JOURNAL OF BACTERIOLOGY, June 1983, p. 1227–1235
0021-9193/83/061227-09$02.00/0

Vol. 154, No. 3

In Vivo Cloning of *Erwinia carotovora* Genes Involved in the Catabolism of Hexuronates

FRÉDÉRIQUE VAN GIJSEGEM* AND ARIANE TOUSSAINT
Laboratoire de Génétique, Université Libre de Bruxelles, Brussels, Belgium

Received 4 October 1982/Accepted 18 February 1983

Using the RP4::mini-Mu pULB113 plasmid, an RP4 derivative carrying a deleted Mu prophage which allows the plasmid to pick up any chromosomal DNA segment to form R′ plasmids, we cloned all of the genes of *Erwinia carotovora* involved in the catabolism of the hexuronates and in the transport of these substrates. With the R′ plasmids we isolated, we performed complementation analysis and found that, in the *Erwinia carotovora* strain we used, the genes involved in the catabolism of the hexuronates are clustered in four regions of the chromosome. This genetic organization is compared with that of *Escherichia coli* K-12.

Erwinia carotovora strains are phytopathogens responsible for soft rot of many plant species (7, 30). The pathogenicity of these enterobacteria seems to be intimately related to their pectinolytic activity, a specific trait of the species which allows them to degrade plant cell walls (4, 6, 7). The pectin is degraded by a battery of enzymes, most of which are secreted by the bacteria. It is demethoxylated into polygalacturonic acid by methylesterase, which may be degraded in two ways: (i) hydrolysis by polygalacturonase of the α-1-4 glycosidic bonds between the galacturonic residues provides saturated oligomers which are subsequently hydrolyzed into galacturonate (GA); (ii) attack by transeliminases generates unsaturated oligomers, which are subsequently cleaved by oligotranseliminase to keto-deoxyuronic acid, which is transformed into 2-keto-3-deoxygluconate (KDG; Fig. 1) (14, 23, 24).

GA and KDG are degraded in *Erwinia carotovora* by a pathway very similar to that in *Escherichia coli*, which has been well characterized (1, 21, 26; Fig. 2). In *Erwinia carotovora*, all enzymes of this pathway have been characterized biochemically (14), but no genetic studies have yet been reported. As a first approach to the genetic study of pectinolysis, we tried to unravel the genetic organization of the genes involved in the hexuronate pathway in *Erwinia carotovora*. We cloned these genes into the transferable plasmid pULB113, a derivative of the broad-host-range plasmid RP4 carrying a deleted Mu prophage which allows the plasmid to pick up any segment of chromosomal DNA by mini-Mu-mediated transposition, to generate R′ plasmids (31; for a detailed discussion of the mechanisms of transposition, see A. Toussaint and A. Resibois, *in* J. A. Shapiro, ed., *Mobile Genetic Elements*, in press). This process works in all bacteria where Mu can grow, including *Erwinia carotovora* (Lejeune et al., submitted for publication). The mini-Mu carried by the RP4 can mediate the transposition of random chromosomal segments onto the plasmid. In most instances the R′ plasmid carries a single chromosomal segment located at the site of insertion of the mini-Mu and flanked by two mini-Mu copies in the same orientation. As a consequence the chromosomal segment can be lost either by homologous reciprocal recombination between the mini-Mu (in Rec^+ bacteria) or by mini-Mu-mediated deletion of part or all of the transposed segment (in Rec^+ and Rec^- bacteria). Since the transposed chromosomal segment can be long (up to at least 100 kilobases [kb]), genes which are clustered on the chromosome can be cotransposed at high frequency. All of the results available so far suggest that, for a given region of the chromosome, the frequency of cotransposition of two markers is inversely related to their distance (9, 5).

We performed heterospecific matings between B374(pULB113), and *E. coli* recipients mutated in the hexuronate genes and selected *E. coli* transconjugants which acquired the corresponding wild-type allele from the *Erwinia* sp. donor. The linkage between the relevant *Erwinia* sp. genes was examined and compared with the organization of the equivalent *E. coli* genes.

MATERIALS AND METHODS

Bacterial strains and plasmids. Bacterial strains and plasmids used in this work are listed in Table 1.

Media. Bacteria were grown in L broth (16) supplemented with 50 μg of thymine per ml when necessary

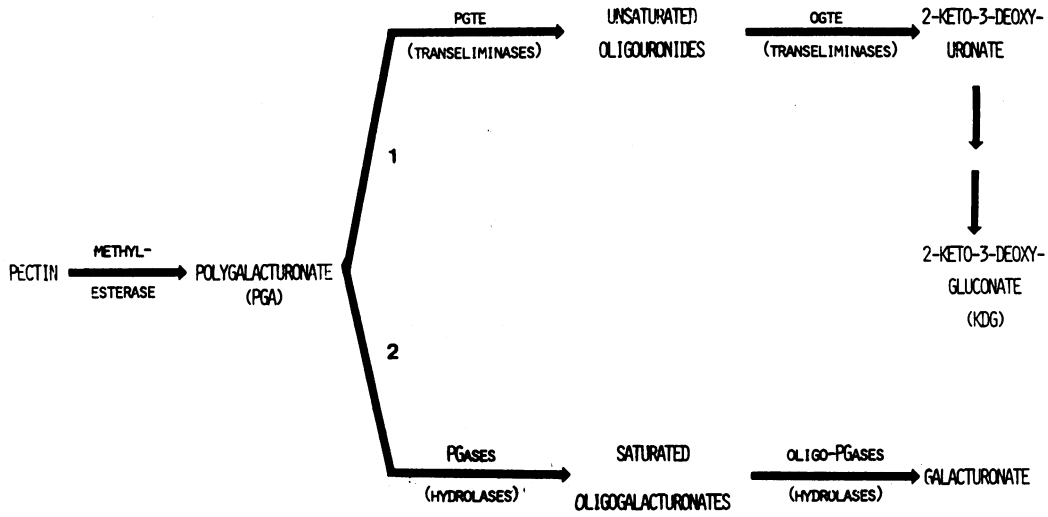

FIG. 1. Degradation of pectin. Pectin may be degraded in two ways: (1) transelimination as described by Preiss and Ashwell (23, 24); (2) hydrolysis as described by Kilgore and Starr (14). Abbreviations: PGTE, polygalacturonate transeliminase; OGTE = oligouronide transeliminase; PGase = polygalacturonase.

and diluted in 10^{-2} M $MgSO_4$. They were scored on L medium (L broth + 1.2% Difco agar), A medium (10), or M63 minimal medium (18) supplemented with 1.4% Difco agar. Carbon sources were added at 0.2%; amino acids, at 40 μg/ml; thymine, at 100 μg/ml; streptomycin, at 200 μg/ml; spectinomycin, at 100 μg/ml; trimethoprim, at 50 μg/ml; kanamycin, at 20 μg/ml; tetracycline, at 20 μg/ml; and ampicillin, at 20 μg/ml.

Chemicals. 2-Keto-3-deoxygluconic acid was kindly provided by M.-A. Mandrand-Berthelot. Glucuronic acid and galacturonic acid were purchased from Sigma Chemical Co.

Nomenclature. The genetic symbols are those used by Bachmann and Low (2). The following abbreviations are used: GU, glucuronate; GA, galacturonate; KDG, 2-keto-3-deoxygluconate; Gur^+, Gar^+, and Kdg^+, ability to catabolize glucuronate, galacturonate, and KDG respectively; Spc, spectinomycin; Str, streptomycin; Tmp, trimethoprim; Kan, kanamycin; Tet, tetracycline; Amp, ampicillin.

Matings. Overnight cultures, 0.2 ml each, of the donor carrying pULB113 and the recipient were spread together on A medium and incubated for 4 to 6 h at 33°C. Bacteria were collected from the growth area, suspended in 1 ml of 10^{-2} M $MgSO_4$, and spread on appropriate selective media. The donor strain was counterselected with streptomycin (200 μg/ml), spectinomycin (100 μg/ml), or trimethoprim (50 μg/ml).

The presence of the plasmid was checked by testing

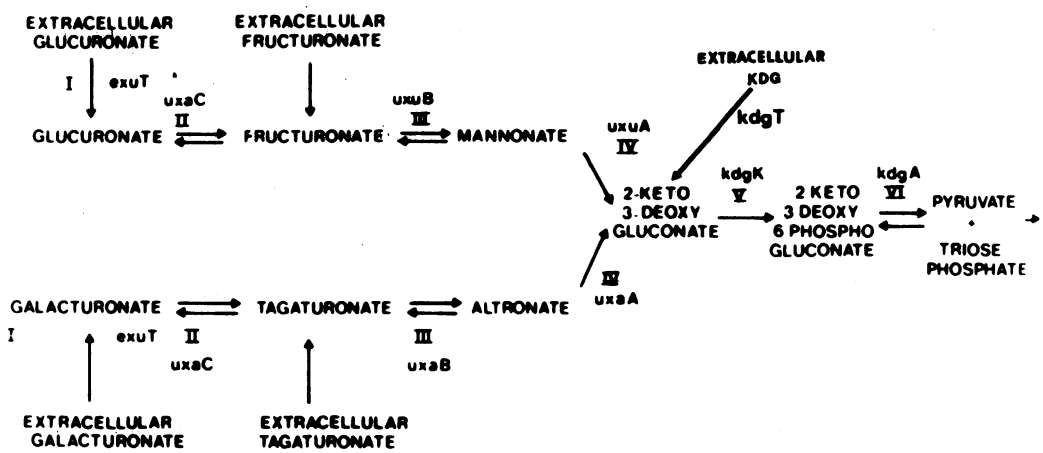

FIG. 2. Catabolism of GU and GA. Taken from Portalier et al. (21).

TABLE 1. Bacterial strains and plasmid

Strain	Description	Source
Bacteria		
Erwinia carotovora		
B374	Subsp. *chrysanthemi* (Lemattre, personal communication)	11
Escherichia coli K-12		
RP1 *rpsE*	Hfr P4X *uxaA1 metB1 relA1 spoT rpsE*	Spontaneous Spc^r mutant of RP1 (20)
1862	*met arg thyA uxaB1 rpsL recA*	KL16-99 × PB3 (19)
PB3	*his-1 tolC uxaC1 rpsL*	19
PB3 *thy*	*his-1 tolC uxac1 rpsL thyA*	Tmp^r, mutant of PB3
1866	Δ(*exuR'-exuT-uxaC-uxaA*) *argG6 rpsL recA*	—[a]
27	Δ(*exuR'-exuT-uxaC-uxaA*) *uxaB3 argG6 rpsL*	N. Cotte-Pattat[a]
RJ27	*uxuA1 thr leu argH rpsL*	25
RJ54	Δ(*uxuA-uxuB*) *thr leu arg rpsL recA*	J. Baudouy and R. Portalier, from RM15 (25)
K146	*kdgK1 metB his-1 argH1 rpsL malA1 lacY gal-6 recA*	Lagarde[a]
A146	*kdgA314 metB argH1 thiA rpsL malA1 lacY gal-6 recA*	Lagarde[a]
CT110 *recA*	*kdgR391 kdgT110 metB ilvD rpsL lac gal mal mtl recA*	*recA* derivative of CT110 (22)
PUT4	*kdgP3 kdgT4*(Ts) *exuT9 metB argG rpsL*	15
NT2	*exuT*::Mu *d* (Ap^r *lac*) *araD139* Δ*lacU169 thi rpsL*	12
NH1	*uxaA*::Mu *d* (Ap^r *lac*) *araD139* Δ*lacU169 thi rpsL*	12
NA1	*uxaB*::Mu *d* (Ap^r *lac*) *araD139* Δ*lacU169 thi rpsL*	12
P4X	Hfr *metB1 relA1 spoT*	13
Plasmid		
pULB113	Tra^r Ap Km Tc (Mu3A)[b] *tra*$^+$ *bla*$^+$ (TEM-2) tn*1* *tet*$^+$ *aphA*$^+$ (Mu3A)[d]	31[c]

[a] Strains received from the collection of the Laboratory of Microbiology, INSA, Lyon, France.
[b] Phenotype.
[c] pULB113 was derived from the RP4 plasmid described by Saunders and Grinsted (27), contrary to what was mentioned by mistake in reference 31.
[d] Genotype.

for resistance to the three antibiotics kanamycin (20 μg/ml), tetracycline (20 μg/ml), and ampicillin (20 μg/ml).

Complementation tests. Three methods were used to perform complementation tests.

(i) Spot tests. Transconjugants carrying an R' plasmid containing a hexuronate gene were suspended in a drop of 10^{-2} M $MgSO_4$ and spotted on a selective medium where 0.1 ml of an overnight culture of the recipient strain had been spread. The recipient was mutated in the hexuronate gene to be tested for complementation.

(ii) Titration after mating. Single drops of overnight cultures of donors carrying R' plasmids and appropriate recipients were incubated for 3 to 6 h at 33°C on A medium and suspended in 1 ml of 10^{-2} M $MgSO_4$. Mating mixtures were titrated on L medium supplemented with kanamycin and the suitable antibiotic to counterselect the donor, to measure the frequency of transfer of the plasmid, and on minimal medium supplemented with GU, GA, or KDG. The hexuronate gene tested for complementation was assumed to be present on the R' plasmid when about the same titer was obtained on the two types of plates (i.e., 10 to 100% of the frequency of transfer of the RP4 resistance markers).

(iii) Test of Kan^r transconjugants. Donor and recipient were mated on A medium as described in (ii). The Kan^r transconjugants were selected, and these Kan^r transconjugants were tested for their Gur, Gar, or Kdg phenotype.

Plasmid extraction and restriction analysis. Plasmid DNA was isolated from cleared sodium dodecyl sulfate lysates by density gradient centrifugation in a CsCl-ethidium bromide gradient as described by Betlach et al. (3). DNA was digested by endonuclease *Pst*I (prepared in this laboratory) according to Sharp et al. (28). Restriction fragments were separated by electrophoresis (5 h, at 50 mA) on a horizontal slab gel apparatus, using 40 mM Tris-acetate (pH 7.8)–5 mM NaAc–1 mM EDTA electrophoresis buffer containing 0.5 μg of ethidium bromide per ml. Gels were photo-

TABLE 2. Frequency at which R' plasmids carrying hexuronate genes are recovered[a]

Recipient strain used	Gene(s) selected	Frequency of recovery of Gar$^+$ (Gur$^+$, Kdg$^+$) transconjugants	Frequency of reversion to Gar$^+$ (Gur$^+$, Kdg$^+$) of the recipient strain
PUT4	*exuT*	10^{-6}	$<2.0 \times 10^{-9}$
NT2	*exuT*	2.0×10^{-7}	$<2.0 \times 10^{-9}$
RP1 *rpsE*	*uxaA*	10^{-5}	4.3×10^{-7}
1862	*uxaB*	3.6×10^{-6}	$<5.0 \times 10^{-9}$
PB3	*uxaC*	1.6×10^{-5}	$<4.0 \times 10^{-8}$
1866	*exuT-uxaC*	1.2×10^{-6}	$<3.6 \times 10^{-9}$
	exuT-uxaC-uxaA	1.2×10^{-6}	$<3.6 \times 10^{-9}$
27	*exuT-uxaC*	8.7×10^{-7}	$<6.0 \times 10^{-9}$
	exuT-uxaC-uxaA-uxaB	7.1×10^{-7}	$<6.0 \times 10^{-9}$
RJ27	*uxuA*	2.3×10^{-5}	$<4.0 \times 10^{-9}$
RJ54	*uxuA-uxuB*	6.0×10^{-5}	$<10^{-8}$
K146	*kdgK*	9.0×10^{-6}	$<1.3 \times 10^{-8}$
A146	*kdgA*	1.9×10^{-5}	1.8×10^{-8}
CT110 *recA*	*kdgT*	6.8×10^{-6}	5.6×10^{-9}

[a] Matings were performed on A medium plates for 6 h at 33°C as described in the text. The frequency of R' plasmid recovery was calculated as number of Gur$^+$ (Gar$^+$, Kdg$^+$) transconjugants per number of recipient cells which received plasmid pULB113. The frequency of transfer of plasmid pULB113 was 20 to 100% per input donor cell.

graphed under UV light. Restriction fragments were measured on a Hewlett-Packard digitizer HP9874A.

Southern transfer of restriction fragments and DNA-DNA hybridization. DNA transfer from agarose gels to nitrocellulose filters was performed essentially as described by Southern (29). ^{32}P-nick translation of Mu DNA was performed according to Maniatis et al. (17). The hybridization was performed according to Denhardt (8).

RESULTS AND DISCUSSION

Isolation of R' plasmids carrying the hexuronate genes of B374. B374, the *Erwinia carotovora* subsp. *chrysanthemi* strain used in this work, does not naturally grow on GU or KDG as a sole carbon source. However, mutants able to use those sugars occur spontaneously at a frequency of 10^{-6}, showing that all genes necessary for the uptake and degradation of those substrates are present in the strain. We chose to clone the various genes of B374 involved in the metabolism of hexuronates by performing heterospecific matings between B374(pULB113) as donor and *E. coli* recipients for two reasons: (i) *E. coli* strains mutated in each of the different hexuronate genes are available, allowing selection of R' plasmids derived from *Erwinia* sp. that are able to complement the *E. coli* defect; (ii) chromosomal recombination is very rare due to nonhomology, so that most of the *E. coli* transconjugants recovered are complemented for their hexuronate defect as a result of acquiring an R' plasmid. B374(pULB113) was mated with different *E. coli* recipient strains mutated in one or more hexuronate genes, and transconjugants able to grow on GA, GU, or KDG were selected on minimal media supplemented with the appropriate substrate, as described in Material and Methods.

Gur$^+$, Gar$^+$, or Kdg$^+$ transconjugants were recovered at frequencies ranging from 10^{-7} to 10^{-5} in all matings (Table 2). This is similar to the frequencies of recovery of R' plasmids carrying other markers in *E. coli*(pULB113) × *E. coli* matings (31).

Since we used *E. coli* mutants in each of the hexuronate genes, this means that B374 possesses genes equivalent to all *E. coli* genes involved in the metabolism of the hexuronates, including those coding for tansport, and that all of these genes can be expressed in *E. coli*. When the Gur$^+$ and Gar$^+$ transconjugants were grown on nonselective medium, some lost the hexuronate marker in both Rec$^+$ and *recA* backgrounds. (The frequencies at which the markers are lost range from <1 to 20%.) This instability favors the hypothesis that the *Erwinia* sp. genes are located on the plasmid; this was confirmed by further physical analysis of the transconjugants (see below).

Genetic organization of the hexuronate genes in B374. Transconjugants able to catabolize GU were recovered at about the same frequency whether B374(pULB113) was mated with a single *uxuA* or Δ(*uxuA-uxuB*) *E. coli* recipient (Table 2). Similarly, transconjugants able to catabolize GA were recovered at the same frequency whether single *exuT*, *uxaA*, *uxaB*, or *uxaC* *E. coli* mutants or Δ(*exuT-uxaC-uxaA*)*uxaB* *E. coli* was used as the recipient. This strongly suggests that *uxuA* and *uxuB*, on the one hand, and *exuT*, *uxaA*, *uxaB*, and *uxaC*, on the other hand, are

TABLE 3. Complementation tests with the R' plasmids selected for the presence of the different hexuronate genes[a]

Gene(s) selected	No. of R' plasmids tested	Classes of R' plasmids recovered	No. of R' plasmids found in each class	Method of testing[b]
exuT	100	R' *exuT-uxaC-uxaB-uxaA*	91	i
		R' *exuT-uxaC*	1	i, ii
		R' *exuT*	8	i, ii
exuT-uxaC	100	R' *exuT-uxaC-uxaB-uxaA*	90	i
		R' *exuT-uxaC*	10	i
uxaA	21	R' *exuT-uxaC-uxaB-uxaA*	20	i, ii for 5 candidates
		R' *uxaC-uxaB-uxaA*	1	i, ii
uxaB	20	R' *exuT-uxaC-uxaB-uxaA*	20	i
uxaC	20	R' *exuT-uxaC-uxaB-uxaA*	6	i
		R' *exuT-uxaC-uxaB*	1	i, ii
		R' *uxaC-uxaB-uxaA*	8	i, ii, and iii for 2 candidates
		R' *uxaC-uxaB*	5	i, ii, iii
uxuA	100	R' *uxuA-uxuB*	67	i
		R' *uxuA-uxuB-kdgK*	23	i
kdgK	20	R' *uxuA-uxuB-kdgK*	20	i–iii for 10 candidates
kdgA	20	R' *kdgA*	20	i, ii for 3 candidates
kdgT	15	R' *kdgT*	15	i, ii, and iii for 6 candidates

[a] R' plasmids selected for their ability to complement one (or more) hexuronate genes were tested for their ability to complement the other hexuronate genes. The different classes of plasmids found are shown in column 3. Only those genes found to be expressed by the plasmid are noted.

[b] i, Spot test; ii, titration after mating iii, test of Kan^r transconjugants. The methods are described in the text.

linked in B374. To know more about the linkage of the different genes cloned, several R' plasmids selected for their ability to complement one hexuronate gene were transferred to *E. coli* strains mutated in other hexuronate genes to test for complementation. The results of this functional analysis are presented in Table 3. They suggest that in B374 the hexuronate genes are clustered in four regions of the chromosome. Of 100 R' plasmids selected for their ability to complement *uxuA*, all complemented *uxuB* as well and about one-third complemented *kdgK*. When R' plasmids complementing *kdgK* were selected, they all also complemented *uxuA* and *uxuB* (20 of 20). The *uxuA*, *uxuB*, and *kdgK* genes are thus clustered in B374. This is not the case in *E. coli*, where the *kdgK* gene is separated from *uxuA-uxuB* by ~20 min on the chromosome (Fig. 3).

A second cluster was found which contains the *exuT*, *uxaA*, *uxaB*, and *uxaC* genes. We found six different classes of R' plasmids complementing one or more genes in that region, *exuT-uxaC-uxaB-uxaA*, *exuT-uxaC-uxaB*, *exuT*, *uxaC-uxaB*, *uxaC-uxaB-uxaA*, and *exuT-uxaC*. The proportion of these different classes varied depending on the gene which had been selected (see Table 3). When R' *uxaC* plasmids were selected, only a minority (6 of 20) complemented the whole *exuT-uxa* region. When R' *uxaA*, R' *uxaB*, or R' *exuT* plasmids were selected, most of them complemented *exuT* and all of the *uxa* genes. The reason for this discrepancy is not yet elucidated. The *uxaB* gene probably lies between *uxaC* and *uxaA* since we found one plasmid which complements *exuT* and *uxaC* but not *uxaB* and *uxaA* and several plasmids which complement *exuT*, *uxaC*, and *uxaB* but not *uxaA*. That we never obtained an R' plasmid which can complement *uxaA* but not *uxaC* and *uxaB* strongly suggests that these three genes belong to a single transcription unit proceeding

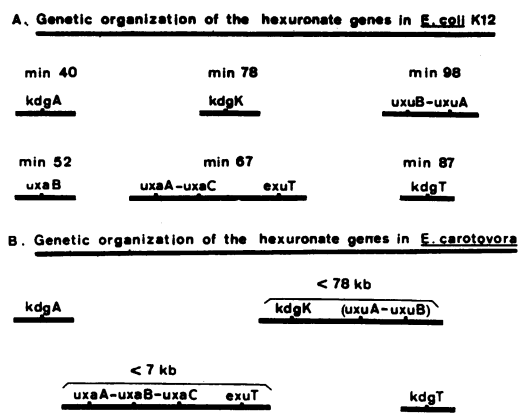

FIG. 3. Comparison of the genetic organization of the hexuronate genes in *E. coli* K-12 and *Erwinia carotovora*. For *E. coli* K-12 the chromosome location of the genes is given according to Bachmann et al (2).

from *uxaC* towards *uxaA*. Since we found R' plasmids complementing only *exuT* and others complementing *uxaA*, *uxaB*, and *uxaC* but not *exuT*, the *exuT* gene does not belong to a transcription unit with *uxaC*, *uxaB*, and *uxaA*. In *E. coli*, the *exuT*, *uxaC*, and *uxaA* genes are clustered, with *uxaC* and *uxaA* belonging to a transcription unit which is different from that of *exuT*; *uxaB* is not linked to these three genes (see Fig. 3).

R' plasmids selected for their ability to complement *kdgA* mutants complement only *kdgA*, and none of the other R' plasmids can complement *kdgA*. In B374 the *kdgA* gene, therefore, seems to be unlinked to any other hexuronate gene.

The fourth region contains the *kdgT* gene, which is not linked to any of the other hexuronate genes.

Physical analysis of the R' plasmids. Two R' plasmids representative of each of the 10 classes described in Table 3 were purified and digested with endonuclease *Pst*I. Restriction fragments were separated by electrophoresis on a 0.7% agarose gel as described in Materials and Methods. If the R' plasmids have indeed been formed by mini-Mu-mediated transposition, they should carry the transposed chromosomal segment inserted at the point of insertion of the mini-Mu prophage and flanked by two prophages in the same orientation. Consequently, since the *Pst*I enzyme cuts twice in the mini-Mu DNA, the restriction pattern of the R' plasmids should exhibit the six restriction fragments of the parental RP4::mini-Mu plasmid, a duplication of the internal mini-Mu fragment, and additional bands corresponding to the transposed bacterial DNA (31). This is schematized in Fig. 4. The digestions patterns of the R' plasmids are shown in Fig. 5. In most cases all parental plasmid fragments are indeed still present. In some cases, however, one of the junction fragments between the RP4 and the mini-Mu DNA (fragments C and D, Fig. 4) suffered a deletion (Fig. 5a, slot 5; Fig. 5c, slots 4, 7, and 8). The band corresponding to the internal mini-Mu fragment is always at least duplicated. A "Southern blotting-DNA hybridization" (29), using ^{32}P-labeled Mu DNA as the probe (Fig. 5b), confirms that (i) the three fragments of pULB113 which contain Mu DNA (C, D, F) are conserved in the R' plasmids except for fragment C in slot 5 which, as mentioned above, has suffered a small deletion; (ii) in all of the R' plasmids except one (slot 3) there are only two Mu-chromosomal DNA fusion fragments and thus only two copies of the mini-Mu and most probably only one segment of transposed chromosomal DNA. The R' *kdgK* plasmids, all of which complement the *uxuA* and *uxuB* genes, are longer than the plasmids which complement *uxuA* and *uxuB* but not *kdgK* (See Fig. 5a). Five bands (noted by →) were shared by the three R' *kdgK* plasmids analyzed but were absent from the R' *uxuA-uxuB* plasmids. These fragments represent about 23 kb of DNA. The two sets of genes, *kdgK* and *uxuA-uxuB*, seem thus rather far from each other. A possible explanation for the fact that all of the R' *kdgK* plasmids carry the *uxu* genes involves the presence of a preferential site of insertion of the mini-Mu near *uxuA-uxuB* with the *uxu* genes between this insertion site and the *kdgK* gene. If that had been the case, one of the mini-Mu bacterial DNA junction fragments should have been the same in the five R' plasmids carrying this region.

As shown in the autoradiograph in Fig. 5b, there is no common junction fragment in the five R' plasmids analyzed. Thus, there is no preferential site of insertion of the mini-Mu in that region. Since *kdgK* and *uxuA-uxuB* are separated by at least 23 kb, they probably do not belong to the same operon, although the existence of a single transcription unit going from *uxuA-uxuB* towards *kdgK* could have explained the 100% linkage found between *kdgK* and *uxuA-uxuB* (5). One possible explanation for the observed discrepancy in linkage would be that a function required for the expression of *kdgK* lies very near *uxuA-uxuB*.

Figure 5c shows the restriction patterns of the R' plasmids carrying the *kdgT* gene and the *exuT-uxa* region. The four genes *exuT-uxaC-uxaB-uxaA* of *Erwinia* sp. are present on a chromosomal DNA segment no longer than ~7 kb (i.e., the size of the chromosomal DNA carried by the shortest R' plasmid carrying that region, slot 5). All but one of the R' plasmids carrying the *uxaC* gene share two fragments of

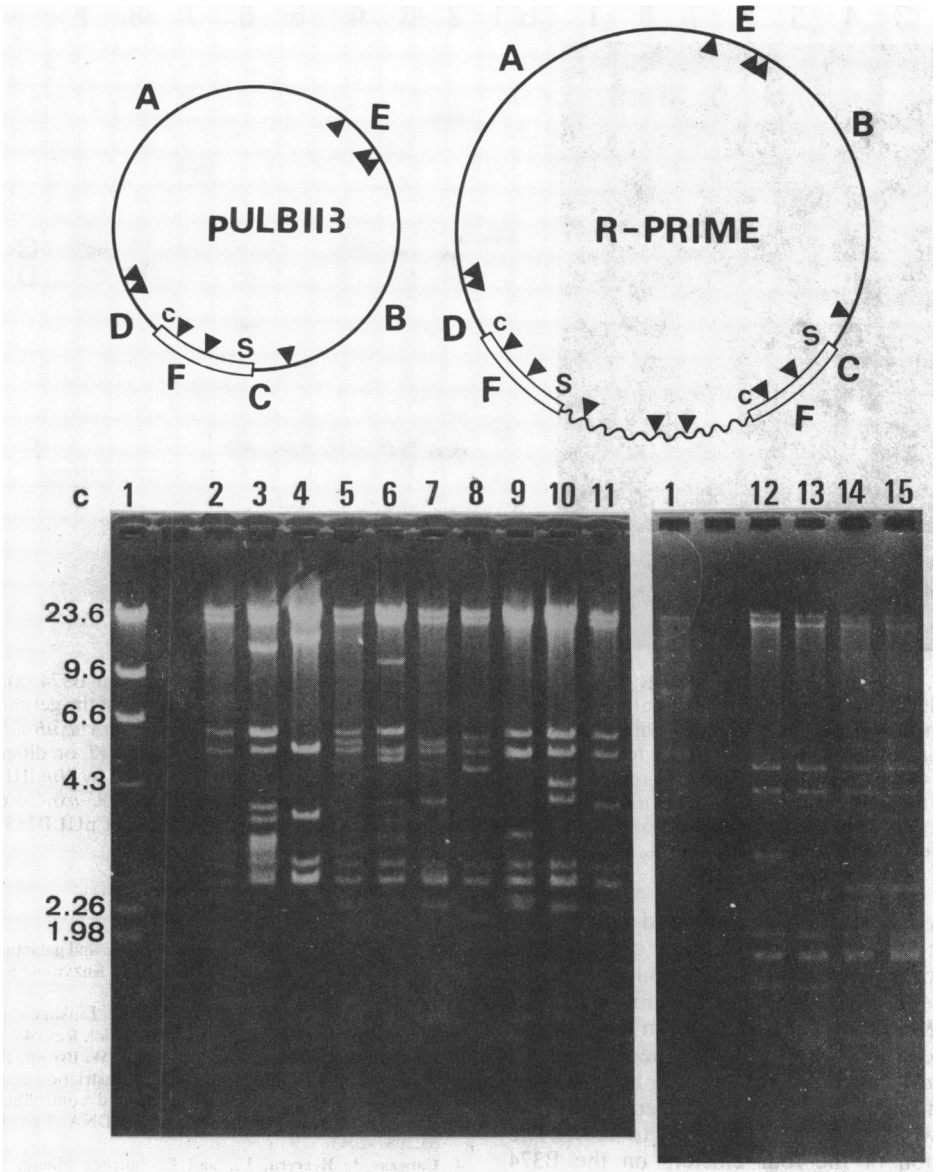

FIG. 4. Physical structure of pULB113 and R′ plasmid derived from pULB113 and formed by mini-Mu-mediated transposition of bacterial DNA. Symbols: ▼ = *Pst*I sites. The six largest fragments of pULB113 are noted A, B, C, D, E, F. F is the internal *Pst*I fragment of the mini-Mu. □ = Mini-Mu; c = immunity end of the mini-Mu; S = S end of the mini-Mu. ⁓ = Bacterial DNA. The maps are drawn according to Van Gijsegem and Toussaint (31).

2.3 and 1.3 kb. These two fragments must be located between the *exuT* and the *uxaB* genes. The only R′ *exuT-uxaC-uxaB* plasmid which does not present these two fragments has to be further analyzed; it probably has suffered rearrangements. Strikingly, the size of the bacterial DNA transposed in pULB113 depends on the chromosomal region selected. Although the R′ *uxuA-uxuB* genes carry 70 to 80 kb, the R′ *exuT-uxa* genes usually carry only ~10 kb.

Conclusion. From all these results we conclude that (i) B374 contains nine genes involved in the transport and degradation of GA and KDG equivalent to the *kdgK*, *uxuA*, *uxuB*, *exuT*, *uxaA*, *uxaB*, *uxaC*, *kdgA*, and *kdgT* genes of *E. coli*; and (ii) these genes in B374 are organized in

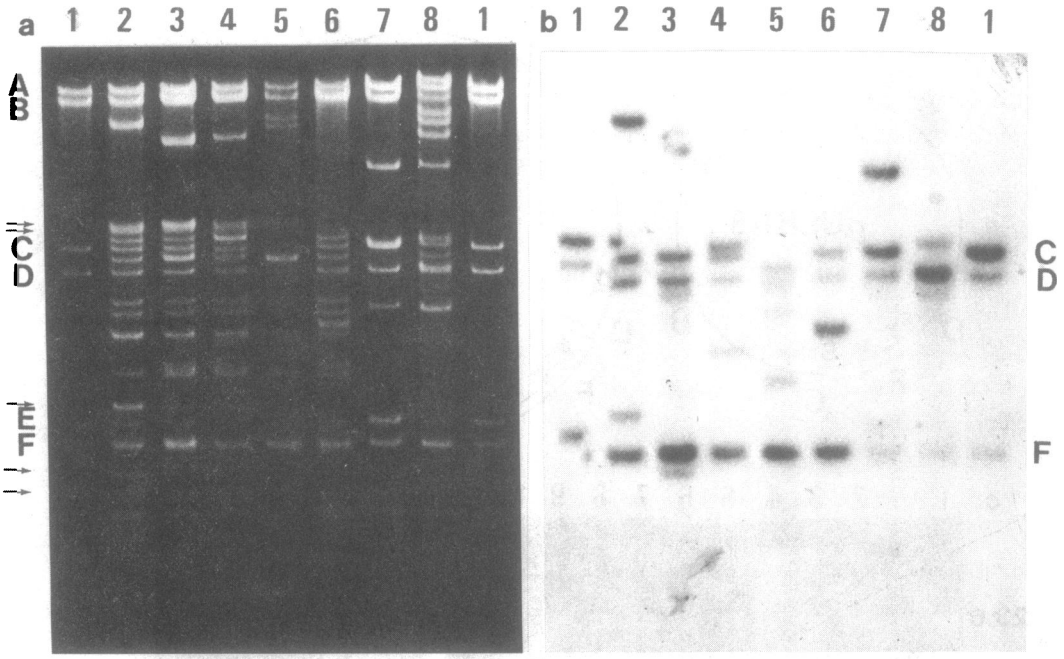

FIG. 5. Digestion with *Pst*I of the R′ plasmids carrying *kdgK*, *uxuA-uxuB*, or *kdgA* genes of B374 (a). (1) pULB113; (2, 3, 4) R′ *uxuA-uxuB-kdgK*; (5, 6) R′ *uxuA-uxuB*; (7, 8) R′ *kdgA*. (b) Autoradiograph of the gel shown in (a) after blotting and hybridization with a Mu ^{32}P-labeled probe. (1) pULB113; (2, 3, 4) R′ *uxuA-uxuB-kdgK*; (5, 6) R′ *uxuA-uxuB*; (7, 8) R′ *kdgA*. (c) Digestion with *Pst*I of the R′ plasmids carrying *kdgT* or different segments of the *exuT-uxaC-uxaB-uxaA* region of B374. (1) Size markers given by λ DNA digested by *Hin*dIII; (2) pULB113; (3, 4) R′ *kdgT*; (5, 6) R′ *exuT-uxaC-uxaB-uxaC*; (7, 8) R′ *exuT-uxaC-uxaB*; (9, 10) R′ *uxaC-uxaB-uxaA*; (11, 12) R′*uxaC-uxaB*; (13) R′ *exuT-uxaC*; (14, 15) R′ *exuT*. The six largest fragments of pULB113 are noted A to F as in the legend to Fig. 4.

a slightly different way as compared with *E. coli*. They lie in four regions of the chromosome instead of five. *uxaB* is srongly linked to *uxaC* and *uxaA* in B374, whereas it is not in *E. coli*, and *kdgK* is closer to *uxuA-uxuB* in B374 than it is in *E. coli* (see Fig. 3). (iii) In the *exuT-uxaC-uxaB-uxaA* cluster, there seem to be two transcription units. One certainly covers *exuT*. The second would proceed from *uxaC* to *uxaA*. The localization of the four clusters on the B374 chromosome is presently in progress (F. Van Gijsegem and N. Hugouvieux-Cotte-Pattat, manuscript in preparation).

ACKNOWLEDGMENTS

We thank J. Robert-Baudouy for initiating us to the hexuronate system and sending strain, M.-A. Mandrand for giving us KDG, A. Herzog for his help in preparing the *Pst*I enzyme, and M. Pato for his critical reading of the manuscript. We wish to especially thank Nicole Hugouvieux Cotte-Pattat for fruitful collaboration, very useful advice, and construction of strain 27.

This work was supported by an ATP Internationale (no. 8105) of the French Centre National de la Recherche Scientifique and by grants of the convention ULB-IRE/NIRCEN/SCK. F.V.G. is Chargé de Recherches and A.T. is Chercheur Qualifié from the Fonds National de la Recherche Scientifique.

LITERATURE CITED

1. **Ashwell, G.** 1962. Enzymes of glucuronic and galacturonic acid metabolism in bacteria. Methods Enzymol. **5**:190–208.
2. **Bachman, G. J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. Microbiol. Rev. **44**:1–56.
3. **Betlach, M. D., V. Hershfield, L. Chow, W. Brown, H. M. Goodman, and H. W. Boyer.** 1976. A restriction endonuclease analysis of the bacterial plasmid controlling the *EcoR*1 restriction and modification of DNA. Fed. Proc. **35**:2037–2043.
4. **Cabezas de Herrera, E., and E. Sanchez Maeso.** 1978. Isolation, purification and estimation of the different pectic enzymes of *Erwinia carotovora*, p. 617–629. *In* Proceedings of the Fourth International Conference on Plant Pathogenic Bacteria, Angers, France.
5. **Cabezon, T., F. van Gijsegem, A. Toussaint, M. Faelen, and A. Bollen.** 1978. Phage Mu-1 mediated transposition: a tool to study the organization of ribosomal protein genes in *E. coli*. Mol. Gen. Genet. **161**:291–296.
6. **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. **142**:111–119.
7. **Chatterjee, A. K., and M. P. Starr.** 1980. Genetics of *Erwinia* species. Annu. Rev. Microbiol. **34**:645–676.
8. **Denhardt, D.** 1976. A membrane filter-technique for the detection of complementary DNA. Biochem. Biophys. Res. Commun. **23**:641–646.
9. **Faelen, M., and A. Toussaint.** 1976. Bacteriophage Mu-1, a tool to transpose and to localize bacterial genes. J. Mol. Biol. **104**:525–539.

10. **Fry, B. A.** 1959. Conditions for the infection of *E. coli* with lambda phage and for establishment of lysogeny. J. Gen. Microbiol. **21**:676–689.
11. **Hamon, Y., and Y. Peron.** 1961. Les proprietes antagonistes reciproques parmi les *Erwinia*. Discusssion de la position taxonomique de ce genre. C. R. Acad. Sci. **253**:913–915.
12. **Hugouvieux-Cotte-Pattat, N., and J. Robert-Baudouy.** 1981. Isolation of fusions between the *lac* genes and several genes of the *exu* regulon: analysis of their regulation, determination of the transcription direction of the *uxaC-uxaA* operon, in *Escherichia coli* K12. Mol. Gen. Genet. **182**:279–287.
13. **Jacob, F., and E. L. Wollman.** 1961. Sexuality and the genetics of bacteria. Academic Press, Inc., New York.
14. **Kilgore, W. W., and M. P. Starr.** 1959. Catabolism of galacturonic and glucuronic acids by *Erwinia carotovora*. J. Biol. Chem. **234**:2227–2235.
15. **Lagarde, A. E., and F. R. Stoeber.** 1977. *Escherichia coli* K-12 structural *kdgT* mutants exhibiting thermosensitive 2-keto-3-deoxy-D-gluconate uptake. J. Bacteriol. **129**:606–615.
16. **Lennox, E. S.** 1953. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190–206.
17. **Maniatis, T., A. Jeffrey, and D. Kleid.** 1975. Nucleotide sequence of the rightward operator of bacteriophage lambda. Proc. Natl. Acad. Sci. U.S.A. **72**:1184–1188.
18. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Portalier, R. C., J. M. Robert-Baudouy, and G. M. Nemoz.** 1974. Etude de mutations affectant les genes de structure de l'isomerase uronique et de l'oxydoreductase altronique chez *Escherichia coli* K12. Mol. Gen. Genet. **128**:301–319.
20. **Portalier, R. C., J. M. Robert-Baudouy, and F. R. Stoeber.** 1972. Localisation genetique et caracterisation biochimique de mutations affectant le gene de structure de l'hydrolyase altronique chez *Escherichia coli* K12. Mol. Gen. Genet. **118**:335–350.
21. **Portalier, R., J. Robert-Baudouy, and F. Stoeber.** 1980. Regulation of *Escherichia coli* K12 hexuronate system genes: *exu* regulon. J. Bacteriol. **143**:1095–1107.
22. **Pouyssegur, J., and A. Lagarde.** 1973. Systeme de transport du 2-keto-3-desoxy-gluconate chez *E. coli* K12: localisation d'un gene de structure et de son operateur. Mol. Gen. Genet. **121**:163–180.
23. **Preiss, J., and G. Ashwell.** 1963. Polygalacturonic acid metabolism in bacteria. I. Enzymatic formation of 4-deoxy-L-threo-5-hexoseulose uronic acid. J. Biol. Chem. **238**:1571–1576.
24. **Preiss, J., and G. Ashwell.** 1963. Polygalacturonic acid metabolism in bacteria. II. Formation and metabolism of 3-deoxy-D-glycero-2,5-hexodiulosonic acid. J. Biol. Chem. **238**:1577–1583.
25. **Robert-Baudouy, J. M., and R. C. Portalier.** 1974. Mutations affectant le catabolisme du glucuronate chez *Escherichia coli* K12. Mol. Gen. Genet. **131**:31–46.
26. **Robert-Baudouy, J., R. Portalier, and F. Stoeber.** 1981. Regulation of hexuronate system genes in *Escherichia coli* K-12: multiple regulation of the *uxu* operon by *exuR* and *uxuR* gene products. J. Bacteriol. **145**:211–220.
27. **Saunders, J. R., and J. Grinsted.** 1972. Properties of RP4, an R factor which originated in *Pseudomonas aeroginosa* S8. J. Bacteriol. **112**:690–696.
28. **Sharp, P., B. Sugden, and J. Sambrook.** 1973. Detection of two restriction endonuclease activities in *Haemophilus parainfluenzae* using analytical agarose electrophoresis. Biochemistry **12**:3055–3063.
29. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments. J. Mol. Biol. **98**:503–517.
30. **Starr, M. P., and A. K. Chatterjee.** 1972. The genus *Erwinia*: enterobacteria pathogenic to plant and animals. Annu. Rev. Microbiol. **26**:389–426.
31. **van Gijsegem, F., and A. Toussaint.** 1982. Chromosome transfer and R-prime formation by an RP4::mini-Mu derivative in *E. coli, Salmonella typhimurium, Klebsiella pneumoniae* and *Proteus mirabilis*. Plasmid **7**:30–44.