Pathovar-Specific Monoclonal Antibodies for *Xanthomonas campestris* pv. *oryzae* and for *Xanthomonas campestris* pv. *oryzicola*

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ABSTRACT

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Monoclonal antibodies (mAbs) were produced that were specific for *Xanthomonas campestris* pv. *oryzae*. Monoclonal antibody Xco-1 reacted with all 178 tested strains of *X. c. oryzae* from diverse geographical locations, including strains isolated from the recent blight outbreak on rice in Texas and Louisiana. A second mAb, Xco-2, reacted with most (87%) strains of *X. c. oryzae*, which included all but one of 92 Philippine strains. A third mAb of *X. c. oryzae* (Xco-5) reacted only with the 29 strains from Texas and Louisiana, and not with any other strain of *X. c. oryzae*; however, Xco-5 also reacted weakly with strains of *X. c. oryzicola*. An mAb specific for *X. c. oryzicola* was generated. None of the mAbs reacted with 130 xanthomonads of other pathovars and species, including 11 epiphytic xanthomonads from rice leaves, or with 89 strains of other genera. Based on immunofluorescence and immunoelectron microscopy, these mAbs detected surface antigens. The Xco-1 and Xco-2 epitopes were heat-sensitive and heat-resistant, respectively, and the Xco-2 epitope was found in the lipopolysaccharide fraction. Culture conditions were critical for expression of the Xco-5 epitope.

Bacterial leaf blight of rice caused by *Xanthomonas campestris* pv. *oryzae* is one of the most serious diseases of rice in many rice-growing countries (16,19). The variation in the virulence of different strains of this organism on differential host cultivars has led to the subdivision of this taxon into races (16–18); however, the host-pathogen interaction is at times equivocal, and a rapid and simple method for the accurate identification of this pathogen and its races is needed. Although the use of polyclonal antisera identified serotypes of the pathovar, such antisera were not race-specific (1,9,10). Therefore we attempted to: produce monoclonal antibodies (mAbs) that react only with strains of *X. c. oryzae*; identify serogroups; identify races; and delineate *X. c. oryzae* from *X. c. oryzicola*, the causal agent of bacterial leaf streak of rice. The feasibility of accomplishing these objectives was based on having generated mAbs previously to several phytopathogenic bacteria that were useful for identification, diagnosis, classification, and epidemiological purposes (3,8,23). Monoclonal antibodies that identified the genus *Xanthomonas* and those that detected antigens associated with virulence of the black rot pathogen, *X. c. campestris*, have been used extensively for seed analysis and field studies (5,23). In addition, we produced mAbs that identified other pathovars of *X. campestris* (unpublished data), and mAbs that were useful for the identification of strains of *X. c. citri* associated with citrus disease outbreaks in Mexico and Florida (4,7). Thus, the potential application of this technique for identification of strains of *X. c. oryzae* was explored. We report here the production of an mAb specific for *X. c. oryzae*, two mAbs that group strains of this pathogen, and one mAb specific for *X. c. oryzicola*.

MATERIALS AND METHODS

Bacterial strains. The Philippine strains (PXO) of *X. c. oryzae* were tested at the International Rice Research Institute for pathogenicity on rice differentials (16–18), and they represented the six known races from the Philippines (Table 1). Other strains, provided by researchers from widely dispersed locations, were pathogenic on rice varieties in their respective regions.

For screening purposes, the bacterial strains reported previously (3) were used for early fusions; in the later fusions the bacterial strains given in Table 2 were employed. These organisms were isolated in Hawaii or were provided by donors from different geographical locations. Cultures of questionable identity and bacteria that were recovered from rice seed and produced yellow colonies were examined by standard bacteriological tests (11) to determine whether or not they were xanthomonads. Bacteria associated with the brown blotch disease of rice, originally described as xanthomonads (15), were more closely related to species of *Pseudomonas* and *Flavobacterium* (21), and in the present study these bacteria did not react with the *Xanthomonas*-specific mAbs, X1 and X11.

Production of monoclonal antibodies. The procedures used were similar to those described previously (2,3). BALB/c mice were immunized twice, 14 days apart, by intraperitoneal injections of formalinized PXO strains (Nos. 40, 79, 86, 99) either individually or as mixtures. Mice also were immunized with a single Texas strain (either X1-5, X1-6, or X1-7), or with *X. c. oryzicola*, strain 101. Three days after the second injections, spleen cells were mixed with P3-X63 myeloma cells and cultured as described previously (3). Supernatant fluids from culture wells with hybridomas were screened by either radioimmunoassay (3) or enzyme-linked immunoassay (ELISA) for antibody reactions to various strains of *X. c. oryzae* and *X. c. oryzicola*. Included as controls for initial selection of mAbs were a strain of *Erwinia herbicola* (Eh1) and a strain of *X. c. campestris* (A249). Selected antibody-producing cultures were cloned twice by the limiting dilution technique. After either the first or second cloning, selected cultures were expanded to obtain a sufficient volume of culture supernatant fluids to screen cultures of most of the strains of *X. c. oryzae* and of many other xanthomonads. Clones that reacted with strains of *X. c. oryzae* and not with other xanthomonads were selected to be grown as ascites in BALB/c mice. The resulting ascites also were screened for reactivity to all strains of *X. c. oryzae*, other xanthomonads, and other bacterial genera. Hybridomas specific for *X. c. oryzae* were selected for further study. Ascites were clarified by centrifugation and stored at −20 C. The isotypes of mAbs were determined by immunoprecipitin using antisera obtained from Litton Bionetics, Inc., Kensington, MD. All

bacterial strains also were reacted with two mAbs (X1, X11) specific for the genus *Xanthomonas* (3).

Enzyme-linked immunosorbent assay. Formalinized bacterial cells were washed three times in phosphate-buffered saline (PBS), resuspended in 0.05 M carbonate-bicarbonate buffer (pH 9.6), and adjusted to an absorbance of 0.1 OD at A_{600nm} in a spectrophotometer. Polyvinyl chloride 96-well plates (Costar, Cambridge, MA, and Dynatech Labs., Inc., Alexandria, VA) were coated with 100 μl of the cell suspensions by drying in a 37 C circulating-air incubator, and stored in the refrigerator until used. The plates were blocked for 15 min with 5% BLOTTO (12) in PBS and washed once with 0.16 M borate buffer (pH 8.3). One hundred microliters of each of the following reagents, each diluted in a 1:3 dilution of 5% BLOTTO in borate buffer was added sequentially, and each was incubated 1 hr at room temperature followed by three washes with borate buffer: mAb, 1:1000 rabbit anti-mouse globulin, 1:1000 Protein-A-horseradish peroxidase (Bio-Rad Labs, Richmond, CA). Finally, substrate consisting of 0.05% 5-amino-salicylic acid, and 0.06% H_2O_2 in phosphate-EDTA buffer (6) was added, and after 1 hr, absorbance was measured at 450 nm with a Titertek Multiskan plate reader.

Immunofluorescence. Drops of cell suspensions, prepared as for ELISA, were placed on slides, air dried, and fixed for 15 min in −20 C absolute methanol. After washing with PBS, various dilutions of mAbs followed by rabbit anti-mouse immunoglobulin-FITC (Miles Scientific, Naperville, IL) were added. For mounting, 70% glycerol-30% PBS was added, the slides were covered with coverslips and examined with a Zeiss IV F1 epifluorescent microscope.

Immunoelectron microscopy. The technique described by Robinson et al (20) was used. In brief, 10–20 μl of washed formalinized bacterial suspension (A_{600nm} = 0.2) was placed on Formvar grids. After 1 min, the grids were washed four times by transferring to PBS. Monoclonal antibodies were added to the grids for 30 min, and after washing, the grids were incubated for 30 min with 15-nm gold spheres conjugated with goat anti-mouse antiserum (Ted Pella, Inc., Tustin, CA). After washing with PBS and removing all PBS, grids were floated on 1% phosphotungstic acid (pH 6.0) for 1 min, washed with distilled water, and air dried. Specimens were examined with a Zeiss 10/A transmission electron

TABLE 1. Strains of *Xanthomonas campestris* pv. *oryzae* tested with monoclonal antibodies

Strains tested (no.)	Strain designations	Origin	Received from[a]
	X. c. oryzae		
26	PXO Race 1: 1, 4, 5, 9, 10, 13, 14, 15, 19, 20, 23, 25, 32, 34, 35, 38, 44, 48, 52, 61, 62. 84, 85, 131, 151, 157	Philippines	4
16	Race 2: 63, 78, 82, 83, 86, 103, 104, 126, 134, 135, 138, 139, 140, 149, 158, 159	Philippines	4
16	Race 3: 18, 22, 79, 81L, 87, 88, 141, 146, 147, 148, 152, 153, 154, 155, 156, 160	Philippines	4
6	Race 4: 69, 70, 71, 113, 125, 129	Philippines	4
14	Race 5: 45, 80, 105, 106, 107, 108, 109, 110, 111, 112, 130, 144, 145, 150	Philippines	4
14	Race 6: 99, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 127, 128	Philippines	4
31	AU-6, B-13, B-18, B-77, BU-1, BU-2, BU-6, BU-010, BU-12, BU-14, BU-15, BU-20, CL4-4, CL-6, H-14, H66, H100, H146, H200, H201A, IG12, IG15, IG24, N1, N3, PA-3, PA-12, P15, TL12, TL23, TW7, TW12	Australia (AU), Bangladesh (B), Burma (BU), Ceylon (CL), India (H), Indonesia (IG), Japan (N), Pakistan (PA), Philippines (PI), Taiwan (TW), Thailand (TL)	1
2	CIAT: 1185, 1186	Colombia	6
14	FXO Race unknown: 33, 34, 35, 36, 39, 40, 50, 54, 60, 61, 62, 63, 64, 65	Australia, Bangladesh, China, India, Taiwan, Thailand	4
5	IRN 109, X03, X016, X020, X024	Taiwan	1
5	X1-5, X1-6, X1-7, X1-8, X1-10	Texas	2, 3
20	X4-1B, X4-1C, X4-2C, X4-3D, X4-4C, X4-4D, X4-8C, X7-2D, X7-3E, X7-5A, X11-1A, X11-1B, X11-2D, X11-4B, X11-5A, X11-5B, X11-6B, X13-2E, X13-3A, X13-5C	Texas	2, 3
4	X8-1A, X8-1B, Ru-8717, Ru-8718	Louisiana	2, 3
2	JXO T7174, T7133	Japan	3, 5
2	PXO 61-SM, PXO 86-R1F	Philippines	3
1	IXOB	Bangladesh	3
178			

[a] 1, I. W. Buddenhagen, California; 2, C. F. Gonzalez, Texas; 3, J. E. Leach, Kansas; 4, T. W. Mew, Philippines; 5, S. S. Wakimoto, Japan; 6, R. S. Zeigler, Colombia.

microscope, operated at 80 kV.

Extraction and identification of xanthomonads from rice seed and leaves. Thirteen seed lots produced from rice (*Oryza sativa* L. 'Lemont') in Texas were examined for the presence of xanthomonads. Thirty-five grams was soaked for 30 min in 50 ml of PBS-Tween 20, after which 25 ml was centrifuged for 10 min, and the pellet was resuspended in 1 ml of sterile saline. After three washes, the pellet was resuspended in 1 ml of 0.05 M carbonate-bicarbonate buffer (pH 9.6), adjusted to $A_{600nm} = 0.1$ and coated on microtiter plates for ELISA. The remaining extract was passed through a 0.22-μm filter, and the bacteria removed by resuspending the filter in 5 ml of PBS were coated on microtiter plates for ELISA. In addition, 10-μl samples were plated by dilution streaking onto three culture media: a differential medium containing tetrazolium chloride at a final concentration of 0.001% (14); modified Wakimoto's medium (13); and Fieldhouse-Sasser medium (23). The cultures were later purified and tested for pathogenicity by needle-prick inoculation onto Taichung Native 1 and Lemont cultivars of rice. Cultures also were tested by immunofluorescence with mAbs. Six controls were prepared by adding known amounts of a pure culture of Texas strain X1-8 in PBS-Tween to 35-g samples of rice seed produced from symptomless rice plants. A 10-fold dilution series of inoculum, adjusted by optical density and confirmed by viable plate counts, ranged from 4×10^2–4×10^7 cfu/ml. One milliliter from each dilution was added to 49 ml of PBS for soaking the seed; subsequently all samples were processed as described.

RESULTS

***X. c. oryzae*-specific mAbs.** Two major specificities were repeatedly observed in testing parental and first clones of thousands of hybridoma cultures resulting from seven fusions with PXO strains: mAbs that reacted with all strains of *X. c. oryzae*

TABLE 2. Xanthomonads and other bacterial genera used to test the specificity of monoclonal antibodies

Strains tested (no.)[a]	Strain designations	Genus/species or pathovar	Received from[b]
1	LS2	*Xanthomonas albilineans*	4
		X. campestris	
1	ICPB163	pv. *aberrans*	29
2	G22, G41	pv. *alfalfae*	15
5	756, XLS-2, G3-27, XLS-4, 417	pv. *armoraciae*	1, 5, 30
4	A915, X45, ICPBXB9, A2050	pv. *begoniae*	1, 7, 14, 29
5	A249, A902, EEXC114, A342, RR68	pv. *campestris*	1, 12, 31
5	ARCO-2, ARCO-4, B70, B75, B103	pv. *carotae*	25, 30
7	XC59, XC62, XC63, XC64, XC69, XC70, XC90	pv. *citri*	10
5	D78-3, D110, D147, D194, D238	pv. *dieffenbachiae*	1, 24
2	A912-2, A875-2	pv. *euphorbiae*	1
4	X17, X24, X25, X37	pv. *hederae*	7
1	413	pv. *holcicola*	20
1	PDCC574	pv. *incanae*	32
5	X10, X27, X108, X203, X204	pv. *malvacearum*	7
5	HMB9, HMB38, HMB55, HMB286, XM105	pv. *manihotis*	21, 29
4	X38, X127, X128, X188	pv. *pelargonii*	7
3	A584, A602, M191	pv. *phaseoli*	1
5	A1779, A1804, PX079, PX087, PX0101	pv. *oryzae*	6, 22
8	B18, B910, B911, B912, 5738, 5739, 256, 101	pv. *oryzicola*	6, 20
5	86-1, 86-2, 86-3, X85-F, X85AY	pv. *raphani*	16
5	X159, X162, X163, X172, X181	pv. *syngonii*	7
5	M01, M03, M05, M06, M07	pv. *translucens*	26
2	X19, X32	pv. *urticae*	7
6	A1757, A1782, A1785, A1786 XCV-1, XCV-2	pv. *vesicatoria*	1, 11
5	10TB10, 7D52, A674, X42, XCV164	pv. *vitians*	1, 7, 19, 29
		X. campestris undescribed pathovars from	
5	A88-3, A206-2a, A226-3, A227-1, A255-4	*Allium cepa* (onion)	1
2	A910-2, A910-3	*Cordyline terminalis* (ti)	1
5	C121-2AR, C130-2A, C130-2AR, C210-2, C260-4A	*Cynodon dactylon* (Bermuda)	1
1	G715	*Polycias guilfoylei* (panax)	1
5	DR34, W46, DR46a, DR52, DR54	*X. maltophilia*	1
		Xanthomonas sp.	
2	PXO 40, 101 (Race 0)	nonpathogenic strains (rice)	22
9	S7-1, S8-1, S8-2, S9-2, S10-2, S11-2, S11-3, S12-1, S12-2	nonpathogenic strains from rice seed	3
7	Ru87-2, Ru87-4, Ru87-5, Ru87-6, Ru87-8, LR28-7, JBG-9	nonpathogenic strains from rice leaes	20
14	1A, 3B, 4B1, 7B1, 8A2, 9A1, 9B1, 9B2, 10A1, 10A2, 11A1, 11B, S3-1, S10-2	nonxanthomonads from rice	1, 3

(continued next page)

TABLE 2. (cont'd)

3	B9, H201, TL-12		1, 6
3	CIAT BB: 1171, 1173, 1192	brown blotch (rice)	20, 33
1	ATCC 27853	*Pseudomonas aeruginosa*	2
1	P24	*P. cattleyae*	7
1	PC25	*P. cepacia*	29
5	C441-3a, C460-3a, C546-2, C606-2, C397-1	*P. chichorii*	1
1	PF1	*P. fluorescens*	1
3	B634-3, N1012-1, SR532	*P. fuscovaginae*	33
1	P10	*P. marginalis*	7
2	Heliconia 8, K60	*P. solanacearum*	1, 28
5	PSS-1, A1790, A1791, M194, ATCC 19310	*P. syringae* pv. *syringae*	1, 2, 11
2	P13, PT5	pv. *tabaci*	7, 29
1	PST2	pv. *tomato*	11
1	PV-1	*P. viridiflava*	11
1	ATCC 19048	*P. viridilivida*	2
5	IPM61, IPM1286, M785, RGB1, 058-3	*Erwinia atroseptica*	13
5	IPM60, RGB1, 057-3, ICPB153, UC176	*E. carotovora*	8, 13, 27, 29
5	A1042, E21A, D6, 1237, EC183	*E. chrysanthemi*	1,7
1	Eh-1	*E. herbicola*	1
1	A414	*Agrobacterium radiobacter*	1
1	TR108	*A. rhizogenes*	29
1	UCBPP388	*A. tumefaciens*	27
1	ATCC 6887	*Corynebacterium flaccumfaciens*	2
1	ATCC 9682	*C. poinsettiae*	2
1	A707	*C. ulcerans*	1
1	ATCC 12975	*Clavibacter fascians*	2
1	ATCC 10253	*C. insidiosum*	2
5	A518-1, A518-8a, B100, CM761, DS414	*C. michiganense*	1, 30
1	ATCC 13659	*C. rathayi*	2
1	ATCC 9850	*C. sepedonicum*	2
1	M110	*Enterobacter aerogenes*	23
2	F2, YP-16	*E. cloacae*	24
1	M111	*Bacillus cereus*	23
1	M112	*B. megaterium*	23
1	M113	*B. subtilis*	23

[a]The hosts and geographical origins of strains are recorded at the Department of Plant Pathology, University of Hawaii.
[b]1, Local isolation; 2, American Type Culture Collection; 3, L. W. Barnes, TX; 4, R. G. Birch, Australia; 5, L. C. Black, LA; 6, I. W. Buddenhagen, CA; 7, A. R. Chase, FL; 8, A. K. Chatterjee, MO; 9, J. J. Cho, HI; 10, E. L. Civerolo, MD; 11, M. A. Cubeta, CA; 12, E. Echandi, NC; 13, G. D. Franc, CO; 14, D. Funck-Jensen, Denmark; 15, D. W. Gabriel, FL; 16, R. D. Gitaitis, GA; 17, P. Y. Hseih, Taiwan; 18, J. B. Jones, FL; 19, C. J. Kado, CA; 10, J. E. Leach, KS; 21, H. Maraite, France; 22, T. W. Mew, Philippines; 23, Microbiology Department, University of Hawaii; 24, W. T. Nishijima, HI; 25, D. J. Robeson, CA; 26, D. C. Sands, MT; 27, M. N. Schroth, CA; 28, L. Sequeira, WI; 29, M. P. Starr, CA; 30, J. C. Watterson, CA; 31, P. H. Williams, WI; 32, J. M. Young, New Zealand; 33, R. S. Zeigler, Colombia.

tested and mAbs that reacted with all but a few strains tested. These mAbs did not react with any other tested xanthomonad or non-xanthomonad. Stable and high-titer hybridomas with these specificities were selected for further studies. An mAb reacting with all strains of *X. c. oryzae* was designated Xco-1, and an mAb reacting with most strains was designated Xco-2. In addition to these specificities, only two clones were found that appeared to delineate some of the races; however, these clones were unstable and were lost.

Examples of binding curves of Xco-1 (clone 139-159, IgMk) and of Xco-2 (clone 138-68, IgG3k) are shown in Figure 1. Binding curves with other strains of *X. c. oryzae* were similar to these, although some strains gave quantitatively lower values. Based on replicate determinations of binding curves, 1:1,000 dilutions of these mAbs (ascites) were used routinely. Positive and negative reactions were easily delineated since negative reactions had ELISA values (A_{450nm}) of 0–0.1, whereas positive reactions usually had values above 0.3.

Three fusions made with either Texas strains X1-5, X1-6, or X1-7 also resulted in numerous hybridomas with Xco-1 specificity; however, hybridoma cultures with Xco-2 specificity were observed only in fusions made with X1-5 and X1-7 strains but not with X1-6. In addition to the Xco-1 and Xco-2 specificities, an mAb was generated that was specific for only the Texas and Louisiana strains. This mAb, designated Xco-5, reacted weakly but significantly with some strains of *X. c. oryzicola*, but not with any other tested strains of *X. c. oryzae*, xanthomonads, or nonxanthomonads. A typical binding curve of Xco-5 (clone 139-39, IgGk) is shown in Figure 1. Based on reactions with these three mAbs, the 178 strains of *X. c. oryzae* formed four groups (Table 3). The major group (I), characterized as Xco-2-positive and Xco-5-negative, consisted of 91/92 PXO strains and 49 strains from widely different geographical regions. A smaller group (II), negative for both mAbs Xco-2 and Xco-5, consisted of one PXO strain (#35) and eight strains from widely different geographical locations (Table 3). All of the Texas and Louisiana strains were exclusively Xco-5 positive, and they formed additional groups (III, IV) which were separated on the basis of reactions with Xco-2 (Table 3).

***X. c. oryzicola*-specific mAb.** The fusion made with *X. c. oryzicola* as antigen yielded an mAb that reacted only with this organism and not with any other tested xanthomonad or non-xanthomonad (Table 3). Although the Texas mAb, Xco-5, reacted weakly with strains of *X. c. orizicola*, to date no mAb that reacted only with *X. c. orizicola* and Texas strains of *X. c. oryzae* has been generated from a fusion made with *X. c. oryzicola*.

Identification of *X. c. oryzae* on rice seeds and leaves with mAbs. Using the three Xco and the *Xanthomonas*-specific mAbs, X1 and X11, we were able to quickly identify colonies of *X. c. oryzae* (X1-8) in artificially infected seed samples and separate them from

nonpathogenic xanthomonads and nonxanthomonads. Nonpathogenic xanthomonads were recovered from seven of the 13 rice seed lots. The presence of nonpathogenic xanthomonads in some of the mixed cultures recovered from the initial seed extraction was indicated by positive ELISA reactions with X1 and X11, and negative reactions with Xco-1, Xco-2, and Xco-5. After purification, culturing on yeast-glycerol agar, and testing by ELISA, these cultures again reacted strongly with X1 and X11 but were negative with the mAbs of *X. c. oryzae*. In contrast, the *Xanthomonas*-like colonies recovered from all of the control samples inoculated with Texas strain X1-8 reacted strongly with Xco-1 and Xco-5 as well as with X1 and X11 mAbs. Orange-yellow colonies on yeast-glycerol agar were recovered from two bacteria rice samples that did not react with any of the mAbs; later these were shown to be nonxanthomonads by bacteriological tests. None of the xanthomonads or nonxanthomonads recovered from rice seed were pathogenic except for the controls infected with Texas strain X1-8. The latter strain produced lesions typical of symptoms produced in the field during the Texas outbreak (L. Barnes, *personal communication*).

Characteristics of the antigens detected with mAbs. For determining heat stability of the epitopes, formalinized, washed suspensions of PXO 86 and Texas strain X1-5 each were heated for 1 hr in a boiling water bath, coated on plates, and reacted with Xco-1, Xco-2, and Xco-5. The epitopes detected with Xco-1 and Xco-5 were heat-sensitive, whereas the Xco-2 epitope was heat-resistant. Furthermore, phenol extracts (22) of PXO strains and Texas strain X1-5 contained Xco-2 antigen that precipitated with Xco-2 in immunodiffusion, but did not contain antigens precipitable with Xco-1 and Xco-5. The Xco-1, -2, -5 epitopes were stable for a period of 8 mo when bacteria were stored at 4 C in Formalin.

Reactions of PXO and Texas strains with Xco-1 gave weak immunofluorescence and no binding in immunoelectron microscopy (Fig. 2A). Xco-2 and Xco-5 showed intermediate and bright immunofluorescence, respectively, and both showed similar distributions on the cell surfaces in immunoelectron microscopy (Fig. 2). The distribution of the Xco-2 and Xco-5 epitopes (Fig. 2B and C) were similar to what we have observed with antigens of *X. c. campestris* associated with lipopolysaccharide (LPS) and was unlike the distribution we have observed of capsular antigens of *X. c. campestris* or of extracellular polysaccharide adhering to cells (unpublished data). The distribution of Xco-2 is consistent with a heat-stable LPS epitope. The pattern of binding of *X. c. oryzicola* mAb in immunoelectron microscopy differed from the patterns seen with the *X. c. oryzae* mAbs (Fig. 2D); the epitope might be associated with the capsule of *X. c. oryzicola*. Further studies on the chemical nature of these antigens are in progress.

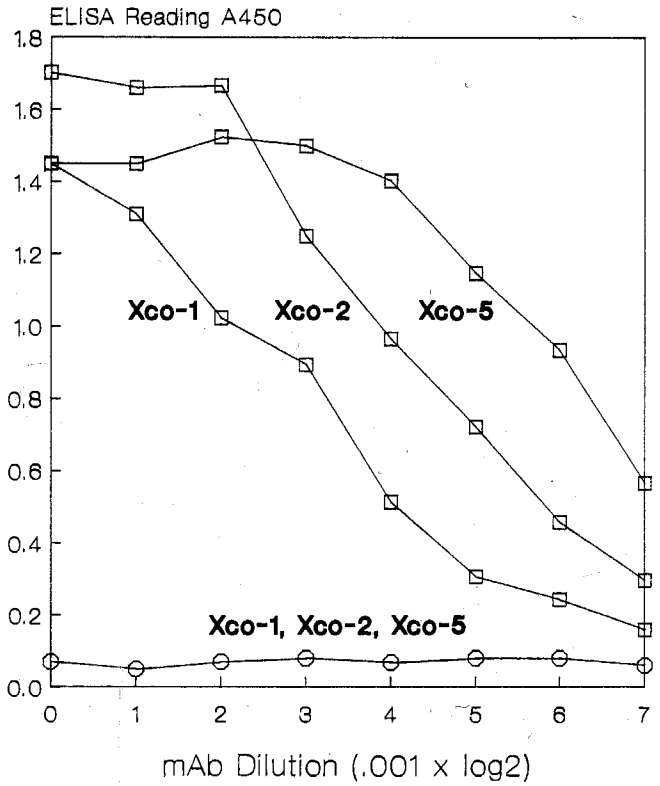

Fig. 1. Binding curves of monoclonal antibodies (ascitic fluids) Xco-1, Xco-2, and Xco-5 reacted with (□) *Xanthomonas campestris* pv. *oryzae*, Texas strain X1-5, and with (o) other pathovars of *Xanthomonas campestris* and other genera.

Microtiter plates coated with the Texas strains grown in nutrient broth were received from J. E. Leach, reacted well to Xco-1 and Xco-2, but did not react to the Texas specific-Xco-5 mAb. However, the same Texas strains cultured on yeast-glycerol agar in our laboratories were Xco-5-positive.

Therefore, the age of the cultures and the influence of various culture media on expression of the Xco-5 epitope were examined (Table 4). Texas strain X1-5 failed to bind Xco-5 when grown in nutrient broth for 1–4 days, whereas weak binding was observed with strain X1-8 on days 3 and 4. In nutrient agar X1-8 cells were Xco-5 positive but X1-5 cells were Xco-5 negative even after 5 days of culturing. The maximum activity of strain X1-8 with mAb Xco5 was obtained when grown in yeast-glycerol broth for 3 days or in yeast-glycerol agar for 2 days; X1-5 only expressed the Xco-5 antigen when grown in yeast-glycerol agar.

DISCUSSION

Among the many mAbs screened for specificity, one (Xco-1) was specific for all strains of *X. c. oryzae* tested. Xco-1 did not react with 130 strains of other *Xanthomonas* species or pathovars or with 89 strains of other phytopathogenic or epiphytic bacterial genera. Moreover, Xco-1 did not react with any of the nonpathogenic xanthomonads recovered from rice seed. Thus, Xco-1 should be useful for detection and identification of the bacterial leaf blight pathogen.

In addition, another mAb (Xco-2) was generated that reacted with most strains of *X. c. oryzae*. The relationship of the few strains that failed to react with Xco-2 is not known. Studies are continuing to determine whether Xco-2-negative strains represent a homogeneous group.

Xanthomonas strains isolated from the recent outbreak of leaf blight in Texas and Louisiana were Xco-1 positive, and thus would be classified antigenically as *X. c. oryzae*. However, fusions made with Texas strains yielded an mAb (Xco-5) that detected an epitope

TABLE 3. Grouping of strains of *Xanthomonas campestris* pv. *oryzae* with monoclonal antibodies

MAb	*X. c. oryzae* group[a] I[b]	II[c]	III[d]	IV[c]	*X. c. oryzicola*	Other xanthomonads	nonxanthomonads
X1	+	+	+	+	+	+	−
Xco-1	+	+	+	+	−	−	−
Xco-2	+	−	+	−	−	−	−
Xco-5	−	−	+	+	+[f]	−	−
Xccola	−	−	−	−	+	−	−
Total no.	140	9	15	14	8[g]	130	89

[a] 178 strains tested.
[b] 91 PXO strains (all 6 races): 12 FXO strains; 35 from Australia, Bangladesh, Burma, Ceylon, Colombia (CIAT 1186), India, Indonesia, Japan, East Pakistan, Taiwan, and Thailand; 2 antibiotic resistant strains, PXO 61-SM and PXO 86-RIF.
[c] PXO 35; FXO strains 61, 631; 1 each from Thailand, India, Japan, Ceylon, Colombia (CIAT 1185), and Taiwan.
[d] Texas strains X1-5, X1-7; X75A, X7-2D, X7-3E, X11-1A, X11-5A, X11-1B, X11-4B, X11-5B, X11-6B, X11-2D, X13-3A, X13-5C, X13-2E.
[e] Texas strains X1-6, X1-8, X1-10; X4-1B, X4-1C, X4-2C, X4-3D, X4-4C, X4-4D, X4-8C; Louisiana strains X8-1A, X8-1B, Ru-8717, Ru-8718.
[f] Weak positives.
[g] All strains had the indicated reactivity patterns.

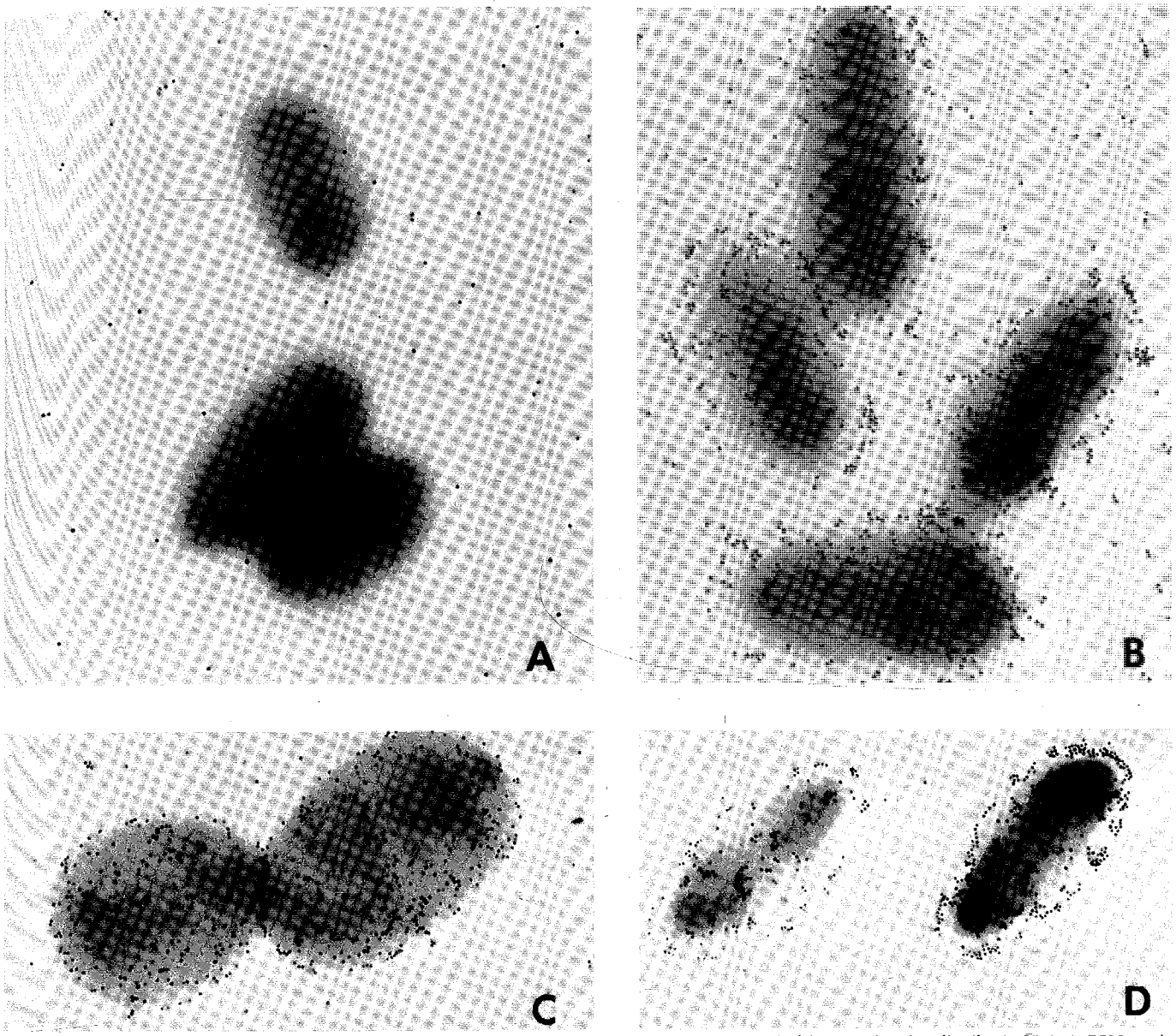

Fig. 2. Immunoelectron micrographs of *Xanthomonas campestris* pv. *oryzae* and *X. c. oryzicola* reacted with monoclonal antibodies (mAbs). **A,** PXO strain 86 + Xco-1; **B,** PXO strain 86 + Xco-2; **C,** Texas strain X1-5 + Xco-5; D, *X. c. oryzicola*, strain 101 + *X. c. oryzicola*-specific mAb. (31,250×).

TABLE 4. Effect of culture conditions on expression of *Xanthomonas campestris* pv. *oryzae* (Xco) antigens detected with monoclonal antibodies

Growth[a] medium	mAb	Days of culture of Xco strain X1-5					Days of culture of Xco strain X1-8				
		1	2	3	4	5	1	2	3	4	5
NB	X1	1.259[b]	1.362	1.515	1.091	...[c]	1.280	0.806	1.366	1.460	...
	Xco-1	0.478	0.547	0.736	0.634	...	0.726	0.557	0.957	1.123	...
	Xco-2	1.419	1.466	1.422	1.337	...	0.031	0.049	0.174	0.195	...
	Xco-5	0.010	0.005	0.003	0.047	...	0.029	0.037	0.194	0.234	...
NA	X1	...	0.922	1.301	1.183	1.216	...	1.182	1.354	1.354	1.358
	Xco-1	...	1.167	1.115	1.082	0.878	...	0.425	1.022	1.089	1.135
	Xco-2	...	1.365	1.305	1.274	1.309	...	0.035	0.186	0.163	0.194
	Xco-5	...	0.041	0.009	0.014	0.016	...	0.414	0.597	0.456	0.598
YGB	X1	1.280	1.220	1.039	1.421	...	0.401	0.943	1.410	1.465	...
	Xco-1	0.583	0.328	0.365	0.490	...	0.475	0.712	0.780	1.171	...
	Xco-2	1.372	1.283	1.252	1.280	...	0.023	0.008	0.167	0.184	...
	Xco-5	0.066	0.059	0.096	0.080	...	0.213	0.675	1.091	1.321	...
YGA	X1	...	0.929	1.323	1.412	...	...	0.770	1.240	1.364	1.366
	Xco-1	...	0.844	0.780	0.816	...	...	0.680	0.940	1.061	1.191
	Xco-2	...	1.261	1.230	1.252	...	...	0.053	0.075	0.116	0.145
	Xco-5	...	0.942	1.083	1.073	...	...	1.124	1.250	1.375	1.435

[a] NB = nutrient broth; NA = nutrient agar; YGB = yeast-glycerol broth; YGA = yeast-glycerol agar.
[b] Elisa values (A_{450}nm).
[c] not determined.

unique for all Texas and Louisiana strains, and which also reacted weakly with *X. c. orizicola*. According to R. K. Jones (*personal communication*), the Texas strains produced a mild form of bacterial leaf blight. The Texas strains affected highly susceptible rice cultivars (Taichung Native 1 and Lemont), but caused no symptoms on the Philippine rice varieties. Based on these observations, interesting questions are raised regarding the relationships among the well-known PXO strains, the Texas strains, and *X. c. oryzicola*.

The value of the genus-specific mAbs (X1, X11) produced in earlier work (3) was particularly apparent when seed extracts were examined for the presence of the leaf blight pathogen. Numerous organisms recovered from extracts were typical xanthomonads on several culture media and reacted with X1 but not with any of the mAbs specific for *X. c. oryzae* (Xco-1, -2, and -5). All such cultures were negative in pathogenicity tests on susceptible rice cultivars. (L. W. Barnes, *personal communication*). A multiple test using this panel of mAbs can thus facilitate the separation of pathogenic from nonpathogenic xanthomonads from rice seed, and the controversial question as to the seedborne nature of this disease (16,19) could thereby be reexamined.

With regard to the Texas mAb (Xco-5), certain caution must be taken to culture test organisms on yeast-glycerol agar, since expression of the Xco-5 epitope was reduced when cultured in broth and on nutrient agar. Other media have not been tested yet; thus, we routinely use yeast-glycerol agar when preparing antigens. Strains may be cultured on nutrient broth or WF-P medium as long as they are transferred to yeast-glycerol agar before testing with Xco-5.

The procedure of generating mAbs with formalinized bacteria has been successful for revealing *Xanthomonas*-specific antigens and antigens that delineated strains within a pathovar (3,7,8). The utility of this procedure was substantiated by generating pathovar-specific mAbs in this study and in studies on *X. c. pelargonii* and *X. c. begoniae* to be reported. Limited chemical studies and examination by immunofluorescence and immunoelectron microscopy revealed that most of these antigens are on the bacterial surface. In studies to be reported, several of the antigens of *X. c. campestris* detected with mAbs appear to be associated with the bacterial outer membrane.

Repeated attempts to produce race-specific mAbs have not been successful. In fact, in addition to Xco-2, other mAbs have been produced that identify groups of *X. c. oryzae* composed of more than one race (unpublished data).

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