Identification of Xanthomonads and Grouping of Strains of *Xanthomonas campestris* pv. *campestris* with Monoclonal Antibodies

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ABSTRACT

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Monoclonal antibodies (MCAs) were produced that identified the genus *Xanthomonas*, distinguished *Xanthomonas campestris* pv. *campestris* (*X.c.* pv. *campestris*) from other pathovars of *X. campestris*, and grouped and subgrouped strains of *X.c.* pv. *campestris*. One antibody, X1, was specific for all 436 xanthomonads that were tested, but it did not react with 104 bacteria of 12 other genera that were tested. Only crucifer strains (*X.c.* pv. *campestris* and two strains of *X.c.* pv. *armoraciae*) reacted with one or more of three MCAs (X9, X13, and X17) whereas 192 xanthomonads of other species and pathovars failed to react with any of these MCAs. The 104 strains of other genera and species also failed to react with these antibodies. Two hundred *X.c.* pv. *campestris* strains from Hawaii, Georgia, California, and Thailand were divided into six groups based on reactions with five MCAs, and into subgroups based on these and other MCAs. Most (83%) strains were in groups 1, 2, and 5; however, the strains from Hawaii and Georgia, the largest sample sizes, were distributed differently among these groups. These MCAs may be applied as markers for rapid identification of *X.c.* pv. *campestris* strains and for tracing strains in epidemiological studies of black rot.

Black rot of crucifers caused by *Xanthomonas campestris* pv. *campestris* (*X.c.* pv. *campestris*) is a seedborne bacterial disease that affects cruciferous crops worldwide (21). Bacteria survive in crop residues in soil (19) and on weed hosts (18) and are spread by windborne rain, aerosols (11), and insects (21). Control measures, other than varietal resistance, are based on reduction of inoculum since susceptible plants may become infected throughout the cropping cycle. This aspect is particularly important in humid tropical areas where short crop rotations on small farms promote a buildup of inoculum in crop residues (1).

Assessment of the relative importance of different inoculum sources of a pathogen depends on the ability to differentiate unique strains of the pathogen. By using phage typing (12) and serology with conventional antisera (3,4), we were able to detect the pathogen in seeds, plant debris, and soil, but we were unable to differentiate unique strains of *X.c.* pv. *campestris* on Hawaiian cabbage farms. Thus, we initiated a study on the use of somatic cell hybridization (10) to generate monoclonal antibodies (MCAs) to identify strains of *X.c.* pv. *campestris* and to differentiate *X.c.* pv. *campestris* from other pathovars and species of *Xanthomonas* (22). The hybridoma technique is used extensively in biology and medicine to produce antibodies specific for single antigenic determinants associated with viruses, bacteria, and a variety of cells (14–16). To date, little use has been made of the technique for the identification and classification of phytopathogenic bacteria. We report here the identification of *X.c.* pv. *campestris* and a key for grouping and subgrouping strains of *X.c.* pv. *campestris* by using selected MCAs.

MATERIALS AND METHODS

Bacterial strains. Strains of *X.c.* pv. *campestris*, other pathovars of *X. campestris*, and unrelated genera and species were isolated in Hawaii or were provided by many donors from different geographical locations.

The genera and species with the numbers and designations of strains tested from each taxon are given in Table 1. The hosts and sources of the 540 strains used in this study (Table 1) are recorded as part of the culture collection maintained at the Department of Plant Pathology, University of Hawaii. In 1982, 160 isolations were made from diseased cabbage plants collected from 12 farms in Kula, Maui, at elevations ranging from 488 to 1,006 m. Five separate sites were selected at random from each of three to four cabbage fields per farm. Samples were taken from wrapper leaves of mature cabbage plants showing V-shaped lesions characteristic of black rot. Cultures were purified by repeated dilution streaking and preserved in sterile distilled water (SDW) at 6 C. Pathogenicity of strains of *X.c.* pv. *campestris* was tested by cutting 1-mm V-shaped notches into vein endings at leaf margins of 3- to 6-wk-old cabbage plants with a razor blade and submerging notched leaves in a water suspension of bacterial cells containing about 10^7 cells per milliliter.

Cultures received from other laboratories were first streaked on tetrazolium chloride medium (9) to check for purity, then grown on yeast-glycerol agar (YGA) at 28 C for 48–72 hr and collected in 0.01 M phosphate-buffered saline (PBS) (pH 7.4) for subsequent assay. If assays were not carried out immediately, cells were stored at 6 C in PBS containing 0.5% formalin and duplicate cultures were stored in SDW for further culturing.

Production of MCA. BALB/c mice were immunized twice, 14 days apart, by intraperitoneal injection of 10^8 living cells of either cabbage strain A249 (Hawaii) or cabbage strain OK2 (Japan). For another fusion, mice were immunized with a mixed suspension of strains A249, OK2, A342, RR68, and A674. A booster injection was given 2–3 days prior to hybridization. The mouse spleens were removed and 5×10^7 spleen cells were mixed with 5×10^7 washed P3-X63-Ag8.653 myeloma cells (8), centrifuged, and fused with 45% polyethylene glycol (MW 1300–1600) (American Type Culture Collection, Rockville, MD). The cells were washed and resuspended in Dulbecco's modified Eagle's medium with 10% fetal calf serum, 1 mM sodium pyruvate, 0.1 mM hypoxanthine, 0.0004 mM aminopterin, 0.016 mM thymidine, and 1% nonessential amino acid solution (Gibco Laboratories, Grand Island, NY), 10%. NCTC 109 lymphocyte growth medium (Microbiological Associates, Bioproducts, Walkersville, MD) and dispensed into at least five 96-well culture plates (Costar, Cambridge, MA). The culture plates were prepared the previous day to contain mouse

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peritoneal macrophage "feeder" cells. The peritoneal washings of one mouse were sufficient to coat four culture plates. After 7–10 days, the supernatant fluids from wells with growing colonies were screened by radioimmunoassay (RIA) for antibody reactions to 11 xanthomonads and a control organism. For screening, 7 strains of *X.c.* pv. *campestris* (A249, OK2, PHW46, A902, EEXC 114, A342, and RR68) were selected to represent the range of phage types and geographical origins based on previous studies (12,13). Three lettuce strains (10TB10, A674, and S4-1) and an onion strain (A88) were included to represent strains obtained from crops rotated with cabbage. A strain of *Erwinia herbicola* (Eh1) was included as a control to represent a common epiphyte on cabbage leaves. Based on the binding assays, selected antibody-producing hybrids were cloned and subcloned by limiting dilutions to ensure monoclonality of hybridomas. Unstable antibodies and those showing reactivity patterns identical to those produced by previously subcloned hybridomas were eliminated. Selected stable clones were grown as ascites by injecting pristane-primed BALB/c mice intraperitoneally with approximately 10^6 hybridoma cells. Resultant ascitic fluids, referred to as MCA, were collected, clarified by centrifugation, and stored at −20 C.

Radioimmunoassay. For solid phase RIA, formalinized bacterial cells were washed three times in PBS, resuspended in 0.05 M carbonate-bicarbonate buffer (pH 9.6), and adjusted to $A_{600\ nm}$ = 0.1 OD on a spectrophotometer. The cells in 50 microliters of cell suspension were adsorbed to the wells of polyvinylchloride microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA) by drying in a 37-C circulating-air incubator. The plates were stored in the refrigerator until used. The plates were washed three times with borate buffer (pH 8.3) and rinsed with PBS saline containing 1% bovine serum albumin to block nonspecific protein binding. Fifty microliters of each of the following reagents were added sequentially, and each was incubated 1 hr at room temperature followed by three washes with borate buffer: MCA, rabbit antimouse globulin, and Protein A (Pharmacia Fine Chemicals, Piscataway, NJ) labeled with ^{125}I. Each microtiter plate well was cut, placed in a counting vial, and counted in a well-type gamma scintillation counter. Radiolabeling was based on enzymatic (lactoperoxidase and glucose oxidase) iodination with Enzymobeads™ (Bio-Rad Laboratories, Richmond, CA).

Isotype determination of MCA. The RIA was modified for this purpose by the addition of 50 μl of rabbit antimouse immunoglobulin heavy chain-specific antisera (Litton Bionetics, Inc., Kensington, MD) to dilutions of MCA that had been adsorbed to bacteria-coated plates. ^{125}I-labeled Protein A was added, and counts were made as above.

Agglutination reactions. To determine the ability of MCA to agglutinate bacteria to which the antibodies have strong binding, 0.5-ml amounts of formalinized bacteria adjusted to a McFarland Standard No. 9, were added to doubling dilutions (1:15 to 1:5,120) of MCA. After incubation for 2 hr at 37 C followed by refrigeration overnight, the agglutination reactions were read.

RESULTS

Pathogenicity tests. Plants inoculated with *X.c.* pv. *campestris* strains produced well-defined V-shaped lesions characteristic of black rot within 7–10 days after inoculation. Strains that did not produce symptoms were not included in the serological study. Four strains, A342, RR68, K85, and K86, produced atypical plant reactions in that blackening of the veins was not accompanied by

	Reactivity with bacterial strains												Antibody characterization	
	Xanthomonas campestris pv. campestris							Xanthomonas campestris pv. vitians						
	cabbage					br	rad	lettuce			on			
MCA Xcc	A249	OK2	PHW46	A902	EEXC 114	A342	RR68	A674	10TB10	S4-1	A88	E. herbicola	Isotype (Ig)	Agglutination
X1	■	■	■	■	▨	■	■	■	■		■		IgG2a	−
X2	■	■	■	■	▨	■	■	■	■				IgM	−
X3	■	■	■	■					▨				IgG3	+
X4	■	■	■	■	▨	■	■	■	▨				IgG2a	−
X8	■	■	■	■	■	■	■	■	■	■	■	■	IgM	−
X9	■	■	■	■			■						IgM	−
X10							■						IgG2a	−
X11	■	■	■	■	■	■	■	■	■		■		IgM	−
X12	▨	▨	▨	▨		▨		■	■				IgM	+
X13					■								IgM	−
X15	■	■	■										IgM	−
X16	▨	▨	▨	▨		■	▨	■	■				IgG3	+
X17	■	▨	■	■	▨								IgM	−

Fig. 1. Reactivity patterns determined by radioimmunoassay of monoclonal antibodies with strains of *Xanthomonas campestris* pv. *campestris* isolated from cabbage, broccoli (br), and radish (rad), strains of *X.c.* pv. *vitians* isolated from lettuce, and an undescribed pathovar that causes leaf blight of onion (on). *Erwinia herbicola* was included to represent an unrelated genus. Isotype characterization and agglutination reactions are described in Materials and Methods. Dark squares represent high reactions, hatched squares represent intermediate reactions, and blank squares are negative reactions.

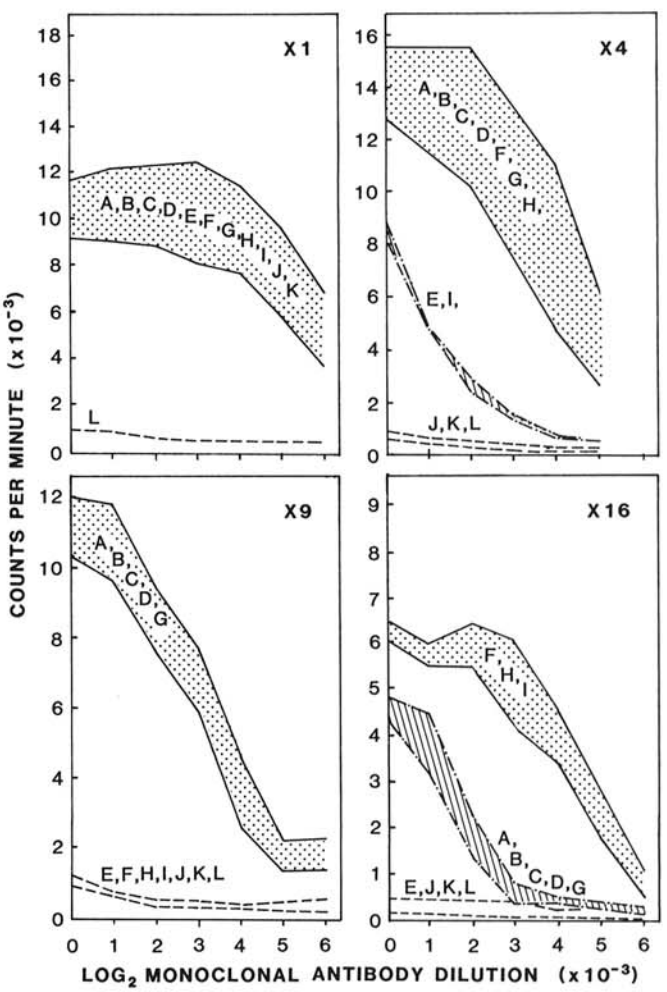

Fig. 2. Examples of binding curves determined by radioimmunoassay for monoclonal antibodies X1, X4, X9, and X16 with intact bacterial cells of the following strains: A249,(A); OK2, (B); PHW46,(C); A902, (D); EEXC 114, (E); A342, (F); RR68, (G); A674, (H); 10TB10, (I); S4-1, (J); A88 (K); *Erwinia herbicola*, Eh1, (L). Dotted areas show range of high reactions, hatched areas are intermediate reactions, and clear areas between dotted lines are low reactions.

V-shaped zones of chlorosis. Furthermore, the blackening extended 0.5–1.0 mm into the parenchymatous tissue surrounding the veins.

Selection and inital screening of MCA. Each of five fusions resulted in more than 50% of the microculture wells with hybrids. An average of 18% of the hybrids in the five fusions produced antibodies to the reference strains used for screening. Of the numerous MCAs produced, only those shown in Fig. 1 were useful for serotyping of *X.c.* pv. *campestris*, and these are referred to as X1, X2, etc. Also shown in Fig. 1 are the immunoglobulin isotypes and the ability of the MCAs to agglutinate appropriate bacteria. Selection of specific MCAs was based on repetitive binding curves, examples of which are shown in Fig. 2. Binding was classified as high, intermediate, weak, or negative. Intermediate values varied from high to low intermediate values.

Antibody X1 reacted with all xanthomonads but not with *E. herbicola*, and X11 reacted with all xanthomonads except a lettuce strain (S4-1) and *E. herbicola* (Fig. 1). Four reference cabbage strains (A249, OK2, PHW46, A902 from Hawaii, Wisconsin, Japan, and Hawaii, respectively) showed identical reactions with all MCA X1 to X13 but not with X15 and X17 (Fig. 1). Strain A902 was distinguished from the other three strains on the basis of a negative reaction to X15. Strain EEXC 114 from North Carolina differed from the other cabbage strains in reactions with MCA X2, X3, X4, X9, X12, and X16, and it was the only reference xanthomonad that reacted with X13. Two strains (A342 and RR68) could be separated from the others on the basis of reactivity patterns. Only RR68 reacted with X10. Antibody X8 reacted with all bacteria tested, and therefore was used as a positive control in RIA to confirm the presence of a sufficient number of bacteria on the assay plates.

Based on these data, 540 bacterial strains were screened with a 1:1,000 dilution of various MCAs. This dilution gave maximum binding of each antibody with all reference strains.

MCAs of *Xanthomonas*. X1 was specific for xanthomonads because it reacted with 436 known strains of *Xanthomonas* tested including different pathovars of *X. campestris*, different *Xanthomonas* species, and undescribed strains of *Xanthomonas* pathogenic to local hosts (Table 2). Antibody X1 did not react with 104 bacterial strains of other genera and species from diverse

TABLE 1. Bacterial strains tested for reactivity with monoclonal antibodies produced against *Xanthomonas campestris* pv. *campestris*

Strains tested (no.)	Representative strains[a]	Genus/species or pathovar	Received from[b]
		Xanthomonas	
6	LS2L, LS2S, LS13, LS14, LS20, LS64	*X. albilineans*	4
2	XF3, XF4	*X. fragariae*	8
		X. campestris	
3	XLS-2, 417, 756	pv. *armoraciae*	5,23
3	A912-2, A915, X45	pv. *begoniae*	1,6
244	A249, OK2, PHW46, A902, EEXC 114, A342, RR68, K1-K87, KC1-KC27	pv. *campestris*	1,7,9,12,24
	Georgia: 1-83 (81-2, 81-6, 82-7, 82-15, E1-E10, F1-F10, G1-G7, A1-A13, B1-B10, C1-C10, D1-D10)	pv. *campestris*	11
	California: 1-20 (370, 389, 402S, 402L, B56, B85, B87, DR1, DR3S, DR3L, DR5, DS135, DS136, DS137A, DS137B, DS141, DS474D, DS474E, DS476, X311)	pv. *campestris*	23
	Thailand: 1-10 (BT1, BT4, BT6L, BT6S, BT16, BT17, BT21, BT27, BT34, BT35)	pv. *campestris*	19
	Mexico: C1-C8, B1, B2	pv. *campestris*	10
6	ARCO, 1-ARCO 6	pv. *carotae*	17
28	XC62, XC63, XC64, XC69, XC70, Xcc7, Xcc18, Xcc36, Xcc43, Xcc45, Xcc49, Xcc51, Xcc52, Xcc62, Xcc63, Xcc70, Xcc84A, Xcc83B, Xcc84B, Xcc85B, Xcc87B, Xcc88B, Xcc89B, Xcc90B, Xcc91B, Xcc93B, Xcc94B, M3	pv. *citri*[d]	8,25
17	A755, A844, A858, A925, A990-1, A990-5, A100-3, A1007, A1007-3, A1024, A1026, B29, B41, B61A, QR33L, QR33S, X29	pv. *dieffenbachiae*	1,3,6
3	ATCC 29088, 29089, 29091	pv. *graminis*	2
1	X24	pv. *hederae*	6
1	XM 106	pv. *malvacearum*	22
3	XM1, XM4, XM6	pv. *manihotis*	8
16	PXO (40, 101, 61, 85, 86, 63, 79, 87, 71, 70, 112, 99, and 124), IRN 395, X03, X016	pv. *oryzae*	13, 15
1	X38	pv. *pelargonii*	6
13	F83-2, F83-6, S84-1, A747, A996-2, A996-3, A1004-2, A1004-4, A1004-5, A868, A869, A127	pv. *phaseoli*	1,21
4	A875-1, A912-2, X54, X3	pv. *poinsetticola*	1,6
12	X58, X87, X22, X25, X45, X155, X156, X157, RG2, RG18, RG20, RG21	pv. *translucens*	18
20	A134, A135-1, A428, A564-2, A913-1, A777-1, MB9, 71-21, 71-34, 72-7, 75-4, 81-27, 82-7, 82-12, 82-14, 82-17, 83-6, 83-14, E3, X6	pv. *vesicatoria*	1,6,14
24	A674, 10TB10, S4-1, A555-1, A558-1, A558-3, A563, A574-5, A592, A674-4A, A676-2A, A680, A782, A1057, S2, 10TB7, 10TB9, 166A, 167A, 170A, ATCC 11525, X31, X42, XY175	pv. *vitians*	1,2,6,7,22
29	A88, A85-1, A30-2A, A94-1C, A95-3, A136, A206, A225, A226, A227, A255, A274, A359, A374, A551, A554, A579, A763, A210, A420-2, A420-3, C121, A910-2, A910-3, A875-1, A912-2, KO-3-1, KO-2-2, A611	*X. campestris* undescribed pathovars[c]	1
		Agrobacterium	
1	UCBPP388	*A. tumefaciens*	20
1	UCBPP604	*A. rhizogenes*	20
		Erwinia	
3	A582-2, A582-3, A582-30	*E. atroseptica*	1
7	A815, A808-2, EC153, UC249-1, UC836-2, UC196-2, UC196-1	*E. carotovora*	1,7
2	A1042, A1073	*E. chrysanthemi*	1
4	Eh-1, Eh-103, A808-2, X022-a	*E. herbicola*	1,22

(continued)

animal and plant origins (Table 2). Attesting to the specificity of X1 for identification of xanthomonads, X1 reacted with only one of 41 yellow, Gram-negative, oxidative strains isolated from leaves and fruits of Mexican lime and other citrus showing symptoms of bacteriosis (2), and only this strain proved to be *Xanthomonas* by bacteriological and other tests including an enzyme-linked immunosorbent assay using antiserum specific for *X.c.* pv. *citri* plasmid profiles, phage sensitivity, and fatty acid analysis (E. L. Civerolo, *personal communication*). Likewise, in numerous screenings of yellow, Gram-negative, oxidative cultures isolated from various other hosts, antibody X1 failed to react with cultures which bacteriologically proved to be non-xanthomonads (5,6). X11 also appeared to be a xanthomonad-specific antibody. However, unlike X1, X11 failed to react with eight X1-positive strains (Table 2).

Crucifer MCA. All except 3 of 244 strains of *X.c.* pv. *campestris* reacted with at least one of three MCAs (X9, X13, and X17), whereas these MCAs did not react with 192 xanthomonads of other species and pathovars. However, two strains (XLS-2 and 756) of *X.c.* pv. *armoraciae* isolated from cabbage, reacted with X9 and X17 (Table 2). Most strains of *X.c.* pv. *campestris* were positive for either X9 and/or X17 (see below) whereas only 32 of 200 strains of *X.c.* pv. *campestris* were X13-positive.

Grouping of 200 crucifer strains with MCA. The strains of *X.c.* pv. *campestris* from Hawaii, Georgia, California, and Thailand (strains from the latter three locations were generously provided by R. Gitaitis, J. Watterson, and N. Schaad, respectively) were reacted with 1:1,000 dilution of each of the 13 MCAs given in Fig. 1. First, the reaction of each strain of *X.c.* pv. *campestris* with each antibody was rated as high (rating 3), intermediate (rating 2), very low (rating 1), and negative (rating 0) based on the numerical value (cpm) of the reference strains (Fig. 1) determined in the same assay. Then the numerical difference between the reaction of the reference strain of *X.c.* pv. *campestris* and the test strain of *X.c.* pv. *campestris* was determined for each antibody. The sum of the absolute differences was a measure of the seriological relationship of the test strain of *X.c.* pv. *campestris* to the reference strain. The lower the number the closer the relationship. A computer program was devised to expedite this calculation.

Three broad groups were evident: namely, strains closely related to strains A249, OK2, PHW, and A902; strain EEXC 114; and neither of these groups. Based on these results, the reactions of all strains of *X.c.* pv. *campestris* were then compared to the reactions of selected representatives from group iii by using the same calculations as given above. This procedure facilitated grouping; however, key MCAs were used to separate groups.

As expected, all strains reacted with the xanthomonad antibody, X1, and all but two strains reacted with at least one crucifer antibody (X9, X13, and X17). Antibody X10 reacted with the radish strain (RR68) and a few cabbage strains, and X12 gave intermediate reactions with numerous crucifer strains but high reactions to numerous lettuce strains; thus, these MCAs were not used for grouping or subgrouping. Finally, serogroups of *X.c.* pv. *campestris* were based on reactions with X3, X4, X11, X13, and X15. A key is presented in Fig. 3.

TABLE 1 (continued). Bacterial strains tested for reactivity with monoclonal antibodies produced against *Xanthomonas campestris* pv. *campestris*

Strains tested (no.)	Representative strains[a]	Genus/species or pathovar	Received from[b]
		Pseudomonas	
1	A715-1	*P. agaricus*	1
5	A909-1, A778-2, A778-3, A779-1, A779-2	*P. cichorii*	1
		P. syringae	
4	HB20, HB36, G50, A798-2	pv. *phaseolicola*	1,20
8	A616, QR85, QR86, QR87, QR88, A613, A476-1, A476-4, A476-8	*P. solanacearum*	1
		Corynebacterium	
1	ATCC 12975	*C. fascians*[c]	2
1	ATCC 6887	*C. flaccumfaciens*	2
1	ATCC 10253	*C. insidiosum*	2
4	A438, A518-5, A714-2, EECM1	*C. michiganense*	1,9
1	ATCC 9682	*C. poinsettiae*	2
1	ATCC 9850	*C. sepedonicum*	2
1	ATCC 13659	*C. rathayi*	2
		Bacillus	
3	M111	*B. subtilis*	16
	M112	*B. megaterium*	
	M113	*B. cereus*	
1	A518-3	Gram-positive soil saprophyte	1
6	A1055, A1056, A1057, A1058, A1059, A1060	Gram-negative soil saprophytes	1
31	M1, M3, M4-M32	Gram-negative, leaf epiphytes[d]	8
1	M114	*Alcaligenes faecalis*	16
2	ATCC 13048, A698	*Enterobacter aerogenes*	1,16
1	M115	*E. cloacae*	16
1	M116	*Escherichia coli*	16
1	ATCC 13883	*Klebsiella pneumoniae*	16
1	M117	*Micrococcus citreus*	16
1	M118	*M. luteus*	16
1	M119	*M. roseus*	16
3	M120(Grp A), M121(Grp C), M122(Grp D)	*Streptococcus* sp.	16
1	ATCC 27853	*Pseudomonas aeruginosa*	2
1	M123	*P. acidovorans*	16
1	M124	*P. fluorescens*	1

[a]The hosts and geographical origins of the strains is recorded at Department of Plant Pathology, University of Hawaii.

[b]1. Local isolation; 2. American Type Culture Collection; 3. M. Aragaki, Hawaii; 4. R. Birch, Hawaii; 5. L. Black, Louisiana; 6. A. Chase, Florida; 7. J. J. Cho, Hawaii; 8. E. L. Civerolo, Maryland; 9. E. Echandi, North Carolina; 10. L. Fucikovski, Mexico; 11. R. D. Gitaitis, Georgia; 12. M. Goto, Japan; 13. S. P. Y. Hsieh, Taiwan; 14. J. B. Jones, Florida; 15. T. W. Mew, Philippines; 16. Microbiology Department, University of Hawaii; 17. D. Robeson, California; 18. D. C. Sands, Montana; 19. N. W. Schaad, Idaho; 20. M. N. Schroth, California; 21. H. F. Schwartz, Colorado; 22. M. P. Starr, California; 23. J. C. Watterson, California; 24. P. H. Williams, Wisconsin; and 25. D. Zagory, Florida.

[c]Identity as *X. campestris* was established by bacteriological tests and pathogenicity was confirmed, but the pathovar status has not been described.

[d]Strains were received as formalin-killed preparations of cultures cultured on yeast-glycerol agar in Beltsville, MD.

[e]Strain was reclassified as *Rhodococcus rhodochrous*.

Most strains were X11-positive and X13-negative, thus forming groups 1–4. These groups were separated on the basis of reactions with X3, X4, and X15. Most of the strains were in groups 1 and 2 (positive for X2, X3, X4, X9, and X17), as were the reference cabbage strains A249, OK2, PHW, and A902, and these two groups were separated on the basis of reactivity with X15 (Fig. 3). Group 3 was separated by negative or very low reactions to X3 plus a positive reaction to X4. Group 4 was separated by a negative reaction to X4. Group 5, the third largest group, represented strains reacting with X13 (as did reference strain EEXC 114). Unlike the major groups 1 and 2, group 5 strains were X3-negative and most representatives were negative to X9. Four strains negative to the relatively common xanthomonad antibody (X11), and negative to most of the other MCAs except X17, were placed in group 6.

The geographical distribution of the 200 strains of *X.c.* pv. *campestris* based on group classification is shown in Table 3.

Subgroups. The reactions used for grouping and subgrouping strains of *X.c.* pv. *campestris* are presented in Table 4. A rapid key to the subgroups is presented in Fig. 4. Reproducible quantitative differences in binding with X16 were used for subgrouping because of the clear distinction between high (rating 3), intermediate (rating 2), and negative (ratings 0 or 1) reactions. Subgrouping with the other MCAs, for which intermediate values were not as distinct, was based on either positive (ratings 2 or 3) or negative (ratings 0 or 1) reactions. Groups 1 and 2 each were subdivided based on binding with X16. Group 3 was subgrouped on the basis of positive or negative responses to X15, and group 4 was subdivided on the basis of positive or negative reactions to X2, X3, and quantitative differences in reactions to X16. Group 5 was subdivided on the basis of reactions to X2, X15, and X16. Six group 5 strains, identified by positive reactions to X13, could not be placed into the four subgroups, and were left unclassified rather than subgrouped individually. Most (198 strains) of the 200 strains reacted with X9, X13, or X17. The two nonreacting cabbage strains (K85, K86) also failed to cause V-shaped zones of chlorosis in pathogenicity tests, and blackening or necrosis also extended into the parenchymatous tissue around the veins resembling symptoms produced by strains A342 and RR68. Three strains of *X.c.* pv. *armoraciae* also produced these symptoms following notch inoculation. The significance of these findings is being investigated.

TABLE 2. Numbers of xanthomonads and other bacteria reacting with monoclonal antibodies

Bacteria	Strains tested (no.)	Monoclonal antibodies		
		X1	X11	X9, X13, or X17
Xanthomonas campestris pv. *campestris*	244	244	240	241
X. campestris: other known pathovars	155	155	153	2[a]
X. campestris: undescribed pathovars	29	29	27	0
X. albilineans, *X. fragariae*	8	8	8	0
Other plant pathogens: *Erwinia*, *Pseudomonas*, *Agrobacterium*, and *Corynebacterium*	43	0	0	0
Bacteria of human, animal, and soil origins; leaf epiphytes	61[b]	0	0	0

[a]Two strains of *X. campestris* pv. *armoraciae* (XLS and 2,756) isolated from cabbage in California and Louisiana, respectively, reacted with X9 and X17.
[b]Eight genera and 15 species were tested.

DISCUSSION

Sufficient numbers of antibody-producing colonies were obtained from five fusions to yield MCAs capable of delineating strains of *X.c.* pv. *campestris*. The MCAs produced probably were those specific for surface antigens since selection was based on RIA in which whole cells were employed as antigen. Ten of the 13 MCAs used in this study failed to agglutinate appropriate bacteria indicating either a low density of surface antigens or low binding affinities of these antibodies, such that lattice formation was not possible. Nevertheless, all the MCAs bound sufficiently to give reproducible results both in solid-phase RIA and enzyme-linked immunosorbent assay (ELISA). The MCAs used in this study have been stable for the past 2 yr.

One antibody, X1, detected a common antigen found in all strains of *Xanthomonas* that were tested including different *Xanthomonas* species and pathovars of *X. campestris*. X11 also detected a common xanthomonad antigen in all except 8 of 436 strains.

Three MCAs (X9, X13, and X17) each detected different common antigens of *X.c.* pv. *campestris*. Only 3 of 244 strains of

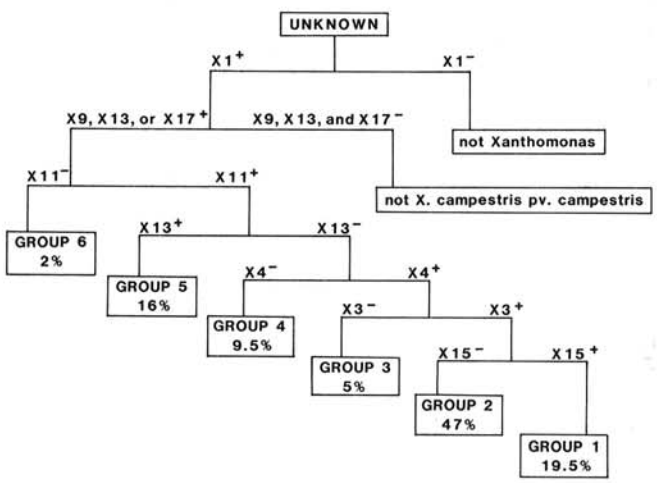

Fig. 3. Key to *Xanthomonas campestris* pv. *campestris* groups based on reactions to monoclonal antibodies. High or intermediate reactions were considered positive (+); very low or negative reactions were considered negative (−). Numbers in boxes are the percentage of 200 *X.c.* pv. *campestris* strains in that group.

TABLE 3. Group classification with monoclonal antibodies of 200 strains of *Xanthomonas campestris* pv. *campestris* from different geographical origins

Group	Hawaii		Georgia[a]		California[b]		Thailand[c]		Total strains	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent
1	19	21.8	12	14.5	7	35	1	10	39	19.5
2	58	66.7	27	32.5	7	35	2	20	94	47.0
3	1	1.2	6	7.2	1	5	2	20	10	5.0
4	4	4.6	12	14.5	3	15	0	0	19	9.5
5	3	3.4	23	27.7	2	10	4	40	32	16.0
6	0	0	3	3.6	0	0	1	10	4	2.0
X[d]	2	2.3	0	0	0	0	0	0	2	1.0
Total strains	87		83		20		10		200	

[a]Strains provided by R. D. Gitaitis, University of Georgia.
[b]Strains provided by J. C. Watterson, Woodland, CA.
[c]Strains provided by N. W. Schaad, University of Idaho.
[d]Strains were untypable since they were negative for X9, X13, and X17.

X.c. campestris isolated from crucifers failed to react to at least one of these antibodies. With the exception of two strains of *X.c.* pv. *armoraciae* that reacted with X9 and X17, the three antibodies appeared to be specific for *X.c.* pv. *campestris*.

Based on additional MCAs (X3, X4, and X15) that were not specific for *X.c.* pv. *campestris*, 200 strains of *X.c.* pv. *campestris* from four different geographical locations formed six serogroups. This grouping does not imply a closer genetic relationship among members of a group than with members of other groups. Most (83%) strains of *X.c.* pv. *campestris* were in groups 1, 2, and 5 (20, 47, and 16%, respectively), and only two MCAs (X13 and X15) were used to separate these groups. Interestingly, the group 5 serotype, characteristic of the reference strain from North Carolina (EEXC 114), was found more frequently among the Georgia cabbage strains. Whereas 28% of the Georgia strains were in group 5, only 3.4% of the Hawaiian strains were in this group. In contrast, 88% of the Hawaiian strains were in groups 1 and 2 and only 47% of the Georgia strains were in these groups. The number of strains tested from California and Thailand was insufficient to make such comparisons; however, 14 of 20 California strains selected from northern California farms were in groups 1 and 2 whereas the strains from Thailand, selected to represent a range of cultures isolated from that region (20), were fairly evenly distributed among five groups.

Antigenic variation must be considered when grouping the strains serologically. The reference strains (Fig. 1) have given essentially the same reactions to the MCAs over a period of 2 yr of subculturing on TZC and YGA media. Whether or not the 200 strains recovered for grouping also will maintain their antigenic stability is not known. The effect on antigenic variation from repeated passage through the host is being studied.

Subgrouping appears to become arbitrary as more and more specific MCAs are produced for individual strains of an organism; nevertheless, with the MCAs of specificities selected in this study, subgrouping seemed reasonable. As with grouping, the antigens used for subgrouping have been stable in the reference strains for 2 yr. As pointed out earlier, antigen-antibody binding curves for X16 indicated clearcut differences between high, intermediate, and negative or low reactions; thus, X16 was used to form subgroups of groups 1, 2, and 5. Such differences were not as well delimited with other MCAs (Table 4); therefore, intermediate reactions were not used for subgrouping. The dilemma of subgrouping becomes particularly apparent with groups 3 and 4, which contain only a small number of strains. Until additional strains are tested that may be classified into the latter groups, subgroups have been provisionally assigned based on positive or negative reactions to X2, X3, and X15. These MCAs also were used in combination with each other and with X16 to form group 5 subgroups.

The utility of the MCAs for epidemiological studies was observed in a survey performed prior to the present study in which the 160 strains of *X.c.* pv. *campestris* obtained from 12 farms in Kula, Maui, were screened with MCAs available at that time: namely, X1, X2, X3, and X4. Most (89%) of the strains were positive for the four MCAs, and therefore would be members of either group 1 or 2. Strains negative to X2, X3, or X4 were later tested with all the MCAs used in the present study and were found to be members of either group 4 or group 6. All strains in group 6 caused blackening of veins and tissues surrounding veins with no chlorosis, resembling the host response to strain A342 and the strains of *X.c.* pv. *armoraciae*.

The MCAs provide convenient markers for tracing the spread of strains of *X.c.* pv. *campestris* on Hawaiian farms and their survival on weeds and alternate crops. In addition, MCAs can be used to determine how rapidly a new strain becomes established in a field after having been introduced on contaminated seed. Serological

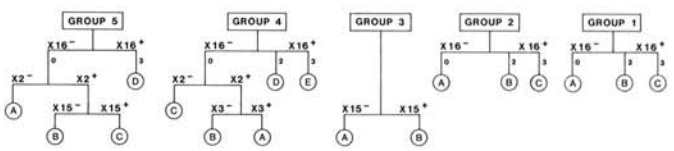

Fig. 4. Keys to the subgroups of *Xanthomonas campestris* pv. *campestris* based on reactions to monoclonal antibodies. Numbers adjacent to vertical lines give quantitative (high, 3; intermediate, 2; and negative, 0) reactions to monoclonal antibody X16. Intermediate and high reactions with other antibodies were considered positive (+) and very low reactions were considered negative (−), based on values shown on Table 4.

TABLE 4. Grouping and subgrouping of 200 strains of *Xanthomonas campestris* pv. *campestris* based on reactions with monoclonal antibodies[a]

Group	Subgroup	Monoclonal antibodies								Strains	
		X1	X11	X13	X2	X3	X4	X15	X16	No.	Percent
1	A	3	3	0	3	3	3	3	(0)	4	2
	B	3	3	0	3	3	3	3	(2)	33	16.5
	C	3	3	0	3	2,3	2,3	2,3	(3)	2	1
2	A	3	3	0	3	3	3	[0,1]	(0)	6	3
	B	3	3	0	3	3	3	[0]	(2)	84	42
	C	3	3	0	3	3	2,3	[0,1]	(3)	4	2
3	A	3	3	0	2,3	[0,1]	[3]	(0,1)	2,3	6	3
	B	3	3	0	2,3	[0,1]	[3]	(2,3)	2,3	4	2
4	A	3	3	0	3	(2,3)	0	0-3	(0)	9	4.5
	B	3	3	0	(3)	(0)	0	0-2	(0)	3	1.5
	C	3	3	0	(0)	(0)	0	0	(0)	2	1
	D	3	3	0	3	3	0	0	(2)	3	1.5
	E	3	3	0	3	0	0	0	(3)	2	1
5	A	3	3	[3]	(0)	0	0,1	0,1	(0)	8	4
	B	3	3	[2,3]	(2,3)	0,1	1,2	0	(0)	7	3.5
	C	3	3	[3]	(2,3)	0	1,2	(2,3)	(0)	7	3.5
	D	3	3	[2]	(3)	0	0	0,1	(3)	4	2
	v	3	3	[2,3]	0-3	0-3	0-3	1-3	0-3	6	3
6		3	[0,1]	0	0-3	0	0	0,1	0	4	2
Untypable		3	3	0	3	0	0	0	3	2	1

[a] Values (counts per minute) were established by radioimmunoassay and are reported as high (3), intermediate (2), low (1), or negative (0) relative to reactivity of control strains included in each assay. Boxes indicated differences used to separate groups, and circles indicate subgroup differences.

markers that can be used to assess the effect of introduced inoculum on a subsequent disease outbreak and to compare its importance relative to the indigenous population will help to establish tolerance levels for seed contamination. A need for quantitative field evaluations of disease resulting from seedborne bacterial pathogens has been mentioned in recent reviews (7,17). Finally, MCAs also permit the rapid identification of xanthomonads associated with unusual symptoms in mixed infections when, for example, black rot symptoms are masked by environmental factors, nutritional disorders, or other diseases (13). We have used these MCAs to distinguish xanthomonad colonies from colonies of other yellow oxidative Gram-negative rods cultured from leaf surfaces (2). The MCAs are being used in an ELISA developed for epidemiological studies (3).

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