# Serological Relationships Among Strains of *Erwinia chrysanthemi*

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## ABSTRACT

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Serological relationships among 27 strains of *Erwinia chrysanthemi* isolated from 18 different hosts were determined by agglutination and Ouchterlony double-diffusion tests of a membrane protein complex (MPC) extracted from whole cells. Antisera were prepared to MPC of five strains of *E. chrysanthemi* and one strain each of *E. carotovora* and *Salmonella typhimurium*. Agglutination tests were useful only for differentiation strains of *E. chrysanthemi* from other *Erwinia* spp. Strains of *E. chrysanthemi* were grouped into four serovars. There was no correlation between host of origin and serovar. Immunoelectrophoretic studies revealed that the MPC of *E. chrysanthemi* contained two immunogens, one neutral and one acidic. Polyacrylamide gel electrophoresis profiles of MPC of *E. chrysanthemi* showed one to four major protein bands with molecular weights ranging from 26,000 to 43,000. Agglutination and agar double diffusion tests with MPC proved to be a rapid, accurate method of identifying *E. chrysanthemi*.

Several attempts have been made to determine the relatedness of *Erwinia chrysanthemi* to other species of the Erwiniae and among strains of *E. chrysanthemi* isolated from different hosts. Biochemical, physiological, and cultural characteristics have been used to establish groupings (3, 6, 9) and to classify these organisms by numerical taxonomy (13), but *E. chrysanthemi* could not be differentiated from other *Erwinia* spp. Deoxyribonucleic acid (DNA) base composition studies (22) revealed that *E. chrysanthemi* strains had guanine plus cytosine (G + C) mol percent values ranging from 55.1 to 57.1 whereas those of other species of Erwinia ranged from 50 to 58. Based on DNA hybridization studies, Brenner et al (4) classified strains of *Pectobacterium* (*Erwinia*) *chrysanthemi* into four groups: those from chrysanthemum and guayule, those from dahlia or dieffenbachia, those from corn or grass, and a single strain from sugar cane. The four groups were highly interrelated and distinct from *Erwinia carotovora*.

Results of several serological studies (12,16,18,20) of *E. chrysanthemi* and closely related species indicated nonspecific relationships except when either a single purified immunogen (eg, pectic lyases) (15) or O antigen was used (19). Numerous common antigens were found between species when immunizing agents consisted of whole cells or heat-treated soluble antigens.

We chose a membrane protein complex (MPC) (8) as the inject antigen because this approach had been successful in typing strains of *Neisseria miningitidis*. Such a complex was easily extracted from cells of *E. chrysanthemi* by a mild extraction procedure (LiCl at 45 C) and it produced high titered antisera in rabbits. The MPC of *E. chrysanthemi* was specific at the species level in agglutination tests and at the subspecies level in Ouchterlony double diffusion tests. Twenty-seven strains of *E. chrysanthemi* were typed into four serovars. There was no correlation between host origin and serovar.

Immunoelectrophoresis separated the MPC into neutral and acidic antigenic groups. Polyacrylamide gel electrophoresis did not reveal any serovar specific protein bands.

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## MATERIALS AND METHODS

**Bacterial strains and growth conditions.** Thirty-five strains of nine bacterial species were used in this study (Table 1). Bacteria were maintained on slants of yeast-dextrose-calcium carbonate (YDC) agar (23) and grown in liquid medium 523 (10) in 2.4-L Fernbach flasks on a rotary shaker at room temperature for 18–21 hr. Cells were harvested by centrifugation in an International B-20 (International Equipment Co., Needham Hts., MA 02194) centrifuge with a model 872 rotor at 10,000 *g* for 5 min at 4 C. When large numbers of cells were needed for antiserum production, the bacteria were grown in aerated cultures in 20-L glass carboys. Cells were harvested with a Sharples continuous flow centrifuge (Sharples-Stokes Div. Pennwalt Corp., Warminster, PA 18974).

**Extraction of MPC.** MPC was extracted as follows (8): Cells were washed once with 0.85% sodium chloride (saline) and resuspended in 200 ml of 0.2 M LiCl/10 g wet weight of cells. This suspension was added to 250-ml Erlenmeyer flasks in 150-ml lots. Each flask contained approximately 3 ml of glass beads (3-mm diameter) and was agitated on a rotary shaker for 2.0–2.5 hr at 45 C to extract the MPC. The suspension was filtered through cheesecloth and the cells were pelleted by centrifuging at 12,000 *g* for 20 min and 4 C. The supernatant fluid was centrifuged at 30,000 *g* for 40 min at 4 C to remove large membranous materials. The resulting supernatant fluid containing the MPC was centrifuged at 100,000 *g* for 2 hr at 4 C. The pellet was washed once with distilled water and centrifuged at 100,000 *g* for 2 hr at 4 C. The pellet containing the MPC was suspended in a solution of 0.02% $NaN_3$ and stored at 2–5 C.

**Preparation of antisera.** MPC from five strains of *E. chrysanthemi* (A-310, A-305, A-312, A-308, and A-17), one strain of *E. carotovora* (A-7), and one strain of *Salmonella typhimurium* (E-26) were used to produce antisera. New Zealand white rabbits were given three graded injections of MPC at 10-day intervals. The injections contained 200, 400, and 1,200 μg of protein, respectively, as determined by the method of Lowry et al (14). The MPC was mixed with an equal volume of Freund's incomplete adjuvant and injected either intramuscularly or intraperitoneally. Rabbits were bled from the marginal ear vein 1 and 2 wk after the last injection. In one case, a fourth injection was given and a third bleeding was

taken 1 wk later.

**Serology.** Antisera titers were determined by agglutination tests. Twofold dilutions of antisera were made from 1:20 to 1:5,120 with 0.5 ml of saline containing 0.02% $NaN_3$ as diluent. Dilutions were made in 10 × 75 mm culture tubes. A tube containing 0.5 ml of saline with 0.02% $NaN_3$ served as a control. To each of the above tubes an equal volume of cell suspension was added. The turbidity of each suspension was adjusted to 30–35% transmittance at 590 nm, which is equivalent to $10^8$–$10^9$ cells per milliliter. Tubes were incubated at 37 C for 2 hr in a water bath and observed for agglutination. Each antiserum was tested against antigens of homologous and several heterologous strains.

Ouchterlony gel double diffusion was performed in a 0.75% purified agar medium containing trypan blue (21). Fifteen milliliters of medium was added to 10-cm plastic petri dishes. An Auto-Gel pattern cutter (Grafar Corp., Detroit, MI 48238) was used to cut a pattern with a center well 7 mm in diameter surrounded by six outer wells each 5 mm in diameter and 5 mm from the center well. Ten microliters of antigen was placed in each outer well and 20 μl of antiserum in the center well. A Piazzi test pattern (17) was used to determine optimal proportions of reagents. When necessary, antisera were concentrated by freeze-drying and resuspending in smaller volumes of saline. Plates were placed in a humidity chamber at 10 C and observed for precipitin lines after 2–3 days.

For cross absorption studies, antisera to MPC of *E. chrysanthemi* strains A-17, A-305, and A-308 were absorbed with sonicates of cells from strains A-17, A-305, and A-308. Cell envelopes were removed by a method modified from that of Ames (2). After 100–200 ml of a 16-hr culture in liquid medium 523 was centrifuged, the pelleted cells were resuspended in 10 ml of distilled water. Suspensions were sonicated at 75 W for 80 sec in four 20-sec intervals separated by 40-sec cooling periods. The resulting suspensions were centrifuged for 20 min at 6,000 *g* and the supernatant fluid was added to an equal volume of the appropriate antiserum. The mixture was incubated for 1 hr at 37 C in a water bath, centrifuged at 1,600 *g* for 10 min, and the pellet was discarded. Antisera were absorbed as above three more times by further additions of sonicate. Absorbed antisera were freeze-dried, resuspended in their original volume of saline containing 0.02% $NaN_3$, and stored at 2–5 C.

Immunoelectrophoretic tests with pH 8.6 barbital buffer (Millipore Corp., Bedford, MA 01730) were performed to determine if the MPC consisted of single or multiple immunogens. One-microliter samples were placed in wells on Millipore Immuno-AgroSlides. One well was charged with 1.0 μl of 0.1% bromphenol blue as tracking dye. Electrophoresis was performed at a constant voltage of 100 V until the tracking dye had migrated 38 mm toward the positive terminal (30–35 min). The slides were transferred to a humidity chamber and the troughs were flooded with 50 μl of the appropriate antiserum. Diffusion was allowed at room temperature for 12–15 hr and precipitin arcs were enhanced by soaking the slides for 5 min in 2% tannic acid.

**Polyacrylamide gel electrophoresis.** Sodium dodecyl sulfate

TABLE 1. Bacterial strains used to investigate the serological characteristics of strains of *Erwinia* spp.

| Laboratory strain No.[a] | Received as: Name | Received as: Source strain no. | Source[b] | Host of origin |
|---|---|---|---|---|
| A-310 | *Erwinia chrysanthemi* | B-102 | 1 | African violet |
| A-311 | *E. chrysanthemi* | 5-A | 1 | African violet |
| A-321 | *E. chrysanthemi* | 244 | 2 | Aglaonema |
| A-322 | *E. chrysanthemi* | 339 | 2 | Banana |
| A-323 | *E. chrysanthemi* | 239 | 2 | Begonia |
| A-313 | *E. chrysanthemi* | 1559 | 3 | Carnation |
| A-314 | *E. chrysanthemi* | 10070 | 3 | Carnation |
| A-324 | *E. chrysanthemi* | 603 | 2 | Carrot |
| A-305 | *E. chrysanthemi* | ICPB EC-17 | 4 | Chrysanthemum |
| A-312 | *E. chrysanthemi* | 1552 | 3 | Chrysanthemum |
| A-304 | *E. chrysanthemi* | ICPB EM-112 | 4 | Corn |
| A-308 | *E. chrysanthemi* | SR80 | 5 | Corn |
| A-317 | *E. chrysanthemi* | 1065 | 6 | Corn |
| A-325 | *E. chrysanthemi* | 590 | 2 | Cyclamen |
| A-326 | *E. chrysanthemi* | 583 | 2 | Dahlia |
| A-316 | *E. chrysanthemi* | 1563 | 3 | Dieffenbachia |
| A-319 | *E. chrysanthemi* | 910 | 6 | Dieffenbachia |
| A-327 | *E. chrysanthemi* | 553 | 2 | Grass |
| A-328 | *E. chrysanthemi* | 431 (ICPB 1849) | 2 | Guayule |
| A-318 | *E. chrysanthemi* | ICPB 1849 | 6 | Guayule |
| A-329 | *E. chrysanthemi* | 266 | 2 | Philodendron |
| A-330 | *E. chrysanthemi* | 589 | 2 | Pineapple |
| A-331 | *E. chrysanthemi* | 438 | 2 | Shasta daisy |
| A-15 | *E. chrysanthemi* | original | 7 | Sweet potato |
| A-17 | *E. chrysanthemi* | original | 7 | Sweet potato |
| A-20 | *E. chrysanthemi* | original | 7 | Sweet potato |
| A-22 | *E. chrysanthemi* | original | 7 | Sweet potato |
| A-332 | *E. chrysanthemi* | 276 | 2 | Syngonium |
| A-7 | *E. carotovora* var. *carotovora* | ATCC 495 | 12 | Carrot |
| A-27 | *E. carotovora* var. *atroseptica* | ATCC 7404 | 8 | Irish potato |
| A-404 | *E. amylovora* | 1269 | 9 | |
| D-1 | *Escherichia coli* | 06 | 10 | |
| E-26 | *Salmonella typhimurium* | RIA | 11 | |
| E-25 | *Enterobacter agglomerans* | Biogroup 1 | 10 | |
| C-51 | *Pseudomonas solanacearum* | K-60 | 5 | Tomato |
| B-65 | *Xanthomonas campestris* | original | 7 | Cabbage |

[a]Strain identification numbers were assigned in the Laboratory of N. W. Schaad, University of Georgia, Experiment.
[b]Coded culture sources: 1 = J. Miller, Florida Department of Agriculture; Gainesville, FL; 2 = R. Dickey, Cornell University, Ithaca, NY; 3 = D. Dye, Auckland, New Zealand; 4 = R. Goodman, University of Missouri, Columbia, MO; 5 = A. Kelman, University of Wisconsin, Madison, WI; 6 = National Collection of Plant Pathogenic Bacteria, Harpenden, England; 7 = N. Schaad, University of Georgia, Experiment, GA; 8 = D. Brenner, Center for Disease Control, Atlanta, GA; 9 = L. Moore, Oregon State University, Corvallis, OR; 10 = W. B. Ewing, Center for Disease Control, Atlanta, GA; 11 = N. Bigley, University of Chicago, Chicago, IL; and 12 = American Type Culture Collection, Rockville, MD.

TABLE 2. Agglutination titers of antisera to membrane protein complex (MPC) against cells of *Erwinia chrysanthemi*, *Erwinia carotovora* var. *carotovora*, and *Salmonella typhimurium*

| | Antisera to MPC of strains | | | | | | |
|---|---|---|---|---|---|---|---|
| | *E. chrysanthemi* | | | | | *E. carotovora* | *S. typhimurium* |
| Cells of strain[a] | A-17 | A-311 | A-305 | A-312 | A-308 | A-7 | E-26 |
| *E. chrysanthemi* | | | | | | | |
| A-15 | 320 | 320 | 160 | 320 | 160 | –[b] | – |
| A-17 | 320 | 320 | 1,280 | 640 | 80 | – | – |
| A-20 | 320 | 640 | 320 | 320 | 160 | – | – |
| A-22 | 160 | 640 | 160 | 320 | 160 | – | – |
| A-311 | 320 | 320 | 640 | 320 | 80 | 20[c] | – |
| A-310 | 640 | 320 | 1,280 | 320 | 80 | – | – |
| A-304 | 640 | 320 | 640 | 320 | 80 | – | – |
| A-313 | 320 | 320 | 640 | 320 | 160 | 20[c] | – |
| A-316 | 160 | 320 | 320 | 320 | 40 | – | – |
| A-319 | 320 | 1,280 | 1,280 | 640 | 160 | 80[c] | – |
| A-314 | – | 320 | 320 | 40 | 80 | – | – |
| A-305 | 640 | 640 | 640 | 640 | 160 | – | – |
| A-312 | 640 | 320 | 640 | 640 | 80 | – | – |
| A-308 | – | 320 | 320 | – | 320 | – | – |
| A-317 | – | – | – | – | – | – | – |
| *E. carotovora* var. *carotovora* | | | | | | | |
| A-7 | – | – | – | – | – | 640 | – |
| *S. typhimurium* | | | | | | | |
| E-26 | – | – | – | – | – | – | 2,560 |

[a]Strain numbers were assigned in the laboratory of N. W. Schaad, University of Georgia, Experiment.
[b]Minus sign indicates no reaction at 1:20 dilution of antiserum.
[c]Weak reaction forming fine granular particles.

TABLE 3. Ouchterlony double diffusion reactions of membrane protein complex (MPC) from *Erwinia chrysanthemi* against antisera to MPC of strains of serovars I, II, and III

| MPC from | | Reactions against antisera to MPC of | | | | |
|---|---|---|---|---|---|---|
| | | Serovar I strains[a] | | Serovar II strains[a] | | Serovar III strain[a] |
| Serovar | Strain[a] | A-17 | A-311 | A-305 | A-312 | A-308 |
| I | A-15 | ++[b] | ++ | + | + | + |
| | A-17 | ++ | ++ | + | + | + |
| | A-20 | ++ | ++ | + | + | + |
| | A-22 | ++ | ++ | + | + | + |
| | A-311 | ++ | ++ | + | + | + |
| | A-310 | ++ | ++ | + | + | + |
| | A-321 | ++ | ++ | + | + | ND |
| | A-323 | ++ | ++ | + | + | ND |
| | A-313 | ++ | ++ | + | + | + |
| | A-314 | ++ | ++ | + | + | + |
| | A-324 | ++ | ++ | + | + | ND |
| | A-304 | ++ | ++ | + | + | + |
| | A-325 | ++ | ++ | + | + | ND |
| | A-326 | ++ | ++ | + | + | ND |
| | A-316 | ++ | ++ | + | + | + |
| | A-319 | ++ | ++ | + | + | + |
| | A-318 | ++ | ++ | + | + | ND |
| | A-332 | ++ | ++ | + | + | ND |
| II | A-305 | ++ | ++ | ++ | ++ | ++[c] |
| | A-312 | ++ | ++ | + | ++ | – |
| III | A-308 | ++ | ++ | +[c] | + | ++[c] |
| IV | A-327 | – | – | + | – | ND |
| | A-330 | – | – | + | – | ND |
| NT | A-322 | – | – | – | – | ND |
| | A-317 | – | – | – | – | – |
| | A-328 | – | – | – | – | ND |
| | A-331 | – | – | – | – | ND |

[a]Numbers were assigned in the laboratory of N. W. Schaad, University of Georgia, Experiment.
[b]Symbols: ++ = reaction of identity (5), + = cross-reaction (5), – = no reaction, ND = not determined, and NT = not typed.
[c]Two precipitation lines formed.

polyacrylamide gel electrophoresis was performed in vertical slab gels on a Bio-Rad electrophoresis apparatus with the discontinuous buffer system of Laemmli (11). Separating and stacking gels were prepared from a stock solution of 30 g of acrylamide (Bio-Rad Laboratories, Richmond, CA 94804) and 0.8 g of N,N′-*bis′* methyleneacrylamide (Eastman Kodak Co., Rochester, NY 14650) in 100 ml of water. Separating and stacking gels contained 10 and 5% acrylamide, respectively. For molecular weight determinations, MPC and standard proteins (0.5 to 2 mg/ml) were dissolved in Laemmli sample buffer (11) for 2 min at 90 C. Ten microliters of sample were applied to each sample well and electrophoresis was performed at a constant current of 12.5 ma for 4 to 4.5 hr.

## RESULTS

**Agglutination tests.** With few exceptions, 14 of 15 strains of *E. chrysanthemi* showed strong cross-agglutination reactions (Table 2) with all five antisera. Groupings among the strains were not possible except for A-317, which did not react with any of the antisera. *E. chrysanthemi* was easily differentiated from *E. carotovora* and *Salmonella typhimurium.*

**Ouchterlony double diffusion.** Antisera to MPC of *E. chrysanthemi* strains A-305, A-311, A-312, and A-17 produced a single precipitin band (Fig. 1/A–D) whereas antiserum to MPC of strain A-308 produced two precipitin bands (Fig. 1-E) when reacted against homologous MPC preparations. When the above five antisera were tested against MPC of 27 strains of *E. chrysanthemi* (Table 3, Fig. 1A–E), strains A-15, A-17, A-20, A-22, A-311, A-321, A-323, A-313, A-314, A-324, A-304, A-325, A-330, A-316, A-319, A-318, and A-332 were serologically identical (bands fuse [4]) and were designated serovar I. All strains of serovar I cross-reacted (bands partially intersect [4]) with serovar II antiserum against strains A-305, and A-312 (Fig. 1C and D). Antisera to MPC of A-305 and A-312 reacted identically with MPC of A-305 or A-312 only. Strains A-305 and A-312 were designated serovar II. Antiserum to MPC of A-308 reacted identically with MPC of A-308 and A-305. When MPC of A-305 was placed between wells containing MPC of A-308 and tested against antiserum to A-308 however, two precipitin bands of identity resulted and they crossed at their confluence (Fig. 1E, wells 1, 2, and 3). Furthermore, MPC of A-308 failed to give a reaction of identity when tested against antiserum to MPC of A-305 (Fig. 1C, wells 1 and 6). Strain A-308 therefore was designated serovar III. Membrane protein complex from strains A-327 and A-330 cross-reacted with antiserum to MPC of strain A-308 (serovar II) but not with antiserum to MPC of serovar I or serovar III. These strains were designated serovar IV. Strains A-317, A-322, A-328, and A-331 could not be typed because their MPC failed to react with antiserum to MPC of any of the five strains.

Groupings of the strains of *E. chrysanthemi* into serovars I, II, and III was substantiated by cross-absorption tests (Table 4 and Fig. 2).

No antisera prepared to MPC of *E. chrysanthemi* reacted with MPC of *E. carotovora* var. *carotovora, E. amylovora, E. carotovora* var. *atroseptica, S. typhimurium, Escherichia coli, Enterobacter agglomerans, Pseudomonas solanacearum,* or *Xanthomonas campestris.* Antiserum to MPC of *S. typhimurium*

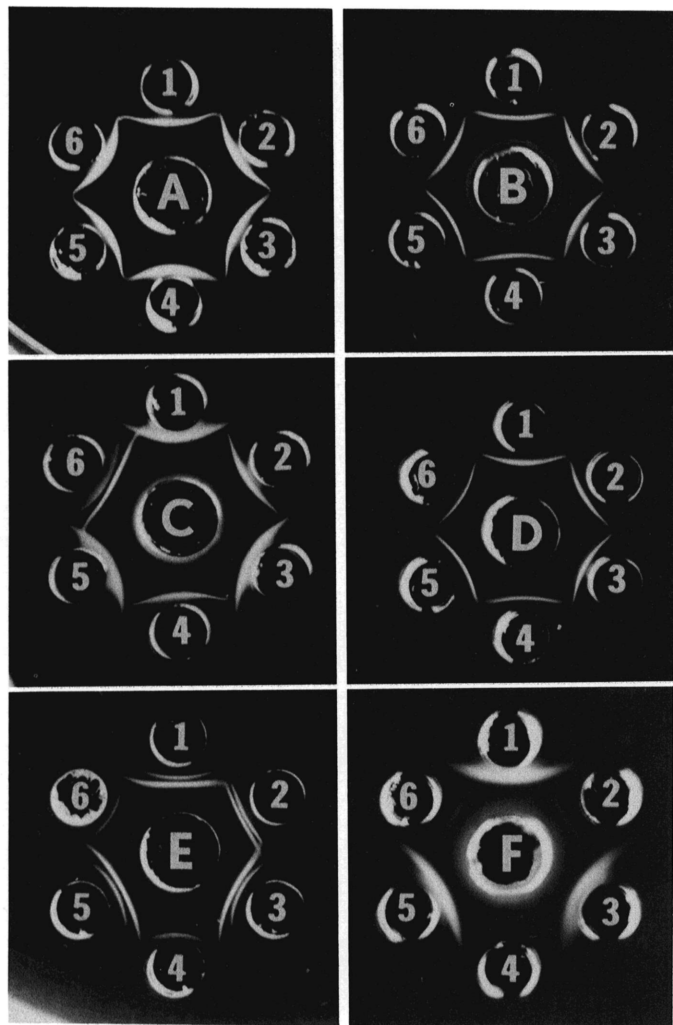


**Fig. 1.** Ouchterlony double diffusion patterns of membrane protein complexes (MPCs) of different strains of *Erwinia chrysanthemi* and *E. carotovora.* Center wells contained antiserum to MPC of *E. chrysanthemi* strains: **A**) A-17, **B**) A-311, **C**) A-305, **D**) A-312, **E**) A-308, and *E. carotovora,* **F**) A-7. Outer wells 1, 3, and 5 of each pattern contained MPC homologous with antiserum. Outer wells of A, B, C, D, and F contained MPC of *E. chrysanthemi:* 2) A-316, 4) A-304, and 6) A-308. Outer wells of E contained MPC of *E. chrysanthemi*: 2) A-305, 4) A-310, and 6) A-311.

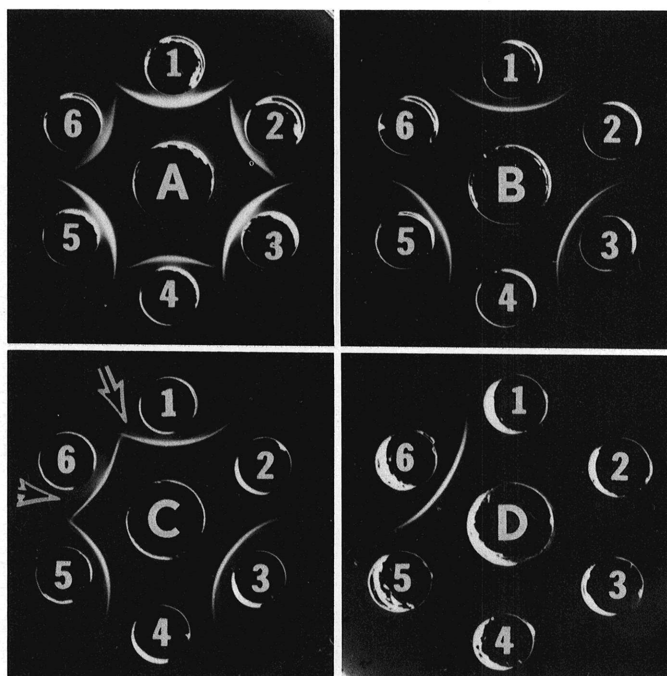


**Fig. 2.** Ouchterlony double diffusion patterns of membrane complex (MPC) of different strains of *Erwinia chrysanthemi* with absorbed antiserum. Center wells contained antiserum to MPC of *E. chrysanthemi* strain A-305: **A**) unabsorbed, **B** and **C**) absorbed with MPC of A-17, and **D**) absorbed with MPC of A-305. Outer wells 1, 3, and 5 of each pattern contained MPC of strain A-305. Outer wells of A and B contained MPC of strains: 2) A-15, 4) A-17, and 6) A-20. Outer wells of C and D contained MPC of strains: 2) A-22, 4) A-304, and 6) A-308. Note reaction of identity (arrows) between weak and strong precipitin lines in pattern C wells, 1, 5, and 6.

reacted with homologous MPC but not with MPC of *E. chrysanthemi.* Antiserum to MPC of *E. carotovora* reacted with MPC of *E. chrysanthemi* after the antiserum was concentrated four times. A weak band formed midway between wells containing antiserum to MPC of *E. carotovora* and antigen to *E. chrysanthemi* strains A-316, A-304, and A-308 (Fig. 1F).

**Immunoelectrophoresis.** One or two immunogens were observed depending on the antiserum (Fig. 3). One immunogen was acidic and migrated toward the anode whereas the other was neutral and remained near the circular sample well. Whereas the acidic immunogen always was observed in reactions between MPCs of *E. chrysanthemi* and antiserum to MPCs of *E. chrysanthemi,* the neutral immunogen was not always evident. A single acidic immunogen was revealed when antiserum to MPC of A-17 was tested against homologous MPC and MPC of A-305. In addition to an acidic immunogen, however a neutral immunogen was revealed when antiserum to MPC of A-305 was tested against its homologous MPC (Fig. 3A). Even though only the acidic immunogen of MPC from A-17 was revealed against homologous antiserum, this same antiserum revealed both acidic and neutral immunogens when tested against MPC of A-311 (Fig. 3C). When MPC of A-311 was tested against antiserum to MPC of A-311, A-17, and A-305, identical acidic and neutral immunogens were revealed (Fig. 3C, D). Testing antiserum to MPC of A-17 against MPC of A-312 also revealed both acidic and neutral immunogens. Antiserum to MPC of both A-311 and A-17 revealed only an acidic immunogen against MPC of A-308.

**Polyacrylamide gel electrophoresis.** MPC from 15 strains representing serovars I, II, and III were resolved into one to four major protein bands with molecular weights ranging from 26,000 to 43,000. No single major protein band was found common to all strains of a given serovar.

## DISCUSSION

The agglutination titers were high, considering that only surface components were used to produce antisera. Surface antigens are not as immunogenic in purified form as when administered as whole cells (7). The failure of our antisera to MPC of *E. chrysanthemi* to agglutinate cells of the closely related *E. carotovora* agreed with results obtained with antisera to whole cells (18, 20) or to heated cells (19) of *E. chrysanthemi.* Lazar (12) also noted that, with few exceptions, antiserum prepared to whole cells of *E. chrysanthemi* agglutinated cells of the same species more strongly than cells of closely related species. Our studies indicated that *E. chrysanthemi* antigens, administered either as MPCs or whole cells, were highly specific at the species level in cross-agglutination studies.

With Ouchterlony double diffusion tests with LiCl-extracted MPC preparations, we were able to type 27 strains of *E. chrysanthemi* into four serovars. Strains of serovar II probably produced the greatest number of immunogens since MPC of serovar I and III strains cross-reacted with antiserum to MPC of strains of serovar II. Whether MPC of strains of serovar IV possess more or fewer immunogens in relation to serovars I through III cannot be determined without further work. Strains A-322, A-312, A-318, and A-331 could not be typed because their MPCs did not react with antiserum to the MPC of any serovar in Ouchterlony double diffusion.

Cross-absorption studies supported the division of *E. chrysanthemi* strains into the four serovars. MPC contained in sonicates from cells of A-308 (serovar III) failed to absorb antiserum to MPC of A-17 (serovar I) and A-305 (serovar II) but successfully absorbed homologous antiserum indicating that strain A-308 had different surface antigens. The failure of MPC of A-305 (serovar II) to absorb antibody to MPC of A-308 (serovar III) from antiserum to A-305 (serovar II) is difficult to explain. One possibility is that there were two immunogens of different concentrations in inject antigen of A-305. A less concentrated immunogen of A-308 was homologous to a more concentrated

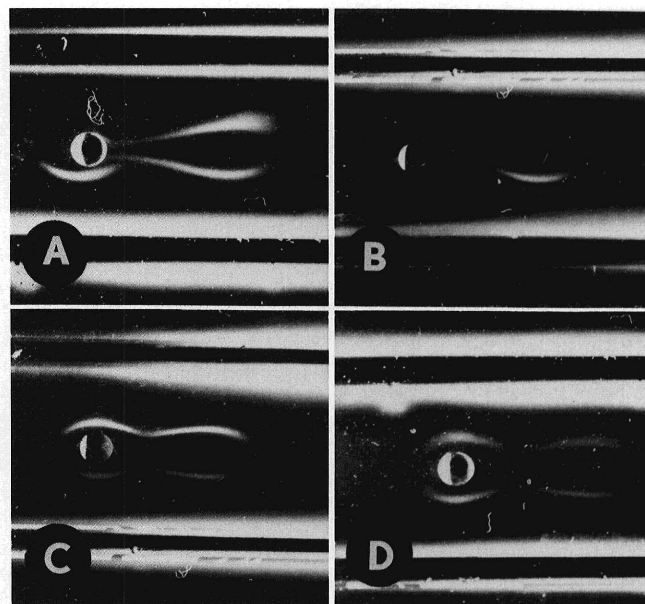


**Fig 3.** Immunoelectrophoresis patterns of membrane protein complex (MPC) of *Erwinia chrysanthemi.* Circular wells contained MPC of the following strains: **A,** A-305; **B,** A-308; **C,** A-311; **D,** A-311. Upper troughs contained antiserum to MPC of the following strains: **A,** A-17; **B,** A-305; **C,** A-17; and **D,** A-311. Lower troughs contained antiserum to MPC of the following strains: **A,** A-305; **B,** A-311; **C,** A-311; and **D,** A-311. Anode is to the right.

TABLE 4. Ouchterlony double diffusion reactions of absorbed antisera (AS) to membrane protein complex (MPC) of *Erwinia chrysanthemi* against MPCs of several different *E. chrysanthemi* strains

| MPC from strains[a] | AS to MPC of A-17 absorbed[b] by MPC of strains | | | AS to MPC of A-305 absorbed[b] by MPC of strains | | | AS to MPC of A-308 absorbed[b] by MPC of strains | | |
|---|---|---|---|---|---|---|---|---|---|
| | A-17 | A-305 | A-308 | A-17 | A-305 | A-308 | A-17 | A-305 | A-308 |
| A-15 | –[c] | – | + | – | – | + | – | – | – |
| A-17 | – | – | + | – | – | + | – | – | – |
| A-20 | – | – | + | – | – | + | – | – | – |
| A-22 | – | – | + | – | – | + | – | – | – |
| A-310 | – | – | + | – | – | + | – | – | – |
| A-313 | – | – | + | – | – | + | – | – | – |
| A-304 | – | – | + | – | – | + | – | – | – |
| A-316 | – | – | + | + | – | + | – | – | – |
| A-319 | – | – | + | + | – | + | – | – | – |
| A-305 | – | – | + | + | – | + | – | – | – |
| A-308 | – | – | – | + | + | – | + | + | – |

[a]Strain numbers were assigned in the laboratory of N. W. Schaad, University of Georgia, Experiment.
[b]Sonicates containing MPC used to absorb sera.
[c]Symbols: + = reaction of identity or cross reaction (5), and – = no reaction.

immunogen of A-305 (reaction of identity, Fig. 2C wells 1, 5, and 6). On the other hand, a less cencentrated immunogen of A-305 was homologous to a more concentrated immunogen of A-308. When we absorbed antiserum to MPC of A-305 with homologous MPC (A-305), a weak precipitin band of A-308 (Fig. 2D, well 6) was removed but, due to concentration differences, the strong precipitin band was not (Fig. 2D, well 6).

The positive reaction of MPC of serovar I strains A-316 and A-319 with antiserum to MPC of strain A-305 (serovar II) absorbed with MPC of A-17 (serovar I) showed that strains A-316 and A-319 had immunogens in common with serovar II but not with serovar I. Strains A-316 and A-319 could possibly be designated a separate serovar, but we chose not to do so. The successful absorption of antiserum to MPC of strain A-17 with MPC of A-305 showed that A-305 had all the immunogens possessed by strain A-17. Absorption data showed that MPC of A-17 possessed fewer immunogens that MPC of A-305 and supported placing these two strains into separate serovars.

Polyacrylamide gel electrophoresis profiles of proteins in MPCs of *E. chrysanthemi* could not be used to type *E. chrysanthemi* strains. Although one or more major bands were sometimes present in common between different MPCs of *E. chrysanthemi* strains, the diversity of patterns was too great for typing purposes. This suggested that the serovar-specific immunogens either were not proteins or were of too high a molecular weight to enter the gel.

Mazzucchi et al (15) placed strains of *E. chrysanthemi* from African violet, dieffenbachia, carnation, chrysanthemum, shasta daisy, guayule, corn, and philodendron in the same serovar based on immunological studies of their pectic lyases. With MPC, we likewise found no correlation between pathogenicity and serovar. Strains from the above hosts were placed in our serovar I. Although Mazzucchi et al (15) placed strains from corn in the same group with the above, our three corn strains were heterogeneous; strain A-304 was placed in serovar I, A-308 in serovar III, and A-317 could not be typed. In contrast, by using O-antigens and tube agglutination tests, Samson (19) found that 31 strains of *E. chrysanthemi* could be classified in five serological subgroups that seemed to be correlated with pathovars. Our results with MPC in Ouchterlony double diffusion tests agree with his conclusions (19) that guayule (*Parthenium argentatum*) strains failed to cross-react with any other strain of *E. chrysanthemi*

Dye (6) considered the guayule strain (*E. carotovora* f. sp. *parthenii* Starr) identical to *E. chrysanthemi* (*E. carotovora* var. *chrysanthemi*). Brenner et al (4) placed this same strain in a group with four chrysanthemum strains based on DNA relatedness. The guayule strains and strains A-331 and A-317 were not tested for pathogenicity so it is possible that they were not the original strains or that their surface antigens changed in culture. We tried to grow guayule but were unsuccessful. Brenner et al (4) concluded that *E. chrysanthemi* strains isolated from corn and grass were closely related but evidence from our study suggests that those capable of causing corn stalk rot do not have identical cell surface antigens.

*E. carotovora* strain A-7 had antigens in common with *E. chrysanthemi.* This was demonstrated by the formation of a faint precipitin band against MPCs of several strains of *E. chrysanthemi* when concentrated antiserum to MPC of *E. carotovora* was used. Antiserum to MPC of *E. carotovora* also agglutinated cellular suspensions of some *E. chrysanthemi* strains. Reciprocal tests were negative in both double diffusion and tube agglutination. Lazar (12) found bands of precipitin in common between strains of *E. chrysanthemi, E. carotovora,* and *E. atroseptica* with bacterial extracts of unknown origin as antigen. Allen and Kelman (1) reported, on the basis of immunofluorescence with antiserum to glutaraldehyde- and heat-fixed cells as antigen, that *E. chrysanthemi* strains were not related to *E. atroseptica* or *E. carotovora.* In our study, antisera prepared to MPC of neither *E. chrysanthemi* nor *E. carotovora* reacted with MPC of *E. atroseptica* strain A-27. The MPC of *E. chrysanthemi* probably is more closely related to *E. carotovora* than to *E. atroseptica,* but additional strains of *E. carotovora* and *E. atroseptica* will need to be tested to determine the relatedness of their MPC to *E. chrysanthemi.*

Ouchterlony double diffusion tests of an extracted MPC of *E. chrysanthemi* appears to be an ideal method for determining the relatedness of strains of this species. The relative ease of extraction and the specificity of the MPC of *E. chrysanthemi* suggests that MPC might be an excellent antigen to use for the rapid identification and serotyping of plant pathogenic bacteria.

## LITERATURE CITED


1. ALLEN, E., and A. KELMAN. 1977. Immunofluorescent stain procedures for detection and identification of Erwinia carotovora var. atroseptica. Phytopathology 67:1305-1312.
2. AMES, G. F. 1974. Resolution of bacterial proteins by polyacrylamide gel electrophoresis on slabs. J. Biol. Chem. 249:634-644.
3. BONNET, P. 1973. Les Erwinia pectinolytiques. I. Diagnostic biochimique rapide. Ann. Phytopathol. 5:355-376.
4. BRENNER, D. J., G. R. FANNING, and A. G. STEIGERWALT. 1977. Deoxyribonucleic acid relatedness among erwiniae and other enterobacteria. II corn stalk rot bacteria and Pectobacterium chrysanthemi. Int. J. Syst. Bacteriol. 27:211-221.
5. CROWLE, A. J. 1973. Immunodiffusion, 2nd ed., p. 259. Academic Press, New York. 545 pp.
6. DYE, D. W. 1969. A taxonomic study of the genus Erwinia. II. The 'carotovora' group. N.Z. J. Sci. 12:81-97.
7. EISEN, H. N. 1974. Immunology. Pages 351-624 reprinted from Davis, Dulbecco, Eisen, Ginsberg, and Wood's Microbiology, 2nd ed. Harper and Row, Hagerstown, MD. 1,562 pp.
8. FRASCH, C. E., and E. C. GOTSCHLICH. 1974. An outer membrane protein of Neisseria meningitidis group B responsible for serotype specificity. J. Exp. Med. 140:87-104.
9. GRAHAM, D. C. 1964. Taxonomy of the soft rot coliform bacteria. Annu. Rev. Phytopathol. 2:13-43.
10. KADO, C. I., and M. G. HESKETT. 1970. Selective media for isolation of Agrobacterium, Corynebacterium, Erwinia, Pseudomonas and Xanthomonas. Phytopathology 60:969-976.
11. LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.). 227:680-685.
12. LAZAR, I. 1972. Serological relationships between the 'amylovora', 'carotovora' and 'herbicola' groups of the genus Erwinia. Pages 131-141 in H. P. Maas-Geesteranus, ed. Proc. Third Int. Conf. Plant Pathogenic Bacteria, Wageningen, The Netherlands. University of Toronto Press, Toronto. 365 pp.
13. LOCKHART, W. R., and K. KOENIG. 1965. Use of secondary data in numerical taxonomy of the genus Erwinia. J. Bacteriol. 90:1638-1644.
14. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.
15. MAZZUCCHI, U., A. ALBERGHINA, and A. GARIBALDI. 1974. Comparative immunological study of pectic lyases produced by soft rot coliform bacteria. Phytopathol. Mediterr. 13:27-35.
16. MUSHIN, R., J. NAYLOR, and N. LAHOVARY. 1959. Studies on plant pathogenic bacteria. II. Serology. Aust. J. Bio. Sci. 12:233-246.
17. PIAZZI, S. E. 1969. A simple method for preliminary immunodiffusion test of antigen-antibody systems having unknown ratios of reaction. Anal. Biochem. 27:281-284.
18. SAALTINK, G. J., D. H. M. VAN SLOGTEREN, and W. KAMERMAN. 1972. Serological identification of bacteria in bulbs. Pages 153-156 in H. P. Maas-Geesteranus, ed. Proc. Third Int. Conf. Plant Pathogenic Bacteria, Wageningen, The Netherlands. University of Toronto Press, Toronto. 365 pp.
19. 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.
20. SAVULESCU, A., and I. LAZAR. 1967. Investigations on bacterium Erwinia cytolytica Chester. Rev. Roum. Biol. Ser. Bot. 12:379-383.
21. 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.
22. STARR, M. P., and M. MANDEL. 1969. DNA base composition and taxonomy of phytopathogenic and other enterobacteria. J. Gen. Microbiol. 56:113-123.
23. WILSON, E. E., F. M. ZEITOUN, and D. L. FREDRICKSON. 1967. Bacterial phloem canker, a new disease of Persian walnut trees. Phytopathology 57:618-621.