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Chromosomal Mapping in *Erwinia carotovora* subsp. *carotovora* with the IncP Plasmid R68::Mu

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Conjugational gene transfer was established in *Erwinia carotovora* subsp. *carotovora* SCRI193 by using plasmid R68::Mu c^+ to mobilize the chromosome into multiply mutant recipients. It was observed that although the plasmid alone mobilized markers randomly at a frequency of ca. 10^{-5} chromosomal recombinants per donor, the presence of a Mu prophage on the chromosome of the donor increased the frequency of mobilization of markers adjacent to the prophage by up to 10-fold. Using this system it was possible to order 17 chromosomal mutations. The behavior of Mu in *E. carotovora* subsp. *carotovora* was also studied.

The *Erwinia* genus is a member of the family *Enterobacteriaceae* and includes phytopathogenic, soft-rotting species which can enzymatically macerate the parenchymatous tissues of many plants. The bacteria secrete several extracellular pectic enzymes which break down the pectin-containing material of the middle lamella. Early taxonomic studies employing phenotypic characters classified the soft-rot *Erwinia* as different subspecies of *Erwinia carotovora* (11). Later work, including DNA renaturation studies (3, 14), suggested that while *E. carotovora* subsp. *carotovora* and *E. carotovora* subsp. *atroseptica* should remain as subspecies of *E. carotovora*, *E. chrysanthemi* and certain other soft-rot strains should be classified as separate species (20, 31). *E. carotovora* subsp. *carotovora* has a broad host range and infects a wide range of temperate and tropical species (25); many of the other soft-rot *Erwinia* species have more restricted host ranges, as, for example, *E. carotovora* subsp. *atroseptica*, which is restricted mainly to temperately grown potatoes.

The present study was initiated to establish genetic techniques in *E. carotovora* with a view to studying the species as a phytopathogen and also as a member of the *Enterobacteriaceae*. The biochemistry of the *E. carotovora* pectic enzymes has been well studied (2, 7, 9, 32); however, a clearer understanding of the regulation of the expression of these enzymes in *E. carotovora* could be achieved if genetic tools were available in this species. Strain SCRI193, used in the present study, was originally isolated from potatoes; it causes soft rot of potato tubers and produces extracellular polygalacturonase and pectate lyase (M. C. M. Pérombelon, unpublished data), protease (K. J. Forbes, unpublished data), and cellulase (J. Hinton, personal communication). Three different plasmid isolation methods (4, 12, 30) were unable to detect plasmids in the wild-type strain, suggesting that the genetic determinants of pathogenicity in this strain are chromosomally located (K. J. Forbes, Ph.D. thesis, University of Edinburgh, Edinburgh, United Kingdom 1983).

By using well-characterized mutations in general metabolism in *E. carotovora* subsp. *carotovora* SCRI193, it will be possible not only to more readily map genes involved in pathogenesis, but also to compare the order of these chromosomal markers with those of other members of the *Enterobacteriaceae*.

Phage Mu has been used to greater or lesser extents in several members of the *Enterobacteriaceae* including *Erwinia* species (18, 22, 24, 29, 36, 41). With this phage, conjugational gene transfer has been established in *E. carotovora* subsp. *carotovora* SCRI193 by using plasmid R68::Mu c^+ to mobilize the chromosome. It was observed that this plasmid mobilized markers randomly at a frequency of ca. 10^{-5} chromosomal recombinants per donor. However, the presence of a Mu prophage on the chromosome of the donor (24) increased the frequency of mobilization of markers adjacent to the prophage by up to 10-fold. Using this system, it was possible to order 17 chromosomal mutations. Further insertions of Mu into the chromosome of SCRI193 could not be isolated.

MATERIALS AND METHODS

Bacterial strains, phages and plasmids. The bacterial strains, phages, and plasmids used in this work are listed in Table 1.

Media. LB broth (Difco Laboratories, Detroit, Mich.) (21) was supplemented with 100 μg of thymine per ml. Minimal medium was M9 (21). Medium A was that of Miller (21). LBCM was LB with 1 mM calcium chloride and 2.5 mM magnesium sulfate. Top agar was LBCM containing 0.7 g of Difco Bacto-Agar per liter. For other solid media, 14 g of agar per liter was added to the liquid medium. Media were supplemented with carbon sources at 2 mg/ml, amino acids at 50 μg/ml, 80 μg of thymine per ml, 20 μg of ampicillin per ml, 20 μg of kanamycin per ml, 50 μg of nalidixic acid per ml (from stock solutions of 5 mg of nalidixic acid per ml of 30 mM sodium hydroxide), 200 μg of streptomycin per ml, 20 μg of tetracycline per ml, or 50 μg of trimethoprim per ml, each as required.

Isolation of mutants. Spontaneous antibiotic-resistant mutants were selected on LB agar containing the appropriate antibiotic. Chlorate-resistant mutants were isolated by the method of Miller (21).

Auxotrophic mutations and mutations in carbohydrate metabolism were selected from ethyl methanesulfonic acid-treated cultures (21). After treatment, the bacteria were washed in LB broth and cultured overnight in duplicate 10-ml volumes of LB broth. A total of 1% of the bacteria in

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TABLE 1. Bacterial strains, phages, and plasmids

Bacterial strains, phages, and plasmids	Relevant genotype	Source or reference
Erwinia carotovora subsp. *carotovora*[a]		
SCRI193	Wild-type strain isolated from potatoes	Kelman (SR44)[b]
KF1006	*rif-1*	
KF1016	*rif-1 thyA1*::Mu c^+	24
KF1017	KF1016 *deo-1* (R68::Mu c^+)	
KF1060	*hisD1 leu-2 nal-1 str-1 thr-1*	
KF1061	*hisD1 nal-1 pheA1 ser-1 str-1*	
KF1068	*gal-1 hisD1 nal-1 proA1 str-1 trp-2*	
KF1069	*crp-1 hisD1 nal-1 proA1 str-1 trp-2*	
KF1072	*aroB1 glyA1 hisD1 manA1 nal-1 str-1*	
KF1075	KF1072(R68::Mu c^+)	
KF1078	KF1006 *chl-1*	
KF1082	KF1061(R68::Mu c^+)	
KF1083	KF1078(R68::Mu cts)	
KF1089	KF1072 *nal-2*	
KF1091	KF1078(R68::Mu c^+)	
KF1092	KF1072 *thyA*::Mu c^+ (R68::Mu c^+)	
Escherichia coli		
ED8812	*hsdS lacZ leu rpsL thi thr*	Murray[c]
GMI3230	*hsdS leu rpsL thi thr* Mu^r	LBM[d]
KF63	ED8812 (Mu c^+)	
Phages		
Mu cts	Mu cts62	LBM
Mu c^+	Mu c^+Δ445-7	LBM
PRR1		LBM
Plasmids		
R68::Mu cts	R68::Mu cts62	LBM
R68::Mu c^+	R68::Mu c^+ΔD445-7	LBM

[a] *E. carotovora* subsp. *carotovora* SCRI193 was used throughout this study. Mutants of this strain were isolated as the result of spontaneous mutation (*chl-1*, *deo-1*, *nal-1*, *nal-2*, *rif-1*, *str-1*), the insertion of a Mu prophage (*thyA1*), or after mutagenesis with ethyl methanesulfonate (all other mutations).

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these cultures carried auxotrophic mutations. Mutants were isolated from these suspensions by replica plating with appropriate media, either directly from the suspensions and later determining the requirement of the isolated mutant (17) or by ampicillin enrichment for particular mutants (21; but with 100 μg of ampicillin per ml) before replica plating. Those mutants which had nonleaky phenotypes and low frequencies of reversion ($<10^{-7}$ revertants per bacterium) were retained and further characterized by syntrophy and by growth on appropriate media (8). Nomenclature of mutations is taken after the equivalent mutation in *E. coli*.

Mating conditions. The donor and recipient strains were cultured without antibiotic selection in LB broth at 30°C (SCRI193) or 37°C (*E. coli*). Unless otherwise stated, matings were performed as follows. Appropriate volumes of each parental culture were added to a sufficient volume of LB broth to give 10^9 bacteria of each parental strain in a final volume of 20 ml. Volumes (4 ml) from this suspension were filtered down onto membrane filters (25-mm Oxoid Nuflow cellulose-acetate membrane filters, 0.45-μm pore size) and incubated on LB agar at 30°C. After 18 h, matings were terminated by vigorously vortexing the membranes for 1 min in 1 to 3 ml of 0.8% sodium chloride. The concentrations of parental strains, transconjugants, and recombinants at the start of the mating were determined from a replicate filter. Initial and final bacterial concentrations were determined on minimal medium with the appropriate supplements (using tetracycline resistance to select for the R68 plasmids and derivatives) and scored after 2 to 4 days of incubation at 28°C. Unless otherwise stated, all inheritance frequencies are per donor at the start of the mating.

The coinheritance of markers was determined by patching recombinants onto master plates of the original selection medium, incubating them for 2 days, replicating them onto suitable selection plates, and scoring them after 1 to 2 days of incubation at 28°C. Preliminary tests showed that purification of the recombinant clones before patching to remove parental bacteria was not necessary. The coinheritance of Mu was ascertained by replicating the master plate onto LBCM, overlaying with a lawn of Mu-sensitive *E. coli* ED8812, and, after overnight incubation at 37°C, scoring for recombinants which had caused lysis of the lawn.

Donor-specific phage susceptibility. The donor-specific RNA phage PRR1 (23) was used to test for IncP pili by spotting 5 μl of a chloroform-sterilized lysate of PRR1 onto a lawn of the strain in top agar and scoring after overnight incubation at 30°C.

Phage Mu. The presence of Mu in a lysate or bacterial culture was tested by using Mu-sensitive *E. coli* ED8812, Mu-immune *E. coli* KF63, and Mu-resistant *E. coli* GMI3230. The concentration of free phage in cultures was determined after chloroform sterilization by titration on LBCM plates with a lawn of Mu-sensitive *E. coli* ED8812 (21); plates were scored after overnight incubation at 37°C.

Temperature induction of lysogens carrying a temperature-sensitive Mu prophage was by the method of Murooka et al. (22). Fresh, vigorously aerated liquid cultures were induced at 43°C for 30 min, followed by incubation at 37°C with continued aeration.

Mu-induced mutations and insertions were selected as follows. (i) Trimethoprim-resistant mutants (ThyA$^-$) (selected on LB-trimethoprim agar) and xylose nonutilizing mutants (selected after cycloserine enrichment [21]) were selected after the zygotic induction of the phage on R68::Mu c^+ in KF1006, subsequent to its transfer from KF1075, during overnight mating at 30°C. (ii) Mu insertions into the chromosome of chromosomal recombinants from crosses between KF1017 donors and nonlysogenic recipients (KF1060, KF1061, KF1068, KF1072) were sought by scoring for the coinheritance of Mu (and the absence of R68::Mu c^+).

RESULTS

Plasmid R68::Mu and phage Mu in SCRI193. R68 plasmids carrying a Mu prophage (R68::Mu c^+ or R68::Mu cts [10, 24]) were readily transferred from *E. coli* K-12 into SCRI193, typically at 10^{-5} transconjugants per donor. The plasmids in these transconjugants were not lost under nonselective growth conditions, were readily detectable by agarose gel electrophoresis by the method of Eckhardt (12), rendered the transconjugants sensitive to the donor-specific phage PRR1, conferred resistance to ampicillin, kanamycin, and tetracycline, produced phage Mu (tested on *E. coli*) both spontaneously (10^{-4} PFU/CFU) and upon temperature induction (of R68::Mu cts lysogens; 100-fold increase in free

TABLE 2. Frequencies of inheritance of markers in KF1060 mobilized from donors carrying R68::Mu plasmids and a chromosomal Mu insertion[a]

Donor	Inheritance frequency[b]			% Coinheritance frequency[c]	
	hisD1$^+$	*leu-2*$^+$	*thr-1*$^+$	*thr-1*$^+$ from *leu-2*$^+$	*leu-2*$^+$ from *thr-1*$^+$
KF1083	9×10^{-6}	8×10^{-6}	8×10^{-6}	16	19
KF1091	3×10^{-5}	2×10^{-5}	3×10^{-5}	12	23
KF1017	1×10^{-4}	2×10^{-5}	3×10^{-5}	23	27

[a] Mating and selection procedures were as described in Materials and Methods, but with streptomycin resistance counterselection against the donors.

[b] Using Student's *t* test, the inheritance frequencies of all the markers obtained with KF1083 and KF1091 donors and between *leu-2*$^+$ and *thr-1*$^+$ when mobilized from KF1017 were not significantly different. The inheritance frequencies of *hisD1*$^+$ when mobilized from KF1017 and KF1091 were significantly different.

[c] The coinheritance frequencies between *leu-2*$^+$ and *thr-1*$^+$ are lower with KF1083 and KF1091 donors compared with KF1017 donors as a consequence of their close linkage to *str-1*.

phage after a 2-h induction), and were transferable to nonlysogenic SCRI193 recipients at 4×10^{-2} transconjugants per donor.

All SCRI193 strains carrying Mu prophage produced phage spontaneously, that is, both plasmid-borne (as noted above) and chromosomally located (*thyA1*::Mu) prophage; spontaneous induction frequencies of 10^{-4} PFU/CFU were observed for all such lysogens. Zygotic induction of both the c^+ and *c*ts prophage was observed; inheritance of the R68::Mu c^+ plasmid was 32-fold less frequent in matings with nonlysogenic recipients (KF1006; 2×10^{-3} transconjugants per donor) as compared with lysogenic recipients (KF1016; 6×10^{-2} transconjugants per donor) (KF1075 donors; 4-h membrane matings at 28°C).

***thyA1*::Mu mutation.** The *thyA1*::Mu mutation was isolated (24) as a mutation conferring trimethoprim resistance in a recipient bacterium after the transfer, zygotic induction, and transposition of the prophage on R68::Mu c^+ into this recipient. The coincidence of trimethoprim resistance (ThyA$^-$) and Mu lysogeny in this mutant strongly implies that these were dependent, not independent, events. Like all Mu insertional mutations (35) and unlike many spontaneous mutations, *thyA1* did not revert ($<8 \times 10^{-11}$ revertants per bacterium); *thyA2*, a spontaneous mutation, reverted at 2×10^{-8} prototrophic revertants per bacterium. Mobilization of the trimethoprim resistance phenotype from KF1017 to KF1072 (5×10^{-8} trimethoprim-resistant recombinants per donor; streptomycin resistance used to counterselect against donors) was, in the case of 25% of the resultant trimethoprim-resistant recombinants, also associated with Mu lysogeny (10^{-8} *thyA1*::Mu recombinants per donor) (there was no coinheritance of the plasmid). As the recipient strain in this mating spontaneously mutated to trimethoprim resistance at a comparable frequency, the nonlysogenic, trimethoprim-resistant recipients probably arose as a result of mutation rather than inheritance. That these trimethoprim-resistant Mu lysogens arose by the transposition of Mu into *thyA*$^+$ after the zygotic induction of a prophage on a mobilized plasmid is unlikely in view of the difficulty in isolating such mutants in a subsequent experiment (see below).

The presence of a second Mu prophage in the chromosome of *thyA1*::Mu strains was tested (see below) by screening chromosomal recombinants isolated from matings between donors carrying this mutation (i.e., KF1017) and nonlysogenic recipients. No recombinants were found which were lysogenic for Mu, implying that over all of the mobilized chromosome there was no second prophage. Had such a prophage existed then the frequencies of inheritance (see Fig. 2) of markers adjacent to this prophage would have been increased in the same fashion as those adjacent to *thyA1*::Mu were.

Chromosomal mobilization. Both R68::Mu *c*ts and R68::Mu c^+ mobilized various chromosomal markers in matings between donors carrying one of these plasmids (KF1083 and KF1091, respectively) and the multiply mutant KF1060 (Table 2). Both plasmids mobilized all of the markers at similar frequencies of inheritance. The coinheritance of closely linked markers (Table 2) (e.g., between *leu-2* and *thr-1*) but not of more distantly linked markers (e.g., between *hisD1* and *leu-2* or *thr-1*) was observed. Chromosomal mobilization by plasmid R68 alone, under similar mating conditions, occurred at a very low frequency ($<5 \times 10^{-8}$ recombinants per donor) and was often outside the experimental limits of detection.

By comparison, while the insertion of a Mu prophage into the chromosome of the donor strain (*thyA1*::Mu [24]) did not alter the frequency of inheritance (Table 2) of *leu-2*$^+$ or *thr-1*$^+$ when mobilized by R68::Mu c^+, the frequency of inheritance of *hisD1*$^+$ increased threefold. Coinheritance of unselected markers mobilized from this donor was observed between the closely linked *leu-2* and *thr-1* (Table 2) but not between more distantly linked markers (*hisD1* and *leu-2* or *thr-1*).

Chromosomal mapping. The frequencies of inheritance and coinheritance of markers on the chromosome of SCRI193 when mobilized from donors carrying *thyA1*::Mu(R68::Mu c^+) were determined in matings between KF1017 donors and mutant recipients (KF1060, KF1061, KF1068, KF1069, KF1072, KF1082); counterselection against the donor was by the omission of thymine from the minimal medium selection plates.

The order of the chromosomal markers in each of the crosses was ascertained by first determining the distance (percent coinheritance) between each unselected marker and the selected marker and then by determining whether unselected markers were on the same or opposing sides of the selected marker (by comparing the percent coinheritance frequencies from two-marker and three-marker selections). When possible, analyses with four markers were made as a further check on the order of the markers in that cross. These linkage groups were combined to give a single linkage map (Fig. 1). The frequencies of coinheritance of the pairs of markers tested are shown in Fig. 1. *trp-2* and *manA1* could not be ordered with respect to each other by using the above strains by virtue of their close linkage and occurrence in different recipient strains, thereby preventing recombinant analysis between them. Accordingly, *thyA1*::Mu and then R68::Mu c^+ were conjugated into the *manA1* KF1072, and this new donor (KF1092) used to mobilize the chromosome into the *trp-2* KF1068. Three-point cross analysis of *trp-2*$^+$ recombinants selected from this cross yielded the marker order given in Fig. 1.

The frequencies of inheritance of selected markers are shown in Fig. 2. Two of the markers (*gal-1*$^+$ and *manA1*$^+$) were inherited at frequencies lower than that which would be expected from their position on the linkage map; indeed, no *gal-1*$^+$ recombinants were isolated when Gal-1$^+$ was directly selected. This could be the result of delayed expression of these recombinant phenotypes with a consequent reduction

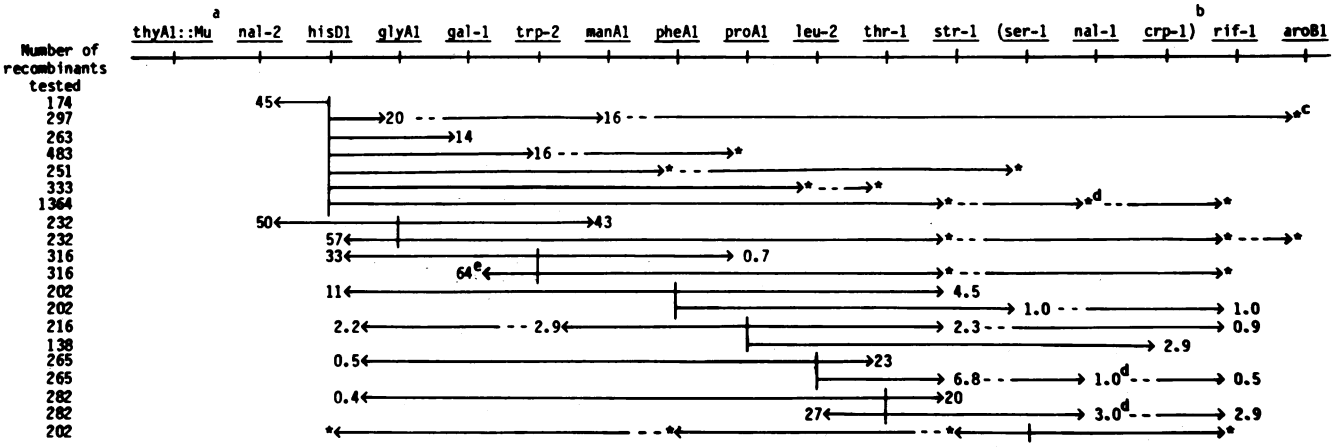

FIG. 1. Percent coinheritance frequencies of markers mobilized from KF1017 donors. Mating and selection procedures were as described in Materials and Methods. Percent coinheritance frequencies between selected markers are indicated by the vertical lines, and coinherited unselected markers are indicated by arrowheads. (a) Origin of polar chromosomal transfer. (b) Probable position of the markers in parentheses. (c) No coinheritance of these asterisked markers detected. Recombinants from three or four experiments were tested, and the total number of these is shown on the left. (d) 100 recombinants from a single experiment. (e) 111 recombinants from two experiments. *gal-1* and *trp-2* are so ordered on the basis of three-point cross analysis of *hisD1*$^+$ and *gal-1*$^+$ coinheritance in *trp-2*$^+$ selected recombinants: 33% coinheritance.

in the viability of the recombinants on the selection plates; this might be due to the accumulation of phosphorylated intermediates (39), as has been observed to be the case with L-sorbose utilization mutants in *E. coli* (38), or possibly through the involvement of these genes in the synthesis of bacterial membrane components. Gal-1$^-$ was found to confer bacteriostatic galactose sensitivity and so could be classified as a mutation in either *galT* or *galU* (34, 39). An approximation of the inheritance frequencies of *gal-1*$^+$ and *manA1*$^+$ can be calculated from the product of the inheritance frequency of *hisD1*$^+$ and the coinheritance of *gal-1*$^+$ or *manA1*$^+$ from *hisD1*$^+$. These estimated frequencies are close

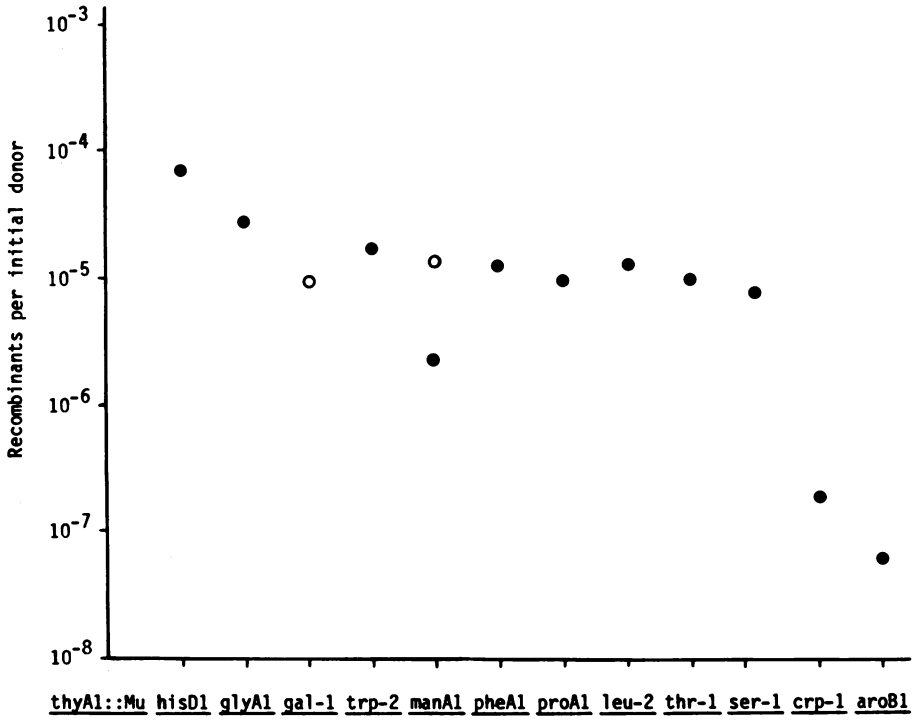

FIG. 2. Frequency of inheritance of chromosomal markers mobilized from KF1017 donors into multiply auxotrophic recipients. Mating and selection procedures were as described in Materials and Methods. Symbols: ●, Frequency of inheritance per donor; ○, frequency of inheritance per donor calculated from the product of the coinheritance frequency of the marker from *hisD1*$^+$ and the frequency of inheritance of *hisD1*$^+$ (discussed in text). Inheritance of *gal-1*$^+$ by direct selection was not observed.

to those which would be predicted from the location of these markers on the linkage map (Fig. 2). As a proportion of the *gal-1*$^+$ or *manA1*$^+$ recombinants would not have inherited *hisD1*$^+$, these frequencies will be underestimates, and this is seen to be the case (Fig. 2).

Selection of Mu insertions on the chromosome. Two methods were used to select chromosomal Mu insertions. (i) KF1075, which carries R68::Mu c^+, was mated to a nonlysogenic recipient (KF1006) to isolate Mu insertional mutations after the zygotic induction of the plasmid-borne prophage on the transfer of the plasmid into the recipient. Trimethoprim-resistant recipients from this mating were recovered at the same frequency as spontaneous mutations of KF1006 itself to trimethoprim resistance. None of the trimethoprim-resistant mutants tested carried Mu ($<5 \times 10^{-3}$ Mu lysogens per trimethoprim-resistant recipient). From a parallel experiment, no xylose nonutilizing mutants were recovered after cycloserine enrichment of the mated culture ($<1 \times 10^{-3}$ Xyl$^-$ mutants per enrichment survivor). (ii) Chromosomal recombinants from crosses between KF1017 donors and nonlysogenic recipients were screened for the presence of Mu since these recombinants provided a population of recipient bacteria which had received Mu and survived the possible zygotic induction of the phage. Recombinants might therefore be expected to carry Mu insertions more frequently than the recipient population in general. Of the 2,716 plasmid-free recombinants screened, none were lysogenic for Mu ($<4 \times 10^{-4}$ lysogens per recombinant).

DISCUSSION

Although SCRI193 has an efficient DNA restriction-modification system (Forbes, Ph.D. thesis), the broad-host-range IncP plasmids used in this study were readily transferred into this strain by conjugation, where they had the expected phenotypes. Lysogens of Mu in SCRI193 all produced phage spontaneously. The prophage on R68::Mu c^+ was induced upon transfer into a nonlysogenic SCRI193 recipient, while the prophage on R68::Mu *c*ts was temperature inducible. Thus, in SCRI193, the Mu genes controlling lysogeny (the *c* gene) and the lytic cycle are expressed. Although a Mu insertional mutation has been isolated in SCRI193 (24), it did not prove possible to isolate further Mu insertional mutations in this strain resulting from the transposition of a plasmid-borne phage, after zygotic induction. In donors carrying R68::Mu, the transposition of Mu, after the spontaneous induction of the phage, integrated the plasmid into the chromosome and thence allowed the mobilization of chromosomal DNA (see below); presumably the completion of the lytic phase in these induced bacteria would result in their eventual lysis and death. It seems, therefore, that although SCRI193 will allow the transposition of Mu in the cases of replicon fusion and in the lytic cycle, it will not readily allow the transposition of single copies of Mu to new sites on the chromosome of the host. Improved selection for Mu insertions into the chromosome of *E. carotovora* subsp. *carotovora* Brig-P1A has recently been reported (18), using Mu phage which carry ampicillin resistance, selection for Mu insertions here relying simply on selection for the antibiotic resistance.

A linear linkage map comprising 17 markers was constructed by using the frequencies of coinheritance of unselected markers (Fig. 1). The pattern of the frequencies of inheritance of these markers (Fig. 2) suggests that there is both polar and nonpolar mobilization of the chromosome from the donor. *hisD1*$^+$, *glyA1*$^+$, *gal-1*$^+$, *trp-2*$^+$, and *manA1*$^+$ were inherited at frequencies decreasing from a maximum of 9×10^{-5} recombinants per donor (*hisD1*$^+$) to 1×10^{-5} recombinants per donor, suggesting that this region of the chromosome was mobilized in a polar manner from a single origin. As this polarity was dependent on the presence of the chromosomal Mu prophage (*thyA1*::Mu) in the donor, the polar chromosomal mobilization presumably occurs by the integration of R68::Mu into the donor chromosome at *thyA1*::Mu by homologous recombination between the two prophage. The mobilization of the *E. coli* chromosome by the same mechanism has been previously reported (10, 40).

pheA1$^+$, *proA1*$^+$, *leu-2*$^+$, and *thr-1*$^+$ were all inherited at frequencies of 10^{-5} recombinants per donor, implying mobilization by a nonpolar, multiorigin mechanism. This mobilization required only the presence of R68::Mu in the donors and presumably resulted from the transposition of Mu on the plasmid onto the chromosome, this replicon fusion forming an Hfr-type donor and presumably R-primes. The nonpolar mobilization of the chromosomes of several species has been reported (29, 37).

Different mobilization mechanisms for the markers in these two groups is further suggested by the frequencies of coinheritance of unselected markers. When pairs of markers mobilized by the polar mechanism are compared for the coinheritance of unselected markers from the selected marker, the frequencies are found to differ greatly: the coinheritance frequency of the earlier marker being greater than the coinheritance frequency of the later marker (Fig. 1). This polarity of coinheritance frequencies between pairs of markers is not observed for markers mobilized by the nonpolar mechanism.

ser-1$^+$, *crp-1*$^+$, and *aroB1*$^+$, although inherited, show little linkage with other markers, generally <1%. *crp-1*$^+$ and *aroB1*$^+$ are inherited at frequencies below that expected for the nonpolar mechanism of transfer, possibly as a consequence of the close linkage of these markers to *thyA1*::Mu, the mutation used for counterselection. *ser-1*$^+$ is inherited at a frequency typical of the nonpolar mechanism, its low linkage to other markers presumably reflecting its location in a poorly marked region of the chromosome. *thyA1*::Mu was observed to be inherited at 10^{-8} recombinants per donor (in the cross between KF1017 and KF1072, noted above, in which mobilization of trimethoprim resistance was selected), a frequency much lower than would be expected for a marker mobilized by the nonpolar mechanism (typically ca. 10^{-5} recombinants per donor). This is presumably a consequence of the transposition of the Mu in *thyA1* in these induced donors which would disrupt the contiguity of the chromosomal sequences flanking the mutation, thereby preventing the recombination of an intact *thyA1* mutation into the recipient chromosome by homologous recombination. The transposition of Mu in *thyA1*::Mu can be implied from the known transposition of the prophage on the plasmid (for the nonpolar mobilization of the chromosome from these donors) and the replication of all prophage in induced polylysogens (13).

Comparison of the chromosomal linkage map of *E. carotovora* subsp. *carotovora* SCRI193 with those of other members of the genus *Erwinia* suggests that there are both conserved and dispersed gene sequences. The maps of the closely related *E. chrysanthemi* that are available (5, 6, 19, 29), between which there are slight differences of gene order, show similarities to that of SCRI193, as does that of the more distantly related *E. amylovora* (26). However, as many of the mutations in these species are not classified beyond gross phenotype, comparison of the orders of the markers in these species must be made with caution. Comparison with the

extensively mapped *E. coli* K-12 (1) and with *Salmonella typhimurium* LT2 (28) shows some notable differences. The order and orientation of the *manA1-trp-2-gal-1* sequence found in SCRI193 is also found in *S. typhimurium* LT2; this region of the *S. typhimurium* LT2 chromosome carries an inversion with respect to the *E. coli* K-12 chromosome (28). Like the *ile-arg* inversion in *E. chrysanthemi* 3937j (19) and the *ilv-rbs* inversion in *E. amylovora* EA178 (26), SCRI193 also has gene orders different from those of *E. coli* K-12 in this region of the chromosome. From their map positions in SCRI193, *nal-1* and *nal-2* are probably homologous with *gyrB* and *gyrA* of *E. coli* K-12. In *E. coli* K-12 *pheA* and *glyA* map adjacent to and on either side of *thyA*; however, in SCRI193, *pheA1* mapped adjacent to *proA1*, and *glyA1* mapped between *hisD1* and *gal-1*.

As more extensive linkage maps of members of the genus *Erwinia* become available it will be of interest to compare the extent of conservation of the gene orders of these species with each other and with those of other species of the *Enterobacteriaceae*, particularly in view of the fluidity of chromosomal gene orders which is now becoming apparent (15, 27, 33, 38). The chromosomal linkage map and the associated chromosomal mutations and gene transfer systems in conjunction with the isolation of nonsense suppressor mutants (16) and pathogenic mutants isolated by chemical (Pérombelon, unpublished data) and transposon (Tn*5*; J. C. D. Hinton, personal communication) mutagenesis in this strain make it eminently suitable for the genetic study of *E. carotovora* subsp. *carotovora* pathogenesis.

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