Comparison of Different Immunogen Preparations for Serological Identification of *Xanthomonas campestris* pv. *campestris*

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ABSTRACT

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Four immunogens—formaldehyde fixed cells, glutaraldehyde fixed cells, trichloroacetic acid extracts, and ribosomal extracts—were compared for identification of *Xanthomonas campestris* pv. *campestris* (XC). In Ouchterlony double diffusion (ODD) tests, antiserum (AS) to each of the immunogens resulted in a single major band and one to three minor bands of precipitin when reacted against homologous cell extracts. A reaction of complete fusion (identity) occurred between the major band of precipitin of the four immunogens. However, the major precipitin was considerably sharper and stronger in the AS to ribosomes. By means of AS to ribosomes and the major precipitin, 25 strains of XC were typed into four serovars. Thirty-two other bacteria, including 16 strains of five other pathovars of XC, 12 strains of five other genera, and four unidentified bacteria from crucifer seeds, were tested by ODD with AS to the four serovars. Three strains of *X. vesicatoria* and one strain of *X. translucens* cross-reacted; all other bacteria failed to react. Two to four immunogens were identified in immunoelectrophoresis. The major band of precipitin was identified as a neutral immunogen. In immunofluorescence (IF) tests, few differences were observed among the four immunogens. The greatest specificity was found with AS to glutaraldehyde and formaldehyde fixed cells. None of the immunogens, however, was specific enough in IF to differentiate XC from other xanthomonads.

Serological techniques have proved useful for identification of plant-pathogenic bacteria (15). Serology is especially well adapted for rapid, accurate identification of bacteria isolated from seeds (2,6) and provides a useful alternative to time-consuming biochemical tests. Such serological tests as immunofluorescence (IF) that are rapid and require little antiserum are especially well adapted to routine assays for seedborne bacteria (2). Many different preparations have been used as immunogens, including untreated cells (4), formaldehyde fixed cells (5), trichloroacetic acid (TCA) extracts (9), LiCl extracts (20), polysaccharides (11), glutaraldehyde fixed cells (1), proteins (10), and ribosomal extracts (12,13). However, no comparative information on the specificity of antisera resulting from these commonly used immunogens is available. The purpose of this investigation was to compare the specificity of antisera to formaldehyde fixed cells, glutaraldehyde fixed cells, TCA extracts, and ribosomal extracts of *Xanthomonas campestris* pv. *campestris* (*X. campestris*). The antisera were tested by Ouchterlony agar double diffusion and indirect IF.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used are listed in Table 1.

Preparation of immunogens. Cultures for immunogen extraction were grown in 2.8-L Fernbach flasks containing 1.5 L of liquid medium 523 (7) on a rotary shaker at 30 C for 18 hr (16). The cells in early to mid exponential phase of growth were harvested by centrifugation at 12,100 *g* for 10 min and washed with 0.01 M phosphate buffered saline, pH 7.2 (PBS).

For formaldehyde fixed cells, the washed cell suspension of strains B-18 and B-24 was adjusted to 100 Klett units (about 10^9 colony-forming units [cfu]/ml) and made to 2% formalin (5). Glutaraldehyde fixed cells of strains B-18 and B-24 were treated as described by Allan and Kelman (1). Briefly, the washed bacteria were resuspended in PBS buffer, dialyzed against 2% glutaraldehyde for 3 hr, then dialyzed against PBS at 4 C for 10 hr with frequent changes of PBS. TCA extracts of strains B-4 and B-18 and preparations of ribosomes of strains B-18, B-24, B-33, and B-90 were prepared as described by Lucas and Grogan (9) and Schaad (13), respectively.

Antisera were prepared in New Zealand white rabbits as described by Schaad (13). Briefly, 1 ml of each immunogen was emulsified with 1 ml of Freund's incomplete adjuvant (Difco, Detroit, MI 48232). Rabbits were injected intramuscularly for formaldehyde fixed cells and glutaraldehyde fixed cells and

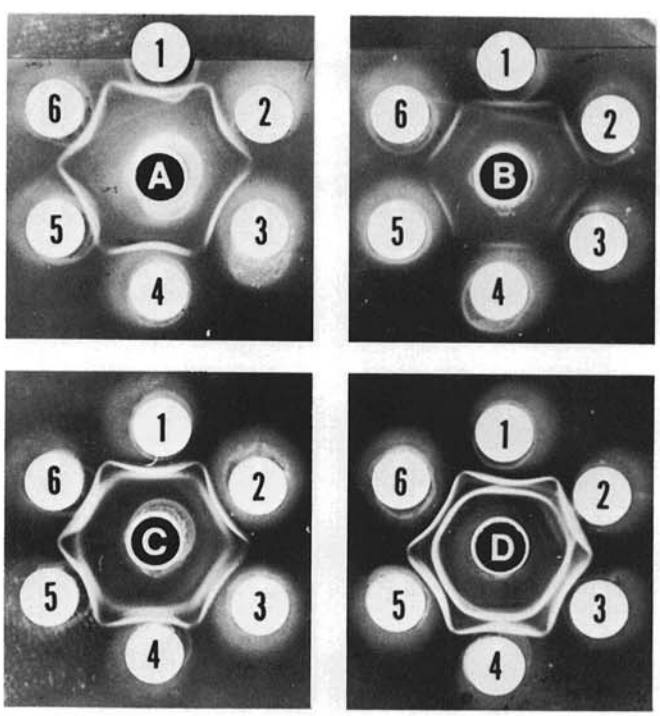

Fig. 1. Ouchterlony double diffusion patterns of different immunogens of *Xanthomonas campestris* pv. *campestris* Ser I strains. Center wells contain 10 μl of antiserum to: **A,** formaldehyde fixed cells, **B,** glutaraldehyde fixed cells, **C,** TCA extracts, and **D,** ribosome preparations. Outer wells contain 10 μl of antigen (containing 3 mg/ml) of 1, B-24; 2, B-28; 3, B-36; 4, B-37; 5, B-38; and 6, B-24.

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intraperitoneally for TCA extracts and ribosomal extracts. Rabbits were injected four times at 10-day intervals and bled 7 days after the last injection. The following schedules were used: formaldehyde, 1 ml for each injection; glutaraldehyde, 0.5, 1.0, 1.5, and 2.0 ml; TCA extracts, 0.75, 1.50, 2.25, and 3.0 mg of protein; and ribosomal extracts, 1.5, 2.5, 3.5, and 4.5 mg of protein. Protein was determined by the method of Lowry et al (8).

Pathogenicity tests. Pathogenicity of all xanthomonads and unknown bacteria from seeds were tested by inoculation with early to mid log phase cultures. Each inoculum was adjusted to 50 Klett units with 0.85% NaCl and diluted to 10^{-4} to yield 10^4–10^5 cfu/ml as described by Vidaver (19). Cabbage seedlings were inoculated as described by Schaad and White (17).

Serological tests. Antigen preparations were obtained from sonicated cells or purified total cell envelopes (3), except that RNase was not used. Ouchterlony double diffusion (ODD) tests were performed as described by Schaad (12). For immunofluorescent staining we used the indirect method (14). Briefly, antiserum to each of the four immunogens was used with commercial goat antirabbit affinity purified IgG (H + L) conjugated to dichlorotriazinyl aminofluorescein (DTAF; Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD 20879). Background stain was reduced by rhodamine conjugated bovine serum albumin (rhodamine-BSA; Microbiological Associates, Walkersville, MD 21793). Block titration tests were run to determine optimum ratios among antisera, antirabbit conjugated fluorescein, and rhodamine-BSA. Smeared bacterial cells were fixed with Kirkpatrick's fixative and treated with rhodamine-BSA, then stained with antiserum and antirabbit fluorescein conjugate as described by Schaad (14). Stained preparations were examined under epifluorescence with a ×100 objective fitted to a Zeiss microscope. A 50W mercury lamp was used as the exciting light source. Immunoelectrophoresis was done as described by Yakrus and Schaad (20).

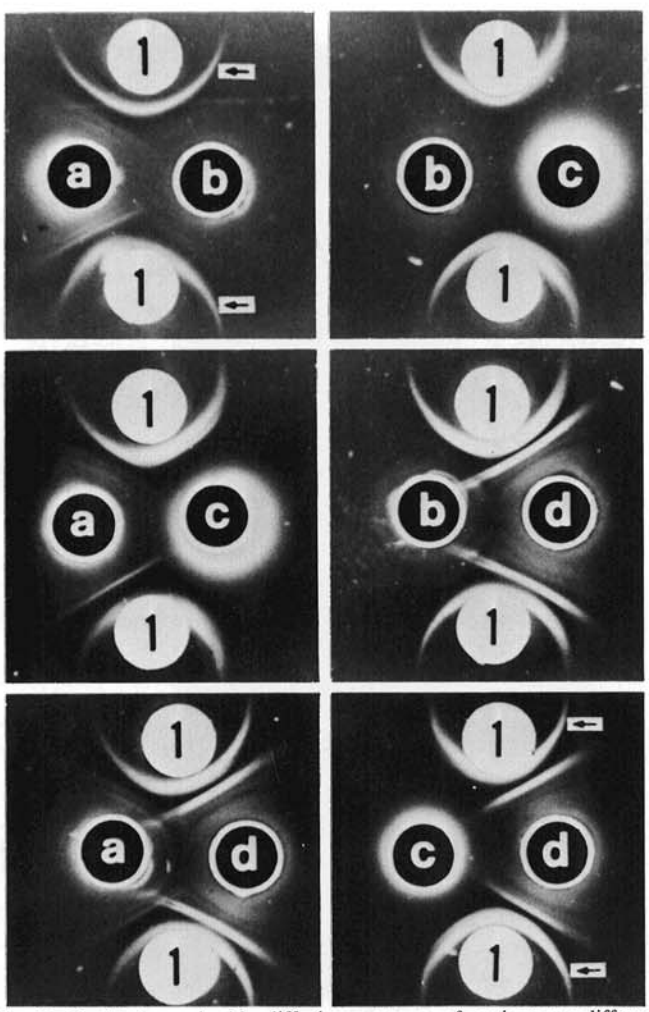

Fig. 2. Ouchterlony double diffusion patterns of antisera to different immunogen preparations of *Xanthomonas campestris* pv. *campestris* Ser II. Antisera to **a,** formaldehyde fixed cells, **b,** glutaraldehyde fixed cells, **c,** TCA extracts, and **d,** ribosome preparations. Wells 1 contain purified total all envelope antigens of strain B-18. Arrows indicate major precipitin band. Each well contains 10 μl of appropriate antiserum and antigen.

RESULTS

Antiserum to each of the four immunogen preparations resulted in a major and minor precipitin band when reacted against homologous antigen in ODD tests (Fig. 1). The major band was always present and a reaction of complete fusion occurred among antisera of the four immunogens (Fig. 2). The minor band, on the other hand, was often weak or missing. The only difference among antisera to the different immunogens was a much sharper and more intense major band of precipitin with the antiserum to ribosomes (Fig. 1D).

In immunoelectrophoresis, two to four bands of precipitin were observed, depending on the immunogen. Two were acidic and two were neutral (Fig. 3). Antisera to TCA extracts (Fig. 3, upper trough), glutaraldehyde, and formaldehyde contained two neutral bands, whereas antiserum to ribosomes contained two neutral bands and two acidic bands (Fig. 3, middle trough). The neutral band was equivalent to the major specific precipitin of ODD tests.

To determine serological specificity among strains, comparative ODD tests were done with antiserum to ribosomes. We chose to use antiserum to ribosomes because antisera to the other immunogen preparations resulted in a major band of precipitin that was somewhat weaker and not as sharp (Fig. 1). The 25 strains of *X. campestris* typed into four serovars (Ser). Ser I, II, III, and IV contained 11, 4, 5, and 2 strains, respectively. Three strains failed to react and therefore could not be typed. Reactions of partial fusion (true spur) (Fig. 4A,B, wells 1 and 8, wells 2 and 3, wells 4 and 5) occurred between most strains of the different serovars (Fig. 4A–D). Antiserum to B-90 (Ser IV) cross-reacted with strains of Ser I but failed to react with strains of Ser II and Ser III (Table 2). Three of four strains of *X. vesicatoria* and one of five strains of *X. translucens* resulted in a reaction of partial fusion with antisera to Ser I and Ser II (Table 3). All other bacteria failed to react (Table 3; Fig. 4E, wells 2–7; Fig. 4F, wells 2–5). Several pseudomonads resulted in a reaction of noninteraction (Table 3; Fig. 4F, wells 7 and 8).

All strains of *X. campestris* except strain B-85 gave a positive fluorescence against antisera to the four immunogens. No consistent difference in intensity of staining was noted among the four serovars (Table 4). In general, however, antiserum to Ser I strains cross-reacted the most. All strains of *X. vesicatoria* resulted

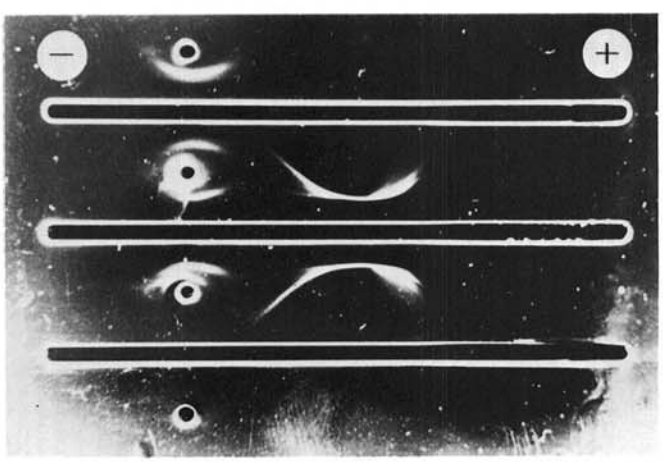

Fig. 3. Immunoelectrophoresis patterns of antisera to TCA extracts and ribosome preparations of *Xanthomonas campestris* pv. *campestris* Ser I. **Upper to lower troughs,** antiserum to strain B-4 TCA, antiserum to strain B-24 ribosomes, and normal serum. Three upper wells contain antigen of strain B-24, and lower well contains tracking dye.

in a positive fluorescence against antiserum to extracts of TCA or ribosomes. Antiserum to formaldehyde fixed cells of Ser I reacted positively with cells of *X. vesicatoria*, whereas antiserum to formaldehyde fixed cells of Ser II did not. Similar results with cells of *X. vesicatoria* were noted with antiserum to glutaraldehyde fixed cells (Table 4). Results with cells of *X. translucens* and *X. malvacearum* varied, depending on the strain. Cells of *X. manihotis* or *X. oryzae* failed to react with any of the four antisera. Cells of *Pseudomonas tomato, P. coronafaciens, P. solanacearum*, and three unknown bacteria from cabbage seeds were negative with all antisera except antiserum to TCA of Ser I. Cells of *P. solanacearum* C-158 and one unknown bacterium resulted in a weak fluorescence with antiserum to TCA of Ser I.

TABLE 1. Strains used in serological studies of *Xanthomonas campestris* pv. *campestris*

Laboratory strain no.	Name	Source strain no.	Location	Source[a]	Host
B-1	*Xanthomonas campestris*	BBS	California	1	Brussels sprouts (*Brassica oleracea* L. var. *gemmifera*)
B-4	*X. campestris*	Original	Georgia	2	Broccoli (*B. oleracea* L. var. *italica*)
B-12	*X. campestris*	Original	Georgia	2	Cabbage (*B. oleracea* L. var. *capitata*)
B-18	*X. campestris*	Original	Florida	2	Cabbage field soil
B-24	*X. campestris*	...	Oregon	3	Broccoli
B-26	*X. campestris*	Original	Georgia	2	Cabbage
B-28	*X. campestris*	Original	Georgia	2	Cabbage
B-30	*X. campestris*	K-2	Germany	4	Rape (*B. napus* L.)
B-32	*X. campestris*	8	New Zealand	5	Cabbage
B-33	*X. campestris*	13	New Zealand	5	Brussels sprouts
B-36	*X. campestris*	Original	Japan	2	Cauliflower seed (*B. oleracea* L. var. *botrytis*)
B-37	*X. campestris*	Original	Japan	2	Cauliflower seed
B-38	*X. campestris*	Original	Japan	2	Cauliflower seed
B-65	*X. campestris*	Original	Brazil	2	Cabbage
B-82	*X. campestris*	...	Australia	6	Cauliflower
B-85	*X. campestris*	Original	Georgia	2	*Lepidium virginicum* L.
B-87	*X. campestris*	Original	California	2	*B. nigra* (L.) Koch
B-88	*X. campestris*	Original	California	2	*B. nigra*
B-89	*X. campestris*	Original	California	2	*Raphanus sativus* L.
B-90	*X. campestris*	Original	California	2	*B. campestris* L.
B-92	*X. campestris*	Original	California	2	*B. geniculata* (Desf.) J. Ball.
B-94	*X. campestris*	Original	California	2	*B. campestris*
B-98	*X. campestris*	Original	California	2	*Cardaria draba* (L.) Desv.
B-107	*X. campestris*	Original	California	2	Cauliflower seed
B-109	*X. campestris*	Original	California	2	Cauliflower seed
B-203	*X. vesicatoria*	069-663	Florida	7	Tomato *Lycopersicon esculentum* Mill.)
B-206	*X. vesicatoria*	...	Florida	7	Pepper (*Capsicum frutescens* L.)
B-214	*X. vesicatoria*	...	Delaware	8	Tomato
B-218	*X. vesicatoria*	Variant of B-214	Delaware	8	Tomato
B-410	*X. translucens*	Xt-104	California	9	...
B-428	*X. translucens*	549	North Dakota	10	Wheat (*Triticum aestivum* L.)
B-430	*X. translucens*	Original	Georgia	11	Rye (*Secale cereale* L.)
B-433	*X. translucens*	Original	Georgia	11	Triticale (*Triticosecale* Whittmack)
B-451	*X. translucens*	973	...	12	...
B-412	*X. malvacearum*	Xm5	California	9	Cotton (*Gossypium hirsutum* L.)
B-414	*X. malvacearum*	R-4	Missouri	13	Cotton
B-441	*X. oryzae*	PXO-63	Philippines	14	Rice (*Oryza sativa* L.)
B-444	*X. oryzae*	PXO-79	Philippines	14	Rice
B-481	*X. manihotis*	Xm-2	Brazil	15	Cassava (*Manihot esculenta* L.)
B-482	*X. manihotis*	Xm-5	Brazil	15	Cassava
B-485	*X. manihotis*	Xm-27	Brazil	15	Cassava
C-7	*Pseudomonas syringae*	B-3	California	16	Peach (*Prunus persica* (L.) Batsch)
C-21	*P. coronafaciens*	Original	Georgia	11	Oats (*Avena sativa* L.)
C-83	*P. coronafaciens*	600	...	12	Oats
C-117	*P. coronafaciens*	Original	Georgia	11	Triticale
C-197	*P. tomato*	Field 3	Georgia	20	Tomato
C-198	*P. tomato*	Field 8	Georgia	20	Tomato
C-158	*P. solanacearum*	51	Ceylon	17	Potato (*Solanum tuberosum* L.)
A-4	*Erwinia carotovora*	EC 105	Missouri	13	...
A-310	*E. chrysanthemi*	B-102	Florida	7	African violet (*Saintpaulia ionantha* Wendel)
D-2	*Escherichia coli*	45-41 (01-6)	Georgia	18	Pig
E-26	*Salmonella typhimurium*	RI-A	Illinois	19	Attenuated
F-3	*Klebsiella pneumoniae*	K-3	Georgia	18	...
...	Unidentified 1	Original	Georgia	2	Cabbage seed
...	Unidentified 2	Original	Georgia	2	Cabbage seed
...	Unidentified 3	Original	Georgia	2	Cabbage seed
...	Unidentified 4	Original	Georgia	2	Cabbage seed

[a] 1 = R. G. Grogan, University of California, Davis; 2 = N. W. Schaad, University of Idaho, Moscow; 3 = L. M. Moore, Oregon State University, Corvallis; 4 = K. Rudolph, University of Göttingen, Germany; 5 = D. Dye, Auckland, New Zealand; 6 = D. S. Trimboli, Narromine, Australia; 7 = J. Miller, Florida Department of Agriculture, Gainesville; 8 = M. Sasser, University of Delaware, Newark; 9 = W. Schnathorst, University of California, Davis; 10 = J. Otta, North Dakota State University, Fargo; 11 = B. Cunfer, University of Georgia, Experiment; 12 = National Collection of Plant Pathogenic Bacteria, Harpenden, England; 13 = R. N. Goodman, University of Missouri, Columbia; 14 = T. W. Mew, International Rice Research Institute, Manilla, Philippines; 15 = A. Takatsu, Universidade de Brasilia, Brazil; 16 = H. English, University of California, Davis; 17 = A. Kelman, University of Wisconsin, Madison; 18 = W. Ewing, Center for Disease Control, Atlanta, GA; 19 = N. Bigley, University of Chicago, IL; 20 = S. M. McCarter, University of Georgia, Athens.

DISCUSSION

Presence of the same antigenic determinant (major band) in ODD tests of the four immunogen preparations was not expected. The results suggest that either the same major specific antigenic determinant is made accessible by formaldehyde, glutaraldehyde, TCA extraction, or ribosomal extraction or that several major antigens share a major specific determinant. Guthrie (6) obtained a single precipitin band from formalized-saline antiserum to *P. phaseolicola* and indicated it was species-specific. In contrast, one or two bands were obtained from formalized-saline antiserum to *Erwinia* spp. (18). For antiserum to TCA extracts, Lucas and Grogan (9) obtained one or two species-specific bands with antisera to *P. lachrymans* and *P. phaseolicola*. Schaad (13) showed that antiserum to ribosomal extracts of *X. vesicatoria* produced one or two precipitin bands. The major specific band was observed to cross-react with *X. campestris*. Allan and Kelman (1) demonstrated that antiserum to glutaraldehyde fixed cells of *E. carotovora* var. *atroseptica* was highly specific in a direct IF test. These results are all in agreement with our results. It seems that a specific determinant is easily extracted by different methods. The number of precipitin bands varied more with bacterial strains than with the immunogen preparation.

In our ODD tests, a very sharp line of precipitin was present with TCA extracts of strains B-4 and B-18 or ribosome preparations of strains B-18, B-24, B-33, and B-90. On the other hand, the line of precipitin was quite diffuse and difficult to interpret with antisera to formaldehyde fixed cells or glutaraldehyde fixed cells of strains B-18 and B-24 when tested against heterologous strains. Since the tests were done with a single rabbit for each immunogen, these qualitative differences could be due to differences in rabbits. However, antisera to each of the four ribosome preparations resulted in a sharp band of precipitin.

The acidic bands in immunoelectrophoresis consisted of two to four antigenic determinants, the exact number being difficult to identify.

When antisera to ribosomes and comparative ODD tests were used, a high correlation resulted between serovar and host of origin. Of the 25 strains studied, 17 originated from cultivated crucifers and eight from cruciferous weeds. Fourteen of the 15 Ser I and Ser II strains originated from cultivated crops. Three of five Ser

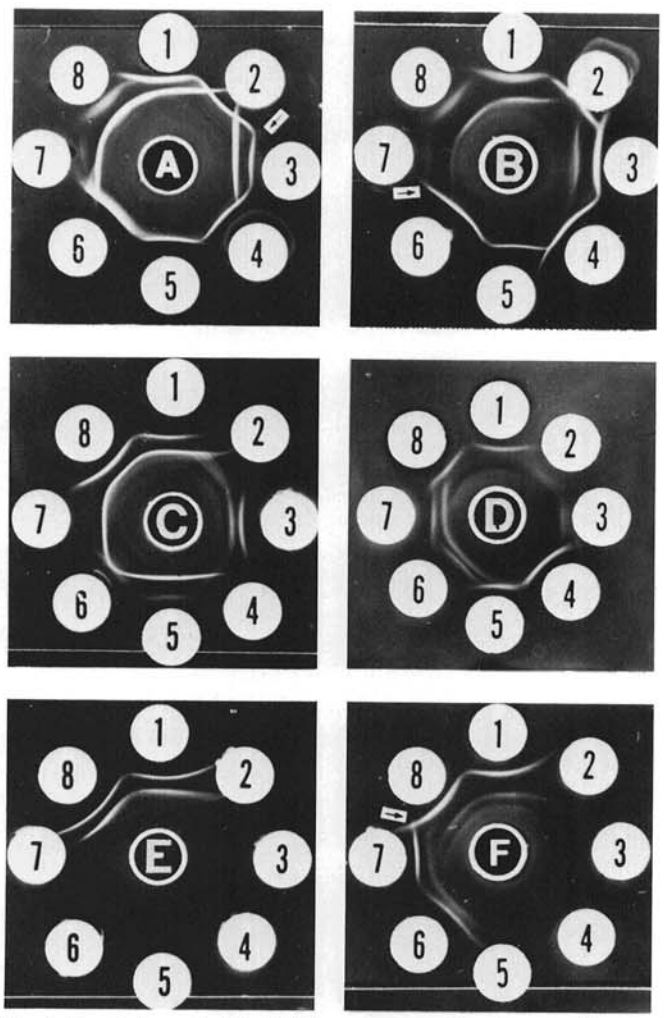

Fig. 4. Ouchterlony double diffusion patterns of antisera to ribosomal extracts of *Xanthomonas campestris* pv. *campestris* Ser I and Ser II. Center wells of **A, C,** and **E** contain antisera to ribosomes of B-24, and center wells of **B, D,** and **F** contain antisera to ribosomes of B-18. Outer wells of **A** contain antigens of 1, B-24; 2, B-1; 3, B-18; 4, B-12; 5, B-28; 6, B-26; 7, B-92; and 8, B-82. Outer wells of **B** contain antigens of 1, B-18; 2, B-12; 3, B-24; 4, B-1; 5, B-26; 6, B-65; 7, B-92; and 8, B-82. Outer wells of **C** contain antigens of 1, B-24; 2, B-33; 3, B-87; 4, B-94; 5, B-82; 6, B-88; 7, B-92; and 8, B-24. Outer wells of **D** contain antigens of 1, B-18; 2, B-88; 3, B-33; 4, B-38; 5, B-28; 6, B-98; 7, B-87; and 8, B-18. Outer wells of **E** contain antigens of 1, B-24; 2, *Erwinia carotovora*; 3, *E. chrysanthemi*; 4, *Escherichia coli*; 5, *Salmonella typhimurium*; 6, *Klebsiella pneumoniae*; 7, unknown from seed; and 8, B-24. Outer wells of **F** contain antigens of 1, B-18; 2, *X. translucens* B-428; 3, *X. manihotis* B-481; 4, *Pseudomonas tomato* C-197; 5, *P. coronafaciens* C-21; 6, *P. syringae* C-7; 7, *P. solanacearum* C-158; and 8, B-18. Arrows indicate weak spur formation. Each center well contains 10 μl of the appropriate antiserum, and each outer well contains 10 μl of the appropriate antigen.

TABLE 2. Ouchterlony double diffusion reactions of antiserum to preparations of ribosomes of different strains of *Xanthomonas campestris* pv. *campestris*

Antigen from:		Antiserum to ribosomes of:			
Serovar	Strain	B-24	B-18	B-33	B-90
I	B-1, B-4, B-24, B-28, B-30, B-36, B-37, B-38, B-89, B-107, B-109	++[a]	+	+	+
II	B-12, B-18, B-26, B-65	+	++	+	−
III-A	B-32, B-33, B-82, B-94	+	+	++	−
III-B	B-92	+	±	++	−
IV	B-90, B-98	+	±	+	++
NT[b]	B-87	+	±	+	−
	B-88	−	+	+	−
	B-85	−	−	−	−

[a] ++ = Reaction of complete fusion (identity), + = reaction of partial fusion (true spur), ± = reaction of noninteraction, − = no line of precipitin.
[b] NT = Not typed.

TABLE 3. Ouchterlony double diffusion reactions of antiserum to preparations of ribosomes of *Xanthomonas campestris* pv. *campestris* and antigens to *X. campestris* and several closely and distantly related bacteria

Antigen from:		Antiserum to ribosomes of *X. campestris* serovar:	
Pathovar	Strain	I	II
Xanthomonas campestris	B-24	++[a]	+
X. vesicatoria	B-206, B-214	+	+
	B-218	+	±
	B-203	−	−
X. malvacearum	B-412, B-414	−	−
X. oryzae	B-441, B-444	−	−
X. translucens	B-428	−	−
	B-410	±	+
X. manihotis	B-481, B-482, B-485	−	−
Pseudomonas syringae	C-7	−	±
P. coronafaciens	C-21, C-83	−	−
	C-117	−	±
P. tomato	C-197, C-198	−	−
P. solanacearum	C-158	±	±
Erwinia carotovora	A-4	−	−
E. chrysanthemi	A-310	−	−
Escherichia coli	D-2	−	−
Salmonella typhimurium	E-26	−	−
Klebsiella pneumoniae	F-3	−	−
Unidentified, from seed	1, 2, 3, 4	−	−

[a] ++ = Reaction of complete fusion (identity), + = reaction of partial fusion (true spur), ± = reaction of noninteraction, − = no line of precipitin.

TABLE 4. Immunofluorescent staining using antiserum to four immunogens of *Xanthomonas campestris* pv. *campestris*

Cells from:			Antiserum[a] to: Formaldehyde fixed cells		Glutaraldehyde fixed cells		Trichloroacetic acid extracts		Ribosomal extracts	
Pathovar	Serovar	Strain	Ser I	Ser II	Ser I	Ser II	Ser I	Ser II	Ser I	Ser II
Xanthomonas campestris	I	B-24	3+[b]	1+	3+	3+	4+	3+	3+	3+
		B-1	3+	1+	3+	3+	3+	2+	3+	3+
		B-28	3+	1+	2+	3+	3+	2+	3+	4+
		B-36	3+	1+	2+	3+	4+	3+	3+	3+
		B-107	3+	1+	3+	4+	3+	3+	4+	4+
	II	B-18	2+	2+	3+	4+	3+	4+	3+	4+
		B-12	4+	3+	2+	3+	4+	4+	3+	4+
		B-26	3+	3+	2+	3+	3+	3+	3+	3+
		B-65	3+	3+	2+	3+	4+	3+	3+	4+
	III	B-32, B-33	1+	1+	1+	1+	1+	1+	1+	1+
		B-82	2+	2+	1+	2+	2+	2+	2+	3+
	IV	B-90	1+	1+	1+	1+	3+	1+	3+	3+
	NT[c]	B-85	−	−	−	−	−	1+	−	−
		B-88	1+	1+	1+	1+	2+	2+	1+	3+
X. vesicatoria		B-203	1+	−	−	−	1+	1+	1+	2+
		B-206	2+	−	1+	−	2+	1+	2+	2+
		B-214	2+	−	−	−	3+	1+	3+	4+
		B-218	3+	−	2+	−	3+	1+	4+	4+
X. translucens		B-410	2+	1+	1+	−	2+	1+	2+	2+
		B-430	−	−	2+	−	−	−	2+	−
		B-433	−	−	1+	−	−	−	1+	−
		B-451	−	−	−	−	−	−	1+	−
X. malvacearum		B-412	1+	1+	1+	−	1+	1+	−	2+
		B-414	1+	1+	−	1+	−	1+	−	−
X. manihotis		B-481	−	−	−	−	−	−	−	−
		B-485	−	−	−	−	−	−	−	−
X. oryzae		B-441	−	−	−	−	−	−	−	−
Pseudomonas tomato		C-198	−	−	−	−	−	−	−	−
P. coronafaciens		C-117	−	−	−	−	−	−	−	−
P. solanacearum		C-158	−	−	−	−	1+	−	−	−
Unidentified, from seed		1	−	−	−	−	−	−	−	−
		3	−	−	−	−	1+	−	−	−
		4	−	−	−	−	−	−	−	−

[a] Staining schedules for antiserum to formaldehyde and glutaraldehyde fixed cells = rhodamine 1:40, antiserum 1:128, DTAF conjugate 1:80. Staining schedules for antiserum to trichloroacetic acid and ribosomal extracts = rhodamine 1:40, antiserum 1:256, DTAF conjugate 1:40.
[b] 4+ = Strong fluorescence, 1+ = weak fluorescence, − = no fluorescence.
[c] NT = not typed.

III strains and all Ser IV and untypeable strains originated from weeds. Furthermore, of the two Ser IV strains and three untyped strains, all but one, B-85, originated from weeds in California. This suggests that the strains of *X. campestris* in weeds may not be the same as those in commercial crops. If this is generally true, one might use serology to study the ecology of *X. campestris*. For example, one might be able to determine if weeds or seeds are the source of infection. ODD tests could therefore be a very useful tool for epidemiological studies.

Antisera to the four immunogens showed little differences in fluorescent staining of cells of *X. campestris*. Antiserum to formaldehyde cells of Ser II did give a weak reaction with cells of Ser I. Cells of Ser I and Ser II reacted stronger than cells of Ser III, Ser IV, and nontyped strains with all four antisera. Antiserum of Ser I cross-reacted more with other xanthomonads than did Ser II. Generally, antisera to TCA and ribosomal extracts cross-reacted more than antisera to formaldehyde and glutaraldehyde fixed cells. Antiserum to glutaraldehyde fixed cells of Ser II showed no cross-reactions in IF tests with other bacteria except strain B-414 of *X. malvacearum*. On the other hand, antiserum to glutaraldehyde fixed cells of Ser I reacted positively with most strains of *X. vesicatoria* and *X. translucens*. The amount of cross-reaction was reduced but not eliminated by using affinity purified DTAF rather than fluorescein isothiocyanate (FITC) conjugated goat antirabbit antibody and rhodamine-BSA. The greatest specificity in IF was found with antiserum to glutaraldehyde and formaldehyde fixed cells of Ser II. However, none of the immunogens was specific enough in IF tests to differentiate among xanthomonads.

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