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Generalized Transduction in the Enterobacterial Phytopathogen *Erwinia chrysanthemi*†

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Bacteriophages induced by mitomycin treatment of *Erwinia chrysanthemi* KS612 produced plaques on lawns of *E. chrysanthemi* EC183 and KS605. Bacteriophage Erch-12, purified from one such plaque, transferred an array of chromosomal genes (*arg, leu, his, ser, thr, trp, ura*) to appropriate recipient strains derived from *E. chrysanthemi* EC183. Recombinants were formed in the absence of cellular contact between donor and recipient bacteria and in the presence of deoxyribonuclease. Ultraviolet irradiation of the bacteriophage stimulated transductional frequency. Linkage was detected in two-factor crosses between the loci *thr* and *ser* and between *rif* and *ade*; several closely linked mutations in *ser* were mapped with respect to *thr*.

Most members of the "carotovora" group in the enterobacterial genus *Erwinia* cause soft rot disease in a wide variety of plants (15, 24, 32). These bacteria produce a battery of extracellular degradative enzymes such as pectinases, proteases, lipases, and cellulases (5, 32), some or all of which are believed to cause tissue maceration and death of plant cells. Recent biochemical (4, 17, 33) and genetic (12) investigations with *E. chrysanthemi* have revealed an essential role of one of the pectolytic enzymes, polygalacturonic acid *trans*-eliminase, in tissue maceration and cell death.

In investigating the genetics of polygalacturonic acid *trans*-eliminase production and the catabolism of polygalacturonic acid by *E. chrysanthemi*, we recognized the need for fine structure mapping of genes that control these processes. In other bacterial systems, such as *Escherichia coli* (3), *Pseudomonas aeruginosa* (35), *Salmonella typhimurium* (31), and *Bacillus subtilis* (20), transduction has proven highly effective in mapping closely linked gene loci. Recently, generalized transducing systems have been developed in several bacterial groups such as *Acinetobacter* (21), *Caulobacter crescentus* (16), *Klebsiella pneumoniae* (18), *Myxococcus xanthus* (8), and *Rhizobium leguminosarum* (7). Although both virulent and temperate bacteriophages are known to infect various phytopathogenic bacteria (28, 32, 34), none has thus far been shown to possess transducing ability. In this report we document that a temperate bacteriophage originating in *E. chrysanthemi* can mediate generalized transduction in a strain of this bacterial species and that this transducing system can be used to order linked gene loci.

MATERIALS AND METHODS

Bacteria and bacteriophage. The *E. chrysanthemi* strains used in this study are listed in Table 1. An *E. coli* strain harboring the R plasmid R68.45 was used to construct conjugational donor strains in *E. chrysanthemi* (10). Mutant strains were derived by ethyl methane sulfonate (EMS) or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) mutagenesis according to previously published procedures (11, 12). Auxotrophic mutants were enriched by ampicillin treatment (26). Rifampin (Sigma Chemical Co., St. Louis, Mo.)-resistant mutants (Rif^r) that were simultaneously auxotrophic were isolated by treating the wild-type strain EC183 with NTG. After mutagenesis and allowing for the phenotypic expression, Rif^r clones were selected on L-agar containing rifampin (50 μg/ml). Drug-resistant clones were then tested for auxotrophy by patching on minimal agar. The nutritional requirements of auxotrophic Rif^r clones were determined by the protocol of Roth (30). Bacterial cultures were maintained on yeast-glucose-$CaCO_3$ (YGC; 10) agar slants at 4°C and were transferred at 3-week intervals. The bacteriophage Erch-12, induced by mitomycin treatment of *E. chrysanthemi* KS612 (Dickey strain 98), was used throughout this study.

Media. L-medium, YGC agar, and minimal medium were prepared as described previously (10). TN-medium consisted of 10 g of tryptone per liter and 8 g of NaCl per liter (pH 7.2). When required, minimal medium was supplemented with amino acids, purines, and pyrimidines (50 μg/ml). An alternate carbon source (L-arabinose), which replaced glucose in the minimal medium, was added to yield a final concentration of 1 mg/ml. Bacteriophage buffer consisted of 10 mM $MgCl_2$ in 10 mM Tris-hydrochloride (pH 7.0).

Mitomycin treatment. Bacterial cultures were grown in L-broth (10 ml contained in a 300-ml-capacity Klett flask) at 30°C on a water bath shaker. Growth of cultures was estimated turbidimetrically by using a

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TABLE 1. *E. chrysanthemi* strains

Strains	Genotype/description[a]	Source[b]
EC183	Wild-type, prototrophic, Pat$^+$ Hpg$^+$ Plc$^+$ Prt$^+$, virulent	ICPB
AC6001	*arg-1*	NTG mutagenesis of EC183
AC6003	*leu-1*	NTG mutagenesis of EC183
AC6005	*his-1*	NTG mutagenesis of EC183
AC6006	*arg-1 his-2*	EMS mutagenesis of AC6001
AC6007	*arg-1 leu-2*	EMS mutagenesis of AC6001
AC6009	*arg-1 leu-2 ara-1*	EMS mutagenesis of AC6007
AC6016	*arg-1 leu-2 ser-1*	EMS mutagenesis of AC6007
AC6017	*arg-1 leu-2 ilv-1*	EMS mutagenesis of AC6007
AC6018	*arg-1 leu-2 thr-2*	EMS mutagenesis of AC6007
AC6019	*arg-1 his-2 trp-1*	EMS mutagenesis of AC6006
AC6020	*arg-1 leu-2 ilv-1 thr-3*	EMS mutagenesis of AC6017
AC6021	*arg-1 leu-2 ilv-1 ser-2*	EMS mutagenesis of AC6017
AC6023	*arg-1 leu-2 ilv-1 ura-1*	EMS mutagenesis of AC6017
AC6026	*arg-1 his-2 trp-1 ser-3*	EMS mutagenesis of AC6019
AC6031	*leu-1 thr-1*	EMS mutagenesis of AC6003
AC6033	*ade-1 rif-1*	NTG mutagenesis of EC183[c]
AC6050[d]	AC6005 (R68.45)	Ap^r Km^r Tc^r transconjugant AC4071 × AC6005
AC6051[d]	AC6009 (R68.45)	Ap^r Km^r Tc^r transconjugant derived from a AC6050 × AC6009
AC4071[d]	*thr$^+$ gal-1 str-1* (R68.45)	10
KS605 (Dickey B-27)	Wild-type prototrophic, virulent	R. S. Dickey 13
KS612 (Dickey 98)	Wild-type prototrophic, virulent	R. S. Dickey 13

[a] Designation of uncommon markers: Pat$^+$, production of polygalacturonic acid *trans*-eliminase; Hpg$^+$, production of hydrolytic polygalacturonase; Plc$^+$, production of phospholipase (lecithinase); Prt$^+$, production of protease. The chromosomal gene symbols are according to those of Bachmann and Low (3), and the plasmid phenotype symbols are according to those of Novick et al. (27).

[b] ICPB, International Collection of Phytopathogenic Bacteria (University of California, Davis).

[c] NTG-induced comutation resulting in rifampin resistance and auxotrophy. See text for the details of isolation procedure.

[d] R68.45 was introduced by conjugation according to Chatterjee (10).

Klett-Summerson colorimeter (red filter no. 66). At early to mid-exponential phase of growth, 10 μg of mitomycin (Sigma) per ml was added. Another culture, which served as a control, received an equal volume of sterile water. Incubation in the dark was continued as before, and culture turbidity was recorded at desired intervals. After 20 to 24 h of incubation, cells were removed by centrifugation (12,000 × *g*, 10 min, 4°C); the supernatant was treated with $CHCl_3$ and assayed for plaque-forming units (PFU).

Preparation and assay of bacteriophage lysate. Bacteriophage particles in a single plaque were picked and resuspended in 1 ml of L- (or TN-) broth. A sample of this suspension (0.2 ml) was mixed with 0.2 ml of a culture (ca. 10^9 cells/ml) of the host and assayed by the standard double-layer technique (1). After overnight incubation (usually 16 h), the plaques that appeared were washed off the plate. A small volume (2 to 3 ml) of L- (or TN-) broth was poured on the soft agar, and the top layer was teased with a pipette tip. The plates were then stored at 4°C for 4 to 6 h. The fluid was collected subsequently, and bacterial cells and debris were removed by centrifugation (12,000 × *g*, 15 min, 4°C). The supernatant was treated with $CHCl_3$ and stored at 4°C. Bacteriophage present in this sample were used for the second cycle of enrichment on agar plates. A titer of 5×10^{10} PFU/ml was usually obtained after three cycles of enrichment.

Bacteriophage adsorption. Equal volumes of the bacteriophage lysate (ca. 5×10^9 PFU/ml) and exponential bacterial culture (ca. 10^9 cells per ml) were mixed and then incubated at 30°C. Samples (0.05 ml) of the mixture were diluted in 5.0 ml in TN-broth. The cells with the adsorbed bacteriophage were removed by centrifugation (12,000 × *g* for 5 min at 4°C). The PFU remaining in the supernatant were assayed as above.

UV irradiation. Stock bacteriophage lysate (titer about 5×10^{10} PFU/ml) in TN-broth was diluted 10-fold in bacteriophage buffer. A sample (usually 15 ml) of the diluted suspension contained in a glass petri dish (100-mm diameter) was exposed to short wave (254 nm) UV rays emitted by a 15-W General Electric germicidal lamp. The contents were stirred while being exposed to the UV rays. The light source was kept at 28 cm from the surface of the liquid, which corresponded to a UV dosage of 40 ergs/mm^2 per s.

Transduction. An equal volume of bacteriophage preparation (in bacteriophage buffer, UV-irradiated or nonirradiated) and exponentially growing bacterial cells were mixed at a multiplicity of infection of 1 to 10 (unless stated otherwise). Bacterial cells treated without the bacteriophage but otherwise similarly were the control. All samples were incubated at 30°C

for 60 min. After cells had been collected by low-speed centrifugation (for 10 min at a setting of 5 on a clinical centrifuge [International Equipment Co., Needham, Mass.]) at room temperature and resuspended in 55 mM potassium phosphate buffer (pH 7.2), a sample (0.1 ml) was spread on appropriate selective agar medium. Colonies appeared on the selective medium after 4 to 5 days of incubation.

DNase treatment. $MgSO_4$ and DNase (Sigma) in 150 mM NaCl were added to a sample of the bacteriophage lysate to yield the final concentrations of 100 μg of DNase per ml and 5 mM $MgSO_4$. The mixture, incubated at 30°C for 2 h, was then assayed directly for PFU and transducing activity by the above procedures.

RESULTS AND DISCUSSION

All 15 wild-type strains of *E. chrysanthemi* tested were equally sensitive to mitomycin (10 μg/ml). In general, after the drug had been added, there was a doubling of cell density followed by cellular lysis. The lysis was gradual and continued for up to 16 to 24 h (detailed data not presented). The cultures that did not receive mitomycin grew into stationary phase and showed no evidence of cellular lysis. The mitomycin-induced culture lysate of strain KS612 produced many plaques (ca. 5×10^4/ml of lysate) on the lawns of strains EC183 and KS605. It is noteworthy that the producing (KS612) as well as the indicator (EC183, KS605) bacterial strains originated on infected dieffenbachia plants. The bacterial isolates from other hosts such as maize or chrysanthemum generally were insensitive to the bacteriophage Erch-12. These preliminary results suggest a differential bacteriophage susceptibility in strains of *E. chrysanthemi* originating on different plant hosts, a notion supported by the findings of Paulin and Nassan (29).

More than 99% of the input bacteriophage was adsorbed rapidly by the host (EC183) cells within the first 30 min, and the remainder was absorbed thereafter more slowly. Host cells continued to adsorb bacteriophages up to 60 min of incubation, albeit at a slower rate. These findings and the results of our preliminary trials on the influence of incubation time on the recovery of transductants led us to choose an incubation period of 60 min in all transductional crosses.

A lysate (titer, 5×10^{10} PFU/ml) of Erch-12 on EC183 was tested for transducing activity by using AC6026 as the recipient strain. A few (5 to 15) prototrophic (Arg^+, His^+, Ser^+, or Trp^+) colonies were detected per 0.2 ml of the lysate. Prompted by the findings on the enhancement of transductional frequency after UV irradiation in other bacterial systems (2, 6–8, 14, 22, 35), we exposed the bacteriophage lysate to UV light and tested for transduction of various markers. UV irradiation of the bacteriophage lysate stimulated transductional frequency of all the tested markers (Table 2). Stimulation (4- to 38-fold) of His^+, Trp^+, and Ser^+ recovery occurred at about 99.9% inactivation of the PFU. The frequency of Arg^+ recombinants was highest at about 45% inactivation of the bacteriophage particles. The data summarized in Table 3 also reveal a stimulatory effect of UV irradiation on the frequency of transfer of *ilv*$^+$, *leu*$^+$, *thr*$^+$, and *ura*$^+$. The UV effect has been attributed to enhanced recombination (6) or to the inactivation of the killing effects of the virulent bacteriophages (7, 16). The data presented for *E. chrysanthemi* do not yet allow us to discount either possibility.

To rule out the possibility that the gene transfer might have been due to transformation or transfection, we preincubated the bacteriophage lysate with DNase and then tested it for plaque-forming ability and transductional activity (Table 3). The frequencies of recombinants of various markers were found to be comparable in the presence or absence of DNase. Moreover, as expected, DNase had no adverse effect on the plaque-forming ability of either irradiated or unirradiated bacteriophage preparations.

Transductants for various markers were tested for lysogeny and the inheritance of unselected markers. Arg^+, Leu^+, Ser^+, and Trp^+ recombinants were purified by three cycles of single-clone isolations on appropriate selective media and tested for susceptibility to Erch-12. Some recombinants were susceptible to the bacteriophage; others were resistant. Two bacteriophage-resistant clones for each recombinant class were grown in the presence or absence of

TABLE 2. *Inactivation of the bacteriophage Erch-12 and stimulation of transductional frequency by UV irradiation*[a]

UV irradiation (s)	Bacteriophage titer (PFU/ml)	Prototrophic colonies/ml of transduction mixture[b]			
		Arg^+	His^+	Trp^+	Ser^+
0	4.0×10^9	90	125	55	960
30	1.8×10^9	3,410	1,700	690	3,670
60	4.5×10^8	2,020	2,320	1,225	3,910
90	1.0×10^8	1,665	1,130	720	1,670
120	2.2×10^7	1,580	730	700	1,000
150	4.0×10^6	400	315	275	385

[a] Refer to the text for the details of the experimental conditions.

[b] Transductional mixture contained 10^9 cells of the recipient *E. chrysanthemi* AC6026. Spontaneous Arg^+ and His^+ revertants were undetected ($<1/10^8$ cells plated); Ser^+ and Trp^+ revertants ranged from 1 to 2 colonies/10^8 cells. Sterility of the bacteriophage preparations was indicated by the lack of colony formation with duplicate 20-μl samples on L-agar.

TABLE 3. *Effect of DNase and UV irradiation on gene transfer*[a]

Recipient[b]	Selection	No. of colonies/PFU[c]			
		Unirradiated phage		Irradiated phage	
		−DNase	+DNase	−DNase	+DNase
AC6026	Arg^+	1×10^{-8}	2×10^{-8}	3×10^{-6}	3×10^{-6}
	His^+	7×10^{-9}	2×10^{-8}	3×10^{-6}	3×10^{-6}
	Trp^+	6×10^{-9}	3×10^{-9}	1×10^{-6}	1×10^{-6}
	Ser^+	1×10^{-7}	3×10^{-7}	6×10^{-6}	5×10^{-6}
AC6020	Leu^+	1×10^{-8}	5×10^{-9}	4×10^{-6}	2×10^{-6}
	Ilv^+	$<2 \times 10^{-9}$	2×10^{-9}	1×10^{-6}	7×10^{-7}
	Thr^+	1×10^{-8}	1×10^{-8}	7×10^{-6}	6×10^{-6}
AC6023	Ura^+	7×10^{-8}	9×10^{-8}	9×10^{-6}	7×10^{-6}

[a] See the text for the details of the experimental conditions.
[b] See Table 1 for the characteristics of the recipient strains.
[c] Bacteriophage titers were as follows: unirradiated, 5×10^9 PFU/ml; irradiated, 8×10^8 PFU/ml. Prototrophic revertants of recipients ranged from 0 to 2 per 10^8 cells plated.

mitomycin, and chloroform-treated culture supernatants were tested for plaque formation on *E. chrysanthemi* (EC183). All transductants tested produced bacteriophages spontaneously and in the presence of mitomycin; the virus titer was invariably higher (about 10^6 PFU/ml) in the presence of mitomycin than in its absence (about 10^3 PFU/ml). In our initial trials, no linkage was detected between *arg-leu*, *arg-his*, *arg-ilv*, *arg-thr*, *arg-trp*, *arg-ser*, *arg-ura*, *his-trp*, *his-ser*, *leu-ilv*, *leu-thr*, *leu-ura*, *ilv-thr*, *ilv-ura*, and *trp-ser*. Possibly those pairs of markers are not closely linked and hence were not carried on the same fragment. To test bacteriophage (Erch-12)-mediated cotransduction of closely linked markers on *E. chrysanthemi* chromosome, we adopted two different approaches, summarized below.

(i) Linkage between *rif* and *ade*. By NTG mutagenesis we isolated Rif^r mutants that simultaneously were auxotrophic and tested linkage between *rif* and the comutated auxotrophic marker. Our rationale for the approach was the finding that NTG causes comutation in closely linked gene loci (19, 25). Of the various pairs of markers (*rif-ade*; *rif-leu*; *rif-met*) tested, linkage was detected between *rif-ade*, but not between *rif* and other markers. The frequency of coinheritance of the markers (*rif* and *ade*) in recombinants from transductional crosses ranged from 3 to 5%. In recombinants from R68.45-mediated conjugational crosses, the coinheritance value was much higher (Table 4). The differences probably could be attributed to the sizes of genomic fragments transferred by these two processes. *thr* and *leu* were not linked in transductional crosses (see above), but are known to be linked in recombinants derived from both Hfr- and R68.45-mediated crosses in a related strain of *E. chrysanthemi* (10, 12).

TABLE 4. *Linkage between rif and ade in E. chrysanthemi by transductional and conjugational crosses*[a]

Mode of gene transfer	Total Ade^+ clones tested	No. of Rif^s clones	% Coinheritance
Conjugation	98	49	50
Transduction	236	8	3.3

[a] Conjugal donor was AC6051 (Table 1; 10); bacteriophage Erch-12 was grown on strain EC183; recipient was strain AC6033 (Table 1). Ade^+ clones were selected on minimal medium and patched on minimal medium containing rifampin (50 μg/ml).

(ii) Linkage between *ser* and *thr*. We further tested contransfer of markers known to be closely linked on chromosomes of other enterobacteria such as *E. coli* and *S. typhimurium*, and we attempted to order independent mutations in these gene loci by two factor crosses. The gene loci, *serB* and *thrABC*, are located within 0.5 map units on both *E. coli* and *S. typhimurium* chromosomes (3, 31). We (9, 10, 12) have some evidence for a homology in gene order on chromosomes of *E. chrysanthemi* and these nonphytopathogenic enterobacteria; so it was reasonable to assume that these markers also were closely linked on *E. chrysanthemi* chromosome. The data from reciprocal crosses reveal that *ser-1*, *ser-2*, and *ser-3* were linked to *thr-1*, *thr-2*, and *thr-3* (Fig. 1). Based on the mean values of the percentage of coinheritance in reciprocal crosses, the tentative order of these gene loci (Fig. 1) was indicated.

It is noteworthy that the pairs of markers (*thr-ser*; *rif-ade*) are also linked on *E. coli* and *S. typhimurium* chromosomes (3, 31). That finding, taken together with our observations on linkage between other markers on the *E. chry-*

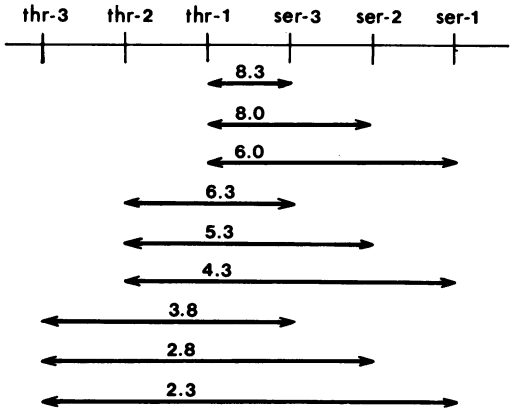

FIG. 1. *Relative order of thr and ser loci on the chromosome of E. chrysanthemi as indicated by two factor transductional crosses. The numbers above the arrows represent means of coinheritance values in percent in reciprocal crosses between strains AC6016, AC6018, AC6020, AC6021, AC6026, and AC6031 (for genotypes see Table 1).*

santhemi chromosome (9, 10, 12), suggests an overall similarity in gene order on the chromosomes of this phytopathogenic bacterial species and other enterobacteria. The generalized transducing system used in conjunction with conjugational systems (10, 12, 23) should allow a critical assessment of the extent of genetic homology between these enterobacteria and elucidation of the organization of genes that specify phytopathogenicity in *E. chrysanthemi.*

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LITERATURE CITED

1. **Adams, M. H.** 1959. Bacteriophages. Interscience Publishers, Inc., New York.
2. **Arber, W.** 1958. Transduction des charactères *gal* par le bactériophage *lambda*. Arch. Sci. (Geneva) **11**:259–338.
3. **Bachmann, B. J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. Microbiol. Rev. **44**:1–56.
4. **Basham, H. G., and D. F. Bateman.** 1975. Relationship of cell death in plant tissue treated with a homogeneous endopectate lyase to cell wall degradation. Physiol. Plant Pathol. **5**:249–262.
5. **Bateman, D. F., and R. L. Millar.** 1966. Pectic enzymes in tissue degradation. Annu. Rev. Phytopathol. **4**:119–146.
6. **Benzinger, R., and P. E. Hartman.** 1962. Effect of ultraviolet light on transducing phage P22. Virology **18**: 614–626.
7. **Buchanan-Wollaston, V.** 1979. Generalized transduction in *Rhizobium leguminosarum*. J. Gen. Microbiol. **112**:135–142.
8. **Campos, J. M., J. Geisselsoder, and D. R. Zusman.** 1978. Isolation of bacteriophage M × 4, a generalized transducing phage for *Myxococcus xanthus*. J. Mol. Biol. **119**:167–178.
9. **Chatterjee, A. K.** 1978. Genetics of phytopathogenic bacteria, p. 3–16. *In* Station de Pathologie Végétale et Phytobacteriologie (ed.), Proceedings of the Fourth International Conference on Plant Pathogenic Bacteria, Angers, France, 1978. Institut National de la Recherche Agronomique, Angers.
10. **Chatterjee, A. K.** 1980. Acceptance by *Erwinia* spp. of the R plasmid R68.45 and its ability to mobilize the chromosome of *Erwinia chrysanthemi*. J. Bacteriol. **142**:111–119.
11. **Chatterjee, A. K., and M. P. Starr.** 1973. Gene transmission among strains of *Erwinia amylovora*. J. Bacteriol. **116**:1100–1106.
12. **Chatterjee, A. K., and M. P. Starr.** 1977. Donor strains of the soft-rot bacterium *Erwinia chrysanthemi* and conjugational transfer of pectolytic capacity. J. Bacteriol. **132**:862–869.
13. **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.
14. **Drexler, H.** 1970. Transduction by bacteriophage T1. Proc. Natl. Acad. Sci. U.S.A. **66**:1083–1088.
15. **Dye, D. W.** 1969. A taxonomic study of the genus *Erwinia*. II. The "carotovora" group. N. Z. J. Sci. **12**:81–97.
16. **Ely, B., and R. C. Johnson.** 1977. Generalized transduction in *Caulobacter crescentus*. Genetics **87**:391–399.
17. **Garibaldi, A., and D. F. Bateman.** 1971. Pectic enzymes produced by *Erwinia chrysanthemi* and their effects on plant tissue. Physiol. Plant Pathol. **1**:25–40.
18. **Goldberg, R. B., R. A. Bender, and S. L. Streicher.** 1974. Direct selection for P1-sensitive mutants of enteric bacteria. J. Bacteriol. **118**:810–814.
19. **Guerola, N., J. L. Ingraham, and E. Cerda-Olmedo.** 1971. Induction of closely linked multiple mutations by nitrosoguanidine. Nature (London) **230**:122–125.
20. **Henner, D. J., and J. A. Hoch.** 1980. The *Bacillus subtilis* chromosome. Microbiol. Rev. **44**:57–82.
21. **Herman, N. J., and E. Juni.** 1974. Isolation and characterization of a generalized transducing bacteriophage for *Acinetobacter*. J. Virol. **13**:46–52.
22. **Holloway, B. W., and M. Monk.** 1961. Influence of ultraviolet irradiation on general transduction in *Pseudomonas aeruginosa*, p. 231–237. *In* P. L. T. Ilbery (ed.), Radiobiology, Proceedings of the 3rd Australian Conference on Radiobiology, Sydney. Butterworths, London.
23. **Lacy, G. H.** 1978. Genetic studies with plasmid RP1 in *Erwinia chrysanthemi* strains pathogenic on maize. Phytopathology **68**:1323–1330.
24. **Lelliott, R. A.** 1974. *Erwinia*, p. 332–340. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
25. **Mekalanos, J. J., R. D. Sublett, and W. R. Romig.** 1979. Genetic mapping of toxin regulatory mutations in *Vibrio cholerae*. J. Bacteriol. **139**:859–865.
26. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 230–234. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
27. **Novick, R. P., R. C. Clowes, S. N. Cohen, R. Curtiss III, N. Datta, and S. Falkow.** 1976. Uniform nomenclature for bacterial plasmids: a proposal. Bacteriol. Rev. **40**:168–189.
28. **Okabe, N., and M. Goto.** 1963. Bacteriophages of plant pathogens. Annu. Rev. Phytopathol. **1**:397–418.
29. **Paulin, J. P., and N. A. Nassan.** 1978. Lysogenic strains and phage-typing in *Erwinia chrysanthemi*, p. 539–545. *In* Station de pathologie Végétale et Phytobacteriologie

(ed.), Proceedings of the Fourth International Conference on Plant Pathogenic Bacteria, Angers, France, 1978. Institut National de la Recherche Agronomique, Angers.

30. **Roth, J. R.** 1970. Genetic techniques in studies of bacterial metabolism. Methods Enzymol. **17a**:3-35.
31. **Sanderson, K. E., and P. E. Hartman.** 1978. Linkage map of *Salmonella typhimurium*, edition V. Microbiol. Rev. **42**:471-519.
32. **Starr, M. P., and A. K. Chatterjee.** 1972. The genus *Erwinia*: Enterobacteria pathogenic to plants and animals. Annu. Rev. Microbiol. **26**:389-426.
33. **Stephens, G. J., and R. K. S. Wood.** 1975. Killing of protoplasts by soft-rot bacteria. Physiol. Plant Pathol. **5**:165-181.
34. **Vidaver, A. K.** 1976. Prospects for control of phytopathogenic bacteria by bacteriophages and bacteriocins. Annu. Rev. Phytopathol. **14**:451-465.
35. **Watson, J. M., and B. W. Holloway.** 1978. Linkage map of *Pseudomonas aeruginosa* PAT. J. Bacteriol. **136**: 507-521.
36. **Wilson, D. E.** 1960. The effects of ultraviolet light and ionizing radiation on the transduction of *Escherichia coli* by phage P1. Virology **11**:533-546.