 5-4A	-	-	-	+
71-4	-	-	-	-

^a Group A strains are nonpectolytic and nonamyolytic; group B strains are pectolytic and amyolytic.

^b +, positive (A_{405} of 0.12 or greater in the ELISA); -, negative. The A_{405} value of 0.12 is an arbitrary threshold which is three times the A_{405} of heterologous strains.

B strains. Specifically, 9 of the 10 group A strains but none of the group B strains utilized *cis*-aconitic acid. D-Mannitol, succinamic acid, alpha-hydroxybutyric acid, and L-aspartic acid also helped distinguish the two groups of strains. Seven, eight, six, and six of the group B strains and 0, 2, 1, and 1 of the group A strains, respectively, used these compounds.

Serology. Three MABs could be used to differentiate most of the strains in the two groups (Table 2). MAB 3 reacted with all but one of the group A strains and none of the group B strains. Either MAB 4H5 or MAB 15 reacted with all but one of the group B strains, but neither of these MABs reacted with any of the group A strains. MAB 5D12 reacted with most strains of both groups.

Fatty acid profiles. The fatty acid profiles of all strains were qualitatively similar and were typical *X. campestris* profiles (22, 25). However, several quantitative differences statistically separated the two groups of strains. The ratio of the 15:0 iso acid to 15:0 anteiso acid for group A strains was near 3:1, compared with a ratio of 2:1 for the group B strains. The two groups of strains formed two clusters with some overlap in a principal component analysis (Fig. 2). A dendrogram (data not shown) of the relationships of the strains produced some clusters that contained both types of strains, apparently because of the overlap of strains in the two groups.

Electrophoresis of soluble proteins. A large number of discrete bands at molecular weights ranging from about 14,000 to more than 100,000 were present in the protein extracts of the strains (Fig. 3). The group A and B strains were clearly different in the low-molecular-weight region. Specifically, the group A strains shared a ca. 32-kDa protein band, whereas the group B strains shared another protein with a lower molecular weight (about 27,000). Of a total of 67

FIG. 2. Principal component analysis of the fatty acid profiles of 38 group A strains (▲) and 38 group B strains (○). The cross-hatched area represents strain profile.

group B strains tested (1a), 3 expressed the 27-kDa band only occasionally. This group of strains is represented by strain BA 29-1 in Fig. 3.

Restriction endonuclease analysis. After digestion of genomic DNAs with *Xba*I, the similarity coefficients ranged from 0.71 to 1.00 in comparisons of the 10 group A strains with each other. The similarity coefficients for the 10 group B strains ranged from 0.29 to 1.00. In comparisons of groups A and B the similarity coefficients ranged from 0.00 to 0.38. Digestion with *Spe*I resulted in similarity coefficients ranging from 0.50 to 0.96 for the group A strains and 0.31 to 1.00 for the group B strains. The similarity coefficients for groups A

FIG. 3. Electropherogram of denatured proteins from SDS-lysed cells of *X. campestris* pv. *vesicatoria*. Lanes 1 through 5, lysates from group B strains 56, 0226, BA 27-1, 71-4, and BA 29-1, respectively; lanes 6 through 9, lysates from group A strains 75-3, 86-46, 89-10, and 87-44, respectively. The following protein molecular weight markers were used (from top to bottom): rabbit phosphorylase B (molecular weight, 97,000), bovine serum albumin (66,000), hen egg white ovalbumin (45,000), bovine carbonic anhydrase (31,000), and soybean trypsin inhibitor (21,000). Silver staining revealed a 32-kDa band (arrow) in the nonamyolytic and nonpectolytic strain lysates (lanes 6 through 9) and a 27-kDa band (arrow) in the amyolytic and pectolytic strain lysates (lanes 1 through 4). In one amyolytic and pectolytic strain (lane 5) the 27-kDa band was not present.

FIG. 4. Bands of DNAs from *X. campestris* pv. *vesicatoria* strains separated by pulsed-field electrophoresis after genomic DNAs were digested with restriction enzyme *SpeI*. Lanes A and J contained yeast chromosomal DNA as markers, and the sizes (in kilobases) of some of the bands are indicated on the left. Lanes B through E contained DNAs from group A strains 75-3, 86-2, 89-10, and 87-21, respectively, and lanes F through I contained DNAs from group B strains 56, BV 5-4A, BV 20-3A, and 0350, respectively. The group B strains (lanes 6 through 9) did not produce bands larger than 600 kb.

and B were 0.08 to 0.26. The group A strains consistently produced bands near 600 to 800 kb, but no bands above 600 kb were present with group B strains (Fig. 4). The two groups of strains formed separate clusters in a tree constructed by using data for pairwise genetic distances. Greater genetic divergence occurred within the group B strains than within the group A strains (Fig. 5).

DNA-DNA hybridization. The levels of hybridization between DNAs of the group A strains and DNA of strain 75-3 (group A) ranged from 79 to 119%, whereas the levels of hybridization between DNAs of group B strains and DNA of strain 75-3 ranged from 0 to 42% (Table 3). The levels of hybridization between DNAs of group A strains and DNA of strain 56 (group B) ranged from 14 to 46%, whereas the levels of hybridization between DNAs of group B strains and DNA of strain 56 ranged from 74 to 108%. The average hybridization values within groups A and B were 98.2 and 90.8%, respectively. The average level of hybridization between DNAs from group A strains and a group B strain was 33%, and the average level of hybridization between DNAs from group B strains and a group A strain was 17%. The levels of DNA homology of strains 75-3 and 56 to the type strain of *X. campestris* were 42 and 32%, respectively.

DISCUSSION

Many discriminating techniques were used to determine the phenetic and genetic characteristics of the two groups of *Xanthomonas* strains isolated from bacterial spot of tomato and pepper, as suggested by Vauterin et al. (26) for work with the genus *Xanthomonas*. Collectively, our results support the conclusion that there are significant genetic differences between the two groups. The differences in pathogenicity between the two groups were consistent, but these differences may be the result of differences in only a few

FIG. 5. Clustering of group A and B strains inferred from similarity coefficients obtained from *SpeI* and *XbaI* restriction endonuclease digestion data. The tree was generated by the KITSCH procedure with the PHYLIP computer package by using the Fitch-Margoliash method. The genetic distances are estimates of the numbers of nucleotide substitutions per site.

genes. Likewise, the genetic basis associated with the differences detected in carbon utilization patterns could have been small. The two groups of strains could be differentiated with a series of MAbs, but some antigens were present in both groups. Fatty acid analysis data differentiated two clusters of strains, but portions of the clusters overlapped. Differences in the profiles of silver-stained proteins were detected, and the DNA fingerprints of restriction enzyme sites helped assess the extent of the differences between the two groups of strains. DNA-DNA hybridization values for the two groups of strains gave the best portrait of the extent of the genetic differences between the groups of strains.

Groups A and B may correspond to subgroups A and B of *X. campestris* pv. *vesicatoria* designated previously by Vauterin et al. (25). In the study of Vauterin et al. a numerical analysis of SDS-PAGE proteins stained with Coomassie blue was completed with 17 strains. Two major clusters were distinguished. Subgroup A was in DNA homology group 1 (26), but subgroup B was not placed in a DNA homology group. Subgroup B contained the pathovar reference strain.

Starch hydrolysis helps distinguish the two groups of strains that can be isolated from lesions on plants with bacterial spot of tomato and pepper. Burkholder and Li (2) pointed out many years ago that strains of the bacterial spot pathogen differed in hydrolysis of starch. Strains obtained from tomato were strongly diastatic, and strains obtained

TABLE 3. Levels of homology between DNAs from *X. campestris* pv. *vesicatoria* group A and B strains and the type strain of *X. campestris* and DNA from a representative of each group

Strain	% Hybridization with:	
	Strain 75-3 DNA	Strain 56 DNA
<i>X. campestris</i> pv. <i>vesicatoria</i>		
Group A strains ^a		
75-3	100	14
87-21	79	39
87-47	101	46
87-44	82	35
86-46	114	15
86-2	85	31
82-4	99	40
85-16	115	29
86-22	119	34
89-10	91	31
Group B strains ^a		
56	25	100
0226	6	104
0350	13	77
71-4	0	108
BA 27-1	42	84
BA 29-1	14	74
BV 20-3A	38	88
BV 5-3A	7	88
BV 5-4A	14	95
BV 5-4B	15	99
<i>X. campestris</i> pv. <i>campestris</i>	42	32
ATCC 33913 ^T		

^a Group A strains are nonpectolytic and nonamylolytic; group B strains are pectolytic and amylolytic.

from pepper were not. These workers may have been dealing with the two groups described here. Schaad also reported that two serotypes of *X. campestris* pv. *vesicatoria* differed in hydrolysis of starch (24). Cultures from the collections used in these two studies will have to be tested with other techniques to determine whether Burkholder and Li (2) and Schaad (24) were dealing with the two groups of strains described here.

Members of both groups are pathogenic for tomato and/or pepper. The host ranges of the groups of strains are not considered to be important because host range is determined by avirulence genes carried on plasmids (3, 21). The majority of the group B strains contain the avirulence gene *avrBsT*, which results in incompatibility in all *Capsicum* species (21). Some group A strains also contain *avrBsT*, and other group A strains contain another avirulence gene, *avrBsP*, which results in incompatibility in *Lycopersicon* species (3). In addition, there may be some solanaceous hosts other than tomato and pepper for one or both groups, but this has not been determined. Other solanaceous hosts of *X. campestris* pv. *vesicatoria* have been described previously (19), and some of them occur only in local geographic regions.

Dye et al. (8) designated a neopathotype for *X. campestris* pv. *vesicatoria* in 1980. Jones and Bouzar (17a) determined that this strain belongs to our group B. This strain may not represent the original description of the bacterium by Doidge (7), because the bacterium causing bacterial spot of tomato in South Africa only weakly hydrolyzed starch after 14 days. Gardner and Kendrick (12), unaware of Doidge's description, also described the pathogen of bacterial spot of tomato

and found that their strains hydrolyzed starch in 2 days. They named their bacterium *Bacterium exitiosum*, a name which was withdrawn because the two bacteria were considered to be the same pathogen and Doidge's description had priority (13). On the basis of starch hydrolysis, the two bacteria represent the two groups described here.

Genetic diversity of strains within pathovars of *X. campestris* is not unusual. Three groups were distinguished among strains of *X. campestris* pv. *citri* on the basis of pathogenicity (9). Strains of the three groups exhibited levels of DNA homology of more than 60% (9, 27), which was interpreted to mean that all of the strains belonged to the same species (27). Strains that exhibited levels of DNA homology greater than 85%, however, corresponded to the three groups.

The nomenclature of the two groups of strains pathogenic for tomato and pepper is a problem. DNA from a strain belonging to each group hybridized to DNA from the type culture of *X. campestris* at a level (<50%) which could be used to justify new species (30). However, determination of levels of DNA homology with representatives of DNA hybridization groups, some of which are listed by Vauterin et al. (26), should be completed before the two groups of strains are classified. The use of two species names for strains that essentially cause the same disease in tomato and pepper may be confusing to researchers working in agriculture. Clearly, the two groups must be distinguished in tomato breeding programs, because tomato resistance differs for the two groups. At present, the two groups of strains are known as race 1 and race 2 of the tomato group of strains (28). However, designation of races is not adequate to distinguish the groups, because race implies differences in strains only at the pathogenicity level. We suggest that these groups should be designated groups A and B of *X. campestris* pv. *vesicatoria*, until the correct taxonomic position of the two groups of strains within the genus *Xanthomonas* has been determined.

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