Bacteriophages of *Erwinia carotovora* and *Erwinia ananas* Isolated from Freshwater Lakes

C. G. Eayre, Research Plant Pathologist, Agricultural Research Service, U.S. Department of Agriculture, Subtropical Agricultural Research Laboratory, 2301 S. International Blvd., Weslaco, Tex. 78596; **J. A. Bartz,** Associate Professor, and **D. E. Concelmo,** Biological Scientist, Plant Pathology Department, 2559 Fifield Hall, University of Florida, Gainesville 32611

ABSTRACT

Eayre, C. G., Bartz, J. A., and Concelmo, D. E. 1995. Bacteriophages of *Erwinia carotovora* and *Erwinia ananas* isolated from freshwater lakes. Plant Dis. 79:801-804.

Bacteriophages for *Erwinia carotovora* subsp. *carotovora* and for *E. ananas* were readily isolated from freshwater lakes in Florida and Texas. Approximately 15% of enrichment cultures with 48 strains of *E. carotovora* yielded phage. Nineteen of 22 phages had distinct host ranges among the 62 strains and up to 10 strains were susceptible to a single phage. Among strains representing 24 serotypes, 12 of 15 phages caused plaques in lawns of 16 serotypes. Each of 13 enrichment cultures with strains of *E. ananas* yielded at least one phage and five distinct host range patterns emerged among host strains and phage isolates. The number of susceptible hosts for each phage ranged from one to six.

Plant pathogenic bacteria currently assigned to the genus *Erwinia* fall into three broad groups (5–7) with dissimilar biochemical, physiological, and pathological properties (12). The distinctly different diseases range from soft rots to necroses, involving tissue rots, wilts, and blights. The bacteria are widespread and can survive epiphytically and endophytically on asymptomatic host plants (15). The most effective control of plant diseases caused by these bacteria has been sanitation to reduce inoculum, particularly initial inoculum. Under favorable environmental conditions, however, small pathogen populations increase rapidly and disease development can be explosive. In addition, chemical treatments, such as applications of fixed copper bactericides or antibiotics, are recommended for control of certain diseases caused by *Erwinia* species (i.e., fire blight), but these treatments are not used for control of diseases such as black leg and soft rot of potato caused by *E. carotovora* (11) or for necroses caused by members of *E. herbicola*. Development of synthetic chemicals that are effective for the control of soft rots or necroses appears unlikely. Biological controls, however, are largely unexplored (3).

Bacteriophages, as an alternative disease control strategy, have not received much attention, in part because they are believed to be too specific and phage-host interaction too unstable for these viruses to be effective biocontrol agents (17). Generally, phages have a narrow host range, each infecting only a few strains of a bacterial pathogen. A diverse collection of phages is needed to address this concern.

The research described here included isolating such a collection of phages, and testing their host range so that future studies to test the potential of bacteriophages as biocontrol agents could be properly designed. Bacteriophages of the pectolytic *Erwinia* species have been isolated from soil and plant material, but the frequency of isolation (number of phage isolations divided by number of samples) has been low, and the phages had relatively restricted host ranges (9,13). Diseased plant material has been cited as a likely source of phages that have narrow host ranges, whereas soil and untreated sewage effluent were likely to have phages with relatively broad host ranges (8). Our initial attempts to isolate erwiniaphages from soft rotted plant material, soil, and untreated sewage by enrichment culture were unsuccessful. But, phages are likely to be found wherever the host bacterium is located (2). The soft rot *Erwinias* are considered to be ubiquitous (10,15) and are particularly common in surface waters (10). Surface waters appear to be an ideal source of phages for several reasons. First, water-based fluids such as surface waters or sewage lack the structural impediments to microbial dispersal associated with the soil or plants. Therefore, bacteria and their phages should be uniformly distributed in the medium, which simplifies the sampling procedure. Second, bacterial populations in surface water should be smaller than those in untreated sewage and more related to plants, particularly those growing in the watershed surrounding the water. Third, surface waters can be selected that receive drainage from croplands where the bacteria of interest are likely to be found. Bacteria and phages from the croplands are likely to be transported into the surface water. Fourth, if diversity of bacterial populations present is responsible for the broader host range of phages found in sewage and soil than that found in plants, then surface waters also should contain phages that have a broad host range. In this paper we describe the isolation of phages for *E. carotovora* subsp. *carotovora* (Jones) Bergey et al., a cause of potato soft rot, and for *E. ananas* Serrano, cause of brown spot of honeydews and cantaloupe (18) from a freshwater lake in Florida (*E. c.* subsp. *carotovora*) and a resaca (oxbow lake) in Texas (*E. ananas*).

MATERIALS AND METHODS

Bacterial strains. Twenty-four strains of *E. carotovora* representative of established serogroups were obtained from M. Powelson (Oregon State University, Corvallis). Twelve strains, including host strains 347 and 394, were obtained from A. Kelman (North Carolina State University, Raleigh). Fifty other strains were isolated from diseased plant material in Florida. Thirteen strains of *E. ananas* were obtained from J. Wells (USDA ARS Plant Science Research, Philadelphia, Pa.). Long-term storage of *Erwinia* strains was in sterile tap water at 25°C. Cultures in use were maintained on nutrient agar (NA) (Difco Laboratories, Detroit, Mich.) in petri plates, incubated at 29°C for 48 h, and stored at 25°C for up to 2 weeks between transfers.

Bacteriophage isolation and purification. Water samples (approximately 3 liters) were taken from a eutrophic lake in north central Florida, and surface water in south Texas. Florida lake water was enriched for phages of *E. c.* subsp. *carotovora*, and Texas surface water was enriched for phages of *E. ananas*. Enrichment flasks, each with a different strain of *Erwinia* species, were prepared by adding 50 ml of lake water, 2 g of $CaCO_3$ and 3 ml of 18-h-old nutrient broth (NB) (Difco) cultures of different *Erwinia* strains to 250-ml Erlenmeyer flasks containing 50 ml of NB. The flasks were incubated at 25°C for 18 h, and subsequently 5-ml samples were removed from each flask and

Accepted for publication 1 May 1995.

centrifuged at 900 *g* for 20 min. Supernatants were decanted, mixed with 0.5 ml of chloroform, vortexed, and centrifuged for 20 min at 900 *g*. The resulting upper-phase supernatants (purified samples) were removed from the chloroform pellet with a sterile glass pipette and stored in sterile containers at 4°C.

Table 1. Strains of *Erwinia* spp. that were hosts for bacteriophage in one or more enrichment cultures

Strain (ATCC number)	Host	Geographic origin
E. ananas[a]		
X4(35396)	Honeydew melon	Guatemala
X5(35397)	Honeydew melon	Venezuela
X9(35400)	Honeydew melon	California
X9R	Honeydew melon	California
P11(11530)	Pineapple	Hawaii
B0	Puskmelon	Texas
B1	Muskmelon	Texas
B11	Muskmelon	Texas
B9	Muskmelon	Texas
B7	Muskmelon	Texas
B13	Muskmelon	Texas
B15	Muskmelon	Texas
B42	Muskmelon	Texas
E. carotovora subsp. *carotovora*[b]		
B5	Crucifer	Gainesville, Fla.
PEP	Green pepper	Florida
12	Muskmelon	Florida
19	Tomato	Quincy, Fla.
45	Potato	Florida
53	Potato	Florida
54	Potato	Florida
68	Tomato	Florida
84	Tomato	Florida
27	Sunflower	Florida
29	Potato	Florida
40	Potato	Florida
52	Potato	Florida
347[c]	Potato seed	Wisconsin
394[c]	Carrot	Wisconsin

[a] Provided by J. M. Wells.
[b] Provided by J. A. Bartz.
[c] Provided by A. Kelman.

Soft agar (0.7% wt/vol) containing the same strains of *Erwinia* species as the enrichment flasks was overlaid on NA (1) and 12 µl of supernatant was spotted on the overlays. The soft agar had been amended with 1 g of $CaCO_3$ per liter and prepared not more than 2 days before use. Plates were incubated overnight at 29°C and observed for plaques. Samples that produced plaques were retained. Phages presumptively responsible for the plaques were purified by three cycles of single-plaque isolation, and designated according to the host strain enrichment from which they were originally isolated.

Preparation of bacteriophage stocks. When up to 3 ml of a phage suspension was needed, 100 to 500 µl of phage suspension was added to an equal volume of an 18-h host cell culture in a sterile microcentrifuge tube. This mixture was vortexed, incubated 20 min, then added to 3.5 ml of NB, vortexed, and incubated on a rotary shaker overnight. Bacteriophages were then purified as described above.

Detection of temperate phage in host strains. To determine if the origins of the phages were their host bacteria, instead of lake water, enrichment procedures were duplicated as above with omission of lake water. Fourteen *E. c.* subsp. *carotovora* and 13 *E. ananas* host strains were incubated overnight in 3.5 ml of NB, treated with 0.2 ml of chloroform, vortexed, and centrifuged 20 min at 900 *g*. Supernatants were assayed for plaque formation on soft agar overlays.

In a separate test, 15-h NB cultures of *E. ananas* were treated with 0.1 µg of mitomycin C (Sigma Chemical Co., St. Louis, Mo.) per ml (2), vortexed, incubated on a rotary shaker for 5 h, and centrifuged 20 min at 900 *g*. Then, 20-µl drops of supernatant were pipetted on soft agar overlays containing host cells. Plates were incubated overnight and observed for plaques.

Subsequently, overnight cultures of *E. ananas* were poured into sterile plastic petri plates and exposed to UV light with a wavelength of 254 nm at a distance of 6.5 cm for 60 s as described by Civerolo (2). Cultures were then incubated in the dark for 6 h, purified and tested for lysogeny as described above.

Host range. Six-microliter drops of phage suspension diluted to the routine test dilution were placed on soft agar overlays containing selected strains of *Erwinia* species. The routine test dilution was determined because use of concentrated phage stocks may cause nonspecific lysis and plaque development in the absence of infection (1). Up to nine phage strains were tested on each host plate and duplicate or triplicate plates were prepared. Plates were incubated at 29°C overnight and formation of plaques was observed. Clear confluent plaques, turbid confluent plaques, or individual plaques were recorded as positive reactions; extremely faint zones were considered negative (4). Host range tests were performed three times with phages for *E. c.* subsp. *carotovora* and twice with those for *E. ananas*. Bacteriophages for *E. c.* subsp. *carotovora* were tested against 62 strains of *E. c.* subsp. *carotovora* and *E. c.* subsp. *atroseptica* (van Hall) Dye and in a separate test on 24 strains representative of different serogroups of *E. c.* subsp. *carotovora*. Bacteriophages for *E. ananas* were tested on 13 strains of *E. ananas* and 26 strains of *E. c.* subsp. *carotovora*.

RESULTS

Bacteriophage isolation. Twenty-two phages of *E. c.* subsp. *carotovora* were isolated by enrichment culture from a eutrophic, freshwater lake in Florida during three separate tests. In each test, 48 strains of *E. c.* subsp. *carotovora* were used in the enrichment cultures, but only 15 strains were hosts for at least one phage in at least one test (Table 1). The recovery rate (number of phages/number of enrichment

Table 2. *Erwinia carotovora* subsp. *carotovora*– erwiniaphage host range[a]

	Host strains																											
Phage	B2	B4	B5	Cel	K11	Pep	9	12	14	24	27	30	34	38	40	45	52	53	54	62	68	84	92	100	150	157	347	392	394
Pep	–	–	+	–	–	+	+	+	–	–	–	+	–	+	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–
B5L2	–	–	+	–	+	+	–	+	–	–	–	–	+	+	–	–	–	–	+	–	+	–	–	+	–	+	+	–	–
12L1	–	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	–	+	+	–	+	–	–	+	–	+	+	–	–
54	–	–	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–	–	+	+	–	–	–	+	–	+	–	–	–
68L1	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	+	–	+	–	–	+	–	+	+	–	–
19	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
27	–	–	–	–	–	–	–	+	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
29L3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–
40L3	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
45	+	+	–	+	+	+	+	–	+	+	–	+	+	+	–	+	+	+	+	+	–	–	–	+	+	+	+	+	+
52L3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
53	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–
84	–	–	–	–	–	+	+	–	–	+	–	+	–	–	+	–	–	–	–	+	–	+	+	–	–	–	+	–	–
347	–	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–
394	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+

[a] Six-microliter drops of phage stocks, adjusted to routine test dilution, were place on soft agar overlays containing the host cells. Following overnight incubation, clear confluent plaques, turbid confluent plaques, or individual plaques were recorded as positive (+), and lack of plaque formation or extremely faint lytic zones were considered negative (–). Phages were tested on a collection of 62 *Erwinia carotovora* subsp. *carotovora* strains of unknown serotype. Strains that supported no plaques are not shown.

cultures) was 15.3%. Twenty-six phages of *E. ananas* were isolated from Texas surface water sampled on two occasions and enriched for phage with 13 strains of *E. ananas* for a recovery rate of 100%. A third Texas water sample added to 13 different strains of *E. c.* subsp. *carotovora* failed to yield phages in enrichment cultures for this bacterium. However, phages for each of five strains of *E. ananas* were recovered from this sample. Temperate phages were not detected in bacterial strains (13 strains of *E. ananas* and 15 of *E. carotovora*) subjected to three different methods for inducing temperate phage.

Phage host range. Nineteen of the 22 bacteriophages from Florida lake water had distinct host ranges in tests with 62 strains of *E. carotovora* (Table 2). One phage, isolate 45, lysed 22 of 62 *Erwinia* strains tested, while another, phage isolate 27, had the widest host range among strains representative of serogroups. Thirty-three of the 62 strains did not develop plaques with any of the tested phages. Six phage isolates had similar, but not identical host ranges and five of these failed to survive storage for 7 weeks at –70°C. All other phages were viable after such storage. In tests with representatives of 24 serotypes of *E. carotovora*, nine of 15 phages caused plaques in lawns of 13 serotypes, and the number of serotype representatives susceptible to a single phage ranged from one to 10 (Table 3). Four different host range patterns were observed with 13 strains of *E. ananas* and their phages (Table 4). Strain X4 formed plaques only on bacterial host strain X4. Bacteriophage X9 formed plaques on host strains X9, X9R, B0, and B7, while phages X9R, B0, and B7 formed plaques on host strains X9R, B0, and B7, but not X9. None of the phages for *E. ananas* formed plaques on any of the strains of *E. c.* subsp. *carotovora* tested.

DISCUSSION

The use of bacteriophages to control diseases caused by *Erwinia* species is likely to require a mixture of strains that have complementary host ranges and that can function independently in bacterial populations. The independent function is critical, as nonspecific lysis of potential host bacteria by incompatible phages might prevent the compatible strain from multiplying as necessary to control the growth of its specific host. Other complex interactions on the surface or inside bacteria could also confound the potential of phage mixtures to control pathogen populations. Before these possibilities can be evaluated, one must have a diverse collection of phages. We have successfully begun to develop a diverse collection of phages for *Erwinia* using surface water as a source.

Lake water and other surface waters appear to have unlimited potential as a source of erwiniaphages. Proctor recently reported that phages are more prevalent in marine environments than previously thought (16). Surface water should be homogeneous with respect to microbial and phage populations. Moreover, lake water is easy to sample and handle, relatively large volumes of lake water can be processed compared with soil or plant tissues, and lake water is safer and more esthetically pleasing to work with than raw sewage. Water samples proved easy to prepare and use and were a relatively rich source of phages. Bacteriophages of *E. ananas* were quite common in the surface water samples from Texas, whereas no phages of *E. carotovora* were detected in those samples. Our yield rate of 15% for phages of *E. carotovora* in Florida lake water compares favorably with the 5% rate of Gross et al. (9) who screened samples of plants and soil in 960 enrichment cultures, using 31 bacterial strains. We used 48 strains, of unknown serotype, in 144 enrichments and isolated phages that lysed all but one of the bacterial strains infected in the study of Gross et al. (9). In addition, three phages in our collection formed plaques on a strain of serogroup 5, one phage caused a plaque on a strain from serogroup 4, two phages caused plaques on a strain of serogroup 8, and two phages caused plaques on strain 503; Gross et al. (9) did not detect phages for these strains. Only 14 of the 52 phages isolated by Gross et al. (9) had unique host ranges; one phage was compatible (infected and lysed leading to plaque formation) with bacterial strains representing six serotypes, another with four serogroups, and the rest with one or two, or only strains representing unknown serotypes or serologically untypable groups. In our tests, 19 of the 22 phages retrieved from enrichment cultures had distinctive host ranges among 62 strains of *E. carotovora*. Of 15 phages tested on

Table 3. *Erwinia carotovora* subsp. *carotovora* phage host range among strains of known serogroup[a]

	Serogroup[b]												
Phage	**18**	**14**	**7**	**3**	**5**	**38**	**4**	**37**	**28**	**33**	**29**	**34**	**34**
B5L2	–	–	–	–	–	–	–	–	–	–	–	–	–
Pep	–	–	–	+	–	–	–	–	–	–	+	+	+
12L1	–	–	–	–	–	–	–	–	–	–	–	–	–
27	–	+	+	–	+	+	+	+	–	+	+	–	+
29L3	–	–	–	–	+	–	–	–	–	–	–	–	–
40L3	–	–	–	–	+	–	–	–	–	–	–	–	–
52L3	–	–	–	–	–	–	–	–	–	–	–	–	–
68L1	–	–	–	–	–	–	–	–	+	–	–	–	–
394	–	–	–	–	–	–	–	–	–	–	–	–	–
54	–	–	–	–	–	–	–	–	–	–	–	–	–
84	–	–	–	+	+	–	–	–	–	–	+	–	–
347	–	–	–	–	–	–	–	–	–	–	–	–	–
53	–	–	–	–	–	+	–	–	–	–	–	–	–
19	+	–	–	+	–	–	–	–	–	–	–	+	+
45	–	–	–	+	+	–	–	–	–	–	–	–	–

[a] Six-microliter drops of phage stocks, adjusted to routine test dilution, were placed on soft agar overlays containing the host cells. Following overnight incubation, clear confluent plaques, turbid confluent plaques, or individual plaques were recorded as positive (+), and lack of plaque formation or extremely faint lytic zones were considered negative (–).

[b] Serogroups 15, 27, 31, 36, 39, were included in the test but did not support plaque formation with any of the phages.

Table 4. *Erwinia ananas* phage host range

	Host strain												
Phage	**X4**	**X5**	**P11**	**X9**	**X9R**	**B0**	**B7**	**B1**	**B9**	**B11**	**B13**	**B15**	**B42**
X4	+	–	–	–	–	–	–	–	–	–	–	–	–
X5	–	+	+	–	–	–	–	–	–	–	–	–	–
P11	–	+	+	–	–	–	–	–	–	–	–	–	–
X9	–	–	–	+	+	+	+	–	–	–	–	–	–
X9R	–	–	–	–	+	+	+	–	–	–	–	–	–
B0	–	–	–	–	+	+	+	–	–	–	–	–	–
B7	–	–	–	–	+	+	+	–	–	–	–	–	–
B1	–	–	–	–	–	–	–	+	+	+	+	+	+
B9	–	–	–	–	–	–	–	+	+	+	+	+	+
B11	–	–	–	–	–	–	–	+	+	+	+	+	+
B13	–	–	–	–	–	–	–	+	+	+	+	+	+
B15	–	–	–	–	–	–	–	+	+	+	+	+	+
B42	–	–	–	–	–	–	–	+	+	+	+	+	+

[a] Six-microliter drops of phage stocks, adjusted to routine test dilution, were placed on soft agar overlays containing the host cells. Following overnight incubation, clear confluent plaques, turbid confluent plaques, or individual plaques were recorded as positive (+), and lack of plaque formation or extremely faint lytic zones were considered negative (–).

strains of 24 known serotypes, the host range of one included 10 serotypes, two included six, two included four, and the rest included one or two, or only strains of unknown or untypable serogroups. Although phages of *E. carotovora* from lake water seemed to have a relatively restricted host range, they have somewhat larger host ranges than phages isolated from plant tissues or soils associates with diseased plants (9). This is consistent with the literature (8,9,17).

It is not clear if individual phages of *E. ananas* had restricted or broad host ranges. These phages collected in the enrichment tests had five distinct host ranges. One phage was compatible with four of the 13 strains and three with three of 13 although the relationship of the three with each other is not clear. The highest rate of compatibility, 30%, is nearly twice that of the broadest host range among carotovoraphages with 10 of 61 or 16%.

One explanation for not finding phages for certain strains of bacteria is that insensitive strains may harbor temperate phages that confer resistance to related lytic phages (1). If lysogenic strains of *Erwinia* species are widespread and prevalent in the environment, then the successful use of phages as biocontrol agents will be confounded as relatedness to existing temperate phages will have to be considered in the selection of phage mixtures. No lysogeny (presence of temperate phage) was detected here among 15 strains of *E. c.* subsp. *carotovora* or 13 strains of *E. ananas* in three different types of induction tests. This does not eliminate the possibility that one or more of the untested bacterial strains may contain a temperate phage. However, the absence of lysogeny in the host strains on which the phages were isolated is strong evidence that the phages isolated were from the surface water used for enrichment and not the bacterial strains themselves.

Previously, phages were considered to have limited potential for biological control because of narrow spectrum of activity and possible mutation to resistance (17). Vidaver (17) wrote, "Specificity against only one or a few strains of a species is useless for control." The host range results indicate that a mixture of five phages potentially could protect fruit against the 13 strains of *E. ananas*, and a mixture of four phages could protect potato tubers against 16 of the 23 serogroups of *E. c.* subsp. *carotovora* tested. The four strains of *E. c.* subsp. *carotovora* from serogroups most often associated with major outbreaks of bacterial soft rot (serogroups 3,5,7, and 37) (14) were susceptible to at least one phage isolate. However, how closely phage susceptibility corresponds with serotype is unknown. An additional consideration in the design of phage mixtures is the presence of strains within a serogroup that are not all susceptible to the same phages. Fortunately, new phages should be easy to find if the appropriate surface waters are sampled. For example, subsequent to the tests with enrichment cultures reported here, phages were detected in the retained liquid after ultrafiltration of drainage ditch water sampled from potato fields in Hastings, Fla. (J. A. Bartz, *unpublished*). The >100,000 molecular weight components of the ditch water sample were concentrated from 2 liters to less than 200 ml and yielded at least three phages when samples of the concentrate were spotted on a limited array of potential host bacteria. With this technique the requirement for enrichment cultures is unnecessary, simplifying the development of phage collections.

LITERATURE CITED

1. Adams, M. H. 1969. Bacteriophages. Interscience Publishers, New York.
2. Civerolo, E. L. 1990. Bacteriophages. Pages 205-213 in: Methods in Phytobacteriology. Z. Klement, K Rudolph, and D. C. Sands, eds. Akadémi Kiadó, Budapest, Hungary.
3. Cook, R. J. 1993. The role of biological control in pest management in the 21st century. Pages 10-20 in: Pest Management: Biologically Based Technologies. R. D. Lumsden, and J. L. Vaughn, eds. Proc. Beltsville Symposium ARS, USDA, Beltsville, Md.
4. Cuppels, D. A. 1983. Isolation and characterization of phages useful for identifying *Pseudomonas syringae* pv. *tomato*. Phytopathology 73:1376-1381.
5. Dye, D. W. 1968. A taxonomic study of the genus *Erwinia* I. The "amylovora" group. N. Z. J. Sci. 11:590-607.
6. Dye, D. W. 1969. A taxonomic study of the genus *Erwinia* II. The "carotovora" group. N. Z. J. Sci. 12:81-97.
7. Dye, D. W. 1969. A taxonomic study of the genus *Erwinia* III. The "herbicola" group. N. Z. J. Sci. 12:223-236.
8. Goto, M. 1992. Fundamentals of Bacterial Plant Pathology. Academic Press, New York.
9. Gross, D. C., Powelson, M. L., Regner, K. M., and Radamaker, G. K. 1991. A bacteriophage-typing system for surveying the diversity and distribution of strains of *Erwinia carotovora* in potato fields. Phytopathology 81:220-226.
10. Harrison, M. D., Franc, G. D., Maddox, D. A., Michaud, J. E., and McCarter-Zorner, N. J. 1987. Presence of *Erwinia carotovora* in surface water in North America. J. Appl. Bacteriol. 62:565-570.
11. Hooker, W. J., ed. 1981. Compendium of Potato Diseases. American Phytopathological Society, St. Paul, Minn.
12. Krieg, N. R., and Holt, J. G., eds. 1984. Bergey's Manual of Systematic Bacteriology, Williams and Wilkins, Philadelphia.
13. Paulin, J. P., and Nassan, N. A. 1978. Lysogenic strains and phage-typing in *Erwinia chrysanthemi*. Pages 539-545 in: Proc. Int. Conf. Plant Pathogenic Bacteria, 4th. Station de Pathologie Végétale et Phytobactériologie, I.N.R.A., Angers, ed. Gibert-Clarey, Tours, France.
14. Powelson, M. L., and Apple, J. D. 1984. Soil and seed tubers as sources of inoculum of *Erwinia carotovora* pv. *carotovora* for stem soft rot of potatoes. Phytopathology 74:429-432.
15. Perombelon, M. C., and Kelman, A. 1980. Ecology of the soft rot *Erwinias*. Annu. Rev. Phytopathol. 18: 361-87.
16. Proctor, L. M. 1990. Viral mortality of marine bacteria and cyanobacteria. Nature 343:60-62.
17. Vidaver, A. K. 1976. Prospects for control of phytopathogenic bacteria by bacteriophages and bacteriocins. Annu. Rev. Phytopathol. 14: 452-465.
18. Wells, J. M., Sheng, W-S., Ceponis, M. J., and Chen, T. A. 1987. Isolation and characterization of strains of *Erwinia ananas* from honeydew melons. Phytopathology 77:511-514.