Erwinia chrysanthemi: Serological Comparisons of Strains from *Zea mays* and Other Hosts

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ABSTRACT

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Four types of reactions were observed in Ouchterlony double diffusion tests when 47 strains of *Erwinia chrysanthemi* originally isolated from *Zea mays* or *Zea mays* var. *rugosa* and 131 strains from 40 different hosts were tested with antisera produced against two glutaraldehyde-treated strains from corn. The reactions observed included: a strong primary precipitin band, a strong primary band plus a weak secondary band, only a weak secondary band, or no bands. A strong primary band was used to designate serovar groups; however, only four strains produced a primary band with either antiserum. Three of the strains, originally isolated from corn, reacted with one antiserum and were included in a new group that was designated serovar V, whereas the fourth strain previously assigned to serovar II group reacted with the other antiserum. Thirty-seven strains produced only secondary bands with the two antisera; 20 of the strains were isolated originally from corn and 17 were isolated from nine different hosts. The secondary bands were shown to be similar, if not identical, by fusion of the bands and by cross absorption tests. Although only corn strains produced primary bands, there was no definite relationship between production of secondary bands and the original host, geographical location, and phenotypic characteristics of the strains. Thirty-three strains of other *Erwinia* species or subspecies did not react with the two antisera. The results demonstrated the specificity of the antisera for strains of *E. chrysanthemi*, the heterogeneity of the antigenic properties of strains and the limitations of the antisera for diagnoses and identification of phytopathogenic strains isolated from corn plants.

Serological methods have been used for the identification of *Erwinia chrysanthemi* Burkholder, McFadden & Dimock 1953 to designate serovars and to determine the relationships among strains of the pathogen from different hosts and with other closely related *Erwinia* species (3,7,13–18,20). It was reported by Dickey et al (7) that 315 of 404 strains of *E. chrysanthemi* originally isolated from 42 different hosts reacted with one or more of nine antisera produced against strains of *E. chrysanthemi* and were separated into four serovars (I, II, III, and IV) and 89 strains failed to react with any of the antisera. A reaction of complete fusion (2) (i.e., identity) between the strong precipitin bands in agar diffusion tests was used to designate serovars. Weaker precipitin bands that did not fuse with the strong band were produced by some strains, but production of weaker bands was not used to distinguish serovars. Other *Erwinia* species or subspecies (103 strains tested) did not react with any of the nine antisera. Forty-six of the 89 strains of *E. chrysanthemi* that failed to react with any of the nine antisera were isolated originally from *Zea mays* L. or *Zea mays* L. var. *rugosa* Bonaf. Although one of the nine antisera was produced against formalin-treated cells of strain W1-1 from *Zea mays*, the antiserum only reacted with the homologous strain and the strain was designated serovar II. It was suggested that production of additional antisera would reduce the number of untyped strains (7), although some evidence indicated that the number of strains assigned to new serovars might be limited by the specificity of the antigenic properties of the strains (15–18).

Subsequently, two antisera have been produced against glutaraldehyde-treated strains of *E. chrysanthemi* originally isolated from *Zea mays*. One strain differed from those used to produce the nine antisera for the previous studies (7), whereas the second strain was a different culture of the strain (W1-1) used to produce the antiserum of serovar II. The latter strain was included to determine whether immunogen preparation or source of culture affected the reaction to antisera. This investigation also was initiated to determine the specificity of antisera produced against strains originally isolated from the same host, the use of antisera for the identification of phytopathogenic bacteria isolated from diseased corn plants, and the significance, if any, of the weak secondary bands produced by some strains of *E. chrysanthemi*.

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MATERIALS AND METHODS

Bacterial strains. The 211 bacterial strains used for the tests included the 89 untyped strains of *E. chrysanthemi* from 14 hosts that did not react with any of the nine antisera previously tested (7); the original number, source, and location of the strains have been reported elsewhere (4–7). Seventeen additional strains also were tested that have failed to react with any of the nine antisera. The seventeen strains were isolated from *Aloe barbadensis* Mill. var. *vera* (A-1, A-2, A-3, A-4, A-5, from E. N. Mulrean, Arizona), *Panicum maximum* Jacq. (0775, from A. C. Hayward, Queensland, Australia), *Solanum tuberosum* L. (DAR 30501, 30505, 30509, 30513, 30514, 30515, 35633, 35634, from E. J. Cother, N.S.W., Australia), *Saccharum officinarum* L. (0801, 0803, from A. C. Hayward, Queensland, Australia) or *Zea mays* L. (SR 172, from J. I. Victoria, Colombia). The nine strains of *E. chrysanthemi* from six hosts used for the production of the nine antisera previously tested (7) were included in the tests, plus 63 typed strains from 27 other hosts that reacted with one or more of the nine antisera. Thirty-three strains of other *Erwinia* species or subspecies also were tested, including five strains of *E. amylovora*, one of *E. ananas*, five of *E. carotovora* subsp. *atroseptica*, four of *E. carotovora* subsp. *betavasculorum*, seven of *E. carotovora* subsp. *carotovora*, two of *E. cypripedii*, one of *E. mallotivora*, two of *E. quercinia*, two of *E. rhapontici*, two of *E. rubrifaciens*, and one of *E. salicis* and *E. uredovora*. The two corn strains of *E. chrysanthemi* for antisera production were obtained from the American Type Culture Collection (Rockville, MD), ATCC 27386 (= C_1B_1-AT) and 29274 (= W1-1-AT).

Preparation of immunogens. *E. chrysanthemi* strains C_1B_1-AT and W1-1-AT were streaked onto yeast extract-dextrose-calcium carbonate medium (YDC) (8) and incubated for 48 hr at 28 C. Bacterial cells were removed from the medium with a sterile rubber spatula and placed in Corex tubes containing sterile, 0.01 M

potassium phosphate-buffered (pH 7.2) saline (0.85% NaCl) (PBS). Cells were washed by centrifuging at 17,600 *g* for 3 min and resuspending the pellet in sterile PBS. This washing procedure was repeated three times. After the last centrifugation, the cells were resuspended in PBS and poured into dialysis tubing (Spectrum Medical Industries, Los Angeles, CA; M. W. Cutoff 12,000–14,000) and dialyzed in 2% glutaraldehyde (Sigma Chemical, St. Louis, MO) solution for 3 hr at room temperature (1). The cells were then dialyzed in PBS for 24–36 hr at 4 C with five to six changes of PBS to remove glutaraldehyde. An equal volume of bacterial suspension (about 3×10^{11} cfu per milliliter) and Freund's incomplete adjuvant (Difco, Detroit, MI) were emulsified with a Spex mixer-mill (Spex Industries, Metuchen, NJ) for 2 min at high speed.

New Zealand white rabbits were injected subcutaneously with 1 ml of the emulsified suspension at weekly intervals. After the fourth injection, the rabbits were bled from the marginal ear vein. Injections continued until agglutination titers exceeded 1:2,560. Rabbits received 7–10 injections. Serum was removed 3–4 hr after bleeding, pipetted into serum bottles, and stored at −20 C without preservatives.

Ouchterlony double diffusion tests: The medium selected for the tests is similar to that reported by Schaad (17). The medium contained 0.75 or 0.95% Oxoid Purified Agar, 0.85% NaCl, 0.025% sodium azide (practical grade; ICN Pharmaceuticals, Life Sciences Group, Cleveland, OH) and 0.01% trypan blue (diamine blue 3B; National Aniline Division, Allied Chemical Corp.). Ten milliliters of the media was distributed into plastic petri dishes (100× 15 mm), and the dishes were kept overnight at room temperature after which they were stored at 9 C. One-half of the plates were placed for 24 hr in an incubator at 30 C before use. Media containing 0.75 or 0.95% agar in plates kept at 9 C or moved to 30 C 24 hr before the tests were used for each test and replication. Circular well patterns consisted of a 4-mm-diameter center well, for antiserum, surrounded by six or eight outer wells, for antigens, each 4 mm diameter, 8 mm from the center well; 14 μl of the antigen or antiserum (AS) were placed in each well. AS C_1B_1-AT was used without dilution, whereas AS W1-1-AT was diluted 1:2 in normal saline. The plates were incubated at 22.5–24.0 C in a humid enclosure and observed with a stereoscopic microscope for precipitin lines at 2 or 3, 5, and 6 or 7 days.

Suspensions of whole bacterial cells and phenol-water extracts were used as antigen preparations. The bacterial cells were grown on YDC (9) for 24 hr at 27 C, and the whole-cell suspensions, prepared in normal saline to provide at least 10^9 cfu per milliliter, were mixed on a vortex mixer for 1 min. Cell suspensions from YDC-cultures were extracted with phenol-water at 65 C for 15 min and dialyzed for 3 days against distilled water (19). Some strains were grown in 100 ml of 523 broth (12) in shake culture, 60 excursions per min, for 16 hr at 28 C. The cells were separated from the broth by centrifugation, suspended in normal saline and extracted in phenol-water as described above. A portion of the dialyzed extracts was heat treated in a boiling water bath for 1 hr or in an autoclave for 30 min at 121 C.

Cross absorption tests. The antisera were mixed 1:1 with a saline suspension (about 10^9 cfu per milliliter) of cells grown for 24 hr at 27 C on YDC slants. The mixtures were kept at 37 C for 2 hr, centrifuged at 6,000 *g* for 30 min, and the pellets were discarded. Antisera also were mixed with phenol-water extracts and kept at 37 C for 2 hr. The treated antisera were tested and, if necessary, the absorption procedures were repeated. The final mixtures of antisera and phenol-water extracts varied from 1:1 to 1:4. The unabsorbed antisera were diluted with a corresponding amount of sterile distilled water.

RESULTS

The results with phenol-water extracts were more distinct than with whole cell suspensions, although the latter antigen preparations were simpler and quicker. Agar concentration and temperature of storage of plates before use had no consistent effects on the production of bands; however, the production and clarity of weak precipitin bands sometimes were enhanced by one or more of the four media preparations. Whole cell suspensions and phenol-water extracts of strains grown in 523 broth produced somewhat weaker and less distinct precipitin bands than the same strains grown on YDC slants. Heat treatment of the phenol-water extracts did not affect the production of precipitin bands.

The 33 strains of other *Erwinia* species or subspecies did not react with AS C_1B_1-AT or AS W1-1-AT.

Primary precipitin bands. Three strains of *E. chrysanthemi* [C_1B_1-CU (Cornell University Collection of Phytopathogenic Bacteria strain 0561), C_2B_2 and 221] produced strong, diffuse primary precipitin bands with AS C_1B_1-AT and a reaction of complete fusion with strain C_1B_1-AT (Fig. 1A, wells 1, 3, 4, and 5). These corn strains were assigned to a new serovar group V (Table 1) because production of a strong primary band was used to designate serovar groups I–IV in previous studies with nine antisera (7), and the strains did not react with any of the nine antisera or AS W1-1-AT.

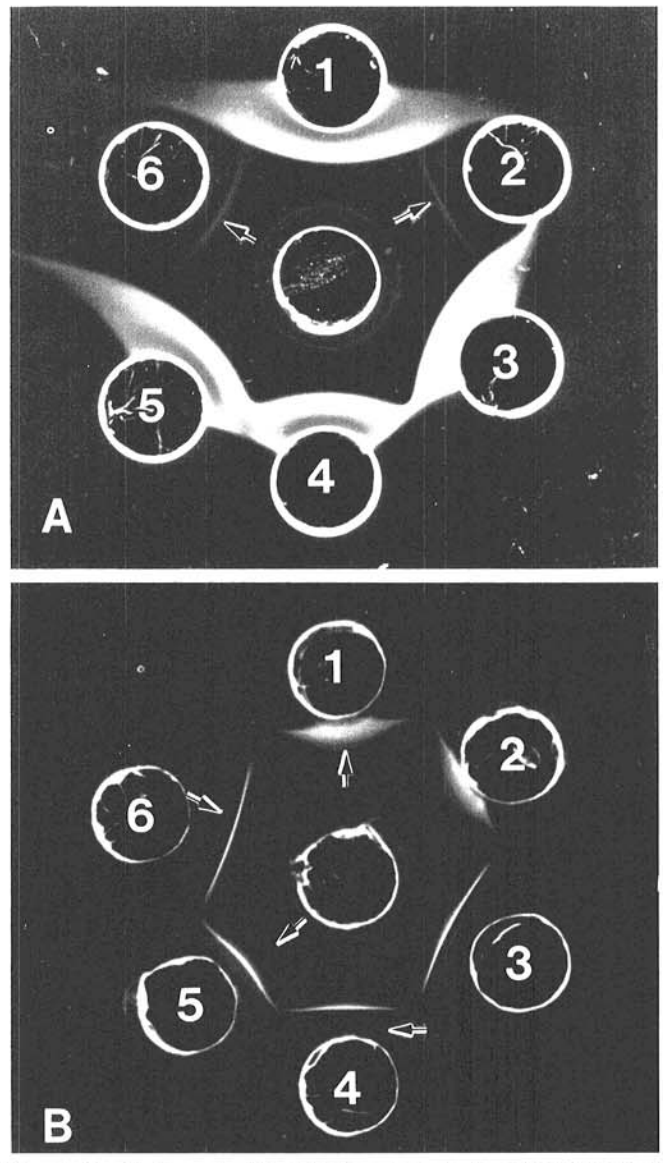

Fig. 1. Ouchterlony double diffusion patterns to different antigen preparations of *Erwinia chrysanthemi*. **A,** Center well contained antiserum C_1B_1-AT. Outer wells contained phenol-water extracts of: 1 and 4, homologous strain C_1B_1-AT (serovar V); 2, strain 362 (serovar III); 3, strain 221 (serovar V); 5, strain C_2B_2 (serovar V); and 6, strain W1-1-AT (serovar II). **B,** Center well contained antiserum W1-1-AT. Outer wells contained: 1 and 2, whole-cell suspensions of strain W1-1-AT; 3 and 6, phenol-water extracts of strain W1-1-AT; 4, phenol-water extract of strain W1-1-CU; and 5, whole-cell suspension of strain W1-1-CU. Arrows indicate secondary bands.

Only strain W1-1-CU (Cornell University Collection of Phytopathogenic Bacteria strain 0661) produced a strong primary band with AS W1-1-AT (Fig. 1B, wells 4 and 5) and a reaction of complete fusion with strain W1-1-AT (wells 3 and 6). Subsequently, strain W1-1-AT was tested with AS W1-1 previously produced against strain W1-1-CU (7), and it produced a primary band and a reaction of complete fusion with homologous strain W1-1-CU. Therefore, the two strains were included in serovar group II (Table 1).

Secondary precipitin bands. Weak secondary bands were produced by some strains as was reported for the previous study (7). The weak secondary bands produced by the homologous strain for AS W1-1-AT and by strain W1-1-CU were located toward the center well from the primary band for whole cell suspensions (Fig. 1B, wells 1, 2, and 5) and toward the outer well from the primary band for the phenol-water extracts (Fig. 1B, wells 3, 4, and 6). The secondary bands produced by strain W1-1-CU were more diffuse and less distinct (Fig. 1B, wells 4 and 5) than those produced by strain W1-1-AT (Fig. 1B, wells 1, 2, 3 and 6). Secondary bands were produced by serovar V strains C_1B_1-CU and C_2B_2 with AS W1-1-AT (see Table 3, unabsorbed column). Stain W1-1-AT (serovar II) produced a secondary band, but no primary band, when tested with AS C_1B_1-AT, whereas strain W1-1-CU failed to produce either a secondary or a primary band with AS C_1B_1-AT (see Table 3, unabsorbed column).

Secondary bands were produced by 37 strains (Table 2) of the 211 strains of *E. chrysanthemi* that were tested with AS W1-1-AT and AS C_1B_1-AT (Fig. 1A, wells 2 and 6; Fig. 2A). The secondary bands coalesced with secondary bands of W1-1-AT, W1-1-CU, C_1B_1-CU, and C_2B_2 and with each other to produce a reaction of fusion (Fig. 2A). The 37 strains, which were collected at 17 locations, were originally isolated from corn and nine other hosts (Table 2). Twelve of the strains also produced primary bands with the antisera for serovar groups I or III, whereas the remaining 25 strains did not produce primary bands with any of the 11 antisera. Although a majority of the strains possess the phenotypic characteristics for biovar (subdivision) IV (4,6), the remaining strains have properties that are characteristic for biovar III.

Cross absorption of AS W1-1-AT and AS C_1B_1-AT with strains that produced secondary bands eliminated the production of secondary bands by all strains that produce secondary bands with unabsorbed antisera (Table 3; Fig. 2B). The production of secondary bands occurred after AS W1-1-AT was absorbed with two strains (C_1B_1-AT and 359) that did not produce secondary bands with the unabsorbed antiserum. A secondary band was not discernible within or outside of the strong, diffuse primary band produced by C_2B_2 with AS C_1B_1-AT (Fig. 1A, well 5). However, secondary bands were not produced by any of the strains when AS C_1B_1-AT was absorbed with strain C_2B_2 (Table 3); this indicated the presence of secondary band antigen(s) in the extract preparation of C_2B_2. The production of primary bands was eliminated only when antisera were absorbed with a strain that belongs to the same serovar as the homologous strain.

One strain, 0775 from *Panicum maximum*, produced a weak band only with AS C_1B_1-AT. However, the antigen(s) of strain 0775 was not completely identical with the antigens of the other strains in this study because a reaction of inhibition (2) occurred between the precipitin band of strain 0775 and the secondary bands of strain W1-1-AT and the other strains in Table 2. In addition, cross absorption of AS C_1B_1-AT with phenol-water extract of strain 0775 did not eliminate the production of secondary bands by the other strains.

TABLE 1. Ouchterlony double diffusion reactions of whole cells and phenol-water extracts of strains tested against antisera to whole cells of 11 strains of *Erwinia chrysanthemi*[a]

	Antisera to strain:				
Serovar	CU 23 NCPPB 568 123 151 387	W1-1-CU[b] W1-1-AT	362	C-15 20-23	C_1B_1-AT[b]
I	+[c]	−	−	−	−
II	−	+	−	−	−
III	−	−	+	−	−
IV	−	−	−	+	−
V	−	−	−	−	+

[a] See reference 7 for additional data concerning serovars I, II, III, IV, and immunogen preparations for antisera CU 23, NCPPB 568, 123, 151, 387, W1-1-CU, 362, C-15, and 20-23. See text for preparation of whole cell suspensions and phenol-water extracts.
[b] W1-1-CU = Cornell University Collection of Phytopathogenic Bacteria strain 0661; W1-1-AT = American Type Culture Collection Strain 29274; C_1B_1-AT = American Type Culture Collection strain 27386.
[c] + = reaction of complete fusion (identity) of primary band; − = no reaction.

TABLE 2. Original host, number, collection location, serovar, and biovar of 37 strains of *Erwinia chrysanthemi* that produced only secondary bands in Ouchterlony double diffusion tests with antisera produced to strains W1-1-AT and C_1B_1-AT

Original host	Original number	Location	Serovar[a]	Biovar[b]
Allium fistulosum L.	LA2	Taiwan	I	IV
Alloe barbadensis Mill.	A-2, A-4	Arizona	UT	IV
Chrysanthemum × *morifolium* Ramat.	NCPPB 427	U.S.A.	I	III
	NCPPB 517	unknown	I	III
Euphorbia pulcherrima Willd.	1, 2	Ohio	I	III
	C192	Connecticut	I	III
Musa sp. 'Cavendish' cv.	362, 368	Panama	III	IV
	364, 365	Panama	UT	IV
Oryza sativa L.	ER 1	Japan	UT	IV
Saccharum officinarum L.	0803	Australia	UT	IV
Solanum tuberosum L.	DAR 30505, DAR 30514	Australia	UT	IV
Syngonium podophyllum Schott	B-87	Florida	I	IV
Zea mays L.	30-3, 40-1, 41-22, 41-32, SR-172	Colombia	UT	IV
	119-14	France	III	IV
	143-B1	France	UT	IV
	I-3, I-4M4, I-5M5, I-7	India	UT	IV
	228, 229	Italy	UT	IV
	C_6, C_7	North Carolina	UT	IV
	NCPPB 377	Zimbabwe	UT	IV
	920	South Dakota	UT	IV
	W3-20	Wisconsin	UT	IV
Zea mays L. var. *rugosa* Bonaf.	85, 88	New York	UT	IV

[a] See Table 1 for serovars I–III. UT = untyped because no primary band produced with any of the 11 antisera listed in Table 1.
[b] See references 4 and 6 for phenotypic characteristics of biovars (subdivisions).

DISCUSSION

This investigation demonstrated the antigenic heterogeneity among strains of *E. chrysanthemi* isolated from a specific host and among strains from different hosts. Only four of 47 strains originally isolated from corn reacted with AS C_1B_1-AT or AS W1-1-AT to produce strong precipitin bands (Fig. 1A and B) indicative of a serovar designation, and 59 untyped strains from 15 other hosts failed to produce strong primary bands. Production of a weak secondary band by some strains, with or without production of a strong primary band, also was not related to the original host of the strain (Tables 2 and 3). Only one (ER 1) of eight strains from rice produced a secondary band with the two antisera, although Goto (10,11) has presented conclusive evidence that the strains are closely related to corn strains in their phenotypic properties and virulence on corn. Eight strains also were included in our tests that had been isolated from potatoes collected in N.S.W., Australia, and identified as *E. chrysanthemi* by Cother and Powell (3). Two of the strains (DAR 30505 and 30514) produced secondary bands with the antisera (Table 2). Results similar to ours were reported by Yakrus and Schaad (20) for Ouchterlony double diffusion tests with immunogen and antigen preparations of extracted membrane protein complex (MPC). They tested two corn strains A-308 (our no. W3-20) and A-317 (our no. 16) and 13 strains of *E. chrysanthemi* from other hosts with an antiserum produced against corn strain A-308. Strain A-317 failed to react with the antiserum, but strain A-308 and a strain (A-305) originally isolated from *Chrysanthemum morifolium* produced a reaction of fusion with the formation of two precipitin lines. If one of the two precipitin lines is comparable to our secondary band, their results are analogous to ours because strain 16 (their no. A-317) did not produce any reaction with our antisera, whereas strain W3-20 (their no. A-308) and two of our strains originally isolated from chrysanthemum did produce secondary bands (Table 2).

Strains W1-1-AT and W1-1-CU currently constitute serovar group II because they were the only strains to react with AS W1-1-AT and with AS W1-1-CU that was prepared previously against W1-1-CU. These results also substantiate those observed previously when 404 strains of *E. chrysanthemi* were tested and only the homologous strain (W1-1-CU) reacted with antiserum AS W1-1-CU (7). Our tests showed that production of primary bands only by the homologous strains is not predicated by the immunogen preparation because formalin-treated cells were used for strain W1-1-CU (7) and glutaraldehyde-treated cells for W1-1-AT.

The new serovar group V includes three strains that produced strong primary bands and reaction of complete fusion with the homologous strain in double diffusion tests with AS C_1B_1-AT (Fig. 1A). Two strains (C_1B_1 and C_2B_2) were isolated originally from corn plants grown in North Carolina, whereas strain 221 was isolated from corn in Italy (4). There were no differences in the primary bands produced by the subcultures of strain C_1B_1 maintained at Cornell University (C_1B_1-CU) and in the American Type Culture Collection (C_1B_1-AT). Although the three strains of serovar V were originally isolated from corn and possess the phenotypic characteristics of biovar (subdivision) IV, there is no definite relationship between antigenic properties and the geographical location of the affected plants.

The fusion of secondary bands (Fig. 2A) and the results of cross absorption tests (Table 3) suggest that the antigens responsible for production of the bands apparently are very similar, although the study does not present any evidence for the identity of the antigenic determinants. The development of secondary bands was influenced somewhat by the medium used for the growth of the bacterial cells. The presence of the antigenic determinants for secondary bands may be dependent on the stability of the strain as evidenced by the differences in the reactions of two subcultures of strains W1-1 and C_1B_1 (Table 3) used in our tests. Victoria (18) also observed differences in reactions of Ouchterlony double diffusion tests between subcultures of corn strains of *E. chrysanthemi* when the subcultures were selected for differences in morphological characteristics. In addition, reactions to his antisera varied, including the production of diffuse precipitin lines, one or more distinctive lines, or no lines.

The results of our tests emphasize some of the problems that are inherent in the use of serology for the diagnosis and identification of strains of *E. chrysanthemi* isolated from one or more hosts. The predominant factor appears to be the variability in the antigenic properties of the strains. The limited number of corn strains that reacted with the two antisera used in the tests may reflect in part a faulty selection of strains for preparation of the antisera. Nevertheless, the results pose the question of how many antisera are necessary to serve as a useful adjunct to the methods used for the proper identification of these phytopathogenic bacteria. Although the relationship of antigenic determinants and virulence of strains is unknown, it seems advisable to use strains originally isolated from corn in a corn breeding program for tolerance to *E. chrysanthemi*. The appropriate selection of strain(s), with an

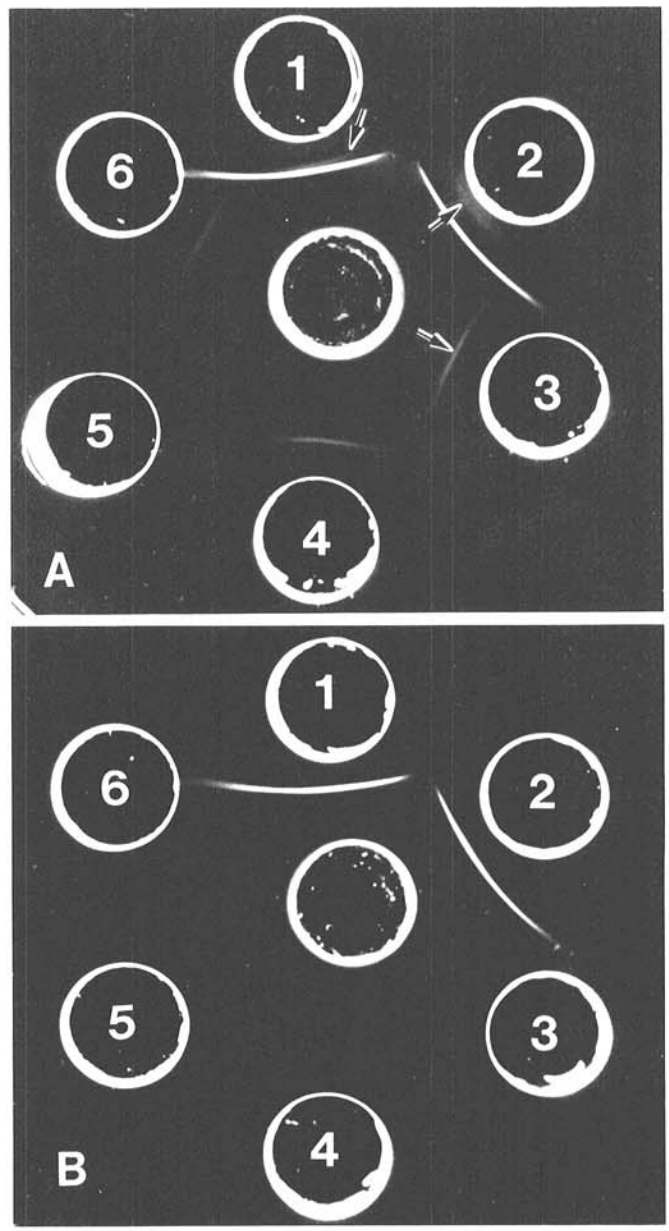

Fig. 2. Ouchterlony double diffusion patterns to phenol-water extracts (PWE) of *Erwinia chrysanthemi* when center wells contained: **A,** Antiserum W1-1-AT absorbed with PWE of strain 359 (serovar III) and **B,** Antiserum W1-1-AT absorbed with PWE of strain 362 (serovar III). Outer wells contained PWE of: 1, strain W1-1-AT (serovar II); 2, strain W1-1-CU (serovar II); 3, strain 365 (untyped); 4, strain 362 (serovar III); 5, strain 368 (serovar III); and 6, strain 119-14 (untyped). Arrows indicate secondary bands.

TABLE 3. Ouchterlony double diffusion reactions of phenol-water extracts (PWE) of *Erwinia chrysanthemi* strains and antisera (AS) W1-1-AT and C_1B_1-AT either unabsorbed or absorbed with PWE of selected strains

			AS W1-1-AT absorbed with PWE of strain								AS C_1B_1-AT absorbed with PWE of strain			
	AS W1-1-AT unabsorbed[a]		C_1B_1-AT 359[b]		C_2B_2 30-3[c] 362[c]		W1-1-CU		AS C_1B_1-AT unabsorbed[a]		W-1-1-AT W1-1-CU 30-3[c] 88[c]		C_2B_2	
Strain	PB[d]	SB[d]	PB	SB	PB	SB	PB	SB	PB	SB	PB	SB	PB	SB
W1-1-AT	+[e]	+	+	+	+	−	−	−	−	+	−	−	−	−
W1-1-CU	+	W+	+	W+	+	−	−	−	−	−	−	−	−	−
C_1B_1-AT	−	−	−	−	−	−	−	−	+	−	+	−	−	−
C_1B_1-CU	−	+	−	+	−	−	−	−	+	−	+	−	−	−
C_2B_2	−	+	−	+	−	−	−	−	+	−	+	−	−	−
37 strains in Table 2	−	+	−	+	−	−	−	−	−	+	−	−	−	−
359[b]	−	−	−	−	−	−	−	−	−	−	−	−	−	−

[a] Unabsorbed AS were diluted with sterile distilled water to equal dilutions with PWE of the selected strains.
[b] Strain 359 (serovar III; biovar IV) was isolated from *Syngonium podophyllum* Schott collected in Florida.
[c] See Table 2 for information concerning serovar III strain 362 and untyped strains 30-3 and 88.
[d] PB = primary band; SB = secondary band.
[e] + = band produced; − = band not produced; W+ = production of a very weak band as compared with bands produced by the other strains.

option of including strains from one or more locations, might avoid an erroneous evaluation of maize germplasm.

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