JOURNAL OF BACTERIOLOGY, Mar. 1987, p. 1223–1231
0021-9193/87/031223-09$02.00/0

Vol. 169, No. 3

Hexuronate Catabolism in *Erwinia chrysanthemi*

NICOLE HUGOUVIEUX-COTTE-PATTAT* AND JANINE ROBERT-BAUDOUY

Laboratoire de Microbiologie, Institut National des Sciences Appliquées, 69621 Villeurbanne, France

Received 5 May 1986/Accepted 16 December 1986

In the phytopathogenic enterobacterium *Erwinia chrysanthemi*, the catabolism of hexuronates is linked to the degradation of pectic polymers. We isolated Mu *lac* insertions in each gene of the hexuronate pathway and used genetic fusions with *lacZ* (the β-galactosidase gene of *Escherichia coli*) to study the regulation of this pathway. Three independent regulatory genes (*exuR*, *uxuR*, and *kdgR*) were found. Galacturonate and glucuronate were converted into 2-keto-3-deoxygluconate (KDG) by separate three-step pathways encoded by the *uxaC*, *uxaB*, and *uxaA* genes and the *uxaC*, *uxuB*, and *uxuA* genes, respectively. The two aldohexuronates entered the cell by a specific transport system, encoded by *exuT*. Wild-type strain 3937 was unable to use glucuronate as a carbon source since glucuronate was unable to induce the *exuT* expression. Mutants able to use glucuronate possessed an inactivated *exuR* gene. The product of the regulatory gene *exuR* negatively controlled the expression of *exuT*, *uxaC*, *uxaB*, and *uxaA*, which was inducible in the presence of galacturonate. The two genes specifically involved in glucuronate catabolism, *uxuA* and *uxuB*, formed two independent transcriptional units regulated separately. *uxuB* expression was not inducible, whereas *uxuA* expression was induced in the presence of glucuronate and controlled by the *uxuR* product. KDG, the common end product of both pathways, is cleaved by the *kdgK* and *kdgA* gene products. KDG enters the cell by a specific transport system, encoded by *kdgT*. The regulatory gene *kdgR* controlled the expression of *kdgT*, *kdgK*, and *kdgA* and partially that of the *pel* genes encoding pectate-lyases. The real inducer of pectate-lyase synthesis, originating from catabolism of galacturonate or glucuronate, appeared to be KDG. The genes of *E. chrysanthemi* affecting hexuronate catabolism are separated into six independent transcriptional units: *exuT*, *uxaCBA*, *uxuA*, *uxuB*, *kdgK*, and *kdgA*, but only three gene clusters were localized on the genetic map: *exuT-uxaCBA*, *uxuA-uxuB-kdgK*, and *kdgA-exuR*.

Erwinia chrysanthemi is a phytopathogenic enterobacterium responsible for soft-rot disease of many plant species. Its pathogenicity is chiefly due to the action of pectate-lyases, which allow the bacteria to digest plant cell walls (5, 28). Pectins are degraded according to the pathway described in Fig. 1. Pectins are first demethoxylated into polygalacturonate (PGA) by a pectin methyl-esterase, encoded by the *pme* gene. Two types of enzymes cleave polygalacturonate (7): the pectate-lyases, encoded by the *pel* genes, which generate unsaturated digalacturonate as their major end product, and the polygalacturonases, encoded by the *peh* genes, which generate saturated digalacturonate as an end product. In the strain used in the present study (strain 3937), five *pel* genes and one *pme* gene have been cloned in a gene library (15). These enzymes acting directly on the pectic polymer are secreted by the bacteria. In contrast, further degradation of the saturated or unsaturated oligogalacturonides appears to be the function of intracellular enzymes (7). The oligogalacturonate-lyase, encoded by the *ogl* gene, cleaves the digalacturonates to give two kinds of monomers: galacturonate and 5-keto-4-deoxyuronate (6). Galacturonate is then converted to 2-keto-3-deoxygluconate (KDG) in a three-step pathway encoded by the *uxaC*, *uxaB*, and *uxaA* genes. KDG is then phosphorylated by the *kdgK* product and cleaved by the *kdgA* product. Galacturonate can enter the cell by a transport system encoded by the *exuT* gene and specific for the two hexuronates, galacturonate and glucuronate (10).

* Corresponding author.

In *Escherichia coli* the catabolism of glucuronate follows a pathway similar to that of galacturonate degradation, leading to the formation of KDG in three steps encoded by the *uxaC*, *uxuB*, and *uxuA* genes (1, 26, 27) (Fig. 1). Some *Erwinia* species also behave in the same manner (14). However, *E. chrysanthemi* B374 and 3937 cannot use glucuronate as a carbon source for growth, probably because this compound does not induce the synthesis of its own transport system (10). Mutants able to use glucuronate constitutively expressed the four genes *exuT*, *uxaC*, *uxaB*, and *uxaA* that are involved mostly in galacturonate utilization.

In *E. coli* and *E. chrysanthemi*, KDG is also unable to enter the wild-type cells. Mutants able to use KDG show a derepressed expression of *kdgT*, the gene encoding a KDG transport system. This phenotype results from a mutation in either the promoter region of *kdgT* or the regulatory gene *kdgR* controlling *kdgT* expression. In *E. coli kdgR* mutants, *kdgK* and *kdgA* are also constitutively expressed (22).

Genetic analysis of hexuronate catabolism in *E. chrysanthemi* B374 has been carried out (30, 32). However, the isolation of insertion mutations in B374 caused difficulties: Tn*9* insertions provoked DNA rearrangements (30), Mu phage lysogenized B374 very poorly (29), and no other tools exist to introduce transposons into this strain. Genetic tools and analysis of pectinolysis have been extensively developed in the more virulent *E. chrysanthemi* 3937 (8, 13, 15, 16, 25). In a *lacZ* mutant of strain 3937 (12), Mu phage and its derivatives have been used to isolate mutations or to construct genetic fusions with the *E. coli lacZ* gene (13).

We describe here the isolation of Mu *lac* insertions in each gene that affect hexuronate catabolism in strain 3937. Some

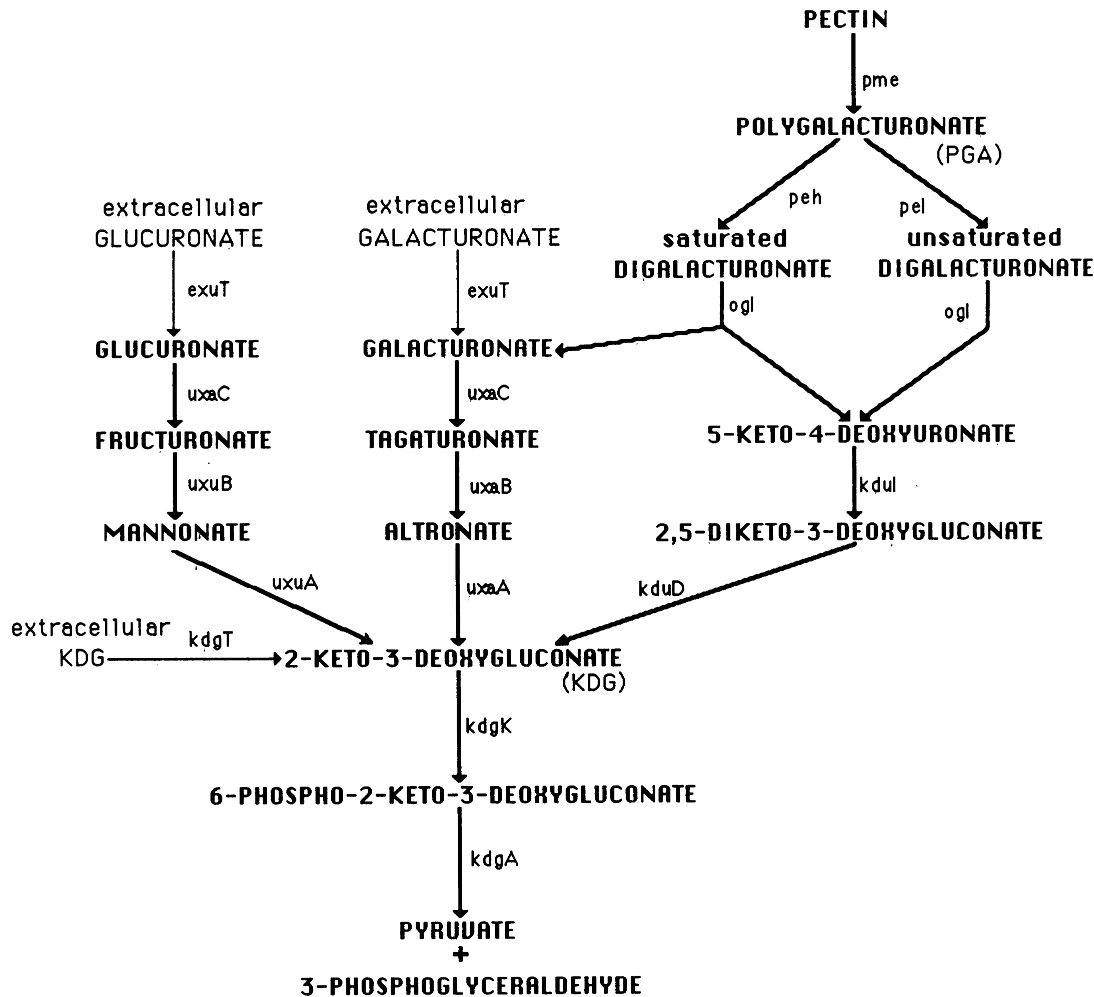

FIG. 1. Degradative pathway of hexuronates and pectin in *E. chrysanthemi*. The different steps are catalyzed by the products of the genes indicated near the corresponding arrows: *pme*, pectin-methyl-esterase; *pel*, pectate-lyase (in fact five genes, *pelA* to *pelE*); *peh*, pectate-hydrolase; *ogl*, oligogalacturonate-lyase; *kduI*, 5-keto-4-deoxyuronate isomerase; *kduD*, KDG oxidoreductase; *uxaC*, uronate isomerase; *uxaB*, altronate oxidoreductase; *uxuB*, mannonate oxidoreductase; *uxaA*, altronate hydrolase; *uxuA*, mannonate hydrolase; *kdgK*, KDG kinase; and *kdgA*, 2-keto-3-deoxy-6-phosphogluconate aldolase. The transport systems mediating the entry of sugars into the cells are also indicated: *exuT*, aldohexuronate transport system, and *kdgT*, KDG transport system. The wild-type strain 3937 cannot grow with glucuronate as a carbon source because of the lack of *exuT* induction. A mutation to glucuronate utilization leads to the constitutive production of genes that are involved mostly in galacturonate utilization (*exuT*, *uxaC*, *uxaB*, and *uxaA*).

of these insertions resulted in genetic fusions to the *E. coli lacZ* gene, which permitted analysis of the regulation of the structural genes of the hexuronate pathway. Four regulatory units and three regulator genes were analyzed. The organization and the precise localization of these genes on the *E. chrysanthemi* 3937 chromosome were determined.

MATERIALS AND METHODS

Phenotypes. The phenotypes referred to in this study are designated as follows: Gar$^+$ Gar$^-$, Gur$^+$ Gur$^-$, and Lac$^+$ Lac$^-$ for the ability (+)/inability (−) to catabolize galacturonate, glucuronate, and lactose, respectively. Antibiotic resistance phenotypes are Ap^r (ampicillin) and Km^r (kanamycin).

Strains and growth conditions. The *E. chrysanthemi* strains used in this study are listed in Table 1. They all originated from the wild-type strain 3937 (strain 3937j in reference 16). Cells were usually grown at 30°C in complete medium L or in synthetic medium M63 (17) supplemented with a carbon source (0.2%) and, when necessary, amino acids (40 μg/ml) and bases (2 to 50 μg/ml).

Enzyme assays. The total pectate-lyase activity was measured after toluene addition to late-log-phase cultures by the method of Moran and Starr (18). β-Galactosidase was assayed by the method of Miller (17).

The assay of altronate hydrolyase, altronate oxidoreductase, uronate isomerase, KDG kinase, and phospho-KDG aldolase (products of *uxaA*, *uxaB*, *uxaC*, *kdgK*, and *kdgA*, respectively) were performed on French press extracts as previously described (13, 30). The hexuronate transport system (the *exuT* product) was measured for whole cells by a rapid filtration technique (10).

The mannonate oxidoreductase (*uxuB* product) was measured by monitoring the increase in A_{340} in a mixture assay consisting of 50 mM glycylglycine buffer (pH 8.4), 10 mM NAD, and 5 mM mannonate. The mannonate hydrolase (*uxuA* product) was measured by monitoring the formation

TABLE 1. *E. chrysanthemi* strains[a]

Strain	Genotype	Reference or origin
3937	Wild type	16
A40	*lmrT*(Con) *exuR2*	12
L37	*lmrT*(Con) *exuR2 lacZ37*	12
L2	*lmrT*(Con) *lacZ2*	12
A430	*lmrT*(Con) *lacZ2 arg-10*	NTG derivative of L2
A555	*lmrT*(Con) *lacZ2 arg-10 met-2*	NTG derivative of A430
AK3727	*his-1 trp-1 strA*	16
A805	*his-1 trp-1 thy-1 strA*	Spontaneous trimethoprim-resistant mutant of AK3727
A853	*his-1 trp-1 thy-1 strA nalA*	Spontaneous mutant of A805 resistant to 100 μg of nalidixic acid per ml
AK38455	*xyl-1 leu-1 met-10 strA*	16
A849	*xyl-1 leu-1 met-10 strA rifR*	Spontaneous rifampicin-resistant mutant of AK38455
A862	*xyl-1 leu-1 met-10 exuT*(Con) *strA rifR*	Spontaneous Gur$^+$ derivative of A849
A863	*xyl-1 leu-1 met-10 exuT*(Con) *kdgK*::Mu dl1681 *strA rifR*	Transduction of A862 by a φEC2 stock made on A825
A1067	*leu-1 xyl-1 ser-2 strA*	Met$^+$ Ser$^-$ derivative of AK38455 (A. Phol)
AK3710	*arg-1 ile-2 strA*	16
A907	*arg-1 ile-2 strA rifR*	Transduction of AK3710 by a φEC2 stock made on A862
A147	*lmrT*(Con) *exuR2 kdgA*	NTG mutant of A40
A576	*lmrT*(Con) *lacZ2 arg-10 kdgK*	NTG mutant of A430
A231	*lmrT*(Con) *lacZ37 exuR2 uxaA*::Mu d(Ap *lac*)	Derivative of L37
A233	*lmrT*(Con) *lacZ37 exuR2 kdgK*::Mu d(Ap *lac*)	13
A235	*lmrT*(Con) *lacZ37 exuR2 uxaC*::Mu d(Ap *lac*)	Derivative of L37
A238	*lmrT*(Con) *lacZ37 exuR2 kdgA*::Mu d(Ap *lac*)	13
A259	*lmrT*(Con) *lacZ37 exuR2 exuT*::Mu d(Ap *lac*)	Derivative of L37
A263	*lmrT*(Con) *lacZ37 exuR2 uxuB*::Mu d(Ap *lac*)	Derivative of L37
A287	*lmrT*(Con) *lacZ37 exuR2 uxuA*::Mu d(Ap *lac*)	Derivative of L37
A288	*lmrT*(Con) *lacZ37 exuR2 uxaB*::Mu d(Ap *lac*)	Derivative of L37
A231k	Same as A231 but *uxaA*::Mu dl1681	Mu dl1681 instead of Mu d(Ap *lac*)
A235k	Same as A235 but *uxaC*::Mu dl1681	Mu dl1681 instead of Mu d(Ap *lac*)
A259k	Same as A259 but *exuT*::Mu dl1681	Mu dl1681 instead of Mu d(Ap *lac*)
A288k	Same as A288 but *uxaB*::Mu dl1681	Mu dl1681 instead of Mu d(Ap *lac*)
A627	*lmrT*(Con) *lacZ2 arg-10 exuR*::Mu d(Ap *lac*)	Derivative of A430
A633	*lmrT*(Con) *lacZ2 arg-10 uxaB*::Mu d(Ap *lac*)	Derivative of A430
A658	*lmrT*(Con) *lacZ2 arg-10 met-2 uxaC*::Mu d(Ap *lac*)	Derivative of A555
A825	*lmrT*(Con) *lacZ2 kdgK*::Mu dl1681	Derivative of L2
A861	*lmrT*(Con) *lacZ37 exuR2 uxuA*::Mu dl1681	Derivative of L37
A837	*lmrT*(Con) *lacZ2 kdgR3*	Spontaneous KDG$^+$ mutant of L2 (G. Condemine)
A952	*lmrT*(Con) *lacZ2 kdgR3 uxaB*::Mu dl1681	Transduction of A837 by a φEC2 stock made on A288k

[a] Genotype symbols are according to Bachmann (2a). In addition, the following designations are used: *strA*, resistance to 100 μg of streptomycin per ml; *nalA*, resistance to 50 μg of nalidixic acid per ml; and *rifR*, resistance to 100 μg of rifampicin per ml. *lmrT*(Con) indicates that the transport system encoded by the gene *lmrT* and able to mediate entry of lactose, melibiose, and raffinose into the cells is constitutively expressed (12). *exuT*(Con) means that the expression of the hexuronate transport system, a product of *exuT*, is derepressed (10). Gur$^+$ and KDG$^+$ are phenotype designations used for the utilization of glucuronate and KDG, respectively. NTG, *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine.

of KDG in a mixture containing 200 mM glycylglycine buffer (pH 8.3), 50 mM 2-mercaptoethanol, 0.8 mM $FeSO_4$, and 3 mM mannonate. The reaction was stopped by adding 3 volumes of 10% trichloroacetic acid plus 20 mM $HgCl_2$. The amount of KDG was then determined by the periodate-thiobarbituric acid method (34).

Chemicals. The various intermediates of the PGA or hexuronate degradation were synthesized in our laboratory by previously published methods: tagaturonate (9), altronate and mannonate (23), fructuronate (2), KDG (20), 6-phospho-KDG (21), 5-keto-4-deoxyuronate and 2,5-diketo-3-deoxygluconate (24), and unsaturated digalacturonate (6).

Isolation of Mu *lac* insertions. Mu d(Ap *lac*) and Mu dl1681 (Km^r) were prepared by heat induction of *E. coli* MAL103 (3) and POI1681 (4), respectively. An overnight culture of the *E. chrysanthemi* recipient strain was infected with the Mu *lac* lysates as previously described (13). Antibiotic-resistant lysogens were selected by plating the appropriate dilution on L agar plates containing ampicillin (10 μg/ml) or kanamycin (20 μg/ml).

Transductions and matings. Transductions with the *E. chrysanthemi* generalized transducing phage φEC2 were carried out by the method of Resibois et al. (25). Matings were performed with plasmid pULB113 (31) or its Km^s derivative pULB110 (33). These plasmids can mobilize the chromosome from any point of origin (31).

RESULTS

Gur$^+$ derivatives. Wild-type *E. chrysanthemi* 3937 cannot use glucuronate as a carbon source for growth. However, glucuronate-fermenting derivatives (Gur$^+$) can be obtained spontaneously at a frequency of about 10^{-6} after a few days of incubation on glucuronate minimal medium. All Gur$^+$ mutants (except A862) constitutively expressed the genes *exuT*, *uxaC*, *uxaB*, and *uxaA* while these genes were inducible in the parental strain (Table 2). We deduced that the regulatory gene controlling the induction of *exuT*, *uxaC*, *uxaB*, and *uxaA* was mutated in these Gur$^+$ strains. This regulatory gene was named *exuR* by analogy with the corresponding gene of *E. coli* (19).

In strain A862, only the *exuT* expression was derepressed. The genes *uxaC*, *uxaB*, *uxaA*, *uxuB*, or *uxuA* remained expressed at the same level as in the wild-type strain, with or

TABLE 2. Expression of the hexuronate genes in a Gur$^+$ mutant

Strain and inducer[a]	Sp act of the product of[b]:								
	exuT	*uxaC*	*uxaB*	*uxaA*	*uxuB*	*uxuA*	*kdgK*	*kdgA*	*pel*
3937									
None	4	15	165	5	70	0.7	15	9	0.11
Galacturonate	30	104	782	32	83	0.6	86	48	1.70
Glucuronate	5	24	157	2	98	2.6	29	17	0.24
A40									
None	24	102	563	15	84	0.3	18	7	0.08
Galacturonate	27	138	672	18	86	0.5	80	45	1.83
Glucuronate	20	86	530	12	89	3.1	64	34	1.40

[a] 3937 is a wild-type *E. chrysanthemi* strain, whereas A40 is a Gur$^+$ derivative. Strains were grown in glycerol minimal medium with or without an inducer (5 mM). Cells were harvested in exponential growth phase, and enzymes were assayed as described in the text.

[b] Specific activities are expressed as micromoles of product per minute per milligram of bacterial dry weight, except for the *exuT* product specific activity, which is expressed as nanomoles of sugar accumulated per minute per milligram of bacterial dry weight.

without inducer. In strain A862, the Gur$^+$ phenotype cotransferred at 85% frequency with the *uxaB* gene, as tested by mating A862(pULB110) with A863. Moreover, Gur$^+$ cotransduced with *uxaB* at 15% with phage ϕEC2. The Gur$^+$ mutation of strain A862 is thus located close to the *exu-uxa* region, whereas the *exuR* mutations giving the Gur$^+$ phenotype were not near this region (Fig. 2). Strain A862 most probably contains a mutation in the operator of *exuT*, which affects the binding of the ExuR protein. The constitutive synthesis of the *exuT* gene product therefore seems to be the only change required for the growth of strain 3937 with glucuronate as a carbon source. Moreover, this result suggests that *exuT* constitutes an independent transcriptional unit since only its expression was affected.

In the Gur$^+$ strains, the expression of the two genes specific for glucuronate catabolism, *uxuB* and *uxuA*, remained unaffected (Table 2): *uxuA* was induced by glucuronate, whereas *uxuB* was expressed constitutively and at a low level. The expression of the *kdgK*, *kdgA*, and *pel* genes became clearly inducible in the presence of glucuronate, in addition to the normal induction by galacturonate (Table 2).

Isolation of Mu insertion mutants. A Gur$^+$ derivative, L37 (*exuR lacZ*), was first mutagenized with Mu d(Ap *lac*) or Mu dl1681. The fact that the parental strain constitutively expressed *exuT*, *uxaC*, *uxaB*, and *uxaA* avoided problems of inducer formation or entry. This also permitted the screening of *uxuA* and *uxuB* mutants specifically affected in glucuronate utilization and allowed identification of mutations affecting both the galacturonate and glucuronate catabolic pathways. After infection with Mu *lac* phages [Mu d(Ap *lac*) or Mu dl1681], antibiotic-resistant lysogens (Ap^r or Km^r) were screened on minimal medium containing either galacturonate or glucuronate. Mutants unable to use PGA were not retained since they most probably represent mutations in *kdgK* or *kdgA*, both of which have previously been

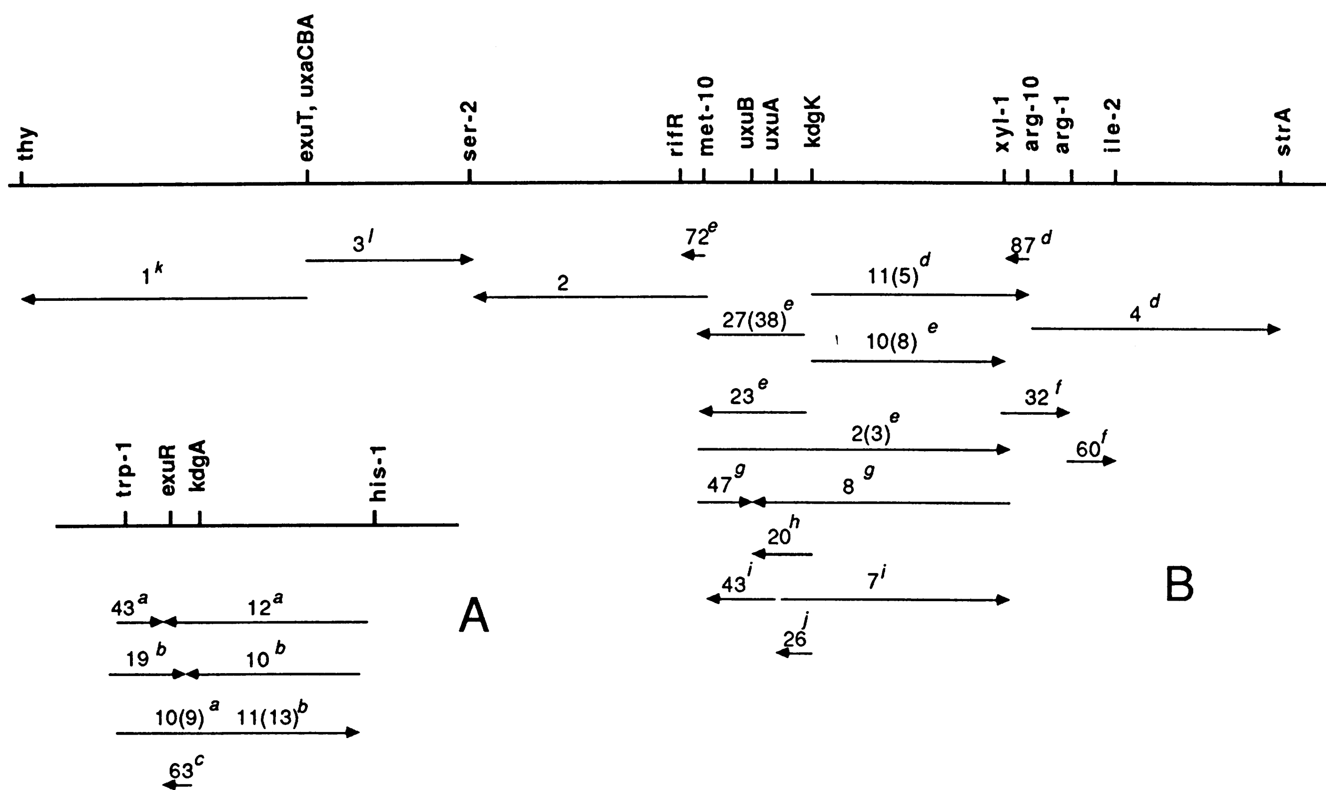

FIG. 2. Genetic organization of the genes affecting hexuronate catabolism in strain 3937. The numbers on the arrows show the percentage of cotransfer of various markers by pULB113 or pULB110. Arrowheads indicate the unselected marker. The results of the cotransfer in the opposite direction are shown in parentheses. The letters in italics refer to the following crosses: (A) *a*, A805 × A40(pULB113); *b*, A805 × A147(pULB113); *c*, A147 × A555(pULB113); (B) *d*, A576 × A849(pULB110); *e*, A863 × 3937(pULB113); *f*, A907 × AK38455(pULB113); *g*, A862 × A263(pULB113); *h*, A863 × A263(pULB113); *i*, A861 × A862(pULB110); *j*, A863 × A861(pULB110); *k*, A288k × A853(pULB110); and *l*, A288k × A1067(pULB110).

TABLE 3. Mutants involved in hexuronate catabolism

Parental strain and mutant[a]	Phenotype[b]		Sp act of the product of[c]:						No. of mutations[d]	No. of fusions[e]	Mutated gene
	Gar	Gur	*exuT*	*uxaC*	*uxaB*	*uxaA*	*uxuB*	*uxuA*			
L37 (*exuR*)											
L37	+	+	29	96	648	17	80	2.2			
A259	−	−	2	58	447	18	87	1.8	1	0	*exuT*
A235	−	−	19	9	15	0.5	91	1.5	3	1	*uxaC*
A288	−	+	21	122	16	0.3	65	2.1	8	4	*uxaB*
A231	−	+	17	81	833	1.2	96	1.7	2	1	*uxaA*
A263	+	−	19	93	406	15	7	1.4	2	1	*uxuB*
A287	+	−	18	106	796	16	107	0.2	2	1	*uxuA*
L2											
L2	+	−	26	95	691	20	86	2.7			
A627	+	+	18	60	948	24	110	2.3	7	4	*exuR*
A658	−	−	25	8	9	1	98	2.1	1	0	*uxaC*
A633	−	−	23	112	3	0.2	72	1.8	7	4	*uxaB*

[a] The *exuR* strains (L37, its derivatives, and A627) were assayed without inducer, whereas L2, A658, and A633 were grown in the presence of 5 mM galacturonate.
[b] The growth phenotype on galacturonate (Gar) and glucuronate (Gur).
[c] Specific activities are expressed as in Table 2.
[d] Mutations were obtained by insertion of either Mu d(Ap *lac*) or Mu dl1681. Each mutant originated from independent mutagenesis.
[e] We retained strains expressing the *lacZ* gene as potential fusions from among the mutants.

studied as *kdgK-lac* and *kdgA-lac* fusions (13). The other mutants could be grouped into six types based on enzymatic analysis (Table 3).

We then mutagenized *exuR*$^+$ (the wild-type allele of this gene) strain L2 (or auxotrophic derivatives). Three types of mutants were obtained (Table 3). The Gur$^+$ mutants correspond to *exuR*::Mu insertions. The constitutive expression of *exuT*, *uxaC*, *uxaB*, and *uxaA* resulted from a Mu insertion; therefore *exuR* most probably acts as a negative regulator gene which was inactivated by the Mu insertion.

Since Mu insertions are known to be polar, the enzymatic analysis of these mutants allowed us to determine the organization of the gene clusters. Some mutants were affected in the expression of only one gene: *exuT*, *uxaA*, *uxuB*, or *uxuA* (Table 3). Thus each of these genes belongs to an independent transcriptional unit or is situated at the end of a polycistronic operon. Fifteen mutants showed reduced expression at both *uxaB* and *uxaA*, and four mutants lost the expression of *uxaC*, *uxaB*, and *uxaA* (Table 3). Therefore *uxaC*, *uxaB*, and *uxaA* constitute one operon which is transcribed from *uxaC* to *uxaA*. All of these mutations (in *exuT*, *uxaC*, *uxaB*, *uxaA*, *uxuB*, and *uxuA*) had no effect on the expression of *kdgK* or *kdgA*. Insertions in *kdgK* or *kdgA* did not affect the expression of the other hexuronate system genes (data not shown). It has been shown that Mu insertion in *kdgK* does not affect the expression of *kdgA* and vice versa (13). Therefore *kdgK* and *kdgA* form two other distinct transcriptional units. Insertions in *exuR* did not abolish the expression of genes of the hexuronate system, and insertions in *exuT*, *uxaC*, *uxaB*, *uxaA*, *uxuB*, *uxuA*, *kdgK*, or *kdgA* did not affect the expression of *exuR*; thus *exuR* constitutes another transcriptional unit.

Identification of the *lac* gene fusions. About 50% of the Mu insertions in genes of the hexuronate system expressed the *lacZ* gene (Table 3) and are a result of Mu insertions in the correct orientation, leading to a fusion of the *lac* genes to the regulatory elements of the gene containing the Mu insertion. We transduced each Gar$^-$ Gur$^-$ mutant to the Gar$^+$ Gur$^+$ phenotype with a stock of the generalized transducing phage φEC2 made on the wild-type strain 3937 and retained only the mutants in which the Gar$^+$ Gur$^+$ phenotype cotransduced at 100% frequency with both antibiotic sensitivity and the Lac$^-$ character.

A stock of φEC2 was then prepared on each MudI1681 insertion mutant and used to transduce strain L2. The Gar$^-$, Gur$^-$, or Gur$^+$ mutations were verified to cotransduce with Km^r (selected marker) and Lac$^+$. The cotransduction frequencies ranged from 36 to 92% depending on the insertion. Low frequencies were probably due to transposition of the phage MudI1681 when introduced into the recipient strain.

Since it is very difficult to transduce Mu d(Ap *lac*) insertions by φEC2-mediated transduction (probably because of the length of the Mu DNA), we transformed the Mu d(Ap *lac*) insertions into Mu dI1681 insertions. A Mu dI1681 lysate was used to infect Mu d(Ap *lac*) lysogens, and we tested recombination between the two phages. Of the Km^r strains, 25 to 75% were Ap^s and kept the Gar$^-$ Gur$^-$ or Gur$^+$ phenotype and *lacZ* expression. These MudI1681 insertions were then transduced into strain L2 or L37 to verify the linkage between the phage and the mutation. This analysis proved linkage between the Gar$^-$ Gur$^-$ or Gur$^+$ mutation and the location of the phage for each Mu insertion.

Expression of the fusions. All of the *uxaCBA-lac* fusions isolated in an *exuR* strain constitutively expressed β-galactosidase (Table 4). In contrast, a fusion in the *uxaCBA* operon in an *exuR*$^+$ strain showed a strong induction of β-galactosidase in the presence of either galacturonate or PGA (induction ratios of about 10). The amount of glucuronate induction was low since this sugar cannot enter the cells in an *exuR*$^+$ strain. The *uxuB-lac* fusion was expressed at a low level without any induction by the hexuronates, whereas the *uxuA-lac* fusion was strongly induced in the presence of glucuronate and weakly by PGA (Table 4). The *exuR-lac* fusion was expressed very weakly and independently of the presence of hexuronate in the growth medium (Table 4).

Various intermediates of hexuronate and PGA catabolism were tested for their ability to induce the *uxaCBA* operon (Table 5). PGA, unsaturated digalacturonate, galacturonate, and tagaturonate gave a good induction of the *uxaCBA-lac* fusion, whereas 5-keto-4-deoxyuronate, 2,5-diketo-3-deoxygluconate, glucuronate, fructuronate, mannonate, altronate, and KDG gave no or poor induction. We cannot exclude the possibility that the absence of induction was due

TABLE 4. β-Galactosidase activity in the fusion strains[a]

Strain	Relevant genotype	Sp act with inducer[b]:			
		None	Galacturonate	Glucuronate	PGA
A235	*exuR uxaC*::Mu d(Ap *lac*)	1,630	1,810	1,700	1,960
A288	*exuR uxaB*::Mu d(Ap *lac*)	418	412	423	438
A231	*exuR uxaA*::Mu d(Ap *lac*)	714	692	829	814
A288k	*uxaB*::Mu d(Ap *lac*)	212	2,420	662	2,680
A263	*exuR uxuB*::Mu d(Ap *lac*)	23	23	25	28
A861	*exuR uxuA*::Mu d(Ap *lac*)	7	9	346	37
A627	*exuR*::Mu d(Ap *lac*)	8	7	10	12

[a] Specific activity is expressed as nanomoles of *o*-nitrophenyl-β-D-galactopyranoside hydrolyzed per minute per milligram of bacterial dry weight.
[b] Strains were grown in glycerol minimal medium with or without 5 mM inducer. β-Galactosidase was assayed in exponential-growth-phase cells as described in the text.

to a failure of the sugar to enter the cell, such as in the case of glucuronate or KDG. It is therefore not possible to state which intermediate(s) is the real inducer of the *uxaCBA* operon. In a *kdgR* mutant, in which KDG can enter the cells, KDG does not induce the *uxaCBA-lac* fusion (Table 5). The real inducer of this operon is thus produced before KDG formation.

Effects of *kdgR* or *exuR* mutations. Fusions in the *uxaCBA*, *uxuA*, *uxuB*, or *kdgK* operons were transferred into strains containing regulatory mutations (*exuR* or *kdgR*) to determine the range of each regulatory system. The *uxuA-lac* and *uxuB-lac* fusions were not affected by *exuR* or *kdgR* mutations (data not shown). The *exuR* mutation affected only the *uxaCBA-lac* fusion, and the *kdgR* mutation affected only the expression of the *kdgK-lac* fusion (Table 6). Furthermore, the *kdgR* mutant synthesized pectate-lyases at a high level without inducer (Table 6). As it was previously shown that the *kdgR* gene partially controlled the expression of some *pel* genes in *E. chrysanthemi* B374 (11), a similar regulation may exist in strain 3937. Pectate-lyase synthesis in the *kdgK* mutants increased about 10-fold in the presence of an inducer (Table 6). This high induced level also exists in *kdgK exuR* and *kdgK kdgR* strains (Table 6). Thus, a *kdgR* mutation gave only a partial derepression of *pel* gene expression since the *pel* expression remained inducible.

Induction of pectate-lyase synthesis in the hexuronate mutants. In the parental strain 3937, pectate-lyase synthesis was induced in the presence of galacturonate or PGA (Table 7). In an *exuR* mutant (A40), pectate-lyase synthesis was also induced by glucuronate. In *exuT* or *uxaC* mutants, pectate-lyase synthesis was no longer induced by galacturonate or glucuronate. In *uxaB* or *uxaA* mutants, pectate-lyase synthesis was no longer induced by galacturonate, and in *uxuB* or *uxuA* mutants this synthesis was no longer induced by glucuronate (Table 7). The catabolism of these two hexuronates is therefore necessary for their inducing power, and the action of the *uxaA* or *uxuA* products is required for the formation of the real inducer of pectate-lyase synthesis.

In a *kdgK* mutant the induction ratio of pectate-lyase synthesis strongly increased, either with galacturonate, glucuronate, or PGA (Table 7). In *kdgA* mutants, a low induction of pectate-lyase synthesis was found, despite the toxicity of the 6-phospho-KDG for the cells. These results indicate that the inducer of pectate-lyase synthesis formed during the hexuronate catabolism is KDG (or a product derived thereof), which accumulates in the *kdgK* mutants.

Localization of the genes affecting hexuronate catabolism. Strain A805 (*thy trp his*) was used as a recipient by mating with the strain A40 (*exuR*), into which the plasmid pULB113 was introduced. The *exuR* mutation (Gur$^+$ phenotype) cotransferred with *trp* and *his* (Fig. 2, cross *a*), indicating

TABLE 5. Expression of the *uxaCBA* operon in the presence of potential inducers[a]

Strain and inducer	Sp act of:	
	β-Galactosidase	Pectate-lyase
A288k		
None	203	0.05
Galacturonate	2,060	0.04
Tagaturonate	2,190	0.02
Altronate	793	0.09
Glucuronate	617	0.24
Fructuronate	304	0.19
Mannonate	176	0.07
Polygalacturonate	2,260	1.36
Unsaturated digalacturonate	2,140	1.75
5-Keto-4-deoxyuronate	501	0.24
2,5-Diketo-3-deoxygluconate	258	0.37
KDG	214	0.12
A952 (*kdgR*)		
None	235	1.43
KDG	273	1.59

[a] Assay conditions and specific activities were as described in Tables 2 and 4, except that inducers (5 mM) were added 3 h before the assays.

TABLE 6. Expression of fusions in various regulatory mutants

Fusion and regulatory mutation[a]	Inducer[b]	Sp act[c] of:	
		β-Galactosidase	Pectate-lyase
uxaB-lac			
None	−	212	0.06
	+	2,420	0.07
exuR	−	418	0.08
	+	412	0.03
kdgR	−	185	1.58
	+	2,510	1.14
kdgK-lac			
None	−	41	0.05
	+	427	15.3
exuR	−	40	0.08
	+	382	13.5
kdgR	−	1,480	1.45
	+	1,510	16.6

[a] Stocks of the transducing phage φEC2 were made on the fusion strains A288k (*uxaB-lac*) and A825 (*kdgK-lac*). The fusions were then transduced into strains containing a regulatory mutation L37 (*exuR*), A837 (*kdgR*), or no regulatory mutation L2.
[b] The inducer used was 5 mM galacturonate.
[c] Assay conditions and specific activities were the same as those described in Tables 2 and 4.

that the *exuR* locus is located between them. This result was confirmed by three-factor analysis (data not shown). The genetic markers resulting in Gur$^+$ and Ap^r (or Km^r) phenotypes of the four presumed *exuR-lac* fusions were found at the same location, confirming that the Mu prophage is inserted in *exuR* in these strains.

When strain A805 was mated with A147(pULB113) (*exuR kdgA*), the *kdgA* marker (Gar$^-$ Gur$^-$) also appeared to be located between *trp* and *his* (Fig. 2, cross *b*). A147 was then mated with A555(pULB113) and 63% of the *kdgA*$^+$ (Gar$^+$) transconjugants were also *exuR*$^+$ (Gur$^-$), indicating a high proximity of these two genes (Fig. 2, cross *c*). When φEC2 stock made on wild-type strain 3937 was used to transduce strain A147 to the Gar$^+$ phenotype, 4% of the transductants also acquired the *exuR*$^+$ allele. The relative order of the four markers could be deduced from the mating between A805 and A147(pULB113) despite the fact that the *kdgA* mutation (Gar$^-$ Gur$^-$) masked the *exuR* allele (Gur$^+$). The three-factor analysis (data not shown) permitted the conclusion that the relative gene order was *his-kdgA-exuR-trp* (Fig. 2).

The *kdgK* gene was located near the *xyl* and *arg* markers by mating strain A576 (*kdgK arg-10*) with A849 (*xyl leu met strA rifR*) containing plasmid pULB110, since *arg-10* and *kdgK* cotransferred. The *arg* and *xyl* markers also strongly cotransferred. We then mated strain A863 (A849 rendered Gur$^+$ and *kdgK*) with 3937(pULB113) (Fig. 2, cross *e*). A three-factor analysis determined that *kdgK* was located between *met* and *xyl* (data not shown). Since the marker *rifR* strongly cotransferred with *met*, we tested their transduction by phage φEC2 and found 42% cotransduction between *rifR* and *met*.

Because *arg-xyl* and *rifR-met* were found to be tightly linked, it was difficult to determine the order of these genes by cotransfer analysis. In the case of *arg* and *xyl*, cotransduction analysis permitted resolution of the problem. The *arg-10* mutation (strain A576) differed from *arg-1* (strain AK3710); *arg-1* did not cotransduce with *xyl*, whereas *arg-10* cotransduced at 85% with *xyl*. Moreover, *arg-1* cotransferred at 7% with *ile-2*, whereas *arg-10* gave no cotransduction. Mating between A907 (*arg-1 ile-2 rifR*) and AK38455(pULB113) (*xyl leu met strA*) gave 60% cotransfer between *arg-1* and *ile-2* and 32% cotransfer between *arg-1* and *xyl* (Fig. 2, cross *f*).

When strain A862 was mated with A263(pULB113), the *uxuB* gene cotransferred with *met* and *xyl* (Fig. 2, cross *g*) and was found to be located between these two markers by three-factor analysis (data not shown). To localize the *uxuB* gene relative to *kdgK*, A263(pULB113) was mated with A863 (Fig. 2, cross *h*). By three-factor analysis, we determined that *uxuB* is located between *met* and *kdgK*. The *uxuA* gene was also found to be located between *met* and *xyl* by mating A861 with A862(pULB110) (Fig. 2, cross *i*), and more precisely between *met* and *kdgK* by mating A863 with A861(pULB110) (Fig. 2, cross *j*). *kdgK* is probably closer to *uxuA* than to *uxuB* since cotransfer was higher for the *kdgK-uxuA* couple than for the *kdgK-uxuB* couple. We found no cotransduction by phage φEC2 between the *kdgK* mutation and either *uxuA* or *uxuB*. The most probable order of the genes of this chromosomal region is shown in Fig. 2.

Strains A231k (*uxuA*::Mu dl1681), A235k (*uxaC*::Mu dl1681), A259k (*exuT*::Mu dl1681), and A288k (*uxaB*::Mu dl1681) were mated with A853(pULB110). In each cross, 0.5 to 1% of the Gar$^+$ transconjugants acquired the *thy* mutation (Fig. 2, cross *k*). Mating of A288k with A1067(pULB110) (*leu ser-2 xyl strA*) gave 3% cotransfer between the *uxaB* gene and the *ser-2* marker (Fig. 2, cross *l*), which has been previously shown to be located near *met* (A. Pohl, unpublished results). The corresponding gene order is shown in Fig. 2.

TABLE 7. Pectate-lyase activity of the hexuronate mutants[a]

Strain	Main genotype	Sp act with inducer:			
		None	Galacturonate	Glucuronate	PGA
3937	Wild-type	0.11	1.70	0.24	1.34
A40	Parental strain (*exuR*)	0.08	1.83	1.40	1.56
A259	*exuT*::Mu d(Ap *lac*)	0.05	0.07	0.09	0.92
A235	*uxaC*::Mu d(Ap *lac*)	0.05	0.04	0.04	0.89
A288	*uxaB*::Mu d(Ap *lac*)	0.08	0.03	0.68	0.96
A231	*uxaA*::Mu d(Ap *lac*)	0.04	0.05	0.85	0.94
A263	*uxuB*::Mu d(Ap *lac*)	0.08	0.64	0.04	1.03
A287	*uxuA*::Mu d(Ap *lac*)	0.12	1.47	0.10	1.12
A233	*kdgK*::Mu d(Ap *lac*)	0.11	17.6	22.4	24.3
A238	*kdgA*::Mu d(Ap *lac*)	0.06	0.15	0.15	0.39

[a] Assay conditions and specific activity are as described in Table 2.

DISCUSSION

In the phytopathogenic bacterium *E. chrysanthemi*, the catabolism of hexuronates is linked to the degradation of pectic polymers. To determine possible connections between the regulatory mechanisms governing these pathways, we studied the regulatory genes acting on galacturonate and glucuronate catabolism in strain 3937.

We show here the existence of three main regulatory systems controlling hexuronate degradation. Genes involved in galacturonate utilization, *exuT*, *uxaC*, *uxaB*, and *uxaA* are under the control of the *exuR* gene product, which probably acts as a negative regulator repressing the expression of the genes belonging to the *exu* regulon. Inactivation of *exuR* by Mu insertion led to constitutive expression of the genes *exuT*, *uxaC*, *uxaB*, and *uxaA*. Intermediates of the pathway (galacturonate, tagaturonate, or altronate) were directly involved in inducing the *exu* regulon. The *exuR* gene product did not affect the expression of either the *uxu* genes (specific for glucuronate catabolism), the *kdg* genes (specific for KDG catabolism), or the *pel* genes (encoding pectate lyases).

The two genes of strain 3937 specifically involved in glucuronate catabolism, *uxuA* and *uxuB*, constitute two independent transcriptional units and are under different controls. In the case of *uxuB*, we found no induction, whereas *uxuA* was induced in the presence of glucuronate, fructuronate, or mannonate but not with KDG. We therefore propose the existence of a regulatory gene, *uxuR*, controlling the expression of *uxuA*. Since the inducers of the *uxuA* gene expression were totally different from those of the *pel*, *uxa*, and *kdg* genes, we deduced that the *uxuR* gene product does not affect the expression of these genes.

The three genes involved in KDG degradation, *kdgT*, *kdgK*, and *kdgA*, are controlled by the *kdgR* gene product. The real inducer of the *kdg* regulon is probably KDG itself. Moreover, the *kdgR* product also acts on other genes of PGA catabolism such as *kduD* (G. Condemine, personal commu-

nication), *ogl* (S. Reverchon, personal communication), and the *pel* genes. Additional analysis is needed to establish the complete role of *kdgR* in the regulation of pectinolysis. This study and previous results (11) suggest that there are additional regulatory genes controlling *pel* expression since after inactivation of *kdgR* the synthesis of pectate-lyase showed an increased basal level but remained inducible. The *kdgR* gene product does not affect the expression of either the *exu-uxa* or the *uxu* genes.

The various intermediates of the hexuronate pathway showed no induction of *pel* gene expression by themselves, but after transformation into KDG they can act as inducers. When accumulated in a *kdgK* mutant strain, KDG gave a high induction of pectate-lyase synthesis. It has already been shown that KDG is one of the direct inducers of *pel* and *kduD* expression formed during pectinolysis (8). KDG is also the real inducer of pectate-lyase synthesis originating from galacturonate or glucuronate catabolism.

The organization and localization of the genes involved in galacturonate degradation were previously analyzed for *E. chrysanthemi* B374 (30, 32). A comparison with the results obtained for strain 3937 reveals great similarities. In both strains these genes are separated into three clusters. The first cluster contains four genes in the order *exuT-uxaC-uxaB-uxaA* and two transcriptional units, one including *exuT* and the second including the three *uxa* genes with a transcription direction from *uxaC* to *uxaA*. In strain 3937 as in B374 the *exuT-uxaCBA* region is weakly linked to the *thy* marker. In 3937 this region is also linked to the *ser-2* marker (Fig. 2). In both strains 3937 and B374, *kdgA* forms an independent transcriptional unit mapping between the *trp* and *his* markers. Moreover, the *exuR* regulatory gene of 3937 is strongly linked to *kdgA* (Fig. 2) since these two loci are cotransduced by phage φEC2. We also found that the *exuR* gene of B374 mapped between *trp* and *his* (data not shown). The *kdgK* gene of 3937 forms an independent transcriptional unit located near the *uxuA* and *uxuB* genes. In B374, these three genes are also next to each other since plasmids bearing the three genes were isolated (32). In strain B374 the *kdgK* gene is located near the *ile* marker, whereas in 3937 it is only weakly linked to *ile*. However, we do not know whether the *ile* mutations of the two strains are in identical or different genes. Not enough markers are available in strain B374 to allow a more precise mapping. In contrast, a detailed map of the region could be established in strain 3937 (Fig. 2).

The genetics of hexuronate catabolism in *E. chrysanthemi* may be compared to that in another enterobacterium, *E. coli* (19, 27). The organization and localization of the genes in these two species show both similarities and differences. The same biochemical pathway exists but its role is probably very different in each organism. In *E. coli*, the *exuR* regulatory gene controls the *exuT*, *uxaCA*, and *uxaB* operons and partially the *uxuAB* operon; *exuR* is located near *exuT*, *uxaC*, and *uxaA* at 67 min (i.e., 6 min from *thyA*) on the chromosome; *uxaB* is not linked to this cluster and lies at 52 min (19). In *E. chrysanthemi*, *uxaB* is gathered with the *exuT-uxaC-uxaA* cluster, but the regulatory gene *exuR* is separated from this cluster and located near *kdgA*.

Our results prove that phage φEC2 can be useful for precise genetic localization in strain 3937. Four examples of cotransducible pairs—*xyl* and *arg-10*, *met* and *rifR*, *exuR* and *kdgA*, and *ile-2* and *arg-1*, which cotransferred at 87, 72, 63, and 60%, respectively, and cotransduced at 85, 42, 5, and 7%, respectively—were found. The order *xyl-arg-10-arg-1-ile* was determined by φEC2 cotransduction analysis (Fig. 2). The detectable limit of transductional linkage with φEC2 requires at least 50% cotransfer by pULB113 (for instance, the couples *met* and *uxu* or *trp* and *exuR* gave no cotransduction). If we suppose that φEC2 can transduce fragments equivalent to the length of its own DNA, i.e., 62 kilobases (25), markers as far apart as 60 kilobases of DNA may be cotransduced. However, additional evidence is needed to establish the correlation between the frequency of cotransfer or of cotransduction and the physical distance separating the two markers.

ACKNOWLEDGMENTS

This work was supported by grants from the Commission of the European Communities (Biomolecular Engineering Program), from an Action d'Intervention sur Programme of the Centre National de la Recherche Scientifique and Association Française pour la Maitrise de l'Energie (Microorganismes d'intérêt industriel, agricole, ou medical), and from the Ministère de la Recherche et de l'Industrie (Aide à la Recherche).

We thank our co-workers, particularly S. Reverchon and G. Condemine, and the teams of J. P. Chambost, A. Kotoujansky, and A. Toussaint for the unpublished information and the strains, phages, and plasmids that we exchanged with them during our work. We gratefully acknowledge S. Roy for adapting the conditions of the assays of the *uxuA* and *uxuB* products to *E. chrysanthemi*. We thank Françoise Brunel for the readings of this manuscript.

LITERATURE CITED

1. **Ashwell, G.** 1962. Enzymes of glucuronic and galacturonic acid metabolism in bacteria. Methods Enzymol. **5**:190–208.
2. **Ashwell, G., A. Wahba, and J. Hickman.** 1960. Uronic acid metabolism in bacteria. I. Purification and properties of uronic acid isomerase in *Escherichia coli*. J. Biol. Chem. **235**:1559–1564.

2a. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.

3. **Casadaban, M. J., and S. N. Cohen.** 1979. Lactose genes fused to exogenous promoters in one step using a Mu-*lac* bacteriophage: in vivo probe for transcriptional control sequences. Proc. Natl. Acad. Sci. USA **76**:4530–4533.
4. **Castilho, B., P. Olfson, and M. J. Casadaban.** 1984. Plasmid insertion mutagenesis and *lac* gene fusion with mini-Mu bacteriophage transposons. J. Bacteriol. **158**:488–495.
5. **Chatterjee, A. K., and M. P. Starr.** 1980. Genetics of *Erwinia* species. Annu. Rev. Microbiol. **34**:645–676.
6. **Collmer, A., and D. F. Bateman.** 1981. Impaired induction and self-catabolite repression of extracellular pectate lyase in *Erwinia chrysanthemi* mutants deficient in oligo-galacturonate lyase. Proc. Natl. Acad. Sci. USA **78**:3920–3924.
7. **Collmer, A., P. Berman, and M. S. Mount.** 1982. Pectate lyase regulation and bacterial soft-rot pathogenesis, p. 395–422. *In* M. S. Mount and G. H. Lacy (ed.), Phytopathogenic prokaryotes, vol. 1. Academic Press, Inc., New York.
8. **Condemine, G., N. Hugouvieux-Cotte-Pattat, and J. Robert-Baudouy.** 1986. Isolation of *Erwinia chrysanthemi kduD* mutants altered in pectin degradation. J. Bacteriol. **165**:937–941.
9. **Ehrlich, F., and R. Guttman.** 1934. Zür Kenntnis der 5-Keto-1-Galaktonsaüre (d-Tagaturonsaüre). Ber. Dtsch. Chem. Ges. **67**:1345–1347.
10. **Hugouvieux-Cotte-Pattat, N., Y. Quesneau, and J. Robert-Baudouy.** 1983. Aldohexuronate transport system in *Erwinia carotovora*. J. Bacteriol. **154**:663–668.
11. **Hugouvieux-Cotte-Pattat, N., S. Reverchon, G. Condemine, and J. Robert-Baudouy.** 1986. Regulatory mutants affecting the synthesis of pectate-lyase in *Erwinia chrysanthemi*. J. Gen. Microbiol. **132**:2099–2106.
12. **Hugouvieux-Cotte-Pattat, N., and J. Robert-Baudouy.** 1985. Lactose metabolism in *Erwinia chrysanthemi*. J. Bacteriol. **162**:248–255.
13. **Hugouvieux-Cotte-Pattat, N., and J. Robert-Baudouy.** 1985. Isolation of *kdgK-lac* and *kdgA-lac* gene fusions in the

phytopathogenic bacterium *Erwinia chrysanthemi*. J. Gen. Microbiol. **131**:1205–1211.

14. **Kilgore, W. W., and M. P. Starr.** 1959. Catabolism of galacturonic and glucuronic acids by *Erwinia carotovora*. J. Biol. Chem. **234**:2227–2236.
15. **Kotoujansky, A., A. Diolez, M. Boccara, Y. Bertheau, T. Andro, and A. Coleno.** 1985. Molecular cloning of *Erwinia chrysanthemi* pectinase and cellulase structural genes. EMBO J. **4**:781–785.
16. **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.
17. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Moran, F., and M. P. Starr.** 1969. Metabolic regulation of polygalacturonic acid trans-eliminase in *Erwinia*. Eur. J. Biochem. **11**:291–295.
19. **Portalier, R., J. Robert-Baudouy, and F. Stoeber.** 1980. Regulation of *Escherichia coli* K-12 hexuronate system genes: *exu* regulon. J. Bacteriol. **143**:1095–1107.
20. **Pouyssegur, J.** 1973. Preparation microbiologique du 2-ceto-3-desoxy-D-gluconate 1-^{14}C ou U-^{14}C. J. Labelled Compd. **9**:3–13.
21. **Pouyssegur, J., and F. Stoeber.** 1970. Production de 2-ceto-3-desoxy-6-phospho-D-gluconate par un mutant d'*Escherichia coli* K-12. Bull. Soc. Chim. Biol. **52**:1407–1417.
22. **Pouyssegur, J., and F. Stoeber.** 1974. Genetic control of the 2-keto-3-deoxy-D-gluconate metabolism in *Escherichia coli* K-12: *kdg* regulon. J. Bacteriol. **117**:641–651.
23. **Pratt, J., and N. Richtmeyer.** 1955. D-Glycero-D-allo-heptose, L-allo-heptulose, D-talo-heptulose and related substances derived from the addition of cyanide to D-allose. J. Am. Chem. Soc. **77**:1906–1908.
24. **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.
25. **Resibois, A., M. Colet, M. Faelen, E. Schoonejans, and A. Toussaint.** 1984. Phi-EC2 a new generalized transducing phage of *Erwinia chrysanthemi*. Virology **137**:102–112.
26. **Robert-Baudouy, J., R. Portalier, and F. Stoeber.** 1974. Régulation du métabolisme des hexuronates chez *Escherichia coli* K-12: modalités de l'induction des enzymes du système hexuronate. Eur. J. Biochem. **43**:1–15.
27. **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 the *exuR* and *uxuR* gene products. J. Bacteriol. **145**:211–220.
28. **Starr, M. P., and A. Chatterjee.** 1972. The genus *Erwinia*: enterobacteria pathogenic to plants and animals. Annu. Rev. Microbiol. **26**:389–426.
29. **Toussaint, A., and E. Schoonejans.** 1982. Production and modification of Mu (G-) phage particle in *Escherichia coli* K-12 and *Erwinia*. Genet. Res. **41**:145–154.
30. **Van Gijsegem, F., N. Hugouvieux-Cotte-Pattat, and J. Robert-Baudouy.** 1985. Isolation and characterization of *Erwinia chrysanthemi* mutants defective in degradation of hexuronates. J. Bacteriol. **161**:702–708.
31. **Van Gijsegem, F., and A. Toussaint.** 1982. Chromosome transfer and R-prime formation by an RP4::mini Mu derivative in *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae* and *Proteus mirabilis*. Plasmid **7**:30–44.
32. **Van Gijsegem, F., and A. Toussaint.** 1983. In vivo cloning of *Erwinia carotovora* genes involved in the catabolism of hexuronates. J. Bacteriol. **154**:1227–1235.
33. **Van Gijsegem, F., A. Toussaint, and E. Schoonejans.** 1985. In vivo cloning of pectate lyase and cellulase genes of *Erwinia chrysanthemi*. EMBO J. **4**:787–792.
34. **Weissbach, A., and J. Hurtwitz.** 1959. The formation of 2-keto-3-deoxyheptonic acid in extracts of *Escherichia coli* B. J. Biol. Chem. **234**:705–709.