Serological Relationships Among Membrane Proteins of Strains of *Xanthomonas campestris* pv. *Translucens*

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ABSTRACT

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The serological relationships among 26 strains of *Xanthomonas campestris* pv. *translucens* from wheat, barley, rye, triticale, wild rice, and weeds were determined with antisera to membrane proteins. One or two major bands of precipitin were observed in Ouchterlony agar double-diffusion tests. The band nearest the antigen well (hereafter referred to as the M_1 band) was consistently present, whereas the other band (M_2) was not. Based on the M_1 band and cross-absorption tests, 25 of the 26 strains tested were grouped into two serovars and two subserovars. One strain was untypable. Of the 44 other bacteria tested, only *X. c.* pv. *begoniae* strain B-935 could not be distinguished from *X. c.* pv. *translucens*. In contrast to the high specificity observed in Ouchterlony tests, immunofluorescence (IF) tests resulted in low specificity. Several other xanthomonads, pseudomonads, and erwiniae could not be differentiated from *X. c.* pv. *translucens* based on IF tests. Although strains of *X. c.* pv. *translucens* were more virulent on their hosts of origin and showed variable pathogenicity reactions on different hosts, there was no correlation between host of origin and serovar.

Xanthomonas campestris pv. *translucens* is the causal agent of bacterial blight of barley (*Hordeum vulgare* L.) (17,18) and other cereals (2,7,9,25,27,41) and black chaff of wheat (*Triticum aestivum* L. em Thell) (17,35,36). The pathogen was first identified in 1917 in the United States on barley (17,18). Economic losses occur in barley, wheat, rye (*Secale cereale* L.), and triticale (*Tritico secale* Wittemack) (7,11,18,25,36). The disease is seed-transmitted and seed is considered the major source of inoculum (2,18,36). Although a seed assay based on a selective medium for detecting contaminated seed lots has been described (33), no rapid laboratory method of identifying *X. c.* pv. *translucens* is available. Present methods of identification require pathogenicity tests, which are time-consuming and expensive.

Serological techniques have proved useful for identifying plant-pathogenic bacteria (3,8,22,32,37) and are especially well adapted for rapid and accurate identification of bacteria isolated from seeds (6,12). The purpose of this study was to determine the serological relationships among strains of *X. c.* pv. *translucens* and other closely and distantly related bacteria. LiCl-extracted membrane protein complex (MPC) (40) was chosen as the immunogen. Such membrane proteins have been shown to be highly specific antigens and useful for identifying plant-pathogenic bacteria (4,24,34,38,40).

MATERIALS AND METHODS

Bacterial strains. Twenty-six strains of *X. c.* pv. *translucens* (Table 1) and 44 other bacterial strains were used in this study. The 44 strains were: B-935 of *X. c.* pv. *begoniae* (XB-8, R. S. Dickey); BT-1, BT-4, and BT-27 (original, N. W. Schaad and N. Thaveechai), BT-31 (12-1, S. Chuenchitt), and B-18 and B-107 (original, N. W. Schaad) of *X. c.* pv. *campestris*; B-800 (XCAR, R. E. Stall), B-804 (UMB-46, A. Takatsu), and B-822, B-836, B-837, and B-846 (original, N. W. Schaad) of *X. c.* pv. *carotae*; B-936 of *X. c.* pv. *incanae* (XI-3, R. S. Dickey); B-497 (S. K. Mohan), B-930, and B-937 (056-1 and 8, respectively, S. Chuenchitt) of *X. c.* pv. *malvacearum*; B-468, B-472 (ENA-2647 and ENA-2648, respectively, O. Kimura), and B-487 (Xm-58, A. Takatsu) of *X. c.* pv. *manihotis*; B-441 (PXO-63, T. W. Mew), B-909, and B-919 (TS 8203 and TB-20, respectively, S. Chuenchitt) of *X. c.* pv. *oryzae*; B-490 (XP-18, M. P. Starr), B-496 (P-60, J. W. Sheppard), and B-702 (1208, S. K. Mohan) of *X. c.* pv. *phaseoli*; B-937 (XP-39, R. S. Dickey) of *X. c.* pv. *pelargonii*; B-202 (L. M. Moore), B-218 (549, M. Sasser), and B-260 (VB-1, S. Chuenchitt) of *X. c.* pv. *vesicatoria*; B-938 (XV-29, R. S. Dickey) of *X. c.* pv. *vitians*; B-939 (LX-103, R. S. Dickey) of an unidentified xanthomonad; C-199 (J. W. Guthrie) and C-200 (DM-123, J. W. Guthrie) of *Pseudomonas syringae* pv. *phaseolicola*; C-34 (25, A. Kelman) of *P. s.* pv. *solanacearum*; C-203 (NAGNAD-29, D. Webster), C-271 (PSS-17, T. P. Hubbord), and C-289 (G-198, S. K. Mohan) of *P. s.* pv. *syringae*; C-198 (Field-8, S. M. McCarter) of *P. s.* pv. *tomato*; A-B6 (B6, L. M. Moore) of *Agrobacterium tumefaciens*; A-4 (EC-105, R. N. Goodman) of *Erwinia carotovora* subsp. *carotovora*; A-310 (B-102, J. Miller) of *E. c.* subsp. *chrysanthemi*; D-2 (01-6, W. Ewing) of *Escherichia coli*; and E-26 (RI-A, N. Bigley) of *Salmonella typhimurium*. Bacterial cultures were maintained on slants of yeast extract-dextrose-calcium carbonate (YDC) agar (39) and King's medium B agar (20) at 2–3 C.

Preparation of membrane proteins. Bacteria were grown in 2.8-L Fernbach flasks containing 1.5 L of liquid medium 523 (19) on a rotary shaker at 30 C for 24 hr. Cells in mid- to late-exponential phase of growth were harvested by centrifugation at 12,000 *g* for 15 min and washed with saline (0.85% sodium chloride). The cells were used immediately or stored frozen for 1 or 2 days. Membrane proteins were extracted with LiCl at 50 C as described (40). Extracts were stored in 0.01% thimerosal at 2–3 C until use.

Preparation of antisera. Membrane proteins from four strains of *X. c.* pv. *translucens* (B-451, B-498, B-499, and B-505), and one strain of *X. c.* pv. *carotae* (B-800) were injected into New Zealand white rabbits as described (40). The injections were given three times at 10-day intervals. The amounts of protein in the first, second, and third injections were 2, 4, and 8 mg, respectively. The method of Lowry et al (21) was used to determine protein concentrations. Rabbits were bled for preimmune sera 1 wk before the first injection and for antisera 1, 2, and 3 wk after the last injection as described (40). After serum processing, antisera were kept at −20 C for long-term storage and at 2–3 C in 0.01% thimerosal for immediate use.

Serology. A Piazzi test pattern (26) was used to determine optimal proportions (concentration) of antiserum and antigen for use in Ouchterlony double-diffusion tests. Ouchterlony double-diffusion tests were made in 10-cm diameter plastic petri dishes containing 15 ml of 0.75% ion agar no. 2-trypan blue medium (30). A stainless steel gel punch (Bio Rad Laboratories, Richmond, CA) was used to cut a pattern with an antiserum well 5 mm in diameter surrounded by six or eight peripheral wells, each 3 mm in diameter. The outer wells were 5 mm from the center well. The center well was filled with 15 μl of antiserum and each outer well with 8 μl of antigen. Petri dishes were sealed with Parafilm, placed at 2–3 C, and observed for precipitin lines after 3–4 days. The precipitin lines were recorded as 1) complete fusion (identity reaction), 2) partial fusion (partial identity or cross-reaction), 3) no fusion (noninteraction, nonidentity), 4) weak reaction (faint precipitin line), or 5) no reaction (no precipitin line) (5). Cross-absorption tests were done as described (40). Briefly, one volume of each antiserum was mixed with an equal volume of MPC antigen (10 mg/ml) in a 2-ml test tube. The mixture was incubated for 1 hr at 37 C in a water bath and centrifuged in a model 5414 Brinkmann microfuge for 5 min, and the pellet was discarded. Antisera were absorbed as above one more time by further addition of MPC antigens.

Immunofluorescent staining. Indirect immunofluorescence (IF) tests were made as described previously (31) using IgGs of strain B-451, B-498, B-499, B-505, and B-800. IgGs were prepared from crude antisera by ion exchange column chromatography on Diethylaminoethyl Sephadex A-50 resin packed in a 1.5 × 15-cm glass column. IgGs were eluted from the column with 0.1 M phosphate buffer (pH 7.6), at the rate of 1 ml/min. Columns were monitored at 280 nm. Preimmune sera were used as controls. Block titration tests were used to determine optimum ratios between IgGs and goat antirabbit IgG-conjugated fluorescein (dichlorotriazinyl aminofluorescein, DTAF; Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD 20879).

Pathogenicity tests. Bacterial strains were grown on YDC agar plates for 24 hr at 30 C. The cells were suspended in sterile saline (0.85% NaCl) and their concentration was adjusted to 0.1 OD at 540 nm. Cell suspensions were diluted 100-fold to approximately 10^6 cell/ml and used to inoculate 2-wk-old wheat, barley, rye, and oat plants. Each plant was inoculated 3 cm away from the leaf tip and at the growing point by pricking the leaf with a 3-ml syringe equipped with a 26-gauge needle. Plants were immediately placed in a dew chamber at 25 C. Five days later, plants were removed from the dew chamber and placed on a laboratory bench at 25 ± 2 C with supplementary lighting. After an additional 15 days, plants were inspected and rated for disease development.

TABLE 1. Strains of *Xanthomonas campestris* pv. *translucens*

Laboratory strain no.	Received as: Source strain no.	Source[a]		Location
B-428	549	1		N. Dakota
B-430, 432	Original	2		Georgia
B-433	Original	2		Georgia
B-451	Xt-1	2	(NCPPB 973)	USA
B-452	Xt-2	2	(NCPPB1836)	USA
B-453	Xt-3	2	(NCPPB1943)	USA
B-454	Xt-4	2	(NCPPB2181)	Japan
B-455	Xt-5	2	(NCPPB1837)	USA
B-456	Xt-6	2	(NCPPB1945)	Canada
B-498	URQIE-12	3		Idaho
B-499	NUG-A9	3		Idaho
B-500	Original	3		Oregon
B-501	Original	3		Utah
B-502, 523, 527, 529	Original	3		Idaho
B-503, 505, 507, 508	Original	3		Idaho
B-504	IDO-172	3		Idaho
B-518	4733	4		Brazil
B-519	4734	4		Brazil
B-934	Xt-8	5		Minnesota

[a] Source names and locations: 1 = J. Otta, Brookings, SD; 2 = B. M. Cunfer and N. W. Schaad, Experiment, GA; 3 = N. W. Schaad, Moscow, ID; 4 = S. K. Mohan, Londrina, Brazil; and 5 = R. L. Bowden, Moscow, ID.
[b] NCPPB = National Collection of Plant Pathogenic Bacteria; Harpenden, Herts, England.

RESULTS

Ouchterlony double-diffusion. *X. c.* pv. *translucens* could be grouped into two distinct serovars based on agar diffusion results (Table 2). Antisera to MPC of strains B-451, B-499, and B-505 (Figure 1A and C) resulted in two sharp precipitin bands (M_1 and M_2) near the antigen well and a diffuse band midway between the antigen and antiserum well when tested against homologous MPC preparations. In contrast, antiserum to MPC of strain B-498 resulted only in the M_1 band when tested against homologous B-498 antigen (Fig. 1B, wells 1 and 3). However, the diffuse band was often present when tested against antigen of another strain of *X. c.* pv. *translucens* (Fig. 1B, well 2).

The M_1 band nearest the antigen well was present among 25 of the 26 strains of *X. c.* pv. *translucens*, whereas the M_2 band was present in only some of the strains. The diffuse inner band was very strong in some reactions (Fig. 1A, wells 1, 4, and 7, and Fig. 1C, wells 1, 4, 7, and 8) and weak in some others (Fig. 1B, well 2, and Fig. 1D, well 2). Because the M_1 band was found to be consistently present, only this band was used for subsequent serotyping within *X. c.* pv. *translucens*.

Twenty of the 26 strains of *X. c.* pv. *translucens* were serologically identical with antisera of B-498 (Fig. 1D, wells 2, 3, 6, and 8) and B-499 and were placed in serovar I (Table 2). Three

TABLE 2. Ouchterlony double-diffusion reactions of membrane protein complex (MPC) of *Xanthomonas campestris* pv. *translucens*

MPC antigen from	Antisera to MPC of strains[a]: Serovar I		Serovar II	
	B-498	B-499	B-505	B-451
B-433, 452, 453, 498, 500, 502, 508, 518, 519, 523	++	++	(−)	−
B-430, 432, 456, 499, 501, 504, 507, 527, 529, 934	++	++	0	−
B-454	+	+	0	0
B-428, 451, 505	−	−	++	++
B-503	−	−	(−)	++
B-455	0	0	0	0

[a] Symbols: ++ = complete fusion (identity reaction); + = partial fusion (cross-reaction, partial identity); − = no fusion of precipitin line with homologous antigen (noninteraction reaction, nonidentity); ( ) = weak reaction, precipitin lines were faint; and 0 = no reaction. Reactions based only on the precipitin line nearest antigent well (= M_1 precipitin).

strains resulted in a reaction of identity only when tested against antisera of B-505 (Fig. 1C, wells 1, 7, and 8) and B-451 (Fig. 2B, wells 4 and 5). One strain (B-503) was identical to strain B-451 (Fig. 2B, wells 1 and 2) but not strain B-505 (Table 2). Therefore, strains B-428, 451, 503, and 505 were designated serovar II (Table 2). Strain B-454 cross-reacted with antigen of serovar I strains (Fig. 2A, well 3 [arrow]) and failed to react with antisera of serovar II (Fig. 1A, well 2, and Table 2). Strain B-454 was, therefore, assigned to serovar IA. Strain B-455 failed to react with any of the antisera and was therefore untypable. Although this strain could not be typed with the present sera, with the proper sera, it undoubtedly is typable.

Strains of serovar I resulted in lines of noninteraction (Fig. 2B, wells 3, 6, and 8) or failed to react (Fig. 1A, wells 5, 6, and 8, and Fig. 1C, wells 2, 3, 5, and 6) with antisera of serovar II (Table 2). Similarly, in reciprocal tests, strains of serovar II resulted in reactions of noninteraction with antisera of serovar I (Fig. 1D, well 5).

Of the other pathovars tested, only *X. c.* pv. *begoniae* strain B-935 could not be distinguished from *X. c.* pv. *translucens*. A reaction of identity occurred between MPC of *X. c.* pv. *begoniae* strain B-935 and antiserum of strain B-451 (Fig. 2C, well 8). An additional band was sometimes observed, however, when B-935 was tested against antiserum to B-451 (Fig. 2C, well 8 [arrow]). In contrast, a reaction of noninteraction (homologous M_2 band, but no M_1 band present) occurred between MPC of B-935 and antisera of serovar I strains B-498 (Fig. 2D, wells 2, 4, and 6), and B-499, and serovar II strain B-505. Two strains of *X. c.* pv. *phaseoli* (B-496 and B-702) and one strain of *X. c.* pv. *malvacearum* (B-930) resulted in reactions of noninteraction against antiserum to strain B-451. No reaction occurred between these strains and antiserum to *X. c.* pv. *translucens* strains B-498, 499, or 505. Antiserum to *X. c.* pv. *carotae* strain B-800 reacted with MPC of *X. c.* pv. *carotae*, but not with that of *X. c.* pv. *translucens* or any other organism tested.

None of the other organisms tested reacted (M_1 antigen) against any of the antisera to *X. c.* pv. *translucens*. However, the M_2 band was observed with some other organisms. For example, strain C-34 of *P. s.* pv. *solanacearum* resulted in a weak M_2 band of precipitin (reaction of noninteraction) when tested against antiserum to B-499. In addition, the M_2 precipitin of strains B-930 and 931 of *X. c.* pv. *malvacearum* and B-202 and 260 of *X. c.* pv. *vesicatoria* was identical to the M_2 band of *X. c.* pv. *translucens* when reacted with antiserum to strain B-451.

Grouping of the strains of pathovar *translucens* into serovars I, IA, and II was substantiated by cross-absorption tests (Table 3). Furthermore, the differentiation of bands of noninteraction (nonidentity) from bands of cross-reactions (partial identity) was confirmed. Absorbing antisera to MPC of strains B-498 (serovar I) and B-505 (serovar II) with MPC from *X. c.* pv. *begoniae* strain B-935 eliminated the noninteraction reactions (Table 3 and Fig. 3A, wells 4 and 6). Reactions of identity (Table 3 and Fig. 3A, well 2) or partial identity (Table 3) were not eliminated. In contrast, absorption of antiserum of B-451 with MPC of B-935 resulted in the elimination of all precipitin bands, proving that *X. c.* pv. *begoniae* and strain B-451 have identical MPC antigens (Table 3). Based on cross-absorption tests, strains B-451 and B-503 were placed into a subserovar of serovar II (serovar IIA). Absorption of antiserum of *X. c.* pv. *translucens* B-499 with MPC of *P. s.* pv. *solanacearum* strain C-34 (reaction of noninteraction) eliminated the band of precipitation with MPC of C-34 (Fig. 3B, well 8) without altering the reaction with *X. c.* pv. *translucens* (Fig. 3B, wells 1–7).

Immunofluorescence. All strains of *X. c.* pv. *translucens* were immunofluorescence-positive against IgG of both strains of

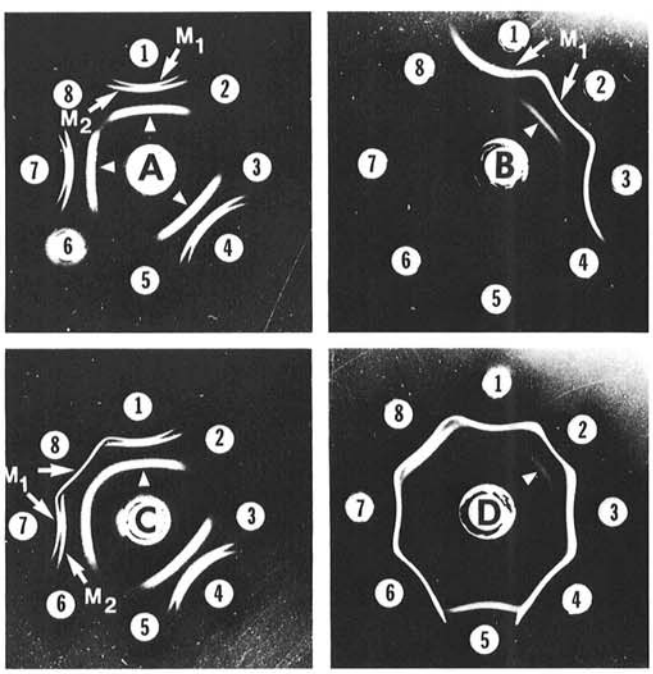

Fig. 1. Ouchterlony double-diffusion reactions of antisera to membrane protein complexes (MPC) of *Xanthomonas campestris* pv. *translucens* against MPC antigens of strains of *X. c.* pv. *translucens*. Center wells contained 15 μl of antiserum to MPC of strains: **A** and **C**, B-505, and **B** and **D**, B-498. Outer wells contained 8 μl of MPC antigens. Outer wells of A contained MPC of strains: 1, 4, and 7, B-505; 2, B-454; 3, B-455; 5, B-456; 6, B-934; and 8, B-499. Outer wells of B contained MPC of strains: 1 and 3, B-498; 2, B-432; and 4–8, no antigen. Outer wells of C contained MPC of strains: 1, 4, and 7, B-505; 2, B-519; 3, B-523; 5, B-527; 6, B-529; and 8, B-428. Outer wells of D contained MPC of strains: 1, 4, and 7, B-498; 2, B-430; 3, B-433; 5, B-451; 6, B-452; and 8, B-453. Note specific precipitin lines (arrows in pattern A, well 1; pattern B, wells 1 and 2; and pattern C, wells 7 and 8) and nonspecific, diffuse precipitin lines (triangle in pattern A, wells 1, 4, and 7; pattern B, well 2; pattern C, wells 1, 4, 7, and 8; and pattern D, well 2).

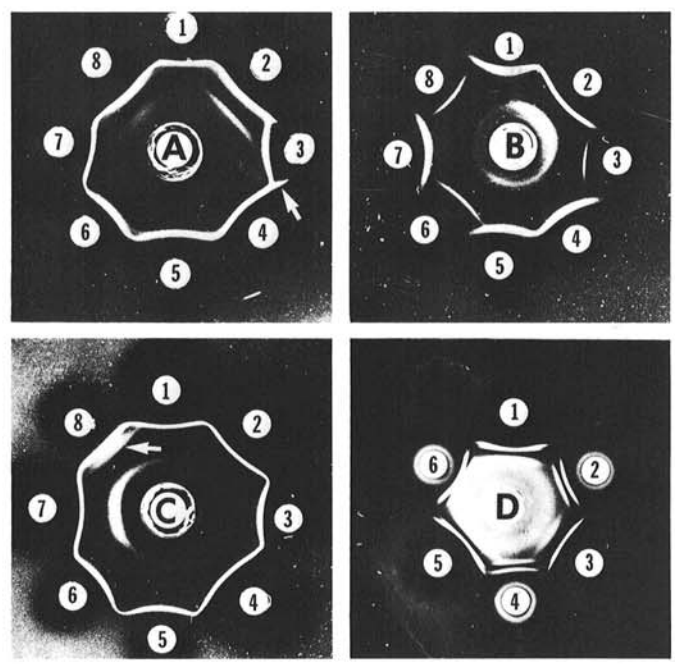

Fig. 2. Ouchterlony double-diffusion reactions of antisera to membrane protein complexes (MPC) of *Xanthomonas campestris* pv. *translucens* against MPC antigens of strains of *X. c.* pv. *translucens* and *X. c.* pv. *begoniae*. Center wells contained 15 μl of antiserum to MPC of strains: **A** and **D**, B-498; **B** and **C**, B-451. Outer wells contained 8 μl of MPC antigens. Outer wells of A contained MPC of strains: 1, 4, and 7, B-498; 2, B-504; 3, B-454; 5, B-507; 6, B-508; and 8, B-518. Outer wells of B contained MPC of strains: 1, 4, and 7, B-451; 2, B-503; 3, B-504; 5, B-505; 6, B-507; and 8, B-508. Outer wells of C contained MPC of strains: 1, 2, and 4, B-451; 3, 5, and 6, B-505; 7, B-503; and 8, B-935. Outer wells of D contained MPC of strains: 1, 3, and 5, B-498; 2, 4, and 6, B-935.

TABLE 3. Ouchterlony double-diffusion reactions between absorbed antisera of *Xanthomonas campestris* pv. *translucens* and MPC antigens of *X. c.* pv. *translucens* and other bacteria

Antigen (MPC) from	Antisera to MPC of[a,b,c]							
	Serovar I B-498		Serovar I B-499		Serovar II B-505		Serovar IIA B-451	
	NA A/B-935		NA A/C-34		NA A/B-935		NA A/B-935	
pv. *translucens*								
B-433, 498, 500, 518	++	++	++	++	(−)	0	−	0
B-430, 499, 507	++	++	++	++	0	0	−	0
B-454	+	+	+	+	0	0	−[d]	0
B-503	−	0	−	−	(−)	0	++	0
B-428, 451, 505	−	0	−	−	++	++	++	0
B-455	−[d]	0[d]	−[d]	−[d]	0	0	0	0
pv. *begoniae*								
B-935	−	0	−	NT[e]	−	0	++	0
pv. *carotae*								
B-800	−[d]	0[d]	0	0	(−)[d]	0	−[d]	0
pv. *oryzae*								
B-909	0	0	−[d]	−[d]	0	0	0	0
Pseudomonas solanacearum								
C-34	0	0	−[d]	0[d]	0	0	0	0

[a] NA = not absorbed.
[b] A/ = absorbed with.
[c] Symbols: ++ = complete fusion (identity reaction); + = partial fusion (cross-reaction, partial identity); − = no fusion of precipitin line with homologous antigen (noninteraction reaction, nonidentity); ( ) = weak reaction, precipitin lines were faint; and 0 = no reaction.
[d] Second precipitin line (= M_2 precipitin).
[e] Not tested.

serovar I of *X. c.* pv. *translucens* (1+ to 5+; 1+ = weak fluorescence, 5+ = strong fluorescence, − = no fluorescence). In contrast, not all strains of *X. c.* pv. *translucens* were positive against IgG of serovar II and IIA (B-498, 499, 500, and 504 were negative against IgG of serovar II, and B-507, 508, 519, 529, 433, 453, 432, 452, and 456 were negative against IgG of serovar IIA). The positive reactions were from weak (1+) to strong (5+), however. In general, no consistent differences in intensity of fluorescence were observed among serovars. However, consistent differences were observed with IgG of *X. c.* pv. *carotae*. Only strains of *X. c.* pv. *carotae* were fluorescent-positive (3+ to 5+) against IgG of *X. c.* pv. *carotae* (strain B-702 of *X. c.* pv. *phaseoli* was weakly positive). Among other *X. campestris* pathovars, cells of *X. c.* pv. *begoniae* strain B-935 gave a positive (5+) fluorescence with two IgGs of *X. c.* pv. *translucens* strains (B-499, serovar I, and B-451, serovar IIA) and rather weak (2+) fluorescence with the other two strains (B-498, serovar I, and B-505, serovar II). Reactions of the strains of *X. c.* pv. *campestris, carotae, incanae, manihotis, pelargonii, phaseoli*, and *vesicatoria* varied greatly from negative to moderately positive (3+), depending on the strain and the IgG involved. Some strains of *P. s.* pv. *syringae* and *phaseolicola, Erwinia chrysanthemi, E. c.* subsp. *carotovora*, and *Salmonella typhimurium* also resulted in weak fluorescence (1+ and 2+) against at least one of the IgGs of *X. c.* pv. *translucens*. Only *E. coli* strain D2 gave a negative reaction with all the IgGs.

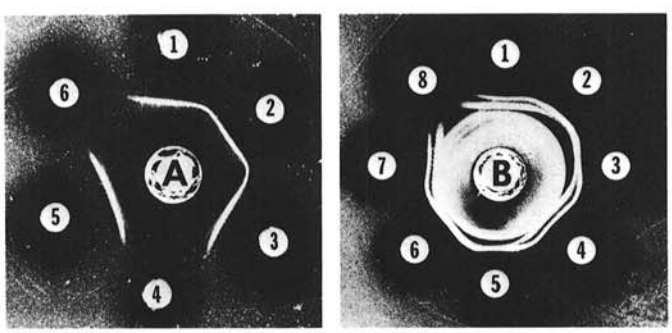

Fig. 3. Ouchterlony double-diffusion reactions of absorbed antisera to membrane protein complexes (MPC) of *Xanthomonas campestris* pv. *translucens* against MPC antigens of strains of *X. c.* pv. *translucens*, other pathovars, and bacteria. Center well contained 15 μl of absorbed antiserum to MPC of strains: **A,** B-498 absorbed with MPC of *X. c.* pv. *begoniae* strain B-935; and **B,** B-499 absorbed with MPC of *Pseudomonas solanacearum* strain C-34. Outer wells contained 8 μl of MPC antigens. Outer wells of A contained MPC of strains: 1, 3, and 5, B-498; 2, B-500; 4, B-503; and 6, B-935. Outer wells of B contained MPC of strains: 1, 4, and 7, B-499; 2, B-500; 3, B-518; 5, B-433; 6, B-430; and 8, *P. solanacearum* strain C-34.

Pathogenicity tests. Strains of *X. c.* pv. *translucens* were generally more virulent on the host from which they were isolated than on the other hosts. All strains except those from grasses and strain B-505 from barley were virulent on wheat (Table 4). The two strains from grasses were pathogenic to barley only. Oats exhibited either no reaction or very slight chlorosis at the inoculation sites

TABLE 4. Host range reactions of small grains to strains of *Xanthomonas campestris* pv. *translucens*[a]

Original host and strain	Wheat[b] (Borah)	Barley[b] (NK425)	Rye[b]	Oats[b] (Anthony)
Wheat				
B-428, 498, 504, 518, 523, 527, 529	+++	+	+	−
B-456	++	+	++	C
B-499, 502, 519	++	+	C	−
Barley				
B-451, 453	+	+++	C	C
B-500, 503, 507, 508	+	+++	−	−
B-501	+	++	C	−
B-505	−	++	−	−
Rye				
B-430, 432	+	++	+++	C
B-452	++	+	++	−
Triticale				
B-433	++	++	++	C
Wild rice				
B-934	+	+	−	−
Orchard grass				
B-454	−	+	C	−
Timothy grass				
B-455	−	+	C	−

[a] Symptom readings are from three plants per treatment replicated three times.
[b] + = mild water-soaking; ++ = moderate water-soaking; +++ = severe water-soaking; − = no symptoms; C = chlorosis at inoculation sites.

(Table 4). Most of the strains from wheat were also pathogenic to rye, whereas none of the strains from barley was virulent on rye (Table 4). In contrast, all rye and triticale strains were pathogenic to barley.

DISCUSSION

One or two precipitin bands in Ouchterlony agar diffusion tests were observed in this study. The number of precipitin bands can vary with the bacterial strain or with the immunogen preparation (37). Normally, the more purified the preparation, the fewer the bands (3).

Using antisera to MPC and comparative Ouchterlony double-diffusion tests, 25 strains of *X. c.* pv. *translucens* were typed into two serovars, of which serovar I strains predominated. One strain (B-455) isolated from a weed plant did not react with any of the antisera and was not typable. This strain was virulent on barley and resulted in typical colonies on YDC agar. Determining whether other strains from grasses (weeds) are serologically distinct will require additional testing of grass strains.

Cross-absorption studies supported our grouping of the *X. c.* pv. *translucens* strains into two serovars. Furthermore, cross-absorption tests revealed the antigenic differences between strains in serovar II and serovar IIA, which were otherwise not evident. These cross-absorption tests also confirmed that *X. c.* pv. *begoniae* strain 935 shares antigens with *X. c.* pv. *translucens* serovar IIA. Therefore, *X. c.* pv. *begoniae* 935 is truly serologically related to serovar IIA of *X. c.* pv. *translucens.*

This investigation was initiated primarily to determine the serological relationships among strains of *X. c.* pv. *translucens* and other closely related pathovars with the goal of using serology for rapid and accurate identification of *X. c.* pv. *translucens.* We found that the antisera produced against MPC from four strains of *X. c.* pv. *translucens* reacted positively in Ouchterlony tests with only one of the 44 other bacteria tested (*X. c.* pv. *begoniae*). This suggests that MPC of *X. c.* pv. *translucens* contains a highly specific immunogen at the pathovar level. A similar specificity of membrane proteins has been reported for *P. s.* pv. *solanacearum* (34), *E. c.* subsp. *chrysanthemi* (40), and *X. c.* pv. *campestris* (38). In contrast to the high specificity observed in Ouchterlony tests, IF tests resulted in low specificity. Several other bacteria, including pseudomonads and erwiniae, could not be differentiated from *X. c.* pv. *translucens.* Therefore, IF tests should be used only as an aid in identifying suspected colonies isolated on specialized media, such as YDC and *Xanthomonas translucens* selective medium (XTS) agars. Diagnosis of black chaff by IF without isolation of *X. c.* pv. *translucens* on media should not be attempted.

The cross-reaction of some bacteria with IgGs of *X. c.* pv. *translucens* strains in IF tests were not expected, because these bacteria did not result in precipitation bands of any type in Ouchterlony tests. One explanation might be that these strains, like *X. c.* pv. *campestris,* may have a common antigen that is shared by serovars I, II, and IIA, but because it is such a small amount it is detected only by the more sensitive IF tests (38). The only pathovar of *X. c.* pv. *campestris* that was both IF- and Ouchterlony-positive was *X. c.* pv. *begoniae* B-935. The strong fluorescence staining of *X. c.* pv. *begoniae* with IgG of *X. c.* pv. *translucens* was supported by results of Ouchterlony tests using absorbed antisera. This serological identity between a strain of pathovars *begoniae* and *translucens* has not been previously reported. However, more strains of *X. c.* pv. *begoniae* will need to be tested before making conclusions about their relatedness. Strain B-935 failed to infect wheat or barley, but we did not test pathogenicity on begonia.

Jones et al (16) separated *P. s.* pv. *tomato* into serogroups by IF tests using antisera to whole cells. In contrast, Thaveechai and Schaad (38) failed to observe any serogroups among strains of *X. c.* pv. *campestris* using MPC antisera and IF tests. Our IF studies using purified IgG and optimum concentrations obtained from block titration tests also failed to distinguish any serovars among strains of *X. c.* pv. *translucens.* These results indicate that IF may not work with sera directed against membrane proteins but it is indispensable under other circumstances. They also emphasize the problems in attempting to type strains or identify unknown bacteria by IF tests alone (31).

Previous studies have supported a high correlation between serological properties and host of isolation for some plant pathogens, such as *E. c.* subsp. *chrysanthemi* (28,29). In contrast, Yakrus and Schaad (40) and Dickey et al (8) were not able to demonstrate such a correlation for strains of *E. c.* subsp. *chrysanthemi.* Our results do not support a high correlation between antigenic properties and original hosts of *X. c.* pv. *translucens* but instead agree with results of Mazzucchi et al (23). They placed strains of *E. c.* subsp. *chrysanthemi* from different hosts in the same serovar based on immunological studies of their pectic lyases.

The variability of pathogenicity reactions of *X. c.* pv. *translucens* strains on different hosts was essentially the same as that reported by Boosalis (2), Bamberg (1), Cunfer and Scolari (7), Fang et al (10), Hagborg (13), Hall and Cooksey (14), and Hall et al (15). These results indicate that strains from individual small-grain species exhibit a variable host range and that a division into forma speciales has no merit. Our results agree with those of Bamberg (1) and Cunfer and Scolari (7) in that some strains of *X. c.* pv. *translucens* are more virulent on their hosts of origin. Some investigators (1,2,7,10,13) have stated that strains from barley exhibit two pathogenicity patterns. Some strains are restricted to barley and are designated f.sps. *hordei* type; other strains are restricted to wheat and are designated f.sps. *undulosa.* Among the barley strains of *X. c.* pv. *translucens* we used, one strain was restricted to barley, whereas seven strains were not restricted. Others report that some strains are restricted largely to rye (f.sps. *secalis*) or triticale (2,13), but our strains (originally from Georgia) from rye and triticale are not restricted to their original hosts.

The results obtained here and those reported by Cunfer and Scolari (7) are similar. However, we found that strains B-455 and B-456 were pathogenic on barley. This difference in pathogenicity may be due to use of different cultivars of barley. Our results do agree that no strain was pathogenic on oats (7).

In conclusion, we have demonstrated that preparations of MPC of *X. c.* pv. *translucens* appear to contain a highly specific immunogen at the pathovar level. Furthermore, Ouchterlony double-diffusion is an effective method for accurate identification of *X. c.* pv. *translucens.*

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