Erwinia chrysanthemi: Serological Relationships Among Strains from Several Hosts

Robert S. Dickey, Cathy H. Zumoff, and Jerry K. Uyemoto

Professor, research technician, Department of Plant Pathology, Cornell University, Ithaca, NY 14853, and associate professor, Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva 14456. Current address of third author: Department of Plant Pathology, Kansas State University, Manhattan 66506.

Accepted for publication 11 June 1984.

ABSTRACT

Dickey, R. S., Zumoff, C. H., and Uyemoto, J. K. 1984. *Erwinia chrysanthemi*: Serological relationships among strains from several hosts. Phytopathology 74:1388-1394.

Nine antisera were produced against formalin-treated strains of *Erwinia chrysanthemi*, and four serovars were distinguished that were designated serovars I, II, III, and IV. Antisera produced to five strains originally isolated from three hosts (sedum, carnation, or philodendron) from different geographical locations were similar when tested by Ouchterlony double diffusion for reaction of identity (serovar I); the two strains from *Musa paradisiaca* produced similar antisera (serovar IV). Three hundred and fifteen (78%) of 404 strains of *E. chrysanthemi* originally isolated from 42 hosts reacted with one or more of the nine antisera, whereas none of the 103 strains of other *Erwinia* species or subspecies reacted with any of the antisera. All strains isolated from 25 of 42 hosts reacted only with the antisera of serovar I, and all strains from *Musa paradisiaca* reacted only with antisera of serovar IV. None of the strains isolated from seven hosts reacted with any of the antisera; some strains from each of the nine remaining hosts reacted with one or more antisera, whereas other strains from the same host did not react with any of the antisera. There was no correlation between reaction to antisera and assignment of strains to five of the six previously described phenotypic subdivisions. Ninety strains of *E. chrysanthemi* (serovar I) from 34 plant species, subspecies, or cultivars were tested for agglutination with unabsorbed antiserum produced against a strain isolated from *Dianthus caryophyllus* and with antisera absorbed with a strain from *Dieffenbachia amoena* or from *Saintpaulia ionantha*. Three kinds of reactions to the absorbed antisera were observed; however, a definite relationship between original host or phenotypic properties and reaction to absorbed antisera was not demonstrated.

Strains of *Erwinia chrysanthemi* Burkholder, McFadden & Dimock 1953 have been isolated from a large and diverse group of infected plants (4). The strains have been classified into biovars (15), infrasubspecific subdivisions (3,5), and pathovars (7) based primarily on phenotypic properties and partially on the original host of the strains. Serological studies of selected strains also have been used as an aid for identification, to designate serovars, and to determine the relationship between phenotypic properties, original host, and antigenic properties.

Several strains of *E. chrysanthemi* from different plant hosts have been used for serological studies. The immunogens that have been used include: untreated whole cells (2,10,13); whole cells treated with heat (14), formalin (12,16), or glutaraldehyde (21); cellular extracts (10); pectic lyases (11); and extracted membrane protein complex (23). Several different tests have been used including microagglutination (13,21), slide agglutination (1,16), tube agglutination (10,12,14,16,23), or gel diffusion (2,10–12,21,22).

The purpose of this study was to determine whether serological techniques might be useful for the diagnoses of diseases caused by *E. chrysanthemi* and to determine whether any relationships exist between antigenic properties, the original plant host, and the phenotypic properties of strains of *E. chrysanthemi*.

MATERIALS AND METHODS

Bacterial strains and growth of bacterial cells. Source and identity of 383 strains of *E. chrysanthemi* isolated from 38 plant hosts were reported previously (3–5). We also tested three strains from *Gymnocalycium "optima rubra"* (MoCa 1, MoCa 2, and MoCa 3 from T. Suslow, California); one from *Nicotiana tabacum* L. (Ech 10 from E. Echandi, North Carolina); five from *Solanum tuberosum* L. (SR5, 030, and 032 from E. R. French, Peru and 256 and 502 from H. P. Maas Geesteranus, The Netherlands); two from *Dieffenbachia* sp. (175 and 208 from H. P. Maas Geesteranus, The Netherlands); one from *Chrysanthemum morifolium* Ramat. (435 from H. P. Maas Geesteranus, The Netherlands); two from *Dahlia pinnata* Cav. (602 and 603 from H. P. Maas Geesteranus, The Netherlands); one from *Anemone* sp. (607 from H. P. Maas Geesteranus, The Netherlands); one from *Dianthus caryophyllus* L. (601 from H. P. Maas Geesteranus, The Netherlands); three from *Zea mays* L. (071-1230 from A. C. Hayward, Hawaii; SA6 from J. P. Mildenhall, South Africa; and CR1 from source unknown, Costa Rica); one from *Phalaenopsis* sp. (173 from H. P. Maas Geesteranus, Germany), and one from *Saintpaulia ionantha* H. Wendl. (175 from H. P. Maas Geesteranus, Germany). In addition, 77 strains of *E. carotovora* subsp. *carotovora* (Jones 1901) Bergey, Harrison, Breed, Hammer, and Huntoon 1923 from 22 hosts (3), 17 of *E. carotovora* subsp. *atroseptica* (van Hall 1902) Dye 1969, three of *E. carotovora* subsp. *betavasculorum* Thomson, Hildebrand and Schroth 1981 (19), two of *E. cypripedii* (Hori 1911) Bergey, Harrison, Breed, Hammer, and Huntoon 1923, one of *E. rhapontici* (Millard 1924) Burkholder 1948, and three of *E. amylovora* (Burrill 1882) Winslow, Broadhurst, Buchanan, Krumwiede, Rogers, and Smith 1920 were used.

Six strains of *E. chrysanthemi* (NCPPB 568, 123, 151, 387, C-15, and 20-23) were grown in 100 ml of 523 broth (9) (shake cultures, 60 excursions per minute) for 16 hr at 28 C. Three strains (CU 23, W1-1, and 362) were grown on a yeast extract-dextrose-calcium carbonate medium (YDC) modified according to Dye (6) for 24 hr at 27 C. Cells from YDC cultures were suspended in sterile 0.85% NaCl (saline). The broth cultures and YDC-cell suspensions were centrifuged at 10,000 *g* for 10 min at 4 C. Pelleted cells were suspended in 100 ml of sterile saline, and the concentration (colony-forming units [cfu]) of viable bacteria per milliliter for each suspension was determined by dilution plate series. All suspensions subsequently were adjusted to a concentration of 10^8 cfu/ml.

Antisera production. Fifty milliliters of each suspension was mixed with 0.64 ml of formaldehyde solution (37.6% HCHO; Fisher Scientific Co., Fair Lawn, NJ) and kept at 27 C for 21 hr. Suspensions were centrifuged and cells were washed three times in sterile saline, after which 1 ml was added to 9 ml of nutrient broth and incubated at 27 C to verify the absence of viable bacteria.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

After normal serum was taken from a rabbit, 4–6 ml of formalin-treated bacterial suspension was homogenated with 2–3 ml of Freund's incomplete adjuvant and intramuscularly injected into the rabbit. This was followed 1 wk later with an intravenous injection (1 ml without adjuvant). After the third week, a second intravenous injection of 0.9 ml of suspension and 0.1 ml of antihistamine (Benadryl®, diphenhydramine hydrochloride; Parke, Davis, and Co., Detroit, MI), to minimize the onset of anaphylactic shock, was given (20). Immunized rabbits were bled by cardiac puncture after 4 wk (short-term bleed [ST]). Additional intravenous injections of 1–2 ml of suspension plus 0.1–0.4 ml of antihistamine were made at 10 and 11 wk, and rabbits were bled at 12 wk (long-term bleed [LT]). Antisera were stored frozen or mixed 1:1 with glycerol and held at −20 C. Unless specified otherwise, serological tests were done with LT antisera. Antibody titers were determined by tube agglutination and Ouchterlony double diffusion (ODD) tests.

Agglutination tests. Tube and drop agglutination methods were used. Bacterial suspensions were prepared by removing cells from a 24-hr YDC slant culture incubated at 27 C and placing the cells in saline. Each suspension was adjusted to OD_{620} = 0.30 in a Spectronic 20 (Bausch and Lomb) spectrophotometer to provide a concentration of $3.5–7.0 \times 10^8$ cfu/ml. One-half of each suspension was heat-treated in a boiling water bath for 1 hr and cooled to room temperature. For the tube agglutination test, 0.5 ml of undiluted or diluted (in saline) antiserum and 0.5 ml of cell suspension were mixed in 13 × 100-mm glass tubes and were held for 2 hr at 37 C in a water bath. The antigen-antibody mixtures were observed for agglutination immediately and after 18–24 hr at 4–6 C. For drop agglutination, one part antiserum was diluted with 19 parts of sterile saline, thoroughly mixed, and centrifuged at 6,000 *g* for 30 min at 4 C. Ten-microliter drops of the centrifuged antiserum were placed in 100 × 15-mm plastic petri dishes and 10 μl of bacterial suspension was added and mixed. The petri dishes were held in a tightly sealed moist chamber for 20–24 hr at room temperature, and each antigen-antibody mixture was observed for agglutination by use of a stereomicroscope at a magnification of ×27.

Ouchterlony double diffusion (ODD) tests. Plastic petri dishes (100 × 15 mm) were prepared by distributing 10 ml of a medium containing 0.75% Oxoid Ionagar #2, 0.85% NaCl, and 0.025% sodium azide (practical grade; ICN Pharmaceuticals, Life Sciences Group, Cleveland, OH) or a medium containing 0.75 or 0.95% Oxoid purified agar, 0.85% NaCl, 0.025% sodium azide, and 0.01% trypan blue (Diamine Blue 3B; National Aniline Division, Allied Chemical Corp., New York). The latter medium is similar to that used by Schaad (17) and was more satisfactory. Media containing 0.75 and 0.95% agar were used for all tests because clearer and less diffused precipitin bands sometimes were produced in medium

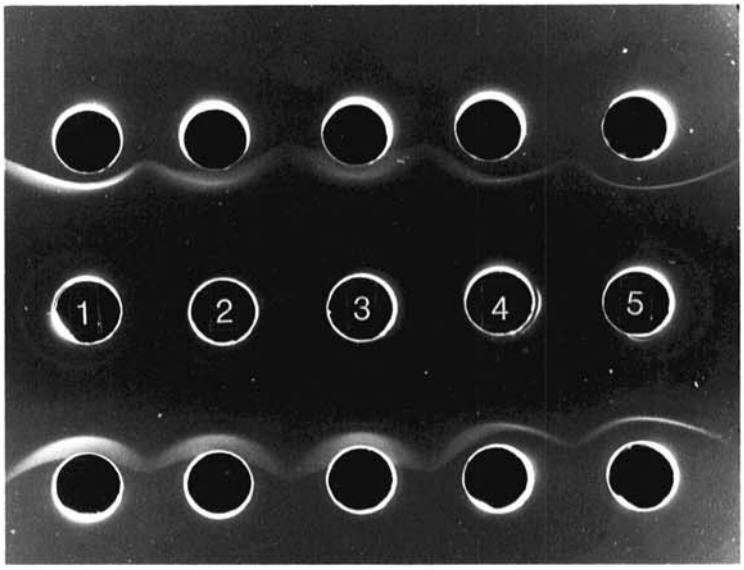

Fig. 1. Agar diffusion patterns for ~10^9 cfu/ml untreated cells of *Erwinia chrysanthemi*. Strain CU 23 (upper wells) and strain 387 (bottom wells) with five undiluted antisera of serovar I strains in the center wells: 1, CU 23; 2, NCPPB 568; 3, 387; 4, 151; and 5, 123. Note production of a single precipitin band for strain CU 23 (upper wells) and a strong primary band plus a weak secondary band for strain 387 (bottom wells) for all serovar I antisera.

TABLE 1. Agglutination titers of antisera (long-term bleed) from rabbits injected with formalin-fixed cells of strains of *Erwinia chrysanthemi* against untreated or heat-treated whole cell suspensions of strains[a]

Strain number	Original host	Source and location	Antisera of strains								
			CU 23	NCPPB 568	123	151	387	W1-1	362	C-15	20-23
CU 23	*Sedum spectabile* Boreau	Heidrick, L. E. New York	640/320[b]	640/640	320/160	640/320	640/320	−/−	−/−	−/−	−/−
NCPPB 568	*Dianthus caryophyllus* L.	Jenkins, J. E. E. UK	640/320	640/640	320/80	320/160	640/320	−/−	−/−	−/−	−/−
123	*D. caryophyllus* L.	Nelson, P. E. Pennsylvania	640/320	640/320	160/80	320/160	640/320	−/−	−/−	−/−	−/−
151	*Philodendron panduriforme* (HBK) Kunth.	Dickey, R. S. Pennsylvania	320/160	320/160	160/80	320/80	640/320	−/−	−/−	−/−	−/−
387	*P. panduriforme* (HBK) Kunth.	Dickey, R. S. Honduras	640/320	640/640	160/80	320/160	640/640	−/−	−/−	−/−	−/−
W1-1	*Zea mays* L.	Kelman, A. North Carolina	−/−	−/−	−/−	−/−	−/−	1,280/20	−/−	−/−	−/−
362	*Musa* sp. 'Cavendish'	Dickey, R. S. Honduras	−/−	−/−	−/−	−/−	−/−	−/−	320/160	−/−	−/−
C-15	*Musa paradisiaca* L.	Victoria, J. I. Colombia	−/−	−/−	−/−	−/−	−/−	−/−	−/−	320/40	320/80
20-23	*M. paradisiaca* L.	Victoria, J. I. Colombia	−/−	−/−	−/−	−/−	−/−	−/−	−/−	160/20	320/80

[a] Expressed as reciprocal of antiserum titer.
[b] Untreated/heat-treated whole cell suspensions; − = no reaction at 1:20 or greater dilution of antiserum.

with 0.95% agar. Circular patterns consisting of either six or eight outer wells (for antigens) surrounding a center well (for antiserum) were cut in the agar; diameters of all wells were 4 mm for the six-well pattern and 5 mm for the eight-well pattern. Distances between centers of adjacent outer wells and between centers of outer wells and center wells were, respectively, 8 and 9 mm for the six-well pattern and 10 and 13 mm for the eight-well pattern; 15 μl and ~20 μl of antigen or antiserum were placed in six-well patterned or eight-well patterned wells, respectively. A third pattern of three parallel rows (well centers between rows were 13 mm apart) of five wells per row (distance between well centers was 10 mm) also was used for agar diffusion tests. The middle row of wells contained antiserum and the outer rows contained antigen. Plates were incubated at room temperature in a humid enclosure and observed for precipitin lines at 3 and 6 days.

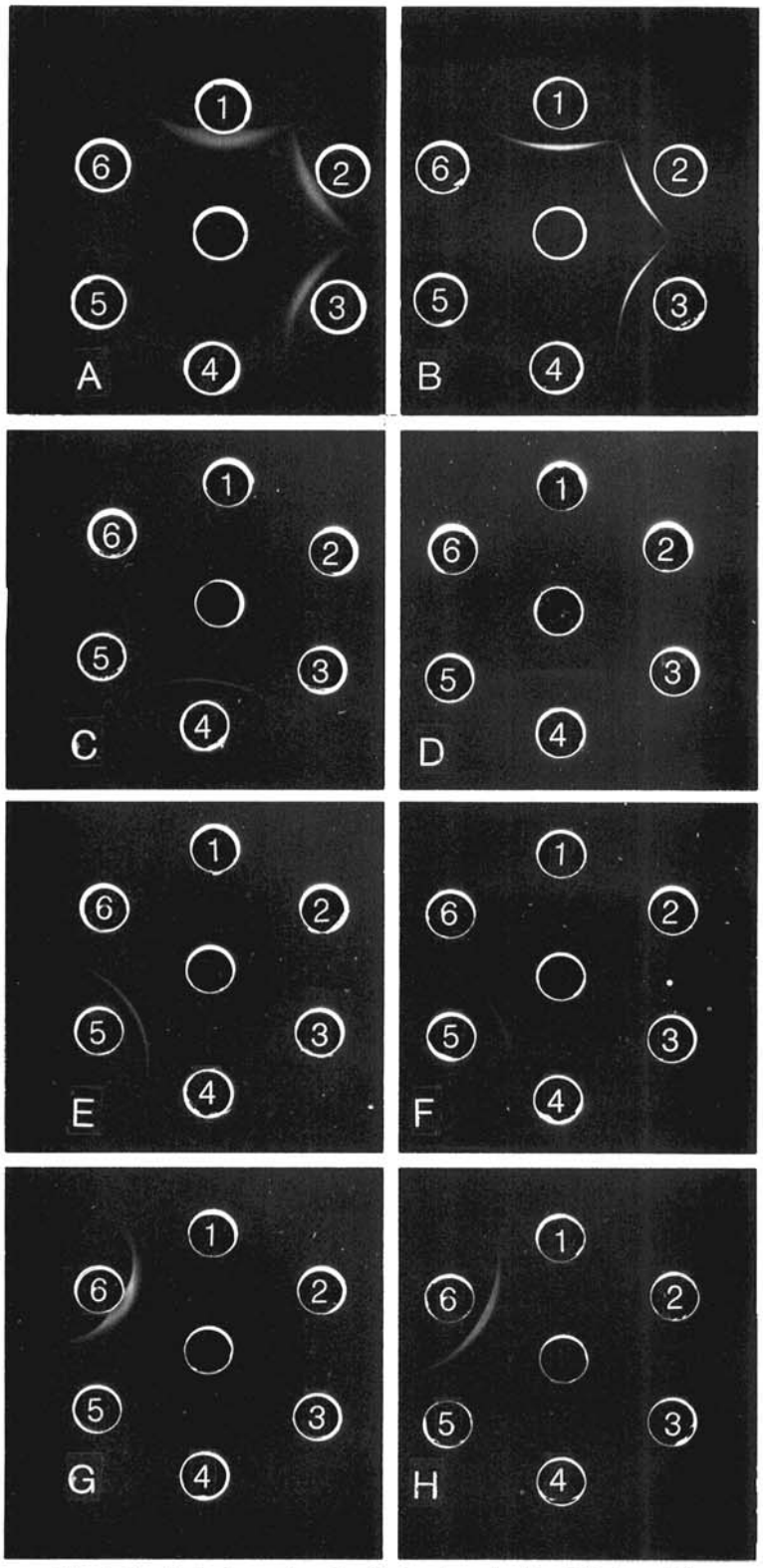

Fig. 2. Serological reactions by Ouchterlony double diffusion of four serovars of *Erwinia chrysanthemi*. Center wells contained antisera prepared against **A,B,** serovar I strain NCPPB 568; **C,D,** serovar II strain W1-1; **E,F,** serovar III strain 362; **G,H,** serovar IV strain 20-23. Outer wells contained either whole-cell suspensions (A, C, E, and G) or phenol-water extracts (B, D, F, and H) of: 1, serovar I strain CU 23; 2, serovar I strain NCPPB 568; 3, serovar I strain 151; 4, serovar II strain W1-1; 5, serovar III strain 362; and 6, serovar IV strain C-15.

Several different antigen preparations were used. Bacterial suspensions containing at least 10^9 cfu/ml were prepared by suspending in 4.5 ml of sterile saline the cells from a YDC slant culture incubated for 24 hr at 27 C. The bacterial suspensions were either untreated, heat-treated in a boiling water bath for 1 or 2.5 hr (18), or extracted in phenol-water at 65 C for 15 min according to Westphal and Jann (22). The water phase containing the lipopolysaccharide and ribonucleic acid was dialyzed for 3 days against distilled water but was not concentrated or freeze-dried.

Cross absorption tests. Cells of the absorbing strains were grown for 24 hr at 27 C on YDC slants and suspended in sterile saline ($\nless 10^9$ cfu/ml). One milliliter of saline suspension was mixed with 1 ml of antiserum, held at 37 C for 2 hr, and centrifuged at 6,000 *g* for 30 min. The pellet was discarded and the cross absorption process was repeated a second time and, if needed, a third time.

RESULTS

Agglutination titers for the nine antisera (LT) ranged from 160 to 1,280 and 20 to 640 for untreated and heat-treated homologous cells, respectively (Table 1). Based on homologous and heterologous reactant combinations, four serovars were clearly established. Serovar I included strains CU 23, NCPPB 568, 123, 151, and 387; II, W1-1; III, 362; and IV, C-15 and 20-23. In agar diffusion tests, antisera to formalin-treated cells of *E. chrysanthemi* produced one or two precipitin bands when reacted against untreated suspensions of homologous cells (Fig. 1). The stronger band was always present and was nearer the antigen well (Fig. 1, bottom wells), whereas the weaker band often was missing (Fig. 1, top wells). The similarity of the antisera produced against the formalin-treated cells of the homologous strains of serovar I group was shown by agar diffusion tests (Fig. 1). In ODD tests, cross reactions did not occur between antisera and somatic or lipopolysaccharide-nucleic acid (LPS-NA) antigens of the homologous strain(s) of a serovar and those of the other serovars (Fig. 2).

Heterologous strains of *E. chrysanthemi* from 34 hosts and representing each of the six subdivisions based on phenotypic properties (3,5) were used to determine the agglutination titers for heat-treated whole cells when tested against six of the nine antisera. Concurrently, untreated whole cells of the strains were tested for antigenic similarity by ODD tests. The results showed that whenever agglutination of heat-treated whole cells of a strain occurred with an antiserum diluted 1:20 or more, a reaction of identity in ODD tests also was produced for untreated whole cells of the strain when reacted against the same antiserum (Table 2). The results also demonstrated that when agglutination and reaction of identity occurred for one of the three antisera of the serovar I group (CU 23, NCPPB 568, and 151), agglutination and reaction of identity also occurred with the other two antisera of the group. None of the strains cross reacted by producing positive reactions with antisera of two or more serovars although some strains did not react against any of the antisera. A consistent correlation between antigenic and phenotypic properties (subdivisions) of the strains was not demonstrated by these results.

Whole untreated cells of an additional 361 strains of *E. chrysanthemi* (for a total of 404) and 103 strains of other *Erwinia* species were tested by ODD for reactions of identity with antisera of each serovar. After completion of tests with the nine antisera and 353 strains, it was deemed advisable to conserve time, effort, and antisera by modifying the procedure for testing the strains against

the five similar serovar I antisera. Therefore, all of the remaining 111 strains were tested against serovar I antiserum 151, and selected strains were tested against one to three of the additional four serovar I antisera. Three hundred and fifteen (78%) of the 404 strains of *E. chrysanthemi* produced a reaction of identity with one or more of the nine antisera, whereas none of the 103 strains of other *Erwinia* species or subspecies reacted with any of the antisera (Table 3). The majority (69.5%) of the strains of *E. chrysanthemi* belong to the serovar I group. Although all strains isolated from 25 of the 42 hosts listed in Table 3 (including several hosts for which only one strain was available) produced a reaction of identity with the antisera of serovar I, and all strains from *Musa paradisiaca* produced a reaction of identity only with the antisera of serovar IV, none of the strains from seven hosts reacted with any of the antisera. The groups of strains isolated from each of the remaining nine hosts (*Allium fistulosum, Chrysanthemum morifolium, Dianthus caryophyllus, Philodendron selloum, Philodendron* sp., *Solanum tuberosum, Syngonium podophyllum, Musa* sp., and *Zea mays*) contained strains that produced reactions of identity with one or more antisera and strains that reacted with none; the majority of the strains from seven of the nine hosts reacted with serovar I antisera. It should be noted that only the homologous strain (W1-1) from *Zea mays* produced a reaction of identity with the antiserum of serovar II.

Agglutination by the drop method was used for absorption tests to ascertain whether any relationship could be detected between phenotypic subdivisions and original host of the strains. Ninety strains of *E. chrysanthemi* (serovar I) from 34 plant species, subspecies, or cultivars were tested for agglutination with unabsorbed antiserum produced by *Dianthus caryophyllus* strain NCPPB 568 (subdivision V) and with the antiserum absorbed with *Dieffenbachia amoena* strain B-27 (subdivision I) or *Saintpaulia ionantha* strain F1 (subdivision IV). Three kinds of reactions to the absorbed antisera were observed (Table 4). The reactions were variable for strains in each of the subdivisions, being somewhat less variable for subdivisions I and V. The results were variable for strains from a specific host (eg, *Philodendron selloum*) or closely related hosts (eg, *Dieffenbachia* 'Exotica') although the strains belong to the same phenotypic subdivision. However, strains with different phenotypic characteristics that were originally isolated from each of three specific hosts (ie, strains from *Dieffenbachia* sp. in subdivisions I and IV, *Chrysanthemum morifolium* in III and IV, and *Dianthus caryophyllus* in III and V) produced different agglutination reactions, although two or more strains with similar phenotypic characteristics from each host did produce the same agglutination reaction.

TABLE 2. Agglutination titers for heat-treated whole cells and production of reaction of identity for untreated whole cells by Ouchterlony double diffusion test for strains of *Erwinia chrysanthemi* from 34 hosts and six phenotypic subdivisions tested against six antisera (CU 23, NCPPB 568, 151, W1-1, 362, and C-15)[a]

Phenotypic subdivision[b]	Strain[b]	Original host	Reciprocal titer[c]/reaction of identity[d]					
			CU 23	NCPPB 568	151	W1-1	362	C-15
I	D1	*Dieffenbachia maculata* [*D. picta*]	320/+	320/+	160/+	−/−	−/−	−/−
	B-27	*D. amoena*	320/+	320/+	160/+	−/−	−/−	−/−
	1b	*Dieffenbachia* sp.	320/+	320/+	80/+	−/−	−/−	−/−
II	NCPPB 1861	*Parthenium argentatum*	−/−	−/−	−/−	−/−	−/−	−/−
III	NCPPB 427	*Chrysanthemum morifolium*	160/+	320/+	160/+	−/−	−/−	−/−
	145	*C. superbum* [*C. maximum*]	−/−	−/−	−/−	−/−	−/−	−/−
	C 191	*Euphorbia pulcherrima*	320/+	160/+	80/+	−/−	−/−	−/−
IV	CU244	*Aglaonema commutatum* 'Treubii'	160/+	160/+	160/+	−/−	−/−	−/−
	LA2	*Allium fistulosum*	320/+	320/+	80/+	−/−	−/−	−/−
	078-255	*Alocasia* sp.	320/+	320/+	160/+	−/−	−/−	−/−
	G18-3	*Ananas comosus*	−/−	−/−	−/−	−/−	−/−	−/−
	3	*Cyclamen* sp.	160/+	80/+	80/+	−/−	−/−	−/−
	B-6	*Dracaena marginata*	160/+	320/+	160/+	−/−	−/−	−/−
	MoCa 2	*Harrisia* sp.	160/+	160/+	80/+	−/−	−/−	−/−
	A-15	*Ipomoea batatas*	320/+	320/+	80/+	−/−	−/−	−/−
	EI 1	*Iris ensata*	−/−	−/−	−/−	−/−	−/−	−/−
	HO-1	*Musa* sp. 'Cavendish'	−/−	−/−	−/−	−/−	160/+	−/−
	Ech 10	*Nicotiana tabacum*	−/−	−/−	−/−	−/−	−/−	−/−
	NCPPB 898	*Pelargonium capitatum*	320/+	320/+	160/+	−/−	−/−	−/−
	ER 1	*Oryza sativa*	−/−	−/−	−/−	−/−	−/−	−/−
	173	*Phalaenopsis* sp.	320/+	320/+	160/+	−/−	−/−	−/−
	B-48	*Philodendron selloum*	320/+	320/+	320/+	−/−	−/−	−/−
	B-101	*P. scandens* subsp. *oxycardium*	160/+	320/+	160/+	−/−	−/−	−/−
	PH 1	*Philodendron* sp.	160/+	160/+	160/+	−/−	−/−	−/−
	F 1	*Saintpaulia ionantha*	160/+	320/+	80/+	−/−	−/−	−/−
	B-73	*Syngonium podophyllum*	160/+	160/+	160/+	−/−	−/−	−/−
	113-2	*Zea mays*	160/+	320/+	160/+	−/−	−/−	−/−
V	607	*Anemone* sp.	320/+	160/+	160/+	−/−	−/−	−/−
	S204-107	*Begonia intermedia* 'Bertinii'	160/+	320/+	160/+	−/−	−/−	−/−
	NCPPB 1385	*Dahlia pinnata*	320/+	320/+	80/+	−/−	−/−	−/−
	F	*Daucus carota* var. *sativus*	160/+	160/+	80/+	−/−	−/−	−/−
	81BA	*Dianthus* sp.	320/+	320/+	80/+	−/−	−/−	−/−
	277-3	*Lycopersicon esculentum*	320/+	320/+	320/+	−/−	−/−	−/−
VI	NCPPB 2511	*Musa paradisiaca*	−/−	−/−	−/−	−/−	−/−	40/+

[a]See Table 1 for original hosts of strains used for production of antisera.
[b]See cited references 2, 3, and 4 for sources of strains and phenotypic characteristics of subdivisions.
[c]− = No reaction at 1:20 or greater dilution of antiserum.
[d]+ = Reaction of identity as produced by the homologous strain (Fig. 1); − = no reaction of identity.

DISCUSSION

This investigation was initiated primarily to determine whether serological techniques would enhance the diagnoses of diseases caused by *E. chrysanthemi* and the identification of the phytopathogen. It was gratifying to find that the antisera produced against nine strains of *E. chrysanthemi* did not react with any of the 103 strains of other *Erwinia* species or subspecies (Table 3). The specificity of antisera prepared against strains of *E. chrysanthemi* also has been demonstrated by others (1,11–14,16,21,23) using cross-reaction studies with strains from other *Erwinia* species and subspecies. Our results showed that 315 (78%) of 404 strains of *E. chrysanthemi* could be grouped into four clearly established serovars (Tables 1 and 3) and that 89 strains did not react with any of the antisera.

An additional purpose of our studies was to determine whether there was any discernible relationship between the antigenic properties, the original plant host, the phenotypic properties (3,5), and/or reactions of selected plants (4) for the strains of *E. chrysanthemi*. Samson (14) used the somatic (O) antigens of 39 strains of *E. chrysanthemi* from 12 host plants for agglutination studies with five antisera produced against heat-treated bacteria and found that 31 of the strains reacted with four antisera. When we tested 29 of the 31 strains by ODD tests, the 29 strains reacted with our serovar I antisera. We also tested by ODD seven of the eight strains for which Samson reported no reaction and found that six strains did not react with any of our nine antisera; the exception was strain B 110 (Samson strain 1245 from *Philodendron selloum*) that reacted with serovar I antisera. Twenty of the 29 strains also were included in our agglutination-absorption tests with heat-treated antigens (Table 4). We used for our tests an antiserum produced against a strain (NCPPB 568) from *Dianthus caryophyllus* that differed from the strain from *D. caryophyllus* used by Samson; however, we absorbed with two strains (B-27 = Samson 1247; F 1 = Samson 1361) used by Samson. The agglutination results for 11 of the 20 strains were the same as those reported by Samson, whereas the results differed for the six strains from *Dieffenbachia*, one from carnation, one from *Saintpaulia*, and one from *Pelargonium*. Samson (14) and Samson and Nassan-Agha (15) have suggested that their results seem to support an obvious correlation between the phenotypic properties, serological properties, and original host from which the strains of *E. chrysanthemi* were isolated. Our results do not support a strong relationship between phenotypic properties (subdivisions) and antigenic properties (Tables 2 and 4) or a relationship between antigenic properties and original host (Tables 2–4) with the

TABLE 3. Reaction of 404 strains of *Erwinia chrysanthemi* and 103 strains of *Erwinia* species to nine antisera in Ouchterlony double diffusion tests

Erwinia spp. and original hosts[a]	Serovars[b] and number of strains				
	I	II	III	IV	Untyped
Erwinia chrysanthemi					
Aglaonema commutatum 'Treubii' (2), *Alocasia amazonica* (1), *Alocasia watsoniana* (1), *Anemone* sp. (1), *Begonia intermedia* (1), *Cyclamen* sp. (2), *Dahlia pinnata* [*D. variablis*] (11), *Daucus carota* var. *sativus* (1), *Dianthus barbatus* (2), *Dianthus* sp. (4), *Dieffenbachia amoena* (8), *Dieffenbachia maculata* [*D. picta*] (8), *Dieffenbachia* 'Exotica' (2), *Dieffenbachia* sp. (7), *Dracaena marginata* (3), *Euphorbia pulcherrima* (10), *Gymnocalycium* sp. (3), *Ipomoea batatas* (3), *Lycopersicon esculentum* (1), *Pelargonium capitatum* (1), *Phalaenopsis* sp. (1), *Philodendron panduriforme* (14), *Philodendron scandens* subsp. *oxycardium* (1), *Saintpaulia ionantha* (10), and *Sedum spectabile* (1).	99	0	0	0	0
Allium fistulosum (5)	2	0	0	0	3
Chrysanthemum morifolium (13)	12	0	0	0	1
Dianthus caryophyllus (56)	55	0	0	0	1
Philodendron selloum (65)	63	0	0	0	2
Philodendron sp. (11)	8	0	0	0	3
Solanum tuberosum (7)	6	0	0	0	1
Syngonium podophyllum (28)	27	0	1	0	0
Musa sp. 'Cavendish' cultivars (12)	6	0	3	0	3
Zea mays (41)	1	1	1	0	38
Musa paradisiaca (30)	0	0	0	30	0
Ananas comosus (13), *Chrysanthemum superbum* [*C. maximum*] (3), *Iris ensata* (1), *Nicotiana tabacum* (1), *Oryza sativa* (8), *Parthenium argentatum* (3), and *Zea mays* var. *rugosa* (8).	0	0	0	0	37
Erwinia amylovora					
Pyrus communis (3)	0	0	0	0	3
Erwinia carotovora subsp. *atroseptica*					
Solanum tuberosum (17)	0	0	0	0	17
Erwinia carotovora subsp. *betavasculorum*					
Beta vulgaris (3)	0	0	0	0	3
Erwinia carotovora subsp. *carotovora*					
Allium cepa (6), *Brassica chinensis* (4), *Caladium* sp. (2), *Chrysanthemum morifolium* (37), *Cucumis sativus* (1), *Cucurbita pepo* var. *melopepo* 'Zucchini' (1), *Cyclamen* sp. (2), *Daucus carota* var. *sativus* (2), *Dieffenbachia* sp. (1), *Dracaena marginata* (1), *Euphorbia pulcherrima* (1), *Hoya carnosa* (1), *Iris* sp. (1), *Lactuca sativa* (1), *Lycopersicon esculentum* (4), *Musa* sp. 'Cavendish' (1), *Nicotiana tabaccum* (1), *Philodendron* sp. (2), *Scindapsus aureus* (2), *Solanum tuberosum* (2), *Zantedeschia aethiopica* (3), *Zea mays* (1)	0	0	0	0	77
Erwinia cypripedii					
Cypripedium sp. (2)	0	0	0	0	2
Erwinia rhapontici					
Rheum rhaponticum (1)	0	0	0	0	1

[a] Numbers in parentheses are the number of strains tested for each host.

[b] Serovars designated by production of reaction of identity by whole untreated cells with one or more of the following antisera: I—CU 23, NCPPB 568, 123, 151, 387; II—W1-1; III—362; IV—C-15, 20-23; and Untyped—none.

exception of phenotypic subdivision VI that includes only the serovar IV strains isolated from *Musa paradisiaca*.

Yakrus and Schaad (23) tested 27 strains of *E. chrysanthemi* for ODD reactions to extracted membrane protein complex (MPC) antigens of the strains against antisera to MPC of five strains. The strains were assigned to four serovars or a nontyped group, and they concluded that a correlation between host of origin and serovar was not demonstrated. Twenty-three of the 27 strains tested by Yakrus and Schaad were included in our studies and the results of reactions for two of their antisera (A-17 and A-311) were similar to our results for the five serovar I antisera, with the exception that strain A-308 (our number W3-20) from *Zea mays* did not react with any of our antisera. This comparison can be extended also to show that there was no correlation between the phenotypic (3) and antigenic properties of the 23 strains as typed by Yakrus and Schaad (23).

Lazar (10) included 12 strains of *E. chrysanthemi* from seven host plants in his comparative serological tests with 66 strains of *Erwinia* species. He used five antisera produced against whole cells for the cross-agglutination tests with cell suspensions and four antisera produced against cellular extracts prepared by two methods for the ODD tests with comparable cellular extracts. Although reactions between the antisera and all strains of *E. chrysanthemi* as well as other *Erwinia* species did occur, he concluded that the antigenic properties among the strains of *E. chrysanthemi* seemed more homogeneous and that the ODD results strongly indicated the existence of distinct serovars. The latter conclusion is substantiated by our results because seven of the 12 strains tested by Lazar were included in our studies and four were typed as serovar I, whereas three did not react with any of our antisera. Mazzucchi et al (11) used pectic lyases produced in polypectate medium as antigens for gel diffusion tests. All antigens

TABLE 4. Agglutination (drop method) of serovar I heat-treated strains of *Erwinia chrysanthemi* by antiserum NCPPB 568 unabsorbed and absorbed with strain B-27 (from *Dieffenbachia amoena*) or strain F-1 (from *Saintpaulia ionantha*)

Phenotypic division[a]	Original host	Strains[a]	Agglutination with antiserum NCPPB 568[b]		
			Unabsorbed	Absorbed with B-27	Absorbed with F-1
I	*Dieffenbachia maculata* [*D. picta*]	28E; D1; 73; 98	+	−	+
	Dieffenbachia amoena	B-1-R; B-27; 68	+	−	+
	Dieffenbachia sp.	1B	+	−	+
	Dieffenbachia 'Exotica'	B-15	+	+	+
III	*Chrysanthemum morifolium*	EC16; EC18; NCPPB 427; NCPPB 517	+	−	−
	Chrysanthemum superbum [*C. maximum*]	140; 145	+	−	−
	Dianthus caryophyllus	NCPPB 1111	+	−	−
	Euphorbia pulcherrima	C 192	+	−	−
		1; 2	+	−	+
		C664; C665	+	+	+
	Solanum tuberosum	502	+	+	+
IV	*Aglaonema commutatum* 'Treubii'	CU244	+	−	−
	Allium fistulosum	LA2; LA4	+	+	+
	Alocasia amazonica	078-3682	+	−	−
	Alocasia watsoniana	078-255	+	−	−
	Chrysanthemum morifolium	435	+	+	+
	Cyclamen sp.	3; 5	+	−	−
	Dieffenbachia sp.	381; 208	+	+	+
	Dracaena marginata	B-6; B-100	+	−	−
	Gymnocalysium sp.	MoCa 1	+	−	−
		MoCa 2; MoCa 3	+	+	+
	Ipomoea batatas	A-15; A-19	+	−	−
	Musa sp. 'Cavendish'	373; NCPPB 2477	+	−	−
	Pelargonium capitatum	NCPPB 898	+	−	−
	Phalaenopsis sp.	173	+	−	−
	Philodendron selloum	CU565; B-8; B-28; B-44	+	−	−
		245; B-63; B-13	+	−	+
		071-3272; 386; B-52; B-59; B-62; B-46; B-48	+	+	+
	Philodendron panduriforme	151	+	−	−
		387	+	+	+
	Philodendron scandens subsp. *oxycardium*	B-101	+	−	−
	Philodendron sp.	PH 1	+	−	−
		158; 169; 525	+	+	+
	Saintpaulia ionantha	F 1; B-102; B-106; B-109; 174	+	−	−
	Syngonium podophyllum	B-73; B-84; B-85; B-87; B-90	+	−	−
	Solanum tuberosum	032	+	−	−
		LG19; 030	+	+	+
	Zea mays	113-2	+	−	+
V	*Anemone* sp.	607	+	+	+
	Begonia intermedia 'Bertinii'	S204-107	+	+	+
	Dahlia pinnata [*D. variablis*]	S187-85; NCPPB 1955	+	+	+
	Daucus carota var. *sativus*	F	+	−	+
	Dianthus caryophyllus	NCPPB 568; NCPPB 429; CN 26	+	+	+
	Dianthus sp.	81 BA; CNBP 1354	+	+	+
	Lycopersicon esculentum	277-3	+	+	+
	Sedum spectabile	CU23	+	+	+

[a] See cited references 2, 3, and 4 for phenotypic characteristics of subdivisions and sources of strains.
[b] + = Agglutination with antiserum; − = no agglutination.

of 78 strains of *E. chrysanthemi* from nine host plants produced precipitin bands with at least one of two pectic lyase isozymes of the homologous strain from carnation; however, antigens for 21 strains of *E. carotovora* subsp. *carotovora* and 14 strains of *E. carotovora* subsp. *atroseptica* did not produce any precipitin bands. These results and those of Lazar (10) demonstrated a similarity of some antigens for all strains designated as *E. chrysanthemi* based on phenotypic properties. The use of whole-cell antigens by us does not reflect this similarity because 29 of the 78 strains tested by Mazzucchi et al (11) were included in our tests and were assigned to the groups designated as serovar I, II, or untyped.

The 89 strains of *E. chrysanthemi* that did not react with any of our antisera (ie, untyped) were originally isolated from 16 hosts (Table 3). The production of additional antisera against several selected untyped strains probably would have resulted in the establishment of more serovars in which some of the untyped strains could be grouped. There is some evidence that tends to substantiate this assumption. Samson (14) prepared an antiserum that was relatively specific for the two strains from *Parthenium* sp. which were included in her 39 strains of *E. chrysanthemi*. Victoria (21) prepared antisera against the cells of four corn strains that had been fixed with glutaraldehyde. He tested the antisera by ODD tests for reaction with 73 corn strains, including 19 strains used in our tests. When phenol-treated bacterial suspensions were used as the antigen, he observed at least one defined precipitin band produced by 13 to 23 of the 73 corn strains tested with the four antisera. He also found that 0 to 13 of the 22 strains supplied by Dickey originally isolated from 17 plant hosts other than corn produced positive reactions when tested against the four antisera. There was no relationship between the 13 strains that produced precipitin bands with one (SR120) of the four antisera and their host of origin or phenotypic properties (3,5). Serological techniques were used by Prasad and Sinha (12) for identification of 16 corn strains that caused maize stalk rot disease. Antisera were prepared against whole cells for four of the strains, and all 16 strains were positive for the antisera when tested by agglutination, microprecipitin and ODD. Two of the strains (I-1M1=84 and I-5M5=88) used by Prasad and Sinha to produce antisera were included in our tests; the two strains did not react with any of our antisera. Goto (8) reported that the strains of *E. chrysanthemi* isolated from rice, *Oryza sativa*, seemed to be closely related to strains from corn in phenotypic properties and ability to produce symptoms in corn. Our results tend to support his conclusions, because his eight strains from rice did not react with any of our antisera as was similarly observed for 46 of 49 strains from corn (Table 3).

LITERATURE CITED

1. Bortels, H., and Sauthoff, W. 1965. Eine bakteriose an *Dieffenbachia* Schott in Deutschland. Phytopathol. Z. 54:285-298.
2. Cother, E. J., and Powell, V. 1983. Physiological and pathological characteristics of *Erwinia chrysanthemi* isolates from potato tubers. J. Appl. Bacteriol. 54:37-43.
3. Dickey, R. S. 1979. *Erwinia chrysanthemi*: A comparative study of phenotypic properties of strains from several hosts and other *Erwinia* species. Phytopathology 69:324-329.
4. Dickey, R. S. 1981. *Erwinia chrysanthemi*: Reaction of eight plant species to strains from several hosts and to strains of other *Erwinia* species. Phytopathology 71:23-29.
5. Dickey, R. S., and Victoria, J. I. 1980. Taxonomy and emended description of strains of *Erwinia* isolated from *Musa paradisiaca* Linnaeus. Int. J. Syst. Bacteriol. 30:129-134.
6. Dye, D. W. 1968. A taxonomic study of the genus *Erwinia*. I. The "amylovora" group. N.Z. J. Sci. 11:590-607.
7. Dye, D. W., Bradbury, J. F., Goto, M., Hayward, A. C., Lelliott, R. A., and Schroth, M. N. 1980. International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathotype strains. Rev. Plant Pathol. 59:153-168.
8. Goto, M. 1979. Bacterial foot rot of rice caused by a strain of *Erwinia chrysanthemi*. Phytopathology 69:213-216.
9. Kado, C. I., and Heskett, M. G. 1970. Selective media for isolation of *Agrobacterium, Corynebacterium, Erwinia, Pseudomonas,* and *Xanthomonas*. Phytopathology 60:969-976.
10. Lazar, I. 1972. Serological relationships between the 'amylovora,' 'carotovora' and 'herbicola' groups of the genus *Erwinia*. Pages 131-141 in: Proc. Third Int. Conf. on Plant Pathogenic Bacteria. H. P. Maas Geesteranus, ed. Wageningen, The Netherlands.
11. Mazzucchi, U., Alberghina, A., and Garibaldi, A. 1974. Comparative immunological study of pectic lyases produced by soft rot coliform bacteria. Phytopathol. Mediterr. 13:27-35.
12. Prasad, M., and Sinha, S. K. 1978. Serologic studies of the maize stalk rot pathogen *Erwinia carotovora* f. sp. *zeae*. Zentralbl. Bakteriol., Parasitenkd., Infektionskr. Hyg. Abt. 2 133:80-85.
13. Saaltink, G. J., Slogteren, D. H. M. Van, and Kamerman, W. 1972. Serological identification of bacteria in bulbs. Pages 153-156 in: Proc. Third Int. Conf. on Plant Pathogenic Bacteria. H. P. Maas Geesteranus, ed. Wageningen, The Netherlands.
14. Samson, R. 1973. Les *Erwinia* pectinolytiques. II. Recherches sur les antigènes somatiques d'*Erwinia carotovora* var. *chrysanthemi* (Burkholder) Dye 1969. Ann. Phytopathol. 5:377-388.
15. Samson, R., and Nassan-Agha, N. 1978. Biovars and serovars among 129 strains of *Erwinia chrysanthemi*. Pages 547-553 in: Station de Pathologie Végétale et Phytobactériologie, ed. Proc. 4th Int. Conf. on Plant Pathogenic Bacteria, Angers, France.
16. Savulescu, A., and Lazar, I. 1967. Investigations on bacterium *Erwinia cytolytica* Chester. Rev. Roum. Biol. Ser. Bot. 12:379-383.
17. Schaad, N. W. 1974. Comparative immunology of ribosomes and disc gel electrophoresis of ribosomal proteins from erwiniae, pectobacteria, and other members of the family Enterobacteriaceae. Int. J. Syst. Bacteriol. 24:42-53.
18. Sinha, R. C., and Peterson, E. A. 1980. Homologous serological analysis of *Rhizobium meliloti* strains by immunodiffusion. Can. J. Microbiol. 26:1157-1161.
19. Thompson, S. V., Hildebrand, D. C., and Schroth, M. N. 1981. Identification and nutritional differentiation of the Erwinia sugar beet pathogen from members of *Erwinia carotovora* and *Erwinia chrysanthemi*. Phytopathology 71:1037-1042.
20. Uyemoto, J. K. 1980. Detection of maize chlorotic mottle virus serotypes by enzyme-linked immunosorbent assay. Phytopathology 70:290-292.
21. Victoria, J. I. 1977. Resistance in corn (*Zea mays* L.) to bacterial stalk rot in relation to virulence of strains of *Erwinia chrysanthemi*. Ph.D. dissertation. University of Wisconsin, Madison. 179 pp.
22. Westphal, O., and Jann, K. 1965. Bacterial lipopolysaccharides. Extraction with phenol-water and further applications of the procedure. Pages 83-91 in: Methods in Carbohydrate Chemistry. Vol. V. R. L. Whistler, ed. Academic Press, New York. 463 pp.
23. Yakrus, M., and Schaad, N. W. 1979. Serological relationships among strains of *Erwinia chrysanthemi*. Phytopathology 69:517-522.