Tn5-Induced Mutations in the Enterobacterial Phytopathogen *Erwinia chrysanthemi*†

ARUN K. CHATTERJEE,* KERRY K. THURN, AND DOROTHY A. FEESE

Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506

Escherichia coli (2492/pJB4JI) matings with *Erwinia chrysanthemi* produced kanamycin resistant (Km^r) transconjugants, a majority of which were gentamicin sensitive (Gm^s). A small proportion (about 0.8%) of the Km^r Gm^s clones were either auxotrophic or failed to catabolize galacturonate (Gtu$^-$). The R plasmid (pJB4JI) DNA was detected in the parent *E. coli* strain and in a Km^r Gm^r transconjugant, but not in Km^r Gm^s *E. chrysanthemi* strains carrying Tn5-induced mutations. In Hfr crosses, Km^r (Tn5) was found linked with most mutations. A majority (>95%) of prototrophic recombinants were Km^s, except for Leu$^+$ and Arg$^+$ recombinants which were 30 to 50% Km^s. Spontaneous revertants were obtained for all markers except *car, gtu, lys, thr*, and *trp*. Prototrophic revertants, with the exception of Met$^+$, Leu$^+$, or His$^+$ clones, were Km^s. We conclude from both genetic and physical data that Tn5 transposed from pJB4JI into different sites on the chromosome of *E. chrysanthemi*.

Transposable elements that encode drug resistance are powerful tools in microbial genetics. Insertion of such transposable elements into genomes can cause mutations in which the mutant allele is linked to the selectable drug resistance marker. Additionally, the use of drug-resistant transposable elements allows gene isolation and construction of deletion mutations and Hfr strains (23, 34). All or some of these possibilities have been realized in the enterobacteria *Escherichia coli, Klebsiella pneumoniae*, and *Salmonella typhimurium* (1, 2, 12, 15, 23, 24, 29, 33) and in other bacteria such as *Caulobacter crescentus* (18), *Pseudomonas aeruginosa* (26, 27), *Rhizobium leguminosarum* (3, 5), *R. meliloti* (17, 28), *Vibrio cholerae* (21), and a *Vibrio* sp. (36).

The use of drug-resistant transposable elements is particularly attractive in studies of pathogenic bacteria. Avirulence resulting from conventional chemical or radiation mutagenesis is a nonselectable phenotype. Aside from the problem of multiple mutations, genetic analysis of radiation- or chemical-induced mutations in pathogenicity genes is impossible unless the mutation is linked to a marker that has a selectable phenotype. In contrast, genetic analysis would be feasible in mutations affecting virulence which result from the insertion of a drug-resistant transposable element. In plant patho-

genic systems, transposable elements have been exploited to some extent in genetic investigations and in the elucidation of pathogenic mechanisms. These include the elucidation of some of the functions specified by plasmids and chromosomes of *Agrobacterium tumefaciens* and *A. rhizogenes* (19, 20, 22, 31, 37), the isolation of auxotrophic mutations in *Pseudomonas solanacearum* (4), the determination of the plasmid location of *iaaM*, a gene locus for tryptophan monooxygenase which is required in indoleacetic acid biosynthesis in *Pseudomonas syringae* pathovar *savastanoi* (13), and the detection of the Tra$^+$ phenotype in a native plasmid of *P. syringae* pathovar *tabaci* (32).

The chromosomal genetics of *Erwinia chrysanthemi* are the most thoroughly investigated among phytopathogenic bacteria (8, 10, 25, 35). However, drug-resistant transposable elements have not been exploited to further genetic knowledge. In this report we provide genetic and physical evidence for mutations in biosynthetic and catabolic genes caused by the translocation of Tn5 from plasmid pJB4JI to many sites on the chromosome of *E. chrysanthemi*.

(A preliminary account of some of this work has been reported [K. K. Thurn and A. K. Chatterjee, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, H79, p. 126].)

MATERIALS AND METHODS

Bacterial strains. The *E. chrysanthemi* strains and plasmid-bearing strains of *E. coli* used in this study are listed in Table 1. Tn5 insertion mutations were ob-

TABLE 1. Bacterial strains

Strain[a]	Characters	Source or reference
EC16	Wild-type, prototrophic, virulent, Pat$^+$, Hpg$^+$, Prt$^+$, Plc$^+$, Lac$^-$	(7)
AC4150	Same as EC16, also Nal^r	Selection for spontaneous resistance to nalidixic acid
AC4052	Hfr-8, prototrophic, Lac$^+$, Str^s	(9)
AC4074	thr-1 his-1 trp-1 Str^r Nal^r	(7)
AC4100	thr-1 his-1 trp-1 met-1::Tn5 (Km^r) Str^r Nal^r	Tn5 transposition in AC4074 (this paper)
AC4104	thr-1 his-1 trp-1 arg-1::Tn5 (Km^r) Str^r Nal^r	Tn5 transposition in AC4074 (this paper)
AC4106	thr-1 his-1 trp-1 gua-1::Tn5 (Km^r) Str^r Nal^r	Tn5 transposition in AC4074 (this paper)
AC4114	car-5::Tn5 (Km^r) Nal^r	Tn5 transposition in AC4150 (this paper)
AC4116	cys-4::Tn5 (Km^r) Nal^r	Tn5 transposition in AC4150 (this paper)
AC4117	his-2::Tn5 (Km^r) Nal^r	Tn5 transposition in AC4150 (this paper)
AC4122	gtu-4::Tn5 (Km^r) Nal^r	Tn5 transposition in AC4150 (this paper)
AC4126	lys-3::Tn5 (Km^r) Nal^r	Tn5 transposition in AC4150 (this paper)
AC4130	ura-4::Tn5 (Km^r) Nal^r	Tn5 transposition in AC4150 (this paper)
AC4131	ade-4::Tn5 (Km^r) Nal^r	Tn5 transposition in AC4150 (this paper)
AC4135	thy-2::Tn5 (Km^r) Nal^r	Tn5 transposition in AC4150 (this paper)
AC4148	trp-7::Tn5 (Km^r) Nal^r	Tn5 transposition in AC4150 (this paper)
AC4168	leu-6::Tn5 (Km^r) Nal^r	Tn5 transposition in AC4150 (this paper)
AC4169	thr-2::Tn5 (Km^r) Nal^r	Tn5 transposition in AC4150 (this paper)
AC4170	Same as AC4150, also Gm^r Km^r (pJB4JI)	Derived from a cross between AC8001 and AC4150 (this paper)
AC8001	arg his lac Str^r/Km^r Gm^r (pJB4JI=pPH1 ::Mu::Tn5)	Derived from cross between 1830/pJB4JI (3) and 2492 (7)

[a] AC8001 is a strain of E. coli; all others are strains of E. chrysanthemi.

tained as described below. R plasmid-bearing strains were kept at 4°C on L agar containing appropriate antibiotics; all other bacterial cultures were maintained at 4°C on yeast-dextrose-CaCO$_3$ agar slants (7). E. chrysanthemi strain AC4052 (Hfr8) (9) was grown immediately before use on minimal lactose agar from lyophilized cells.

Media. L, yeast-dextrose-CaCO$_3$, and minimal media were described previously (7). The nutritional requirements of putative auxotrophic mutants were determined by using the protocol of Davis et al. (16). The ability to ferment carbohydrates was tested on indicator media (BTB-carbohydrate agar) composed of proteose peptone no. 3, 10 g/liter; NaCl, 5 g/liter; beef extract, 1 g/liter; carbohydrate, 10 g/liter; bromthymol blue, 25 mg/liter; and agar, 15 g/liter. Extracellular enzymatic activities were scored on the following media: polygalacturonate-yeast extract agar (pH 7.5) (9) for polygalacturonic acid trans-eliminase (Pat); lecithin agar (11) for phospholipase C (Plc); and nutrient gelatin (Difco Laboratories, Detroit, Mich.) agar for protease (Prt). When required, media were supplemented with amino acids, purines or pyrimidines (50 μg/ml), or kanamycin (50 μg/ml), nalidixic acid (50 μg/ml), or gentamicin sulfate (10 μg/ml).

Mating conditions. Crosses were done on membranes or on L agar (7). The markers (auxotrophy or drug susceptibility) used in counterselection of donor and recipient cells and the procedures for the determination of the coinheritance of unselected markers are noted in the text and in footnotes to Table 2.

Transpositional mutagenesis. The transposon Tn5, which codes for kanamycin resistance, was introduced into E. chrysanthemi (strain EC16 and its derivatives) by using the R plasmid pJB4JI. This conjugative

plasmid, constructed and described by Beringer et al. (3), encodes gentamicin and kanamycin resistance and carries the Mu genome which has been inactivated by Tn5 insertion. Although pJB4JI has a broad host range, it is not maintained in many bacteria (3–5, 17–19, 28, 37). Thus, in crosses involving pJB4JI, the occurrence of Km^r Gm^s transconjugants indicates the transposition of Tn5 and the loss of the plasmid. In contrast, Km^r Gm^r transconjugants are due to the presence of the plasmid.

To obtain Tn5 transposition in E. chrysanthemi, crosses were performed with the donor E. coli strain (AC8001), harboring pJB4JI, and the E. chrysanthemi recipient strain (AC4150 or AC4074). Cells were grown in L broth for approximately 6 h at 30°C. Mating was initiated by spotting an appropriate volume (usually 0.1 ml) of cultures on L agar to yield a ratio of 1:1 of donor to recipient cells. L agar containing only the recipient or the donor cells served as control. After 18 to 24 h of incubation at 30°C, cells were removed and suspended in 55 mM potassium phosphate buffer, pH 7.2. A sample (usually 0.1 ml) of the cell suspension was spread on L agar plus kanamycin and nalidixic acid or BTB-carbohydrate agar plus kanamycin and nalidixic acid. Controls were treated similarly. Donor cells were counterselected by nalidixic acid, and recipient cells were counterselected by kanamycin. After 3 to 4 days of incubation at 30°C, Km^r transconjugants were patched on L agar plus kanamycin and nalidixic acid, L agar plus kanamycin, nalidixic acid, and gentamicin, polygalacturonate-yeast extract agar plus kanamycin and nalidixic acid, nutrient gelatin agar plus kanamycin and nalidixic acid, lecithin agar plus kanamycin and nalidixic acid, and minimal agar plus kanamycin and nalidixic acid. After 12 to 16 h of incubation

TABLE 2. Frequency of prototrophic recombinants in crosses between donor strain E. chrysanthemi Hfr8 (AC4052) and Tn5-induced auxotrophs, linkage between auxotrophy and kanamycin resistance, and reversion of Tn5 insertion mutations[a]

Recipient strain (relevant characters)[b]	Phenotype studied	Frequency of transfer (no. of recombinants/input donor cell)	Coinheritance of Km^s in prototrophic recombinants		Spontaneous reversion	
			No. tested	% Km^s	Frequency[c]	Proportion Km^s (no. tested/ no. Km^s)
AC4100 (met-1::Tn5 Nal^r)	Met^+	1×10^{-5}	180	0	1×10^{-9}	5/0
AC4104 (arg-1::Tn5 Nal^r)	Arg^+	2×10^{-6}	15	53	3×10^{-10}	7/7
AC4106 (gua-1::Tn5 Nal^r)	Gua^+	2×10^{-6}	90	99	3×10^{-9}	29/29
AC4114 (car-5::Tn5 Nal^r)	Car^+	5×10^{-6}	90	99	$<3 \times 10^{-10}$	
AC4116 (cys-4::Tn5 Nal^r)	Cys^+	7×10^{-8}	84	99	6×10^{-8}	90/90
AC4117 (his-2::Tn5 Nal^r)	His^+	2×10^{-6}	87	100	2×10^{-10}	1/0
AC4122 (gtu-4::Tn5 Nal^r)	Gtu^+	8×10^{-9}	8	100	$<3 \times 10^{-10}$	
AC4126 (lys-3::Tn5 Nal^r)	Lys^+	5×10^{-6}	90	98	$<3 \times 10^{-10}$	
AC4130 (ura-4::Tn5 Nal^r)	Ura^+	1×10^{-6}	12	100	2×10^{-10}	1/1
AC4131 (ade-4::Tn5 Nal^r)	Ade^+	2×10^{-6}	102	100	6×10^{-9}	43/41
AC4135 (thy-2::Tn5 Nal^r)	Thy^+	2×10^{-8}	14	100	9×10^{-7}	19/19
AC4148 (trp-7::Tn5 Nal^r)	Trp^+	6×10^{-7}	133	99	$<3 \times 10^{-10}$	
AC4168 (leu-6::Tn5 Nal^r)	Leu^+	7×10^{-6}	93	35	1×10^{-9}	7/0
AC4169 (thr-2::Tn5 Nal^r)	Thr^+	8×10^{-6}	90	100	$<3 \times 10^{-10}$	

[a] Crosses were done on membranes for 6 h at 30°C. For additional details, see Chatterjee (7). Donor cells were counterselected by nalidixic acid (50 µg/ml). Auxotrophic requirement or the inability (of AC4122) to utilize D-galacturonate as a carbon source was used in counterselecting recipient cells. Ade^+, Car^+, Cys^+, His^+, Leu^+, Lys^+, Thr^+, Thy^+, Trp^+, and Ura^+ recombinants were selected on minimal glucose agar plus nalidixic acid. Arg^+, Gua^+, and Met^+ recombinants were selected on minimal glucose agar plus histidine, threonine, tryptophan, and nalidixic acid. Gtu^+ recombinants were selected in minimal galacturonate agar plus nalidixic acid. Prototrophic or Gtu^+ recombinants were patched on selective agar media in the presence or absence of kanamycin (50 µg/ml). Growth was scored visually after overnight incubation at 30°C. The frequency of spontaneous reversion was determined by growing about 10^6 cells of test bacteria to saturation (ca. 5×10^9 cells per ml) in L broth at 30°C, collecting the cells by centrifugation, washing the cells in 55 mM phosphate buffer, pH 7.0, suspending the cells in approximately one-fifteenth of the original volume in phosphate buffer, and spreading a sample of the suspension on selective media without nalidixic acid. To determine the number of viable cells, the suspension was serially diluted in phosphate buffer, and samples (0.1 ml) of appropriate dilutions were plated on L agar. The frequency of reversion is expressed as the number of revertants per input viable cell of the test strain. Kanamycin susceptibility in revertants was determined by patching prototrophic or Gtu^+ clones on media (see above) in the presence or absence of kanamycin (50 µg/ml).

[b] See Table 1 and text for the details of construction and properties of the strains.

[c] A value of $<3 \times 10^{-10}$ means that no colonies appeared on the selection plates. This value represents the lower limit for the detection of prototrophic revertants under the experimental conditions used.

at 30°C, the colonies were scored for Plc (11) and Pat activity (9). Prt activity was scored by flooding nutrient gelatin agar plates with acidic mercuric chloride (14) after 48 h of incubation. Growth of colonies on drug-supplemented L or minimal agar was assessed by visual examination after 24 h of incubation. Clones that were Km^r Gm^s and either auxotrophic, Pat^-, Plc^-, Prt^-, or altered in any other recognizable phenotype were purified by single-colony isolation on L agar plus kanamycin and nalidixic acid. Several single clones of each putative mutant were tested for Km^r, Gm^s, and the mutant phenotype. One mutant clone per phenotype was retained for further studies.

Plasmid isolation. Plasmid DNA was extracted by a slightly modified procedure described by Casse et al. (6). Bacteria were grown overnight (ca. 16 h) in L broth (5 ml) at 30°C and harvested by low-speed centrifugation at room temperature. Cells were suspended in 150 µl of TES (0.05 M Tris-hydrochloride, 0.005 M EDTA, 0.05 M NaCl, pH 8.0), and 2.9 ml of lysing buffer (1% sodium dodecyl sulfate in TES

buffer, pH 12.45) was added and gently mixed. After incubating for 30 min at 34°C, 180 µl of 2 M Tris-hydrochloride, pH 7.0, was added. The sample was mixed by gentle inversions of the tube for 3 min. Sodium chloride (final concentration, 3%) was added, and the contents were gently mixed. Three milliliters of phenol saturated with 3% NaCl was then added, the contents were mixed by repeated inversions of the tube for 3 min, and the resulting suspension was centrifuged for 10 min (10,000 × g) at 4°C. The aqueous phase was collected, and 0.1 volume of 3 M sodium acetate was added. After gentle mixing, 2 volumes of cold ethanol (−20°C) was added, and the sample was stored overnight at −20°C. The precipitated DNA was recovered by centrifugation (15,000 × g, 0°C) for 10 min, and the supernatant was discarded. The residual ethanol was allowed to evaporate and the DNA was suspended in 100 µl of TES buffer. DNA samples were analyzed by agarose gel electrophoresis either immediately or after storage at 4°C.

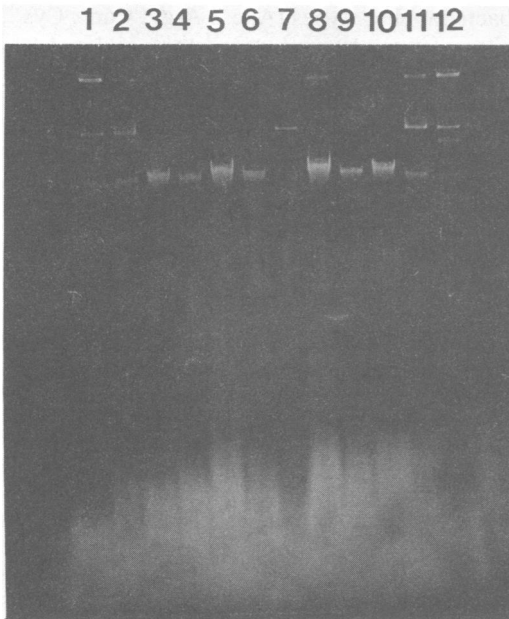**Agarose gel electrophoresis.** Ethanol-precipitated

FIG. 1. Agarose gel electrophoresis of ethanol-precipitated DNA preparations of *E. coli* strain AC8001 (pJB4JI) and *E. chrysanthemi* recipients and transconjugants. Lanes 2 and 11, *E. coli* strain AC8001 (pJB4JI); lane 3, the parent *E. chrysanthemi* strain, AC4150; lanes 4, 5, 6, 8, 9, and 10, Km^r Gm^s transconjugants AC4114, AC4116, AC4122, AC4126, AC4131, and AC4169, respectively; lane 7, a Km^r Gm^r *E. chrysanthemi* transconjugant, AC4170. Lanes 1 and 12 contain molecular size markers (38, 60, and 120 megadaltons). The plasmid isolation procedure and conditions of agarose gel electrophoresis are described in the text.

DNA preparations from cell lysates were examined by electrophoresis in 0.7% agarose (Sigma Chemical Co., St. Louis, Mo.) dissolved in a Tris-borate buffer (89 mM Tris base, 2.5 mM disodium EDTA, 8.9 mM boric acid), as described by Meyers et al. (30). A drop of dye solution consisting of 20% (wt/vol) glycerol, 5 M urea, 0.25 M disodium EDTA, 10% (wt/vol) sodium dodecyl sulfate, and 0.025% (wt/vol) bromophenol blue was added to each DNA sample just before electrophoresis. Horizontal gel electrophoresis was done at 150 V for 2 to 3 h. The gel was stained in a solution of ethidium bromide (0.2 g/ml) for a minimum of 30 min before photographing.

RESULTS

In crosses with an *E. coli* (AC8001) donor, kanamycin-resistant transconjugants of *E. chrysanthemi* were obtained at a frequency of about 7×10^{-6} per recipient cell; conversely, Km^r Gm^r clones were detected at a lower frequency ($\leq 2 \times 10^{-8}$ per recipient cell). A proportion (1 to 2%) of the Km^r Gm^s transconjugants were either auxotrophic or altered in another phenotype.

From several independent crosses a number of Pat$^-$, Plc$^-$, Prt$^-$, Gtu$^-$ (i.e., failed to utilize galacturonate as a carbon source), and auxotrophic mutants were obtained. These auxotrophs were characterized as Ade$^-$, Arg$^-$, Car$^-$, Cys$^-$, Gua$^-$, His$^-$, Leu$^-$, Lys$^-$, Met$^-$, Thr$^-$, Thy$^-$, Trp$^-$, or Ura$^-$. The nutritional requirements of a small proportion of auxotrophic mutants could not be determined.

We then examined cell lysates from representatives of transconjugants that were Km^r Gm^s and concomitantly auxotrophic, Gtu$^-$, Pat$^-$, Plc$^-$, or Prt$^-$ for the presence of pJB4JI DNA. Figure 1 shows that plasmid DNA was present in the *E. coli* donor strain (AC8001) and a Km^r Gm^r clone of *E. chrysanthemi* but not in auxotrophic or Gtu$^-$ strains of *E. chrysanthemi* that were Km^r Gm^s. Plasmid DNA was also not detected in Km^r Gm^s strains that were Pat$^-$, Plc$^-$, or Prt$^-$ (detailed data not presented). This observation strongly suggested that the plasmid, pJB4JI, was unstable in *E. chrysanthemi* and that the Km^r transconjugants resulted from transposition of Tn5 from pJB4JI into the chromosome of *E. chrysanthemi*, producing either various classes of mutations (viz., auxotrophy, Pat$^-$, Prt$^-$, Plc$^-$, or Gtu$^-$) or causing no detectable phenotypic change (i.e., silent transposition).

To confirm this hypothesis, we determined the linkage between Km^r and the mutational phenotype. A majority of prototrophic recombinants derived from Hfr crosses also were Km^s (Table 2), indicating linkage between Tn5 and the mutational phenotype. We might note that although 98% of the prototrophic recombinants for *ade, car, cys, gtu, gua, his, lys, thr, thy, trp*, or *ura* were Km^s (Table 2), only about 53% of the Arg$^+$ recombinants and 35% of the Leu$^+$ recombinants were Km^s. An exception to that pattern was noted with the strain AC4100, which was isolated as Met$^-$ and concomitantly Km^r (Table 1). In this instance, none of the Met$^+$ recombinants tested was Km^s (Table 2).

Reversion to prototrophy was detected with Ade$^-$, Arg$^-$, Cys$^-$, Gua$^-$, His$^-$, Leu$^-$, Met$^-$, Thy$^-$, and Ura$^-$ mutants but not with Car$^-$, Gtu$^-$, Lys$^-$, Thr$^-$, or Trp$^-$ mutants; the frequency of reversion varied considerably (in some cases up to 1,000-fold) depending on the mutation (Table 2). Although a majority of the Ade$^+$ revertants and all of the Arg$^+$, Cys$^+$, Gua$^+$, Thy$^+$, or Ura$^+$ revertants tested were Km^s, the His$^+$, Leu$^+$, Met$^+$, and some of the Ade$^+$ revertants remained Km^r. The possible genetic mechanisms for the Km^r phenotype of these revertants and some of the prototrophic recombinants (see above) are discussed below.

The data summarized in Table 2 show that the frequency of prototrophic recombinants in crosses between the Hfr donor and various

recipient strains varied depending on the marker. For example, Met$^+$ recombinants occurred at high frequency (ca. 10^{-5} per input donor cell) compared with Gtu$^+$ or Thy$^+$ recombinants (ca. 10^{-9} per input donor cell). In an earlier report (9), it was shown that this Hfr strain (Hfr8) transferred chromosomal markers in a polarized manner; *leu$^+$*, a proximal marker, was transferred at a high frequency and *gtu$^+$*, a terminal marker, was transferred at a low frequency. Thus, the gradient of marker transfer (Table 2) suggests that Tn5 insertion produced mutations in gene loci that were scattered over the entire chromosome of *E. chrysanthemi*.

DISCUSSION

A primary objective of this work was to determine whether the transposition of Tn5 from the plasmid, pJB4JI, into the genome of *E. chrysanthemi* occurred. We showed that the plasmid, transferred from an *E. coli* strain (AC8001), produced Km^r Gm^s transconjugants of *E. chrysanthemi* at a high frequency. In such crosses, Km^r Gm^r transconjugants occurred at a much lower frequency (about 100-fold). Although Km^r Gm^r transconjugants of *E. chrysanthemi* maintained pJB4JI as a plasmid (Fig. 1) under selective conditions, the loss of the plasmid occurred at a high frequency in the absence of drug selection (detailed data not presented). These observations collectively indicate the inability of the bacterium to maintain the plasmid. Similar observations have been noted in a variety of bacterial species such as *A. tumefaciens* (19), *A. rhizogenes* (37), *C. crescentus* (18), *P. solanacearum* (4), *R. leguminosarum, R. phaseoli, R. trifolii* (3), and *R. meliloti* (17, 28). Moreover, in these species, Tn5 inserted into either plasmid or chromosomal genomes. The instability of pJB4JI in these bacterial species apparently results from the presence of the Mu genome (3, 18). A similar situation may prevail in *E. chrysanthemi*. However, since we have not investigated this aspect any further, at present we cannot explain the molecular basis for the inability of *E. chrysanthemi* to maintain pJB4JI.

Despite the uncertainty of the mechanism of replication (or segregation) of pJB4JI in *E. chrysanthemi*, our data demonstrate transposition of Tn5 into various sites on the chromosome of this bacterial species. (i) Km^r Gm^s clones, regardless of the mutational phenotype, lacked pJB4JI DNA, whereas the parent *E. coli* donor and the Km^r Gm^r transconjugant of *E. chrysanthemi* possessed the plasmid DNA (Fig. 1). (ii) Km^r was linked with the mutational phenotypes, since most prototrophic recombinants or revertants were simultaneously Km^s (Table 2). (iii) Insertions of Tn5 caused changes in an array of

bacterial phenotypes (Ade$^-$, Arg$^-$, Car$^-$, Cys$^-$, Gtu$^-$, Gua$^-$, His$^-$, Leu$^-$, Lys$^-$, Met$^-$, Thr$^-$, Thy$^-$, Trp$^-$, or Ura$^-$). Based upon our observations on the gradient in the frequency of recombinants of Tn5-induced markers and by taking into consideration the location of various chromosomal markers (7–10, 25), the data are best explained by Tn5 insertions into different regions on the chromosome of *E. chrysanthemi*. The linkage data from Hfr crosses (Table 2) also indicate that 11 of 14 different classes of mutations resulted from single-site insertions of Tn5.

The data (Table 2) also show that although most markers spontaneously reverted to prototrophy, others (i.e., *car, gtu, lys, thr*, and *trp*) did not revert. In other bacterial systems (see reference 18), Mu insertion mutations do not revert. With pJB4JI, Meade et al. (28) observed that a certain proportion of putative Tn5 insertion mutants of *R. meliloti* also carried the Mu sequence. Thus, the possibility remains that the nonreverting Tn5 insertion mutations in *E. chrysanthemi* may also carry the Mu sequence. This possibility is currently under investigation.

The Km^r phenotype of the His$^+$ or Ade$^+$ revertants is somewhat unusual since all of the prototrophic recombinants for these markers were Km^s (Table 2). One possible explanation could be that the spontaneous excision of Tn5 DNA in certain cases is accompanied by the insertion of the transposon into a second site, thereby preserving the Km^r phenotype among prototrophic recombinants.

The basis for the segregation of the Km^r phenotype among Arg$^+$ and Leu$^+$ recombinants (Table 2) and the preservation of the Km^r phenotype in Leu$^+$ revertants (Table 2) is also not clear. It is possible that the *arg-1* (AC4104) and *leu-6* (AC4168) strains may carry double Tn5 insertions that are partially linked; in the *arg-1* strain, Tn5 copies may be more closely linked (perhaps in tandem) than in the *leu-6* strain. This hypothesis is supported by coinheritance frequencies in the Hfr crosses (Table 2): a higher proportion (about 53%) of Arg$^+$ recombinants become concomitantly Km^s compared with Leu$^+$ recombinants, about 35% of which were Km^s. Similarly, the *met-1* (AC4100) strain may contain at least two copies of Tn5 that are unlinked; one may have inserted into a *met* gene causing the Met$^-$ phenotype, and the other may have inserted into a region which did not produce a discernible phenotypic change (a silent mutation). This hypothesis is also supported by the phenotype of Met$^+$ revertants which, like the Met$^+$ recombinants, remained Km^r (Table 2). In the same way, our inability to determine the nutritional requirements of certain auxotrophs may be related to multiple insertions of Tn5. Clearly, additional studies will be required

to clarify the genetic mechanisms of these unusual phenotypes.

The scattered insertion of Tn5 into the chromosome of *E. chrysanthemi* offers new possibilities for genetic analyses of this important bacterial phytopathogen. For example, Tn5-induced mutations should prove valuable in elucidating the functions of extracellular enzymes (Prt, Plc, and the pectolytic enzyme, Pat) in plant disease. We have already detected the occurrence of Tn5-induced nonpectolytic or nonproteolytic mutants (A. K. Chatterjee and K. K. Thurn, manuscript in preparation). By exploiting Tn5 mutagenesis, it also should be possible to identify additional pathogenic determinants, such as bacterial cell surface components. The use of transposable elements to isolate different Hfr strains, as in *S. typhimurium* (12), should now be possible in *E. chrysanthemi* by using a transmissible plasmid carrying the Tn5 DNA. Indeed, we have constructed an F' plasmid that carries both Tn*10* and Tn5 (Chatterjee and Thurn, in preparation). This plasmid has enabled us to obtain Hfr-type donor strains with different origins of chromosome transfer in *E. chrysanthemi* strains carrying chromosomal Tn5 insertions. These developments should facilitate the construction of a gene linkage map and genetic analysis of the pathogenic determinants of the soft-rot bacterium, *E. chrysanthemi*.

LITERATURE CITED

1. Berg, C. M., K. J. Shaw, J. Vender, and M. Borucka-Mankiewicz. 1979. Physiological characterization of polar Tn5-induced isoleucine-valine auxotrophs in *Escherichia coli* K-12: evidence for an internal promoter in the *ilvOGEDA* operon. Genetics 93:309–319.
2. Berg, D. E., A. Weiss, and L. Crossland. 1980. Polarity of Tn5 insertion mutations in *Escherichia coli*. J. Bacteriol. 142:439–446.
3. Beringer, J. E., J. L. Beynon, A. V. Buchanan-Wollaston, and A. W. B. Johnston. 1978. Transfer of the drug-resistance transposon Tn5 to *Rhizobium*. Nature (London) 276:633–634.
4. Boucher, C., B. Message, D. Debieu, and C. Zischek. 1981. transposons into *Pseudomonas solanacearum*. Phytopathology 71:639–642.
5. Buchanan-Wollaston, A. V., J. E. Beringer, N. J. Brewin, P. R. Hirsch, and A. W. B. Johnston. 1980. Isolation of symbiotically defective mutants in *Rhizobium leguminosarum* by insertion of the transposon Tn5 into a transmissible plasmid. Mol. Gen. Genet. 178:185–190.
6. Casse, F., C. Boucher, J. S. Julliot, M. Michel, and J. Denarie. 1979. Identification and characterization of large plasmids in *Rhizobium meliloti* using agarose gel electrophoresis. J. Gen. Microbiol. 113:229–242.
7. Chatterjee, A. K. 1980. Acceptance by *Erwinia* spp. of R

plasmid R68.45 and its ability to mobilize the chromosome of *Erwinia chrysanthemi*. J. Bacteriol. 142:111–119.
8. Chatterjee, A. K., M. A. Brown, J. S. Ziegle, and K. K. Thurn. 1981. Progress in chromosomal genetics of *Erwinia chrysanthemi*, p. 389–402. *In* J. C. Lozano (ed.), Proceedings of the Fifth International Conference on Plant Pathogenic Bacteria, Cali, Colombia, 1981. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.
9. Chatterjee, A. K., and M. P. Starr. 1977. Donor strains of the soft-rot bacterium *Erwinia chrysanthemi* and conjugational transfer of the pectolytic capacity. J. Bacteriol. 132:862–869.
10. Chatterjee, A. K., and M. P. Starr. 1980. Genetics of *Erwinia* species. Annu. Rev. Microbiol. 34:645–676.
11. Chrisope, G. L., C. W. Fox, and R. T. Marshall. 1976. Lecithin agar for detection of microbial phospholipases. Appl. Environ. Microbiol. 31:784–786.
12. Chumley, F. G., R. Menzel, and J. R. Roth. 1979. Hfr formation directed by Tn10. Genetics 91:639–655.
13. Comai, L., and T. Kosuge. 1982. Cloning and characterization of *iaaM*, a virulence determinant of *Pseudomonas savastanoi*. J. Bacteriol. 149:40–46.
14. Crabtree, K. T., and R. D. Hinsdill. 1974. Fundamental experiments in microbiology, p. 122. The W. B. Saunders Co., Philadelphia.
15. Danilevich, V. N., Y. G. Stepanshin, N. V. Volozhantsev, and E. I. Golub. 1978. Transposon-mediated insertion of R factor into bacterial chromosome. Mol. Gen. Genet. 161:337–339.
16. Davis, R. W., D. Botstein, and J. R. Roth. 1980. Advanced bacterial genetics. A manual for genetic engineering, p. 209–210. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. Duncan, M. J. 1981. Properties of Tn5-induced carbohydrate mutants in *Rhizobium meliloti*. J. Gen. Microbiol. 122:61–67.
18. Ely, B., and R. H. Croft. 1982. Transposon mutagenesis in *Caulobacter crescentus*. J. Bacteriol. 149:620–625.
19. Garfinkel, D. J., and E. W. Nester. 1980. *Agrobacterium tumefaciens* mutants affected in crown gall tumorigenesis and octopine catabolism. J. Bacteriol. 144:732–743.
20. Hernalsteens, J. P., H. De Greve, M. van Montagu, and J. Schell. 1978. Mutagenesis by insertion of the drug resistance transposon Tn7 applied to the Ti plasmid of *Agrobacterium tumefaciens*. Plasmid 1:218–225.
21. Johnson, S. R., and W. R. Romig. 1979. Transposon-facilitated recombination in *Vibrio cholerae*. Mol. Gen. Genet. 170:93–101.
22. Klapwijk, P. M., J. Van Breukelen, K. Korevaar, G. Ooms, and R. A. Schilperoort. 1980. Transposition of Tn*904* encoding streptomycin resistance into the octopine Ti plasmid of *Agrobacterium tumefaciens*. J. Bacteriol. 141:129–136.
23. Kleckner, N., J. Roth, and D. Botstein. 1977. Genetic engineering *in vivo* using translocatable drug-resistance elements. New methods in bacterial genetics. J. Mol. Biol. 116:125–159.
24. Kleckner, N., D. A. Steele, K. Reichardt, and D. Botstein. 1979. Specificity of insertion by the translocatable tetracycline-resistance element Tn10. Genetics 92:1023–1040.
25. Kotoujansky, A., M. Lemattre, and P. Boistard. 1982. Utilization of a thermosensitive episome bearing transposon Tn*10* to isolate Hfr donor strains of *Erwinia carotovora* subsp. *chrysanthemi*. J. Bacteriol. 150:122–131.
26. Krishnapillai, V. 1979. DNA insertion mutagenesis in a *Pseudomonas aeruginosa* R plasmid. Plasmid 2:237–246.
27. Krishnapillai, V., P. Royle, and J. Lehrer. 1981. Insertions of the transposon Tn1 into the *Pseudomonas aeruginosa* chromosome. Genetics 97:495–511.
28. Meade, H. M., S. R. Long, G. B. Ruvkun, S. E. Brown, and F. M. Ausubel. 1982. Physical and genetic characterization of symbiotic and auxotrophic mutants of *Rhizobium meliloti* induced by transposon Tn5 mutagenesis. J. Bacteriol. 149:114–122.

29. Merrick, M., M. Filser, R. Dixon, C. Elmerich, L. Sibold, and J. Houmard. 1980. The use of translocatable genetic elements to construct a fine-structure map of the *Klebsiella pneumoniae* nitrogen fixation (*nif*) gene cluster. J. Gen. Microbiol. 117:509–520.

30. Meyers, J. A., D. Sanchez, L. P. Elwell, and S. Falkow. 1976. Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. J. Bacteriol. 127:1529–1537.

31. Ooms, G., P. M. Klapwijk, J. A. Poulis, and R. A. Schilperoort. 1980. Characterization of Tn*904* insertions in octopine Ti plasmid mutants of *Agrobacterium tumefaciens*. J. Bacteriol. 144:82–91.

32. Sato, M., B. J. Staskawicz, N. J. Panopoulos, S. Peters, and M. Honma. 1981. A host-dependent hybrid plasmid suitable as a suicidal carrier for transposable elements. Plasmid 6:325–331.

33. Shaw, K. J., and C. M. Berg. 1979. *Escherichia coli* K-12 auxotrophs induced by insertion of the transposable element Tn5. Genetics 92:741–747.

34. Sherratt, D. 1981. *In vivo* genetic manipulation in bacteria, p. 35–47. *In* S. W. Glover and D. A. Hopwood (ed.), Genetics as a tool in microbiology, symposium 31, The Society for General Microbiology. Cambridge University Press, New York.

35. Starr, M. P., and A. K. Chatterjee. 1972. The genus *Erwinia*: enterobacteria pathogenic to plants and animals. Annu. Rev. Microbiol. 26:389–426.

36. Thomson, J. A., M. Hendson, and R. M. Magnes. 1981. Mutagenesis by insertion of drug resistance transposon Tn7 into a *Vibrio* species. J. Bacteriol. 148:374–378.

37. White, F. F., and E. W. Nester. 1980. Hairy root: plasmid encodes virulence traits in *Agrobacterium rhizogenes*. J. Bacteriol. 141:1134–1141.