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Transposon Tn*5* Mutagenesis in *Erwinia carotovora* subsp. *carotovora* and *E. carotovora* subsp. *atroseptica*†

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In matings between *Escherichia coli* 2492(pJB4JI) and *Erwinia carotovora* subsp. *carotovora* Ecc71 and *E. carotovora* subsp. *atroseptica* Eca12, Km^r Gm^s transconjugants were obtained at high frequencies, indicating instability of the Mu-containing plasmid pJB4JI and transposition of Tn*5* into the recipient genome. This was verified by Southern blot hybridization with pRZ102 DNA containing Tn*5* as the ^{32}P-labeled probe. Examination of Km^r Gm^s transconjugants of Ecc71 and Eca12 disclosed that a proportion (2 to 3%) were either auxotrophic or defective in catabolism of specific carbohydrates. Spontaneous prototrophic revertants were obtained for all markers with the exception of *ilv*, *tyr*, and *suc*. Genetic and physical data indicate that scattered insertions of Tn*5* from pJB4JI into the chromosome of Ecc71 and Eca12 produced a variety of altered phenotypes due mostly to single insertions of Tn*5* not accompanied by Mu DNA.

Transposable elements that encode drug resistance are becoming important tools in genetic and biochemical studies of phytopathogenic bacteria. In *Agrobacterium tumefaciens* (13, 14, 18, 23), *Agrobacterium rhizogenes* (30), and *Pseudomonas* spp. (1, 7, 17, 27), Tn elements have been used to elucidate plasmid and chromosomal gene functions. Recently Tn*5* has been used in *Erwinia chrysanthemi* to develop mutants that are either auxotrophic or unable to catabolize polygalacturonate (6) and in *Erwinia herbicola* to associate a bacteriocin with an indigenous plasmid (12). In the construction of genetic maps of *E. chrysanthemi* (Chatterjee et al., Phytopathology **72**:934, 1982) and *A. tumefaciens* (26), Tn*5* homology was utilized to facilitate polarized transfer of chromosomal markers.

To date, however, Tn elements have not been exploited in genetic studies of either *Erwinia carotovora* subsp. *carotovora* (Ecc strains) or *E. carotovora* subsp. *atroseptica* (Eca strains). Both subspecies are important soft rot pathogens of potato and cause substantial economic losses during preharvest and postharvest periods (25). At present, the only report of any genetic system in *E. carotovora* is that by Pérombelon and Boucher (24), who showed R68.45 mediated transfer of several chromosomal markers including *leu*, *trp*, and *his*. Although such a genetic system has potential, a more powerful and direct approach to elucidating gene function is transpositional mutagenesis with Tn*5* (3). One means of introducing Tn*5* into a bacterial strain is by conjugation with the suicide plasmid pJB4JI, which confers gentamycin resistance and carries Tn*5* inserted into prophage Mu. As demonstrated in *A. tumefaciens* (13) and *Pseudomonas solanacearum* (1), pJB4JI is not stable after transfer from *Escherichia coli*, presumably due to the presence of Mu. This results in loss of the plasmid and segregation of markers, thereby allowing selection of Km^r Gm^s transconjugants in which Tn*5* has potentiality transposed. Likewise, this situation exists in certain strains of *E. carotovora*. In this report we provide genetic and physical data substantiating that transposition of Tn*5* does occur from the plasmid pJB4JI into the chromosomes of *E. carotovora* subsp. *carotovora* and *atroseptica* causing mutations in biosynthetic and catabolic genes.

(A preliminary account of part of this work has been presented previously [R. T. Zink et al., Phytopathology **72**:933–934, 1982].)

MATERIALS AND METHODS

Bacterial strains. *E. carotovora* subsp. *carotovora* and *atroseptica* strains and plasmid-bearing strains of *E. coli* are listed in Table 1. Cultures were maintained at 4°C, R plasmid-carrying strains were maintained on L agar containing appropriate antibiotics, and all other strains were maintained on YDC agar slants (5).

Media. L, YDC, and minimal media were as described by Chatterjee (5). Nutritional requirements of putative auxotrophic mutants were determined by the procedure of Davis et al. (9). The ability to ferment carbohydrates was tested on minimal salts medium containing 1 g of the appropriate carbohydrate per liter. When required, media were supplemented with amino acids, purines, or pyrimidines (50 μg/ml) or the following drugs: kanamycin (Km), 50 μg/ml; nalidixic acid (Nal), 50 μg/ml; gentamycin sulfate (Gm), 10 μg/ml; tetracycline (Tc), 10 μg/ml; or ampicillin (Ap), 50 μg/ml.

Bacterial matings. Bacterial matings were made on L agar (5). Donor and recipient cells were counterselected by use of auxotrophy or drug susceptibility (or both).

Transpositional mutagenesis. Bacterial matings were done as previously described (5). Donor cells were counterselected by nalidixic acid, and recipient cells were counterselected by kanamycin. After 2 to 3 days of incubation at 30°C, Km^r transconjugants were replicate patched on L agar-kanamycin-nalidixic acid, L agar-kanamycin-nalidixic acid-gentamycin, and minimal agar-kanamycin-nalidixic acid with either acetate, L-arabinose, galactose, galacturonate, gluconate, glucose, α-ketoglutarate, lactose, melibiose, succinate, sucrose, or xylose as a carbon source. Colonies were examined visually for growth after 24 h of incubation at 30°C. Transconjugants that were Km^r Gm^s and either auxotrophic or unable to utilize a tested substrate were purified on L agar-

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TABLE 1. Bacterial strains

Bacterial species and strain	Characters	Source derivation or reference
E. carotovora subsp. *carotovara*		
Ecc21	Wild type, serogroup II	S. H. DeBoer
Ecc26	Wild type, serogroup V	S. H. DeBoer
Ecc61	Wild type, serogroup X	S. H. DeBoer
Ecc63	Wild type, serogroup IX	S. H. DeBoer
Ecc71	Wild type, serogroup III	S. H. DeBoer
Ecc166	Wild type, serogroup III	S. H. DeBoer
Ecc189	Wild type, serogroup VI	S. H. DeBoer
Ecc190	Wild type, serogroup IV	S. H. DeBoer
Ecc192	Wild type, serogroup XVIII	S. H. DeBoer
Ecc193	Wild type, serogroup XI	S. H. DeBoer
Ecc380	Wild type, serogroup XXIX	S. H. DeBoer
AC5001	Ecc71, also Nal^r	Spontaneous for Nal
AC5101	*ura-1*::Tn*5* (Km^r) Nal^r	This paper
AC5102	*ilv-1*::Tn*5* (Km^r) Nal^r	This paper
AC5103	*met-1*::Tn*5* (Km^r) Nal^r	This paper
AC5104	*suc-1*::Tn*5* (Km^r) Nal^r	This paper
AC5105	*xyl-1*::Tn*5* (Km^r) Nal^r	This paper
AC5106	*xyl-2*::Tn*5* (Km^r) Nal^r	This paper
AC5107	*tyr-1*::Tn*5* (Km^r) Nal^r	This paper
AC5108	*ura-2*::Tn*5* (Km^r) Nal^r	This paper
AC5109	*car-1*::Tn*5* (Km^r) Nal^r	This paper
AC5110	*trp-1*::Tn*5* (Km^r) Nal^r	This paper
AC5111	*ade-1*::Tn*5* (Km^r) Nal^r	This paper
AC5112	*thr-1*::Tn*5* (Km^r) Nal^r	This paper
AC5113	Revertant of AC5111 (Km^r) Nal^r	This paper
AC5114	Revertant of AC5111 Nal^r	This paper
AC5115	Revertant of AC5103 (Km^r) Nal^r	This paper
AC3001	Ecc193, also Km^r Gm^r (pJB4JI)	AC8001 × Ecc193
E. carotovora subsp. *atroseptica*		
Eca3	Wild type, serogroup I	S. H. DeBoer
Eca5	Wild type, serogroup I	S. H. DeBoer
Eca6	Wild type, serogroup XVIII	S. H. DeBoer
Eca12	Wild type, serogroup I	S. H. DeBoer
Eca15	Wild type, serogroup I	S. H. DeBoer
Eca16	Wild type, serogroup I	S. H. DeBoer
Eca17	Wild type, serogroup I	S. H. DeBoer
Eca19	Wild type, serogroup I	S. H. DeBoer
Eca20	Wild type, serogroup I	S. H. DeBoer
Eca31	Wild type, serogroup I	S. H. DeBoer
Eca180	Wild type, serogroup I	S. H. DeBoer
Eca196	Wild type, serogroup XX	S. H. DeBoer
Eca198	Wild type, serogroup XXII	S. H. DeBoer
AC9001	Eca12, also Nal^r	Spontaneous for Nal
AC9002	*tyr-1*::Tn*5* (Km^r) Nal^r	This paper
AC9003	*pro-1*::Tn*5* (Km^r) Nal^r	This paper
AC9004	*ura-1*::Tn*5* (Km^r) Nal^r	This paper
AC9005	*ade-1*::Tn*5* (Km^r) Nal^r	This paper
E. coli		
AC8001	*arg his* Δ*lac* Str^r Km^r Gm^r (pJB4JI = pPHlJI::Mu::Tn*5*)	2492(5) × 1830(pJB4JI)(6)
AC8002	*arg his* Δ*lac* Str^r Ap^r Km^r Tc^r (RK2)	2492(5) × J531(RK2)(2), D. C. Coplin
AC8003	*arg his* Δ*lac* Str^r Ap^r Km^r Tc^r (RK2::Mu)	2492(5) × 960(RK2::Mu)(2), D. C. Coplin
AC8004	*arg his* Δ*lac* Str^r Gm^r (pPHlJI)	2492(5) × 2174(pPHlJI), T. C. Currier
AC8100	Wild-type K-12	ICPB[a]
HB101	*hsdR hsdM pro leu thr lacY endOI*$^-$ *recA* Str^r Km^r (pRZ102)	S. V. Beer
LA1016	F$^-$ *ara*D139 Δ(*lacIPOZYA*) U169 *rps*L *thi ara*::Mu *cts*	L. Heffernan (19)

[a] International Collection of Phytopathogenic Bacteria (Department of Bacteriology, University of California, Davis).

kanamycin-nalidixic acid. Several single clones of each putative mutant were tested to confirm their phenotype, and then one clone was retained for further analysis.

Isolation of DNA. For rapid screening of bacterial strains for plasmids, DNA was extracted by a modification of the procedure of Casse et al. (4) as described by Chatterjee et al. (6). Total cellular DNA used for restriction endonuclease digestion was prepared as described by Shepard and Polisky (28). In strain Eca12, however, which harbors a nuclease-like activity, additional purification steps were required before restriction digestion. After the second phenol-chloroform extraction the crude cell lysate of Eca12 was treated with proteinase K (0.1 mg/ml, final concentration) for 1 h at 37°C followed by an ethidium bromide-cesium chloride equilibrium density gradient centrifugation, which effectively eliminated the nuclease activity from the DNA preparation.

The plasmid pRZ102 (17) containing Tn5, used in hybridization analysis, was isolated by the procedure of Humphreys et al. (16) and purified by two ethidium bromide-cesium chloride equilibrium density gradient centrifugations. Mu DNA used in hybridization analysis was prepared from Mu particles (2) obtained by heat induction (15) of LA1016 (19).

Electrophoretic analysis of DNA. DNA preparations were examined by electrophoresis in 0.8% agarose gel with Tris-acetate buffer (9). Restriction digestion was accomplished by treating 2 μg of DNA with 10 units of *Eco*RI (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) for 4 h at 37°C. Horizontal gel electrophoresis was done at 65 V for 15 h, and gels were stained with ethidium bromide (0.5 μg/ml).

Detection of Tn5 DNA homology. After agarose gel electrophoresis, DNA was transferred to nitrocellulose by the method of Southern (29) and prehybridized for 24 h at 65°C in 30 mM sodium citrate–0.3 M sodium chloride–0.1% sodium dodecyl sulfate–0.2% Ficoll 400–0.2% bovine serum albumin–0.2% polyvinylpyrrolidone. DNA hybridization was performed at 65°C for 24 h with fresh solution containing either 2×10^7 cpm of single-stranded pRZ102 DNA (1 μg) or Mu DNA (1 μg) labeled with ^{32}P by nick translation (20). Nonhybridized probe DNA was removed from the nitrocellulose by several washes in 15 mM sodium chloride–1.5 mM sodium citrate–0.1% sodium dodecyl sulfate at 68°C before autoradiography at −70°C.

RESULTS

Transfer of pJB4JI to subspecies of *E. carotovora*. Three of the 11 *E. carotovora* subsp. *carotovora* strains and six of the 13 *E. carotovora* subsp. *atroseptica* strains tested acquired Km^r in crosses with *E. coli* strain AC8001 carrying the plasmid pJB4JI (Table 2). The frequencies of Km^r transconjugants ranged from 5×10^{-8} in strain Eca19 to 2×10^{-4} in strain Ecc71, per input donor cell. To determine the coinheritance of the other plasmid marker, Gm^r, Km^r transconjugants were patched to gentamycin-containing medium. In strains Ecc63 and Ecc71 only Km^r Gm^s transconjugants could be detected, whereas in strains Eca19 and Ecc193 all Km^r transconjugants tested were also Gm^r. In the other five strains acquiring Km^r, the proportions of clones with both Km^r and Gm^r were as follows: strain Eca6, 81%; strain Eca31, 41%; strain Eca12, 21%; strain Eca15, 73%; strain Eca180, 35% (Table 2). The proportion of Km^r Gm^s to Km^r Gm^r transconjugants resulting from a given cross was found to vary by not more than 14% between expriments.

To estimate the stability of pJB4JI in Km^r Gm^r *E. carotovora* clones, strains AC3001, AC7004, and AC8001 were grown under nonselective conditions for approximately 25 generations, after which individual colonies were tested for Km^r and Gm^r. The proportion of Km^r Gm^r clones of the total clones tested was 70 of 90, 78 of 90, and 85 of 90 for AC3001, AC7004, and AC8001, respectively. Km^r Gm^s clones were detected at frequencies of 1 of 90 in strain AC7004 and 5 of 90 in strain AC7501, but not in strain AC3001. Under the same experimental conditions, segregation of plasmid markers did not occur in *E. coli* AC8001.

TABLE 2. Transfer of Km^r from *E. coli* (pJB4JI) to strains of *E. carotovora* subsp. *carotovora* and *atroseptica* and coinheritance of Gm^r among the transconjugants

Strain[a]	Frequency of transfer of Km^r/input donor cell[b]	Proportion of Km^r Gm^r Transconjugants[c] (no. Km^r Gm^r/no. tested)
Eca6	5×10^{-4}	73/90
Eca12	6×10^{-5}	108/500
Eca15	1×10^{-6}	66/90
Eca19	5×10^{-8}	50/50
Eca31	4×10^{-4}	37/90
Eca180	7×10^{-7}	32/90
Ecc63	6×10^{-5}	0/90
Ecc71	2×10^{-4}	0/1,800
Ecc193	1×10^{-5}	90/90

[a] No Km^r transconjugants were detected in crosses with the following strains: Eca3, Eca5, Eca16, Eca17, Eca20, Eca196, Eca198, Ecc21, Ecc26, Ecc61, Ecc166, Ecc189, Ecc190, Ecc192, and Ecc380.

[b] Matings were done as previously described (5). The donor and recipients were counterselected on minimal glucose agar containing kanamycin (50 μg/ml). See Table 1 for the phenotypes of bacterial strains.

[c] Transconjugants were tested concurrently on L agar containing kanamycin (50 μg/ml) with and without gentamycin sulfate (10 μg/ml).

Effect of bacteriophage Mu on plasmid transfer. The instability of the plasmid pJB4JI in *Rhizobium* spp. (10) and *Caulobacter crescentus* (11) is reported to be due to a property of bacteriophage Mu causing either killing of recipient cells, restriction of incoming DNA, or interference with plasmid replication. To determine whether Mu had a similar effect on plasmid transfer in strains of *E. carotovora* subsp. *carotovora* and *atroseptica*, a number of different crosses were made. The strains of *E. carotovora* subsp. *carotovora* and *atroseptica* used as recipients were selected on their varied ability to acquire pJB4JI observed in earlier crosses (Table 2). In crosses with Eca12 as the recipient, R-factor transfer was decreased by 10^8 with the plasmid RK2 when Mu was present (RK2::Mu) and by 10^3 with the plasmid pPH1JI when Mu and Tn5 were present (pPH1JI::Mu::Tn5). Similar results were obtained with strain Ecc193. In strain Ecc71, only transfer of RK2 was detected at a frequency of 10^{-7} per input donor cell (Table 3). These transfer frequencies of Mu containing plasmids to *E. carotovora* parallel those reported by Murooka et al. (22). In general, no change in recipient cell survival could be detected due to the presence or absence of Mu in any of these crosses. Thus, it can be concluded that in *E. carotovora*, as in *C. crescentus* (11), Mu does have a limiting effect on R-factor transfer and stability of plasmids that contain it, but no effect on recipient survival.

Analysis of Tn5 insertion mutants. Strains Ecc71 and Eca12 were selected for mutagenesis experiments due to the instability of pJB4JI after its transfer from *E. coli*, resulting

TABLE 3. Transfer of Mu-containing and non-Mu-containing R plasmids to *E. carotovora* subsp. *carotovora* and *atroseptica* and *E. coli*

Donor	Recipient	Selection	Frequency of transfer/input donor cell[a]
AC8002(RK2)	Eca12	Ap^r Km^r Tc^r	10^{-1}
	Ecc71		10^{-7}
	Ecc193		10^{-1}
	AC8100		10^{-1}
AC8003(RK2::Mu)	Eca12	Ap^r Km^r Tc^r	10^{-9}
	Ecc71		$<10^{-10}$
	Ecc193		10^{-5}
	AC8100		10^{-1}
AC8004(pPH1JI)	Eca12	Gm^r	10^{-2}
	Ecc71		$<10^{-10}$
	Ecc193		10^{-2}
	AC8100		10^{-2}
AC8001(pPH1JI::Mu::Tn*5* = pJB4JI)	Eca12	Km^r Gm^r	10^{-5}
	Ecc71		$<10^{-10}$
	Ecc193		10^{-5}
	AC8100		10^{-2}

[a] Matings were done as previously described (5). The donor and recipient cells were counterselected by use of auxotrophy drug susceptibility (or both). See Table 1 for phenotypes of bacterial strains.

in high frequencies of transconjugants that were Km^r Gm^s (Table 2). In matings between donor strain AC8001 and recipient strains AC5001 (Ecc71, Nal^r) and AC9001 (Eca12, Nal^r), Km^r transconjugants were observed at frequencies of 10^{-4} and 10^{-5}, respectively. Phenotypic examination of 1,800 Km^r clones of AC5001 showed that 2 to 3% were either auxotrophic or unable to utilize a specific carbohydrate. For the carbon sources tested (acetate, L-arabinose, galacturonate, gluconate, α-ketoglutarate, galactose, melibiose, lactose, sucrose, succinate, and xylose) mutants defective in catabolism of succinate and xylose were identified. Auxotrophs were classified as Ade^-, Met^-, Ilv^-, Thr^-, Trp^-, Tyr^-, Car^-, and Ura^- (Table 4). Within strain AC9001, 500 Km^r Gm^s clones were examined for phenotypic changes; 2% were found to be auxotrophic. Auxotrophs were classified as Ade^-, Pro^-, Tyr^-, and Ura^- (Table 4). Each of the classified Tn*5* insertion mutants of AC5001 and AC9001 was also pectolytic and proteolytic and macerated potato tuber tissue like the parent strains.

To confirm the absence of the Tn*5* vector plasmid pJB4JI in Km^r Gm^s AC5001 transconjugants, cell lysates of seven representative mutants were examined by agarose gel electrophoresis (Fig. 1). The plasmid pJB4JI, present in cell lysates of Km^r Gm^r strains AC8001 and AC3001 was not detectable in cell lysates of transconjugants of AC5001 that were Km^r Gm^s and either Ura^- Ilv^-, Met^-, Xyl^-, Ade^-, or Thr^-. Identical results were obtained with Km^r Gm^s transconjugants of AC9001 (data not shown). It should be noted that strain AC5001 harbors at least one small cryptic plasmid (Fig. 1). Of the 23 other *E. carotovora* strains surveyed in this study, only one contained plasmids, strain Ecc63, which contained three (Fig. 1).

To determine the status of Tn*5* in Km^r mutants, Southern blot analysis with ^{32}P-labeled pRZ102 DNA containing Tn*5* was done on *Eco*RI-treated and untreated total cellular DNA. The restriction endonuclease *Eco*RI was used because Tn*5* lacks its recognition site (17). The autoradiographs in Fig. 2 and 3 show that Tn*5* hybridized with the donor strain AC8001, but not with either of the Km^s Gm^s parent strains AC5001 or AC9001. Hybridization of Tn*5* did, however, occur with chromosomal, but not plasmid, DNA of the six Km^r transconjugants of AC5001 (Fig. 2). Single insertions of Tn*5* were detected in all but one (AC5111) of the AC5001 Tn*5* mutants tested, each occurring in a different size fragment (Fig. 2). Analysis of two Km^r transconju-

TABLE 4. Frequency of reversion and linkage between prototrophy and kanamycin sensitivity in Tn*5* mutants of *E. carotovora* subsp. *carotovora* AC5001 and *E. carotovora* subsp. *atroseptica* AC9001[a]

Mutant strain	Marker	Reversion frequency	Proportion of Km^s clones (no. Km^s/no. tested)
AC5101	Ura^+	10^{-8}	19/21
AC5102	Ilv^+	$<10^{-10}$	
AC5103	Met^+	10^{-6}	0/90
AC5104	Suc^+	$<10^{-10}$	
AC5105	Xyl^+	10^{-6}	78/88
AC5106	Xyl^+	10^{-6}	80/88
AC5107	Tyr^+	10^{-6}	0/24
AC5108	Ura^+	10^{-9}	11/76
AC5109	Car^+	10^{-8}	0/31
AC5110	Trp^+	10^{-8}	0/24
AC5111	Ade^+	10^{-8}	24/29
AC5112	Thr^+	10^{-8}	32/33
AC9002	Tyr^+	$<10^{-10}$	
AC9003	Pro^+	10^{-9}	15/15
AC9004	Ura^+	10^{-9}	50/50
AC9005	Ade^+	10^{-8}	50/50

[a] See Table 1 for phenotypes of mutant strains, see the text for methods.

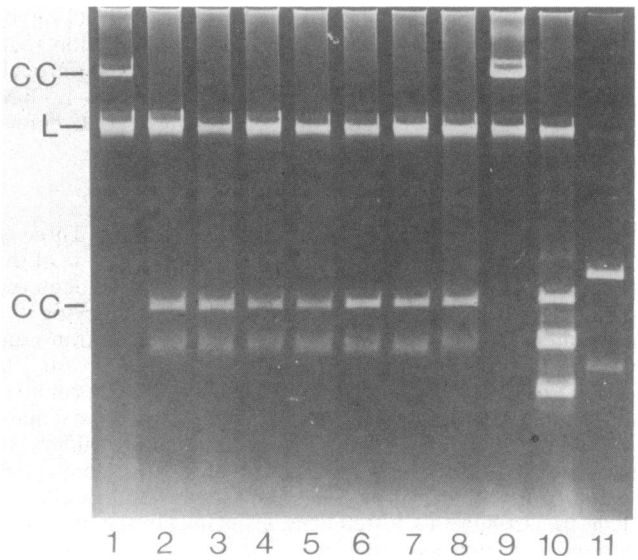

FIG. 1. Electrophoresis on 0.8% agarose gel of DNA preparations (6). Lanes: 1, *E. coli* AC8001(pJB4JI); 2, parent *E. carotovora* subsp. *carotovora* AC5001; 3, 4, 5, 6, 7, and 8, Km^r Gm^s transconjugants AC5101, AC5102, AC5103; AC5105, AC5111, and AC5112, respectively; 9, AC3001, a Km^r Gm^r transconjugant of strain Ecc193(pJB4JI); 10, Ecc63; 11, plasmid pBR322 as a size marker, 4.3 kilobases (2). L, Linear DNA; CC, closed circular DNA.

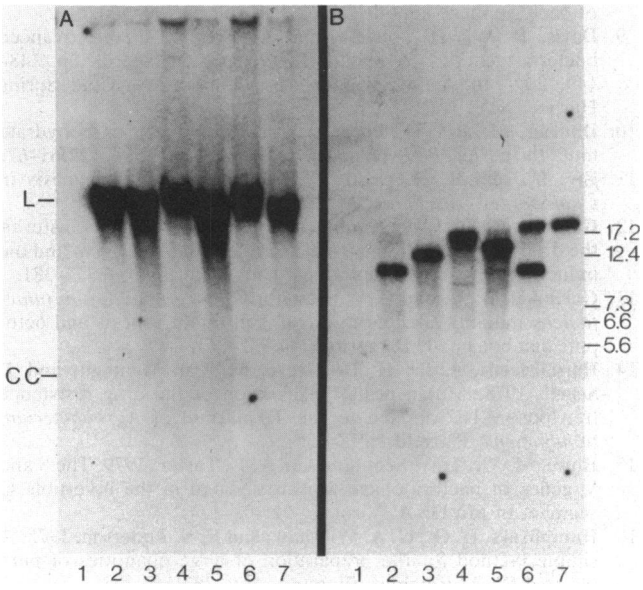

FIG. 2. Autoradiograph of a Southern blot of total cellular DNA from Km^r Gm^s transconjugants of strain AC5001 hybridized with nick-translated pRZ102 DNA containing Tn*5*. (A) Lanes: 1, parent *E. carotovora* subsp. *carotovora* AC5001; 2, 3, 4, 5, 6, and 7, Km^r Gm^s transconjugants AC5101, AC5102, AC5103, AC5105, AC5111, and AC5112, respectively. L, chromosomal DNA; CC, the position of the indigenous plasmid in strain AC5001. (B) The same analysis of the DNA after *Eco*RI digestion. Lanes: 1, AC5001; 2, AC5101; 3, AC5102; 4, AC5103; 5, AC5105; 6, AC5111; 7, AC5112. The weights in kilobases of fragments obtained by *Bam*HI digestion of bacteriophage lambda DNA are shown at right (8).

gants of AC9001 (Pro^- and Tyr^-) revealed a single insertion of Tn*5* in each, at different sites (Fig. 3).

Spontaneous reversion to prototrophy was detected for all markers, excluding Ilv^- and Suc^- in strain AC5001 and Tyr^- in strain AC9001 (Table 4). Reversion to prototrophy and concomitant loss of Km^r was consistent for all markers in strain AC7003. However, in strain AC5001 prototrophic revertants were often Km^r, indicating maintenance of Tn*5*. Hybridization analysis of a Km^r prototrophic revertant of strain AC5111 (Ade^-) and AC5103 (Met^-) revealed that Tn*5* was, in fact, still present within the chromosome (Fig. 3). The Km^r revertants of strains AC5111 and AC5103 contained one and two insertions of Tn*5*, respectively, each in DNA fragments that were of a different size than those in the original mutants. On the other hand, analysis of a Km^s revertant of strain AC5111 confirmed that Tn*5* was no longer present.

To determine whether segments of bacteriophage Mu DNA were associated with Tn*5*, all strains previously probed with Tn*5* were probed with nick-translated Mu DNA. Hybridization was observed with AC8001, but not with any of the Tn*5* mutants (data not shown).

DISCUSSION

The objective of this research was to determine whether Tn*5* mutagenesis with pJB4JI could be utilized in genetic studies of *E. carotovora*. The 24 strains surveyed showed a gradient of acquisition of R determinants conferred by pJB4JI, ranging from 100 to 21% cotransfer of Km^r and Gm^r to transfer of only Km^r to no transfer of either plasmid marker (Table 2).

In the strains AC5001 and AC9001, pJB4JI was not efficiently maintained, producing Km^r Gm^s clones at high frequencies; therefore these strains were ideally suited for transpositional mutagenesis. Transconjugants of AC5001 and AC9001 that were Km^r Gm^s were shown by physical analysis not to contain the plasmid pJB4JI; Km^r was due to transposition of Tn*5* not accompanied by Mu. Scattered insertions of Tn*5* resulted in a range of altered bacterial phenotypes (Ade^-, Car^-, Ilv^-, Met^-, Pro^-, Suc^-, Thr^-, Trp^-, Tyr^-, Ura^-, or Xyl^-). Most of these Tn*5* insertion mutations reverted to prototrophy with the exception of *ilv* (AC5102), *suc* (AC5104), and *try* (AC9002). Based upon findings in *Rhizobium meliloti* (21), these exceptions are likely attributed to imprecise excision of Tn*5* after transposition. The lack of Mu in these mutants, however, contrasts with observations made in *R. meliloti* (21) and recently in *E. chrysanthemi* (unpublished data) wherein Mu sequences are present in a proportion of putative Tn*5* insertion mutants.

Southern blot analysis of classified Tn*5* insertion mutants was essential to determine the status of Tn*5*. In most cases only a single insertion of Tn*5* was detected; however, strain AC5111 contained two insertions (Fig. 2). Such multiple Tn*5* insertions are difficult to detect without an elaborate genetic system, and relying upon reversion data alone can be misleading. An example is strain AC5103, in which all prototrophic revertants were Km^r, suggesting multiple Tn*5* insertions, even though it contained only one. In contrast to

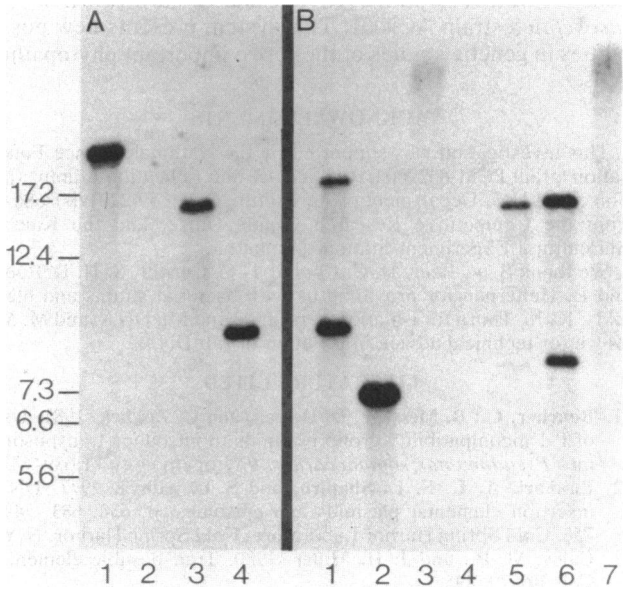

FIG. 3. Autoradiograph of a Southern blot of total cellular DNA digested with *Eco*RI and hybridized with nick-translated pRZ102 DNA containing Tn*5*. (A) Location of Tn*5* in Km^r Gm^s transconjugants of strain AC9001. Lanes: 1, *E. coli* strain AC8001 (pJB4JI) as control; 2, the *E. carotovora* subsp. *atroseptica* AC9001; 3 and 4, Km^r Gm^s transconjugants AC9002 and AC9003, respectively. (B) Location of Tn*5* in Km^r revertants. Lanes: 1, *E. carotovora* subsp. *carotovora* AC5111 (Ade^- Km^r); 2, a revertant of AC5111 (Ade^+ Km^r), AC5113; 3, AC5113 undigested; 4, revertant of AC5111 (Ade^+ Km^s), AC5114; 5, *E. carotovora* subsp. *carotovora* AC5103 (Met^- Km^r); 6, a revertant of AC5103 (Met^+ Km^r), AC5115; 7, AC5115 undigested. The weights in kilobases of fragments obtained by *Bam*HI digestion of bacteriophage lambda DNA are shown at left (8).

this situation is strain AC5111, which has two Tn*5* insertions even though there was an 83% linkage to Km^s upon reversion to prototrophy.

Southern blot data of Km^r spontaneous revertants of strains AC5111 and AC5103 revealed that in recipient strain AC5001 Tn*5* is somewhat unstable and can readily translocate to new sites within the chromosome. From this observation it can be concluded that the lack of complete linkage between reversion to prototrophy and Km^s consistently observed in Tn*5* mutants of AC5001 (Table 4) is likely due to spontaneous reinsertion of Tn*5*, thus maintaining Km^r.

The instability of pJB4JI in some strains of *E. carotovora* may be associated with bacteriophage Mu. When Mu is present in a given plasmid, the frequency of transfer of R determinants conferred by that plasmid is reduced (Table 3), suggesting either a lack of transfer or loss of the plasmid. It has been proposed that abortive replication of Mu results in this inefficient maintenance of the plasmid (11). Another factor possibly involved in the stability of pJB4JI in *E. carotovora* is recipient strain restriction endonuclease systems. Based on numerous matings involving several other plasmids that do not contain Mu other than those reported here, certain strains of *E. carotovora* were consistently able to acquire and maintain plasmids transferred from *E. coli* more efficiently than other strains. This type of response would seem to be characteristic of restriction systems in the recipient strains.

Based on the data we have presented, transposon mutagenesis with the plasmid pJB4JI has potential as a tool for constructing single gene Tn*5* insertions in *E. carotovora* subsp. *carotovora* strain AC5001 and *E. carotovora* subsp. *atroseptica* strain AC9001. This system presents new possibilities in genetic studies of these two important phytopathogens.

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