Physiological, Chemical, Serological, and Pathogenic Analyses of a Worldwide Collection of *Xanthomonas campestris* pv. *vesicatoria* Strains

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ABSTRACT

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Xanthomonas campestris pv. *vesicatoria* strains from a worldwide collection were assigned to the A or B group by testing for amylolytic activity and the presence of the α or β protein band. Cluster analyses of serological, carbon substrate utilization, and fatty acid composition data resulted in dendrograms with interspersed clusters of A and B strains, suggesting that the strain populations within each group are heterogeneous. However, further analysis of the data identified features unique to members of each group. Two monoclonal antibodies reacted only with group A strains, and two reacted only with group B strains. Reactivity patterns further subdivided each group into three serovars. Differentiation of A and B strains by their fatty acid composition was primarily based on lower amounts of 15:0 ante-iso in A strains. The only substrate that discriminated between strains of the two groups was *cis*-aconitate, which was utilized by A but not by B strains. According to these assays, the neopathotype strain of *X. c. vesicatoria* (ATCC 35937) belongs to the B group. The bacterial pathogen *Pseudomonas gardneri* shares characteristics of both A and B strains; thus, it is apparently related to *X. c. vesicatoria*. The dendrograms generated from cluster analyses of carbon substrate utilization patterns and fatty acid profiles of *X. c. vesicatoria* strains included several pathovars, indicating that strains making up this taxon were as diverse in their carbon substrate utilization ability and fatty acid composition as the strains of the different *X. campestris* pathovars tested. The race affiliations of the *X. c. vesicatoria* strains tested were determined with tomato and pepper differential genotypes. The reaction on tomato lines distinguished the two groups as separate races: A strains were tomato race 1 (i.e., T1), and B strains were T2. Except for 14 T2P3 strains, pepper race strains were affiliated with the A group.

Additional keywords: bacterial spot, taxonomy.

Xanthomonas campestris pv. *vesicatoria* is the causal agent of bacterial spot of tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicum annuum* L.). This disease can cause significant losses, particularly in warm and humid environments (16,27). In a recent taxonomic study of 20 strains of *X. c. vesicatoria,* two genetically distinct groups (i.e., A and B) were identified (31). DNA homology between A and B strains was less than 50%, indicating that they likely represent two distinct species (31). Phenotypic differences between A and B strains were observed in utilization of carbon compounds, reaction to monoclonal antibodies (MAbs), fatty acid composition, hypersensitive reaction (HR) on tomato differentials, and amylolytic activity (31). In another study of *X. c. vesicatoria* strains pathogenic on tomato, unique protein bands were identified in each group; most A strains had a 32- to 35-kDa (α) band, whereas B strains had a 25- to 27-kDa (β) band (4). The presence of the α or β protein band was correlated with pathogenicity and, more significantly, with amylolytic activity. Group A strains were unable to hydrolyze starch, whereas group B strains were strongly amylolytic (4,31).

Historically, amylolytic activity has been described as a variable characteristic of *X. c. vesicatoria* (12). In the original description of the causal agent in Pretoria, South Africa, Doidge (9) indicated that the pathogen *Bacterium vesicatorium* was able to hydrolyze 0.1% starch in a broth only after 14 days. However, at about the same time, Gardner and Kendrick (14) reported bacterial spot on tomato in Indiana caused by *B. exitiosum,* which produced halos in starch-containing medium in just 5 days. Gardner and Kendrick (15) compared representative strains of the two pathogens and concluded that the pathogens were identical and by priority rules retained *B. vesicatorium;* however, they failed to report amylolytic activity, a key characteristic to differentiate strains within this taxon (4,31). Burkholder and Li (5) reported that strains isolated from tomato were strongly amylolytic, while those from pepper were not. Later, a similar trend was reported by Dye (10). Several races of this pathogen have since been identified; some infect only one of the two plant species, while others infect both (7,25). Another nonamylolytic bacterial spot pathogen of tomato was described by Sutic (33) as *Pseudomonas gardneri;* this pathogen has since been proposed as a pathovar of *X. campestris* (35) and even as a synonym of *X. c. vesicatoria* (11).

The objective of this research was to expand our knowledge of the variability of *X. c. vesicatoria* strains. In this study, we characterized 160 pepper and tomato strains from around the world. Strains were first assigned to the A or B group by testing for their amylolytic activity and the presence of the α or β protein band. The strains were subsequently characterized as to their pathogenicity on differential tomato and pepper genotypes, reaction to a panel of MAbs, fatty acid composition, and ability to utilize 95 carbon compounds.

MATERIALS AND METHODS

Bacterial strain characterization. The affiliation of the *X. c. vesicatoria* strains with group A or B (sensu Stall et al [31]) was

based on silver-stained protein profiles and amylolytic activity. Strains of group A contain the 32- to 35-kDa α band and are unable to hydrolyze 1% starch in 2 days, whereas B strains have the 25- to 27-kDa β band and are strongly amylolytic (4). The present study included both tomato and pepper strains; tomato strains were typed to the A or B group in an earlier study (4). Relevant characteristics of strains of *X. c. vesicatoria* are listed in Table 1. For comparison purposes, strains from other pathovars of *X. campestris* and species were included (Table 1). Strains were recovered from storage at −70 C and grown on nutrient

TABLE 1. Bacterial strains

Strain[a]	Location	Host[b]	Source[c]
Xanthomonas campestris pv. *vesicatoria* group A			
BA26-1, BA30-1	Argentina	P	1
89-10	Australia	P	14
597 to 601	Bahamas	P	17
IAPAR 9696, IAPAR 9699, IAPAR 9700, IAPAR 9701	Brazil	P	19
8, 21, 31, 35	Canada	T	11
CNBP 1604	Guadeloupe	P	21
CNBP 2484	Guadeloupe	T	21
PAP32 (= ICMP 9087), XV1596, XV12411	Hungary	P	18
XCV1-A, XCV1-C, XCV1-D	India	T	6
90-27, 90-29, 90-30, 90-39	Korea	P	16
B95	Mexico	P	31
91-66 to 91-80	Mexico	T	12
LMG 913	Senegal	P	23
859-8, 985-A1, 985-B7, A1 to A3, P2, P6	Spain	P	20
87-28, 87-37, 87-40, 87-44, 87-47, 87-48, 87-56, 87-65	Taiwan	P	29
87-21	Taiwan	T	29
72-9	Tonga	P	10
LMG 667	Unknown	T	23
B108	California	P	31
LMG 929	Florida	T	23
82-8	Florida	P	31
37, 62, 63, 125	Florida	P	17
69-1, 76-4, 80-5, 81-18, 82-4, 86-22, 86-46, 87-13	Florida	P	27
18, 39, 58, 67, 85, 116, 118, 121, 122, 127, 128, 820, 821, 915, 929, 936	Florida	T	17
75-3, 75-4, 83-4, 85-16, 86-2	Florida	T	27
B82	Florida	T	31
71-1	Hawaii	P	8
A1782, EWCII	Hawaii	P	2
A1074, B111	Hawaii	U	2
ATCC 11633	Indiana	P	3
XV29, XV31, XV32	Oklahoma	P	4
X. c. vesicatoria group B			
BA21-1, BA27-1	Argentina	P	1
BA23-1, BA28-1, BA29-1	Argentina	T	1
BV1-1, BV3-1, BV3-5, BV4-1, BV5-1, BV5-3A, BV5-4A, BV5-4B, BV6-1, BV7-3A, BV8-1, BV20-3A	Argentina	T	5
79-2	Argentina	T	27
0226, 0350	Australia	T	14
IAPAR 8020	Brazil	P	19
X525-85	Brazil	T	22
X. c. vesicatoria group B (*continued*)			
546, IAPAR 8012, IAPAR 8013, IAPAR 8015, IAPAR 8016, IAPAR 8071, IAPAR 9697	Brazil	T	19
XV8035 (= ICMP 8035)	Brazil	T	18
CNBP 30, CNBP 1545-92	France	T	21
LMG 925	Hungary	T	23
LAB2	Hungary	T	18
ATCC 35937	New Zealand	T	3
LMG 916, LMG 917	New Zealand	T	23
71-4 (= ICPPB 167)	New Zealand	T	28
CNBP 2625	Réunion Island	T	21
643	Spain	T	20
B61, B122, XV-1	California	T	31
A571-1	Hawaii	P	2
A135-1	Hawaii	T	2
ATCC 11551	Indiana	T	3
81-6	Indiana	U	8
B80	Louisiana	T	31
XV10, XV15	Oklahoma	T	4
X. c. alfalfae A			27
X. c. armoraciae A			27
X. c. begoniae ATCC 11725			3
X. c. campestris ATCC 33913			3
X. c. carotae B-814			26
X. c. celebensis ICPB XC145			7
X. c. dieffenbachiae X14			7
X. c. glycines ICPB XP175			7
X. c. juglandis X1029			7
X. c. papavericola X1115			7
X. c. pelargonii X1027			7
X. c. phaseoli 826			32
X. c. pisi ICPB XP171			7
X. c. poinsettiaecola ICPB XP137			7
X. c. raphani XCR5			27
X. c. secalis ICPB XT129			7
X. c. '*syngonii*' X161			7
X. c. taraxaci ICPB XT11			7
X. c. translucens B-960			26
X. c. vignicola ICPB XV18			7
X. fragariae UCPPB X861			7
X. oryzae pv. *oryzicola* S58			24
Pseudomonas andropogonis NCPPB 934			30
P. gardneri IPCB XG101			15
P. solanacearum PS4			17
P. syringae pv. *phaseolicola* SAII			25
P. s. syringae 88-22			13
P. viridiflava PV1			17
P. viridiflava PV3			17
Erwinia herbicola 1184-13			9

[a]Group A = nonamylolytic strains expressing the 32-kDA α protein, and group B = amylolytic strains expressing the 27-kDa β protein (4,31).
[b]Host of origin: P = pepper, T = tomato, and U = unknown.
[c]1 = A. Alippi, Universidad Nacional de la Plata, La Plata, Buenos Aires, Argentina; 2 = A. M. Alvarez, University of Hawaii; 3 = American Type Culture Collection, Rockville, MD; 4 = C. L. Bender, Oklahoma State University, Stillwater; 5 = B. Canteros, Est. Exp. Agropecuaria, INTA, Bella Vista, Corientes, Argentina; 6 = R. Chand, Indian Institute of Horticultural Research, Sadashwanagar, Bangalore, India; 7 = A. Chase, University of Florida, Apopka; 8 = A. A. Cook, University of Florida, Gainesville; 9 = D. A. Cooksey, University of California, Riverside; 10 = G. Daft, Department of Agriculture, Nukin Alofa, Tonga; 11 = B. Dhanvantari, Agriculture Canada Research Station, Harrow, Canada; 12 = R. Felix-Gastelum, Campbell Research & Technology, Guasave Sinaloa, Mexico; 13 = R. D. Gitaitis, University of Georgia, Tifton; 14 = A. Hibberd, Redlands Research Center, Cleveland, Queensland, Australia; 15 = D. C. Hildebrand, University of California, Berkeley; 16 = B. K. Hwang, Korea University, Anamdong, Sungbukku, Seoul; 17 = J. B. Jones, University of Florida, Bradenton; 18 = Jozsef Nemeth, Station of County Baranya, Pécs, Hungary; 19 = R. M. V. B. C. Leite, Instituto Agronomico do Parana, Londrina, Parana, Brazil; 20 = M. M. Lopez, Instituto Valenciano de Investigaciones Agrarias, Moncado, Valencia, Spain; 21 = J. Luisetti, Insitut National de la Recherche Agronomique, Station de Pathologie Végétale et de Phytobactériologie, Angers, France; 22 = H. Nagai, Instituto Agronomico, Sao Paulo, Brazil; 23 = Y. Ping, LMG Culture Collection, Universiteit Gent, Belgium; 24 = D. A. Roth, University of Wyoming, Laramie; 25 = M. Sasser, MIDI, Newark, DE; 26 = N. W. Schaad, USDA-ARS, Frederick, MD; 27 = R. E. Stall, University of Florida, Gainesville; 28 = M. Starr, International Collection of Plant Pathogenic Bacteria, University of California, Davis; 29 = A. T. Tschanz, Asian Vegetable Research & Development Center, Shanhua, Tainan, Taiwan; 30 = A. K. Vidaver, University of Nebraska, Lincoln; 31 = J. C. Watterson, Petoseed Research Center, Woodland, CA; 32 = R. Wilkinson, Cornell University, Ithaca, NY.

agar (Difco Laboratories, Detroit, MI) before testing.

Pathogenic race determination. Strains of *X. c. vesicatoria* were tested for race determination on different pepper and tomato genotypes. Pepper (P) races were determined according to reactions on plants of the cultivar Early Calwonder (ECW) and the near-isogenic derivative lines ECW-10R, ECW-20R, and ECW-30R, which carry the *Bs1, Bs2,* and *Bs3* genes, respectively (17), for resistance to *X. c. vesicatoria* strains carrying the avirulence genes *avrBs1, avrBs2,* and *avrBs3,* respectively (25,34). Tomato (T) races were determined according to reactions on plants of the susceptible tomato cultivar Walter and the genotype Hawaii 7998 (38). Strains of tomato race 1 (T1) contain the gene *avrRxv* (38), which is responsible for the induction of HR in Hawaii 7998 plants. Strains of tomato race 2 (T2) do not contain this gene, thus causing disease on Hawaii 7998 (37,38). Procedures to grow plants and bacteria and to test for HR were described previously (25). Strains that caused ambiguous HR on tomato plants were screened for the avirulence gene, *avrRxv,* present in race T1 as described previously (4).

MAb production and serological typing of strains. Representative strains of *X. c. vesicatoria* as immunogen were used to produce 16 MAbs with different specificities (Table 2). MAbs Xv5, Xv6, Xv7, Xv8, and Xv10 were produced according to Kao and Klein (21), and the remaining MAbs were obtained according to Alvarez et al (1). Initially, 36 strains were tested with 16 MAbs to *X. c. vesicatoria* and the genus-specific MAb X1 by procedures previously described (1). Enzyme-linked immunosorbent assay (ELISA) reactions were converted to positive (net readings above background >0.1) and negative values (net readings 0.1 or less). Comparative data for each MAb-strain combination were tabulated, and the similarity coefficient between two strains was calculated with SIMQUAL, NTSYS-PC version 1.70 (Exeter Software, Setauket, NY). Cluster analysis by the unweighted pair group method was performed, and NTSYS was used to construct a dendrogram. After examination of the groups derived from cluster analysis, six MAbs (i.e., Xv1, Xv5, Xv8, Xv10, Xv15, and Xv21) were tested for reactivity with other bacterial genera, *Xanthomonas* species, and pathovars of *X. campestris.* These six MAbs were used for the serotyping of 160 *X. c. vesicatoria* strains by indirect ELISA (24) as modified by Jones et al (20).

Carbon substrate utilization. Bacteria were grown and tested for utilization of the 95 carbon sources available on the GN Microplate (Biolog Inc., Hayward, CA) as previously described (3). The resulting utilization patterns were read with a microplate reader and subjected to cluster analysis to determine relationships among *X. c. vesicatoria* strains (3). Strains within a dendrogram distance of 10 units were grouped in the same cluster. Relationships to other pathovars of *X. campestris* and selected species of plant-pathogenic bacteria (Table 1) were also determined.

TABLE 2. Monoclonal antibodies used to characterize strains of *Xanthomonas campestris* pv. *vesicatoria*

Antibody	Clone	Immunogen	Isotype
Xv1	106-41	A1074	IgG2b
Xv3	131-39	82-17[a]	IgG2b
Xv5	2H10-4C5	75-3	IgG1
Xv6	5D12-1A	75-3	IgM
Xv7	5E9-2D2	BA29-1, BV20-3A, X525-85	IgM
Xv8	4H5-3B1	BA29-1, BV20-3A, X525-85	IgG1
Xv10	3G10-3G12	BA29-1, BV20-3A, X525-85	IgM
Xv11	208-B2	EWCII	IgG2
Xv12	208-C12	EWCII	IgM
Xv13	208-D4	EWCII	IgM
Xv14	209-B5	B61, B80	IgG2a
Xv15	209-C15	B61, B80	IgM
Xv16	218-9	X525-85	IgG2a
Xv17	218-12	X525-85	IgG2a
Xv20	219-137	BA27-1	...[b]
Xv21	220-72	BV5-3A	IgG2b

[a]Strain 82-17 (group A, race P2) from Florida had a reactivity pattern identical to that of strain 83-4.
[b]Information not available.

Fatty acid composition. Extraction and differentiation of whole-cell fatty acid methyl esters (FAMEs) have been described (29). The qualitative and quantitative differences in the fatty acid patterns were used to compute the Euclidian distance to each strain. All the numerical analyses and the calculations for the FAME dendrogram were performed with the Microbial Identification System software, version 3.60 (Microbial ID, Newark, DE). Strains within six Euclidian distance units, the cut-off for subspecies (28), were grouped in the same cluster. Relationships to selected pathovars of *X. campestris* and species of plant-pathogenic bacteria (Table 1) were also determined.

RESULTS

Affiliation to *X. c. vesicatoria* group A or B and distribution of strains. On the basis of silver-stained protein profiles and amylolytic activity, *X. c. vesicatoria* strains were typed to the A or B group (Table 1). The number of A strains originating from tomato was similar to the number of A strains originating from pepper, whereas most B strains originated from tomato. The neopathotype strain of *X. c. vesicatoria* (ATCC 35937) belonged to the B group. The type strain of *P. gardneri* had a protein band that migrated at the position of the β band; however, this strain did not hydrolyze starch. Strains from groups A and B were distributed in many locations (Fig. 1). Both were found where a large number of strains were available; however, in Florida, Korea, Mexico, and Taiwan, only group A strains were identified, whereas group B strains were more prevalent than group A strains in Argentina and Brazil.

Pathogenic race determination and distribution. The HR on differential tomato lines distinguished *X. c. vesicatoria* strains according to the A and B groups that make up this pathovar (Tables 3 and 4). Group A strains were race T1, and B strains were race T2. All strains pathogenic on pepper but not on tomato (i.e., P0–P4) belonged to the A group. The only B strains able to infect pepper were from a group of 14 strains pathogenic to both hosts; these strains were T2P3. None of the 160 strains was characterized as P2, T1P4, T2P0, T2P2, or T2P4. The neopathotype strain had a T2 phenotype. The only T2P1 strain was the type strain of *P. gardneri.* Two B strains (A135-1 and A571-1) were not pathogenic (Table 3).

Geographical distribution of both tomato races was worldwide; however, race T2 was more prevalent in South America and was not present in our cultures from Asia. The predominant pepper races were P1, P2, and P3 (Table 3). P1 and P3 strains were ubiquitous, whereas P2 strains originated from hosts grown in the Americas only (Tables 1 and 4).

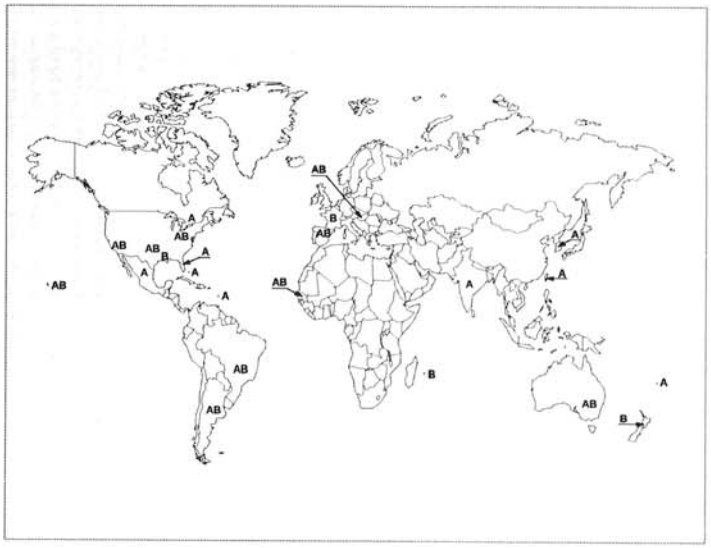

Fig. 1. Distribution of *Xanthomonas campestris* pv. *vesicatoria* phenotypic groups A and B.

TABLE 3. Differential reaction[a] of pepper and tomato genotypes to *Xanthomonas campestris* pv. *vesicatoria* races

	X. c. vesicatoria							
	Group[b]		Pepper[c]				Tomato	
Race	A	B	ECW	ECW-10R	ECW-20R	ECW-30R	Walter	Hawaii 7998
T1	20		+	+	+	+	−	+
T2		34	+	+	+	+	−	−
P0	1		−	+	+	+	+	+
P1	15		−	−	+	+	+	+
P3	4		−	−	+	−	+	+
P4	1		−	−	−	+	+	+
T1P0	2		−	+	+	+	−	+
T1P1	9		−	−	+	+	−	+
T1P2	34		−	+	+	−	−	+
T1P3	24		−	−	+	−	−	+
T2P1	*Pseudomonas gardneri*		−	−	+	+	−	−
T2P3		14	−	−	+	−	−	−
Nonpathogenic		2	nr	nr	nr	nr	nr	nr

[a]+ = Hypersensitive reaction; − = compatible reaction; and nr = no reaction.
[b]The value under the A (α band, nonamylolytic) or B (β band, amylolytic) column indicates the number of A or B strains expressing the race phenotype.
[c]ECW = Early Calwonder; ECW-10R, ECW-20R, and ECW-30R = ECW with incorporated resistance genes *Bs1*, *Bs2*, and *Bs3*, respectively.

TABLE 4. Phenotypic characteristics of *Xanthomonas campestris* pv. *vesicatoria* strains

Strain	Race[a]	Serovar[b]	FAME[c]	Biolog[d]
81-18	T1	A1	V	III
599	T1	A1	VI	III
8, 31, 35, 128, B82, B111, LMG 929	T1	A1	VIII	III
75-4	T1	A2	VI	I
63	T1	A2	VI	VIII
121	T1	A3	VI	III
39, 67, 85, 116, 118, 127, 820, 821	T1	A3	VIII	III
71-4	T2	B0	I	XI
CNBP 30	T2	B0	VIII	XI
B61, BV5-3A	T2	B1	VII	U.Ph.
643, 0350, LMG 925	T2	B1	VII	IV
0226, B122, X525-85, XV-1	T2	B1	X	IV
BV5-4B, BV20-3A	T2	B1	X	V
ATCC 35937, LMG 916	T2	B1	VII	VI
IAPAR 8012, IAPAR 8071, IAPAR 9697, BV4-1, XV8035	T2	B1	VII	VII
BV1-1	T2	B1	X	VII
LAB2, LMG 917	T2	B1	VII	XI
BV6-1	T2	B1	X	XI
BV7-3A	T2	B2	X	U.Ph.
BV5-4A	T2	B2	VII	IV
BV8-1, CNBP 1545-92, XV10, XV15	T2	B2	VII	VII
BV3-1, BV5-1	T2	B2	X	VII
B80, BV3-5	T2	B2	VII	XI
IAPAR 9696	T1P0	A1	VI	III
IAPAR 9700	T1P0	A1	VIII	III
87-40	T1P1	A1	VII	I
A3, 87-56	T1P1	A1	IX	I
EWCII	T1P1	A1	I	III
37, CNBP 2484	T1P1	A1	VIII	III
A1	T1P1	A1	IX	III
80-5	T1P1	A2	II	III
985-B7	T1P1	A2	I	VIII
936	T1P2	A1	VIII	U.Ph.
91-72	T1P2	A1	I	I
ATCC 11633	T1P2	A1	X	I
86-46	T1P2	A1	I	II
75-3	T1P2	A1	II	III
91-68, 91-77	T1P2	A1	IV	III
91-76	T1P2	A1	V	III
CNBP 1604	T1P2	A1	VI	III
58, 122, 915, IAPAR 9699, IAPAR 9701, 76-4, 91-66, 91-67, 91-71, 91-74, 91-78, 91-79, 91-80, B95	T1P2	A1	VIII	III
18	T1P2	A1	U.Ph.	VIII

(*continued on next page*)

[a]Race based on reaction of pepper and tomato lines (Table 3).
[b]Serovar according to enzyme-linked immunosorbent assay reaction patterns (Table 5).
[c]Clusters and unique phenotypes (U.Ph.) based on cluster analysis of fatty acid methyl ester (FAME) composition (Fig. 4 and Table 6).
[d]Clusters and unique phenotypes (U.Ph.) based on cluster analysis of Biolog carbon substrate utilization assay (Fig. 3).
[e]Nonpathogenic strain.

Serological characterization by use of MAbs. All 36 strains of *X. c. vesicatoria* tested in preliminary screenings reacted with *Xanthomonas*-specific MAb X1, but they were heterogeneous with respect to reactions with the 16 newly generated *X. c. vesicatoria* MAbs. No single MAb reacted with all strains. Strains were evenly distributed among four clusters with similarities greater than 80% (Fig. 2). With the exception of one group B strain (BV20-3A), clusters I and III included only group A strains, whereas clusters II and IV were made up of group B strains.

On the basis of this analysis, MAbs Xv1, Xv5, Xv8, Xv10, Xv15, and Xv21 were selected for serotyping the 160 strains. Only three strains representing other pathovars of *X. campestris* reacted with these MAbs, but none had reaction profiles similar to those of *X. c. vesicatoria*. MAbs Xv1 and Xv21 reacted specifically with strains of the A group, whereas MAbs Xv8 and Xv15 reacted with group B strains. Reaction profiles obtained from the screening of *X. c. vesicatoria* strains with the six MAbs identified six serovars, three in each group (Tables 4 and 5). Serovar A1 was common, making up 70% of the A strains. In group B, more than half of the strains typed to serovar B1. Both the neopathotype and the *P. gardneri* strains belonged to serovar B1 (Tables 4 and 5); three B strains did not react with any of the MAbs used for serotyping (serovar B0).

Carbon substrate utilization. Thirteen carbon sources (Tween 40, fructose, α-D-glucose, maltose, sucrose, D-trehalose, methyl pyruvate, mono-methylsuccinate, α-ketoglutaric acid, succinic acid, bromosuccinic acid, alaninamide, and L-glutamic acid) were metabolized by all *X. c. vesicatoria* strains tested. None of the following 45 carbon sources was metabolized by these strains: α-cyclodextrin, *N*-acetyl-D-galactosamine, adonitol, L-arabinose, D-arabitol, *i*-erythritol, *m*-inositol, α-lactose, β-methyl-D-glucoside, D-raffinose, L-rhamnose, D-sorbitol, turanose, xylitol, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketovaleric acid, quinic acid, sebacic acid, glucuronamide, L-asparagine, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-pyroglutamic acid, D-serine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, DL-α-glycerophosphate, glucose 1-phosphate, and glucose 6-phosphate. The only substrate that discriminated between strains of the A and B groups was *cis*-aconitate; 97% of the A strains and none of the B strains oxidized this compound.

The dendrogram, generated from cluster analysis of carbon substrate utilization patterns, presented distinct clusters of A and B strains (Fig. 3). However, clusters of A strains were interspersed

TABLE 4. (*continued from preceding page*)

Strain	Race[a]	Serovar[b]	FAME[c]	Biolog[d]
91-69, 91-73	T1P2	A1	VIII	VIII
91-70	T1P2	A1	VIII	IX
83-4, 91-75	T1P2	A2	VI	I
87-13	T1P2	A2	V	III
86-22, 87-48	T1P2	A2	VIII	III
85-16	T1P2	A3	VIII	II
929	T1P2	A3	VIII	III
XV1596	T1P3	A1	VIII	U.Ph.
87-21	T1P3	A1	VIII	I
82-4	T1P3	A1	U.Ph.	III
87-47	T1P3	A1	V	III
90-30, XCV1-A, XCV1-C, XCV1-D	T1P3	A1	VI	III
21, 985-A1, BA30-1, LMG 667	T1P3	A1	VIII	III
859-8	T1P3	A1	IX	VIII
90-39	T1P3	A1	I	IX
86-2	T1P3	A1	VI	X
90-29	T1P3	A1	VIII	X
69-1	T1P3	A2	I	I
PAP32	T1P3	A2	VIII	II
62	T1P3	A2	II	III
87-37, LMG 913	T1P3	A2	VIII	III
71-1	T1P3	A2	I	VIII
87-44	T1P3	A2	VI	VIII
90-27	T1P3	A2	IX	VIII
546, IAPAR 8015, IAPAR 8016, IAPAR 8020	T2P3	B1	VII	VII
CNBP 2625	T2P3	B1	VI	XI
ATCC 11551	T2P3	B1	X	XI
BA23-1, BA27-1, BA28-1	T2P3	B2	III	IV
BA21-1	T2P3	B2	VII	IV
IAPAR 8013	T2P3	B2	VII	VII
79-2	T2P3	B2	U.Ph.	XI
BA29-1	T2P3	B2	X	XI
81-6	T2P3	B3	VII	IV
XV32	P0	A1	VIII	III
125	P1	A1	I	III
597, 598, 601	P1	A1	VI	III
600, P2, 87-28, XV29, XV31	P1	A1	VIII	III
87-65	P1	A1	VI	VIII
A2	P1	A1	VIII	VIII
72-9	P1	A1	IX	VIII
XV12411	P1	A2	I	VIII
P6	P1	A2	VIII	VIII
BA26-1	P1	A3	IX	III
A1074, A1782	P3	A1	VIII	III
82-8, B108	P3	A2	VIII	III
89-10	P4	A1	VI	VIII
A571-1	...[e]	B0	II	IV
A135-1	...	B1	X	IV
Pseudomonas gardneri ICPB XG101	T2P1	B1	I	XI

with clusters of B strains. The *X. c. vesicatoria* clusters spanned the range of *X. campestris* pathovars tested. The neopathotype strain of *X. c. vesicatoria* belonged to cluster VI, whereas the *P. gardneri* type strain fell into cluster XI.

Analysis of cellular fatty acids. Fatty acids present in only one strain or in low concentration (<2%) were not included in Table 6; however, they were taken into consideration in the generation of the dendrogram (Fig. 4). The fatty acid detected in highest concentration was 15:0 iso followed by 16:1 ω7c (Table 6). Except for cluster IV, which consisted of two strains, group A strains had significantly lower amounts of 15:0 ante-iso than B strains did. This fatty acid was useful for differentiating the two groups of *X. c. vesicatoria.*

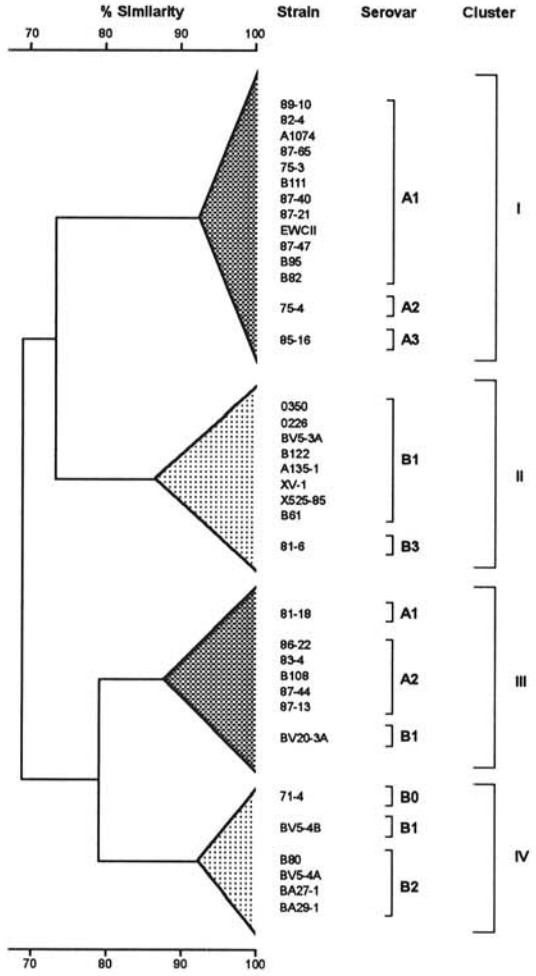

Fig. 2. Dendrogram showing the serological relationships among 36 strains of *Xanthomonas campestris* pv. *vesicatoria*. The dendrogram was derived from cluster analysis of enzyme-linked immunosorbent assay reaction with 16 monoclonal antibodies (MAbs). The serovar designation represents the serological typing of each strain with six selected MAbs (Table 5).

Most of the *X. c. vesicatoria* strains used in this study separated into 10 clusters at Euclidian distances above 6 (Fig. 4). Most B strains were clustered together and were separate from A strains. The A strains fell into clusters I, II, IV, V, VI, VIII, and IX, whereas B strains were grouped into clusters III, VII, and X; however, clusters I, II, VI, VII, VIII, and X contained a few strains of the other group (Fig. 4 and Tables 4 and 6). The *X. c. vesicatoria* clusters spanned the range of *X. campestris* pathovars tested. The neopathotype strain of *X. c. vesicatoria* belonged to cluster VII, while the type strain of *P. gardneri* fell into cluster I.

DISCUSSION

Analysis of our worldwide collection of tomato and pepper *X. c. vesicatoria* strains by protein profile analysis and amylolytic activity confirmed the presence of the two groups (i.e., A and B) described by Stall et al (31); however, further characterization showed that the A and B groups were very diverse phenotypically. The clear separation between the A and B groups obtained by DNA-DNA hybridization (31) was not possible when cluster analyses were performed on data generated by ELISA (Fig. 2), carbon substrate utilization (Fig. 3), and fatty acid (Fig. 4) assays. However, group A and B strains generally formed separate, albeit interspersed, clusters, suggesting the existence of common features among strains of the same group. This interspersing of A and B clusters in the different dendrograms suggests that the strain populations within each group are heterogeneous. In addition, there is apparently no concordance between serovars, biovars, and chemovars, because there was no correspondence in the strain composition of clusters obtained by the three different assays. This lack of correspondence suggests a high level of phenotypic diversity within each *X. c. vesicatoria* group. The interspersing of A and B clusters renders useless these techniques for identification of the two genetic groups. However, fine analysis of the data revealed characteristics useful for distinguishing members of the two groups.

Of 95 carbon substrates tested, only *cis*-aconitate discriminated between strains of the two groups. This substrate may have diagnostic value or prove useful as a selective carbon source in media for the isolation of A strains. FAME analysis confirmed that 15:0 iso and 16:1 ω7c are the two fatty acids detected in highest concentrations among xanthomonads (40). Although Yang et al (40) reported that *X. c. vesicatoria* strains were relatively homogeneous in their fatty acid composition, our collection of strains

TABLE 5. Serological reaction patterns defining serovars of *Xanthomonas campestris* pv. *vesicatoria*

Serovar	ELISA reaction[a] with monoclonal antibody					
	Xv1	Xv5	Xv8	Xv10	Xv15	Xv21
X. c. vesicatoria						
A1 ($n = 77$)	+	+	−	+	−	+
A2 ($n = 21$)	−	−	−	−	−	+
A3 ($n = 12$)	+	−	−	+	−	−
B0 ($n = 3$)	−	−	−	−	−	−
B1 ($n = 29$)	−	+	+	+	−	−
B2 ($n = 17$)	−	−	−	−	+	−
B3 ($n = 1$)	−	−	+	+	−	−
Cross-reacting strain from other taxa						
Pseudomonas gardneri ICPB XG101	−	+	+	+	−	−
X. c. alfalfae A	+	+	−	+	−	−
X. c. armoraciae A	−	+	+	+	−	+
X. c. campestris ATCC 33913	−	−	−	+	−	−
Strains from other taxa[b]	−	−	−	−	−	−

[a]ELISA = enzyme-linked immunosorbent assay. A reaction of 0.12 (arbitrary threshold three times higher than absorbance of heterologous strains) or greater was considered positive.
[b]Listed in Table 1.

presented a range of fatty acid profiles, and we have identified one major fatty acid (i.e., 15:0 ante-iso) that was present in amounts different in the two groups of strains. Although two A strains that make up cluster IV had levels of this acid similar to those of B strains, amounts of 15:0 ante-iso in *X. c. vesicatoria* strains may be used to help differentiate the two groups. The dendrograms generated from cluster analysis of carbon substrate utilization and FAME patterns included the different pathovars tested, indicating that *X. c. vesicatoria* strains were as diverse in their fatty acid content and carbon substrate utilization ability as the strains of the different *X. campestris* pathovars tested.

Serological reaction profiles obtained by ELISA with selected MAbs distinguished strains according to their group affiliation; four MAbs were helpful in identifying group-specific antigens. The group A strains could be identified with MAbs Xv1 and Xv21; all but three of the B strains could be identified with MAbs Xv8 and Xv15. There would be some reaction with other pathovars, but with the heterogeneity of this pathogen, this cannot be avoided. Our data confirmed the relationship between the presence of certain epitopes and amylolytic activity in this group of bacteria (30). A similar observation was reported in another *X. campestris* pathovar (22). Although a clear correlation exists between pathogenicity on tomato and group affiliation, no such correlation can be drawn between pathogenicity on pepper and serovar. Conflicting information on the correlation between serovars and pathogenicity on tomato or pepper occurs in the literature (6,13,23,26,30). These discrepancies are probably caused by the use of different sets of strains; at the time, races were not defined, and the A and B groups that make up the *X. c. vesicatoria* population have only recently been identified (4,31,36).

Strains of the A and B groups differed in pathogenicity. Strains that infected only pepper were members of the A group, whereas the less common B strains were more likely to infect tomato. T1 strains comprised group A, while T2 strains were assigned to group B. By considering amylolytic activity as an indicator of the strain affiliation with the A or B group, our data confirmed the observation made by other authors (5,12) that B (i.e., amylolytic) strains are more likely to be recovered from tomato than pepper. With the exception of the 14 T2P3 strains, none of the strains that infected pepper was of the B group. The absence of T2P0, T2P2, and T2P4 strains in this diverse collection is peculiar and may be caused by the nonrepresentative nature of our culture collection or the lower fitness of strains with these pathogenic reactions. Another explanation for the absence in any of the B strains tested of the plasmid-borne *avrBs1* (32) and *avrBs3* (2) genes may be the presence of native plasmids in B strains that are incompatible with plasmids carrying these avirulence genes. The distribution of the predominant pathogenic races was worldwide. This is not surprising, considering the international traffic of seeds and the seedborne nature of the pathogen (16). Exceptions to this wide distribution of races was the prevalence of T2 strains in South America and the limited distribution of P2 strains, which were found only in the Western Hemisphere.

Careful scrutiny of the serological, physiological, and fatty acid data revealed further information that supports the presence of two distinct *X. c. vesicatoria* subpopulations previously identified in a limited study of 20 strains (31). Strains in the A group were unable to hydrolyze starch. They utilized *cis*-aconitate; contained lower amounts of the fatty acid 15:0 ante-iso; expressed the α protein band; reacted with MAbs Xv1, Xv5, Xv10, or Xv21 but not with MAbs Xv8 and Xv15; and were generally pathogenic on pepper but not on the tomato genotype Hawaii 7998. On the other hand, the B group strains were strongly amylolytic and unable to oxidize *cis*-aconitate. They contained higher amounts of the fatty acid 15:0 ante-iso; expressed the β protein band; reacted with MAbs Xv5, Xv8, Xv10, or Xv15 but not with MAbs Xv1 and Xv21; were rarely pathogenic on pepper; and were pathogenic on Hawaii 7998.

These two groups of pathogens causing bacterial spot may represent the strains originally isolated by Doidge (9) and Gardner and Kendrick (14) that differed in their amylolytic ability. Our B strains were strongly amylolytic, hydrolyzing 1% starch in 2 days, whereas A strains did not. When tubes with nutrient broth were supplemented with 0.1% starch and inoculated by Doidge's protocol, we found that A strains were able to hydrolyze starch after 14 days (*unpublished*), as described for Doidge's *B. vesicatorium* strain. Thus, the A strains had very weak amylolytic

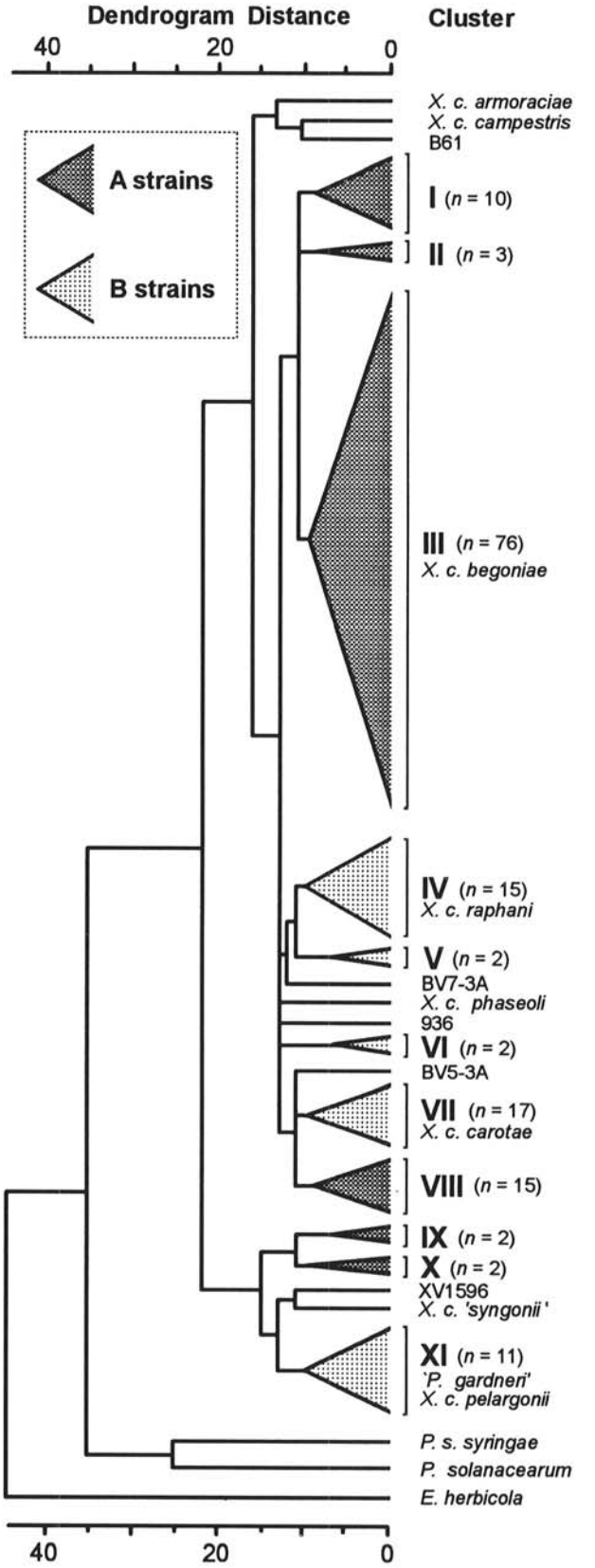

Fig. 3. Relationships among strains of *Xanthomonas campestris* pv. *vesicatoria* on the basis of utilization patterns of carbon substrates available on the GN Microplate (Biolog Inc., Hayward, CA). In parentheses is the total number of strains in the cluster.

TABLE 6. Proportion (mean percentage) of major (>2%) fatty acids in clusters and outlier strains of *Xanthomonas campestris* pv. *vesicatoria*[a]

	Unique phenotype			Cluster[b]									
Fatty acid	79-2	82-4	18	I	II	III	IV	V	VI	VII	VIII	IX	X
11:0 iso	3.0	2.8	2.0	2.8 ± 0.5	3.8 ± 0.8	3.5 ± 0.5	4.3 ± 0.2	3.9 ± 0.7	3.5 ± 0.4	3.9 ± 0.4	3.8 ± 0.6	2.9 ± 0.4	3.3 ± 0.4
Unknown 11.798	1.4	1.8	2.7	1.7 ± 0.6	1.7 ± 0.1	1.2 ± 0.1	1.6 ± 0.1	1.7 ± 0.2	1.8 ± 0.2	1.5 ± 0.1	1.7 ± 0.1	1.6 ± 0.3	1.5 ± 0.1
11:0 iso 3-OH	1.4	1.7	3.2	1.6 ± 0.2	2.0 ± 0.4	2.3 ± 0.3	2.1 ± 0.2	2.0 ± 0.3	1.9 ± 0.2	2.2 ± 0.2	1.9 ± 0.2	1.5 ± 0.1	2.0 ± 0.2
12:0 3-OH	5.2	4.2	6.7	4.1 ± 0.8	2.7 ± 0.3	2.8 ± 0.3	2.2 ± 0.3	2.7 ± 0.3	3.2 ± 0.4	tr[c]	2.6 ± 0.3	3.1 ± 0.3	3.8 ± 0.5
14:0	18.8	4.3	2.4	2.3 ± 0.6	1.2 ± 0.2	tr	tr	tr	1.6 ± 0.3	1.1 ± 0.2	tr	1.2 ± 0.2	1.8 ± 0.5
13:0 iso 3-OH	2.1	3.2	6.4	3.8 ± 0.4	4.3 ± 0.4	2.8 ± 0.4	4.8 ± 0.1	4.5 ± 0.6	4.2 ± 0.5	3.3 ± 0.3	4.2 ± 0.5	3.3 ± 0.3	2.9 ± 0.5
15:0 iso	17.2	35.0	26.6	23.9 ± 1.5	33.0 ± 0.7	19.4 ± 0.9	26.9 ± 1.4	23.0 ± 0.9	27.9 ± 2.0	25.4 ± 2.1	26.3 ± 1.5	21.4 ± 1.1	24.5 ± 1.9
15:0 ante-iso	7.8	3.2	6.7	7.1 ± 1.5	8.9 ± 1.9	16.3 ± 0.9	12.9 ± 1.0	10.6 ± 0.7	9.5 ± 1.5	15.0 ± 1.4	11.6 ± 0.8	11.7 ± 0.9	13.3 ± 2.0
16:0 iso	...[d]	...	1.3	1.3 ± 0.4	1.8 ± 1.0	2.0 ± 0.2	2.8 ± 0.2	1.8 ± 0.7	1.5 ± 0.4	2.2 ± 0.4	1.9 ± 0.4	2.3 ± 0.4	1.7 ± 0.4
16:1 ω9c	2.9	1.0	tr	1.6 ± 0.3	1.3 ± 0.4	1.4 ± 0.1	tr	1.2 ± 0.2	1.7 ± 0.2	1.3 ± 0.2	1.5 ± 0.2	1.4 ± 0.3	1.6 ± 0.2
16:1 ω7c	24.5	27.5	23.5	23.5 ± 1.7	17.1 ± 2.6	15.2 ± 0.5	12.7 ± 1.0	15.7 ± 1.3	20.9 ± 1.4	15.7 ± 1.1	17.9 ± 1.1	20.0 ± 1.1	19.3 ± 1.0
16:0	3.4	6.8	4.6	6.8 ± 1.2	2.6 ± 0.2	3.8 ± 0.3	1.7 ± 0.2	2.7 ± 0.5	4.2 ± 0.7	3.3 ± 0.5	3.0 ± 0.7	5.4 ± 1.4	5.1 ± 0.8
iso 17:1 ω9c	...	3.9	5.5	6.4 ± 1.0	8.9 ± 2.1	9.1 ± 0.3	13.0 ± 1.2	12.3 ± 1.0	7.5 ± 1.0	8.3 ± 0.7	9.9 ± 1.0	8.3 ± 1.2	6.5 ± 0.9
17:0 iso	...	3.5	5.2	7.6 ± 1.2	5.9 ± 0.4	9.2 ± 0.5	7.9 ± 0.3	10.9 ± 1.3	7.3 ± 1.0	7.3 ± 1.0	7.7 ± 0.9	9.2 ± 1.2	6.9 ± 1.1
17:1 ω8c	...	...	...	tr	tr	2.2 ± 0.1	tr	tr	tr	tr	tr	tr	tr

[a]Clusters and unique phenotypes based on cluster analysis of fatty acid content.
[b]Mean percentage ± standard deviation.
[c]Trace amounts (i.e., <1% of cellular fatty acid content).
[d]Not detected.

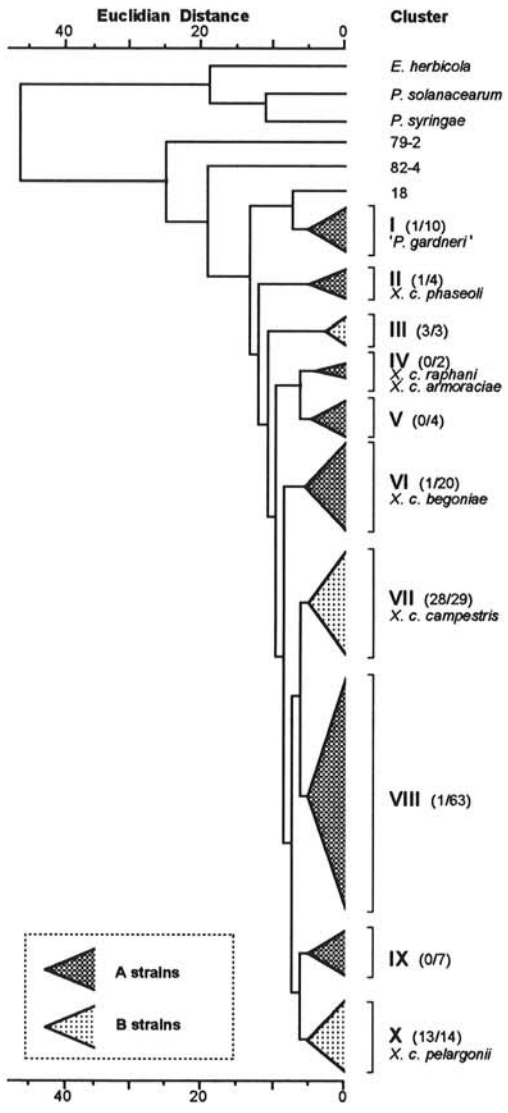

Fig. 4. Relationships among strains of *Xanthomonas campestris* pv. *vesicatoria* on the basis of cellular composition in fatty acids as analyzed by gas-liquid chromatography. In parentheses is the number of group B strains out of the total number of strains in the cluster.

ability, which was similar to that of *B. vesicatorium,* whereas the B strains, with their strong amylolytic activity, resembled Gardner and Kendrick's *B. exitiosum*. Direct comparison between our strains and the original *B. vesicatorium* and *B. exitiosum* strains is not possible, because we do not possess the original strains. The neopathotype strain of *X. c. vesicatoria* (ATCC 35937) is amylolytic and does not appear to represent Doidge's bacterium.

The type strain of *P. gardneri* resembled *X. c. vesicatoria* group B on the basis of serology, carbon substrate utilization (i.e., inability to oxidize *cis*-aconitate), pathogenicity on Hawaii 7998, and the presence of a protein band that migrated at the position of the β band. However, the *P. gardneri* strain differed from group B strains on the basis of its inability to hydrolyze starch and its fatty acid profile (i.e., 5.3% of 15:0 ante-iso). Other authors have reported that *P. gardneri* differed from the neopathotype strain (i.e., B group) by DNA-DNA hybridization (19) and by cluster analysis of carbon sources utilization data (18). These data provide further evidence that *P. gardneri* is a xanthomonad (39), which is different from the neopathotype strain of *X. c. vesicatoria* (8,19,35).

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