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Isolation and Characterization of *Erwinia chrysanthemi* Mutants Defective in Degradation of Hexuronates

FRÉDÉRIQUE VAN GIJSEGEM,[1]* NICOLE HUGOUVIEUX-COTTE-PATTAT,[2] AND JEANINE ROBERT-BAUDOUY[2]

Laboratoire de Génétique, Université Libre de Bruxelles, Rhode-Saint-Genèse, Belgium,[1] and Laboratoire de Microbiologie, Institut National des Sciences Appliquées, Villeurbanne, France[2]

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Spontaneous and Tn*9*-induced mutants of *Erwinia chrysanthemi* were isolated which affect the degradative pathway of galacturonate and ketodeoxygluconate. The mutations were characterized both biochemically and functionally by complementation analysis and localized in the *E. chrysanthemi* chromosome. The *kdgK* gene mapped very close to *ile*, the *kdgA* gene was between *trp* and *his*, and the *exuT-uxaC-uxaB-uxaA* cluster was linked to *thy*. The different types of mutants obtained were consistent with an organization of the *exu-uxa* cluster into two transcription units, one containing the *exuT* gene, and the other containing the three *uxa* genes, with the transcription going from *uxaC* to *uxaA*.

A specific trait of the species *Erwinia chrysanthemi* is its ability to degrade pectin, a major constituent of the vegetal cell wall (2). In a first approach to the understanding of pectinolysis in *E. chrysanthemi*, we undertook a genetic study of the catabolic pathways of GA and KDG, which are two of the degradation products of pectin, and its demethylated product PGA (15, 16). This pathway was established for *Erwinia carotovora* by Kilgore and Starr (8) (see Fig. 1). The same degradative pathway exists in *Escherichia coli* and has been extensively analyzed (12, 19).

We isolated R-prime plasmids carrying different chromosomal segments from *E. chrysanthemi* B374 and established the genetic organization of the genes encoding the enzymes implicated in this pathway (24). They are organized in three clusters. One contains the four genes *exuT*, *uxaC*, *uxaB*, and *uxaA*; *exuT* and *uxaC,B,A* seem to belong to two different transcription units. A second locus contains the *kdgK* gene, and a third contains the *kdgA* gene. In this paper, we report the isolation of mutants defective in the GA-degradative pathway, their characterization, and the localization of these mutations in the B374 chromosome.

MATERIALS AND METHODS

Nomenclature and abbreviations. The genetic symbols are those used for *E. coli* by Bachmann (1). The following abbreviations are used: GU, glucuronate; GA, galacturonate; KDG, 2-keto-3-deoxygluconate; KDGP, 2-keto-3-deoxy-6-phosphogluconate; PGA, polygalacturonate. Phenotypes are designated as follows: Gur^+/Gur^-, Gar^+/Gar^-, and Pga^+/Pga^- for the ability/inability to catabolize GU, GA, and PGA respectively. Antibiotic resistance phenotypes are designated as follows: Spc for spectinomycin, Str for streptomycin, Tmp for trimethoprim, Kan for kanamycin, Tet for tetracycline, Amp for ampicillin, and Cml for chloramphenicol.

Strains. Bacterial strains, plasmids, and bacteriophages are shown in Table 1. All *E. chrysanthemi* strains derive from the wild-type strain B374.

Media. Bacteria were grown in L broth (9) supplemented with 50 μg of thymine per ml when necessary and diluted in 10 mM $MgSO_4$. The colonies were scored on L medium (LB medium plus 1.2% Difco agar), A medium (10), or M63 minimal medium (10) supplemented with 1.4% Difco agar. The following final concentrations were used: carbon sources, 0.2%; amino acids, 40 μg/ml; thymine, 100 μg/ml; streptomycin, 200 μg/ml; rifampin, 100 μg/ml; chloramphenicol, 20 μg/ml; kanamycin, 20 μg/ml; tetracycline, 20 μg/ml; ampicillin, 20 μg/ml.

Chemicals. The intermediate substrates of the GA-degradative pathway were synthesized in the Institut National des Sciences Appliquées laboratory. The method of Ehrlich and Guttmann (4) was adapted to D-tagaturonate synthesis (20). The method of Pratt and Richtmeyer (14) was used for D-altronate preparation.

Isolation of mutants. Ampicillin enrichment was performed as described by Miller (10).

Transposon mutagenesis. To introduce into strain B374 the Tn*9* transposon which confers Cml^r, we used the phage Mu *S*::Tn*9*. Because of the insertion of Tn*9*, the DNA of this phage grown upon induction is too long to be packaged entirely. The mature phage genome is deleted from the right end and is therefore unable to integrate into the chromosome of the newly infected host. Consequently, most of the Cml^r clones obtained after infection with that phage result from the transposition of Tn*9* at different sites into the chromosome, with the phage DNA being lost. This method can, of course, only be applied on bacteria which adsorb MuG(-) phages which are infectious despite the absence of an active *S* gene (22).

Liquid infection was done by mixing 0.1 ml of a phage lysate with 0.9 ml of an overnight culture of strain ERH200. After 20 min of adsorption at 32°C, the mixture was spread on LB plates containing 20 μg of chloramphenicol per ml. A few thousand Cml^r clones were collected and grown overnight in minimal medium supplemented with glycerol. Ampicillin enrichments were performed starting from this overnight culture.

Selection of GA-resistant derivatives. Isolated colonies of the *kdgA* mutant ERH201 were suspended in drops of 10 mM $MgSO_4$, and about 20 μl of each drop was spotted on minimal medium supplemented with glycerol and GA. A few colonies (1 to 10) grew in the spot. They were tested on minimal medium supplemented with either GA alone or GU

* Corresponding author.

TABLE 1. Bacterial strains, plasmids, and bacteriophages

Strain, plasmid, or bacteriophage	Description	Source or reference
Strain		
B374	*E. chrysanthemi*[a]	6; Lemattre, personal communication
ERH200	B374 *his-5* GurA$^+$ Rif^r	
ERH201	ERH200 Tn*9* *kdgA1*	
ERH202	ERH200 *kdgA2*	
ERH203	ERH200 *kdgK1*	
ERH204	ERH200 *kdgK2*::Tn*9*	
ERH205	ERH200 *kdgK3*::Tn*9*	
ERH206	ERH200 *kdgK4*::Tn*9*	
ERH207	ERH200 *uxaA1*::Tn*9*	
ERH208	ERH200 *uxaB1*::Tn*9*	
ERH209	ERH201 *uxaA2* *kdgA*$^+$	
ERH210	ERH201 *uxaA3* *kdgA*$^+$	
ERH213	ERH201 *uxaCBA1* *kdgA*$^+$	This work
ERH214	ERH201 *uxaCB1* *kdgA*$^+$	
ERH215	ERH201 *exuT1* *kdgA*$^+$	
ERH216	ERH201 *exuT2* *kdgA*$^+$	
ERH217	ERH201 *exuT3* *kdgA*$^+$	
ERH218	ERH200 *exuT4*::Tn*9*	
ERH219	ERH200 Δ(*exu-uxa*)*1*::Tn*9*	
ERH220	ERH201 Δ(*exu-uxa*)*2* *kdgA*$^+$	
ERH221	ERH200 *exuT uxaA* Tn*9*	
ERH223	ERH200 Δ(*exu-uxa*)*3*::Tn*9*	
ERH224	ERH200 *uxaA4*::Tn*9*	
RH6010	*thr-1 leu-1 pro-1 his-5 trp-1 thyA ile-1* Str^r Rif^r	21
ERH80		His$^+$ transductant of RH6010
Plasmids		
pULB113	*tra*$^+$ *bla*$^+$ (TEM-2) Tn*1* *tet*$^+$ *aphA*$^+$ (Mu3A)	23
pULB114	pULB113 carrying *exuT*	
pULB115	pULB113 carrying *exuT-uxaC*	
pULB116	pULB113 carrying *uxaC-uxaB*	24
pULB117	pULB113 carrying *uxaC-uxaB-uxaA*	
pULB118	pULB113 carrying *exuT-uxaC-uxaB-uxaA*	
pULB119	pULB113 carrying *kdgK*	
pULB120	pULB113 carrying *kdgA*	
Bacteriophages		
Mu *cts62 S*::Tn*9*		17
φEC2		Resibois et al., in press

[a] Strain B374, which was first reported to be *E. carotovora* (6), was recently retested and identified as *E. chrysanthemi* (Lemattre, personal communication; Perombelon, personal communication).

and glycerol or PGA and glycerol. The Gar$^-$ clones were assumed to be *kdgA* mutants which acquired a secondary mutation. Among them, clones growing on GA plus glycerol and PGA plus glycerol were assumed to be *kdgK kdgA* mutants and were discarded. Among the clones still poisoned by PGA, two types of clones were found: those which were still poisoned by GU and those which were not poisoned by either GA or GU.

To transduce the *kdgA*$^+$ allele in these clones, we mixed 0.1 ml of a lysate of the generalized transducing phage phiEC-2 (A. Resibois, M. Colet, M. Faelen, E. Schoonejans, and A. Toussaint, Virology, in press) grown on strain B374 with 0.1 ml of an overnight culture of the bacterium and spread this mixture on minimal medium supplemented with PGA (multiplicity of infection, 1 to 3).

Complementation tests. A first screening of the mutants was performed by suspending a colony of the mutants in 1 drop of 10 mM $MgSO_4$ and spotting it on a selective medium seeded with 0.1 ml of an overnight culture of an *E. coli thyA* strain carrying the R-prime plasmid to be tested. The mutants described here were also tested by titration after mating. Single drops of overnight cultures of donors carrying the R-prime plasmid and the Gar$^-$ mutants to be tested were mixed on an A plate, incubated for 4 to 6 h at 33°C, and suspended in 1 ml of 10 mM $MgSO_4$. Mating mixtures were titrated on minimal medium supplemented with glucose and kanamycin and lacking thymine (to counterselect the donor) to measure the frequency of transfer of the plasmid and on minimal medium supplemented with GA. The mutant was assumed to be complemented by the R-prime plasmid tested when about the same titer was obtained on the two types of plates (i.e., 5 to 100% of the frequency of transfer of the RP4 resistance marker).

Mapping. The Gar$^-$ mutations were localized by measuring the frequency of cotransfer of the mutation with different markers available on the chromosome of strain B374. Plasmid RP4::mini-Mu pULB113 was transferred in the different mutants, and these strains were mated with the polyauxotrophic strain ERH80 or RH6010 by mixing on A medium 0.1 ml of overnight cultures of the mutants carrying pULB113 and the recipient. The plates were incubated for 4 to 6 h at 33°C. Bacteria were collected from the growth area, suspended in 1 ml of 10 mM $MgSO_4$, and spread on minimal medium supplemented with glucose, streptomycin (to counterselect the donor), and all but one of the amino acids required for growth of ERH80 or RH6010. The transconjugants which acquired one prototrophic marker from the donor were further tested for their Gar phenotype.

Preparation of cell extracts and enzyme assays. Enzyme activities were measured in cells grown exponentially in glycerol minimal medium alone or supplemented with GA. Cell extracts were prepared by breaking the cells suspended in 10 mM phosphate buffer (pH 7.0) with a French press. Extracts were then centrifuged at 15,000 × *g* for 20 min to remove whole cells and cellular debris. Hydrogenase activity was measured by monitoring the decrease in absorbance at 340 nm in a mixture assay consisting of 50 mM phosphate buffer (pH 6.3), 0.4 mM NADH, and 10 mM tagaturonate (13).

Hydrolyase activity was measured by monitoring the formation 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 D-altronate (18). The reaction was stopped by adding 3 volumes of 10% trichloroacetic acid plus 20 mM $HgCl_2$. The amount of KDG was then measured by the method of Weissbach and Hurwitz (25).

TABLE 2. Genetic characterization of Gar$^-$ mutants

Mutant	Growth with C source:			Complementation with:							Most likely phenotype(s)	Origin[a]
	GA	GU	PGA	pULB114 *exuT*	pULB115 *exuT uxaC*	pULB116 *uxaC,B*	pULB117 *uxaC,B,A*	pULB118 *exuT uxaC,B,A*	pULB119 *kdgK*	pULB120 *kdgA*		
ERH200	+	+	+									
ERH201	−	−	−	−	−	−	−	−	−	+	KdgA	b
ERH202	−	−	−	−	−	−	−	−	−	+	KdgA	a
ERH203	−	−	−	−	−	−	−	−	+	−	KdgK	a
ERH204	−	−	−	−	−	−	−	−	+	−	KdgK	b
ERH205	−	−	−	−	−	−	−	−	+	−	KdgK	b
ERH206	−	−	−	−	−	−	−	−	+	−	KdgK	b
ERH207	−	+	+	−	−	−	+	+	−	−	UxaA or UxaB	b
ERH208	−	+	+	−	−	−	+	+	−	−	UxaA or UxaB	b
ERH209	−	+	+	−	−	−	+	+	−	−	UxaA or UxaB	c
ERH210	−	+	+	−	−	−	+	+	−	−	UxaA or UxaB	c
ERH213	−	−	+	−	−	−	+	+	−	−	UxaCBA	c
ERH214	−	−	+	−	−	+	+	+	−	−	UxaCB	c
ERH215	−	−	+	+	+	−	−	+	−	−	ExuT	c
ERH216	−	−	+	+	+	−	−	+	−	−	ExuT	c
ERH217	−	−	+	+	+	−	−	+	−	−	ExuT	c
ERH218	−	−	+	+	+	−	−	+	−	−	ExuT	b
ERH219	−	−	+	−	−	−	−	+	−	−	ExuT-UxaCBA	b
ERH220	−	−	+	−	−	−	−	+	−	−	ExuT-UxaCBA	c
ERH221	−	−	+	−	−	−	−	+	−	−	ExuT-UxaCBA	c
ERH223	−	−	+	−	−	−	−	+	−	−	ExuT-UxaCBA	b
ERH224	−	+	+	−	−	−	+	+	−	−	UxaA or UxaB	b

[a] a, Spontaneous mutants recovered after ampicillin enrichment; b, Tn*9*-induced mutants; c, mutants isolated from a *kdgA* strain as GA resistant.

Isomerase activity was detected by measuring the tagaturonate formed with an excess extract of altronate oxidoreductase. The assay mixture contained 50 mM phosphate buffer (pH 7.6), 0.4 mM NADH, an excess of purified altronate oxidoreductase, and 10 mM GA. Activity was monitored by the decrease in absorbance at 340 nm (11).

Enzyme activity was always expressed in nanomoles of product per min per mg (dry weight) of bacteria. The substrate was omitted from the blank mixture in each assay. All the reactions were started by adding the substrate.

The hexuronate transport system was measured by monitoring the uptake of [^{14}C]GU in whole cells (7). Cells harvested at the late-exponential phase of growth were washed in M63 minimal medium and suspended at a concentration of approximately 10^9 cells per ml in the same medium. [^{14}C]GU was added to 1 ml of cells, and at timed intervals, 0.1-ml samples were removed, diluted, and immediately filtered. The filters were washed twice, dried, and counted. The results were expressed as nanomoles of GU accumulated per min per mg (dry weight) of cells.

RESULTS

Isolation of Gar$^-$ mutants. Mutants unable to use GA as carbon source (Gar$^-$ mutants) were isolated from ERH200 which is a His$^-$ Rif^r Gur$^+$ derivative of strain B374. Wild-type B374 is Gur$^-$ (unable to use GU), but spontaneous Gur$^+$ derivatives are found at a relatively high frequency (10^{-6}) (7, 24).

Gar$^-$ mutants were isolated in different ways. A first set was isolated without mutagenesis after ampicillin enrichment by screening the survivors after three or four cycles. These mutants are shown in Table 2.

A second set of Gar$^-$ mutants (Table 2) was isolated after transposon mutagenesis with Mu *S*::Tn*9* (see above) and subsequent ampicillin enrichment.

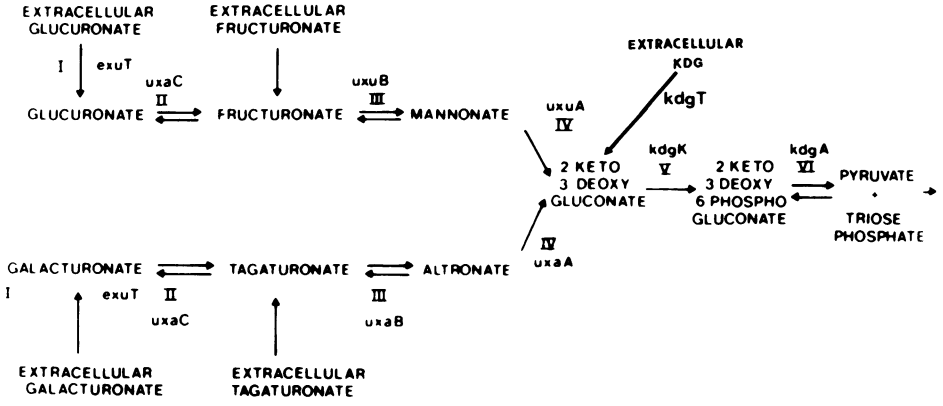

FIG. 1. GA and GU degradation pathways in *E. coli* K-12.

TABLE 3. Enzymatic assays of Gar⁻ mutants

Strain	Sp act (nmol of product/min per mg [dry wt])[a]				Gene(s) affected
	THU *exuT*	ISO *uxaC*	AOR *uxaB*	HLA *uxaA*	
B374[b]	1	14	17	1	
B374[c]	7	175	175	14	
ERH200	14	190	384	13	Constitutive
ERH201	13	165	214	12	*kdgA*
ERH202	13	205	409	13	*kdgA*
ERH203	12	193	278	14	*kdgK*
ERH204	16	136	114	9	*kdgK*
ERH205	16	124	227	14	*kdgK*
ERH206	15	179	222	12	*kdgK*
ERH207	14	186	252	0.1	*uxaA*
ERH208	15	182	18	0.2	*uxaB uxaA*
ERH209	13	156	116	0.2	*uxaA*
ERH210	13	168	136	0.1	*uxaA*
ERH213	12	5	5	0.3	*uxaC uxaB uxaA*
ERH214	9	1	2	7	*uxaC uxaB*
ERH215	2	98	129	10	*exuT*
ERH216	1	180	334	10	*exuT*
ERH217	1	190	294	12	*exuT*
ERH218	3	186	156	17	*exuT*
ERH219	1	5	2	0.6	*exuT uxaC uxaB uxaA*
ERH220	1	1	1	0.4	*exuT uxaC uxaB uxaA*
ERH221	1	204	122	0.3	*exuT uxaA*
ERH223	1	4	9	0.3	*exuT uxaC uxaB uxaA*
ERH224	15	101	290	0.1	*uxaA*

[a] THU, Hexuronate transport system; ISO, uronate isomerase; AOR, altronate oxidoreductase; HLA, altronate hydrolase.
[b] Grown in minimal glycerol medium.
[c] Grown in minimal GA medium.

Finally, we took advantage of the fact that a mutant inactivated in the *kdgA* gene is poisoned by GA because of the accumulation of the toxic compound KDGP (Fig. 1). From a *kdgA* mutant of ERH200 we isolated clones able to grow on minimal medium supplemented with glycerol and GA. Most of these clones carry a secondary mutation in the GA degradative pathway which allows them to escape the poisoning by KDGP. In these mutants, the *kdgA* mutation was removed by phiEC2 transduction of the *kdgA*⁺ allele. Mutants which belong to this third set are indicated in Table 2.

Characterization of Gar⁻ mutants. The fact that the parental strain used in this work is Gur⁺ allowed us to easily screen the mutants isolated. The *exuT* and *uxaC* genes which code for the transport of GA and the first step of GA catabolism are involved in degradation of both GA and GU, while the *uxaA* and *uxaB* genes are specific for GA degradation (Fig. 1). Since *E. chrysanthemi* degrades PGA by a transeliminative process which also produces KDG, mutants unable to grow on GA, GU, and PGA must be affected in either the *kdgK* or the *kdgA* gene. Thus, Gar⁻ Gur⁺ Pga⁺ mutants must be impaired in expression of either *uxaA* or *uxaB* or both. Gar⁻ Gur⁻ Pga⁺ mutants must be impaired in the expression of at least the *exuT* or *uxaC* gene, and Gar⁻ Gur⁻ Pga⁻ mutants must be impaired in the expression of at least the *kdgK* or *kdgA* gene. Moreover, *kdgA* mutants are easily characterized by their sensitivity to GA, GU, and PGA. The Gar, Gur, and Pga phenotypes of the different mutants are summarized in Table 2.

The Gar⁻ mutants were further analyzed by complementation tests. We previously isolated R-prime plasmids carrying one or several genes of strain B374 involved in GA degradation. One plasmid of each type obtained (R′ *exuT*, R′ *exuT-uxaC*, R′ *uxaC-uxaB*, R′ *uxaC-uxaB-uxaA*, R′ *exuT-uxaC-uxaB-uxaA*, R′ *kdgK*, and R′ *kdgA*) was transfered into each of the Gar⁻ mutants to test whether the presence of the R-prime plasmids restored the ability to grow on GA. The results of these complementation tests (Table 2) allowed a final characterization of *kdgK*, *kdgA*, and *exuT* mutants. The situation was more complex for the three *uxa* genes. Indeed, as inferred from the fact that no R-prime plasmid carrying either the *uxaA* or the *uxaB* gene alone was found, the *uxaC*, *uxaB*, and *uxaA* genes are most probably arranged in one transcription unit proceeding from *uxaC* to *uxaA* (24). To determine which of these three genes is affected in the Gar⁻ mutants complemented by the R′ *exuT-uxaC-uxaB-uxaA* plasmid, we measured the enzymatic activities encoded by *exuT* (hexuronate transport system), *uxaC* (uronate isomerase), *uxaB* (altronate oxidoreductase), and *uxaA* (altronate hydrolyase).

Assays of hexuronate enzymes and the hexuronate transport system. Strain ERH200, the parent of the Gar⁻ mutants, is a Gur⁺ derivative of B374. Table 3 shows measurements of the hexuronate transport system and of the different enzymatic activities of the GA-degradative pathway on extracts prepared from B374, ERH200, and the Gar⁻ mutants grown in minimal glycerol medium and B374 grown in the same medium supplemented with GA. GA induced the four proteins assayed in B374, whereas in ERH200 they were expressed constitutively. As expected, these proteins were also constitutively expressed in mutants ERH201 to ERH206, which by complementation were determined to be either *kdgA* or *kdgK*. Four mutants (ERH207, ERH209, ERH210, and ERH224) were only defective for the product of the *uxaA* gene (pUxaA, altronate hydrolase), and four others (ERH215 to ERH218) were only defective in the transport system (pExuT). On the contrary, in the other mutants, the expression of more than one gene was affected. ERH208 was defective in pUxaA and pUxaB (altronate oxydoreductase), ERH213 was defective in the products of genes *uxaC*, *B*, and *A* (uronate isomerase), ERH214 was defective for pUxaB and pUxaC, ERH221 for pExuT and pUxaA. Mutants defective in the four genes *exuT*, *uxaA*, *uxaB*, and *uxaC* (ERH219, ERH220, and ERH223) have also been found both spontaneously and after Tn*9* mutagenesis.

Localization of the genes involved in GA degradation. Mapping of the different Gar⁻ mutations was achieved by using RP4::mini-Mu pULB113. This plasmid can promote the transfer of the chromosome of its host from random points of origin (23). This property was used to construct the first map of B374 (21). pULB113 was transfered in each Gar⁻ mutant, which was then mated with the polyauxotrophic strain RH6010 (*thr leu pro trp his thy ile*) or its His⁺ derivative ERH80. Transconjugants which acquired the prototrophic allele for one of these markers were selected and tested for simultaneous acquisition of the Gar⁻ phenotype. Table 4 shows the percentage of cotransfer of the different Gar⁻ mutations and the prototrophic marker to which they are linked. The *exuT-uxa* region lies near the *thy* locus (1 to 14% cotransfer). As the *thy* marker is far from all the other known markers localized, we could not determine the respective order of these two markers.

kdgK was located very close to *ile* (about 40% cotransfer). To determine the relative order of the *kdgK*, *ile*, and *thr* loci, we reasoned that if a marker is located between two others, transconjugants selected for the simultaneous inheritance of the two external markers should in most cases also have

acquired the central marker. We tested this assumption with the three markers *thr*, *leu*, and *pro*, which have been ordered previously (21). *leu* was cotransferred with *thr* at 38%, whereas *pro* was cotransfered with *thr* at about 2%. We selected Thr$^+$ Pro$^+$ transconjugants and tested their Leu phenotype; 95% of them were Leu$^+$. *kdgK* cotransferred with *ile* at 40%, and *thr* contransferred with *ile* at about 1%. When we selected Ile$^+$ Thr$^+$ transconjugants in the mating ERH203(pULB113) × ERH80, only 34% were Gar$^-$. This suggests that the order of the markers in this region is *kdgK ile thr* (Fig. 2).

The *kdgA* gene cotransferred at 60% with the *trp* marker. *trp* was previously shown to cotransfer at about 30% with *his* (21). The *kdgA* mutant ERH201 is Trp$^+$ His$^-$. As expected, we found that 30% of the Trp$^+$ transconjugants recovered from the mating between ERH80 and ERH201(pULB113) were His$^-$. Moreover, all these Trp$^+$ His$^-$ transconjugants were *kdgA*, strongly suggesting that *kdgA* is located between *trp* and *his*.

In the mutants which were isolated after Tn*9* mutagenesis, the matings also allowed us to check the 100% linkage between the Gar mutation and the transposon which is expected if the mutation is indeed induced by Tn*9* insertion. In mutant ERH201 we found no linkage between the Gar$^-$ phenotype and Cml^r. This mutant must therefore carry Tn*9* outside the *kdgA* gene, and the *kdgA1* mutation was not induced by Tn*9*. For the other mutants isolated after Tn*9* mutagenesis, all the Cml^r transconjugants tested in the matings with ERH80 as a recipient acquired the Gar mutation; these mutations are thus really caused by the insertion of Tn*9*.

After mating RH6010 or ERH80 with the Tn*9*-induced mutants as donors, we observed that a variable proportion of the Gar$^-$ transconjugants were Cml^s. This led us to test for the stability of the Cml phenotype in the mutants. We found that indeed after a few weeks, a majority (sometimes more than 95%) of the isolated colonies that were recovered from stabs containing the mutants were Cml^s. In strains ERH207, ERH224 (*uxaA*), and ERH208 (*uxaB-uxaA*), the majority of the Cml^s segregants had in addition become Gur$^-$, suggesting that a deletion had occurred around Tn*9*, eliminating both the transposon gene *cat* and some adjacent genes, such as *uxaC* or *exuT*.

This instability of the Cml marker was also observed in other Tn*9*-induced mutants of B374, such as auxotrophs. It can, of course, be avoided by growing the mutants in the presence of chloramphenicol.

TABLE 4. Localization of Gar$^-$ mutations[a]

Mutant	Marker linked	% of cotransfer (ratio)
ERH201	*trp*	60 (59/97)
ERH202	*trp*	59 (52/88)
ERH203	*ile*	39 (59/150)
ERH204	*ile*	44 (44/100)
ERH205	*ile*	42 (30/71)
ERH206	*ile*	35 (35/100)
ERH207	*thy*	4 (4/99)
ERH208	*thy*	2 (2/100)
ERH209	*thy*	10 (5/48)
ERH210	*thy*	8 (8/100)
ERH213	*thy*	1 (1/100)
ERH214	*thy*	5 (5/99)
ERH215	*thy*	5 (10/195)
ERH216	*thy*	4 (4/100)
ERH217	*thy*	4 (4/100)
ERH218	*thy*	5 (5/100)
ERH219	*thy*	14 (11/80)
ERH220	*thy*	4 (3/79)
ERH221	*thy*	2 (2/92)
ERH223	*thy*	2 (2/94)
ERH224	*thy*	8 (4/49)

[a] The procedure is given in the text. The frequencies of cotransfer of the Gar$^-$ mutation with the selected marker are given by the ratio of the number of Gar$^-$ mutants among the transconjugants which received the selected marker. These numbers are shown within parentheses.

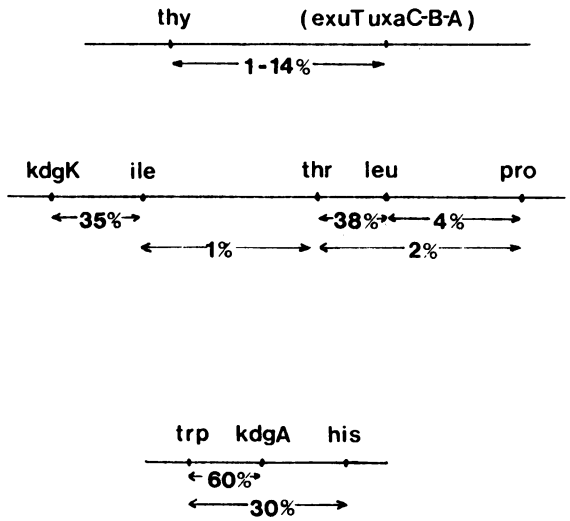

FIG. 2. Genetic organization in strain B374 of the genes involved in the degradation of GA and KDG. See the text for details. The numbers on the arrows show the percentage of cotransfer by RP4::mini-Mu between the different markers.

DISCUSSION

The genetic organization of strain B374 genes involved in galacturonate degradation was previously partially determined by in vivo cloning of these genes on the RP4::mini-Mu plasmid pULB113 (24). The *kdgK* and *kdgA* genes were found to be unlinked and separated from a cluster containing genes *exuT*, *uxaC*, *uxaB*, and *uxaA*, which itself was suggested to contain two transcriptional units, one including *exuT*, the other including the three *uxa* genes and proceeding from *uxaC* to *uxaA*.

To confirm these results and to map the different genes on the B374 chromosome, we isolated and characterized spontaneous and Tn*9*-induced Gar$^-$ mutants. Tn*9* was used because it is known to generate polar mutations. Consistent with previous results, none of the *kdgA* and *kdgK* mutants isolated (including those induced by Tn*9*) showed any defect in expression of other genes involved in the GA pathway.

We found *uxaA*::Tn*9* and *exuT*::Tn*9* mutants which were not affected for the expression of any other gene of the *exu-uxa* cluster, suggesting that these genes are either each the last gene of a polycistronic operon or form distinct transcription units. This again is in agreement with the organization of the *exu-uxa* cluster proposed previously. This gene order was also confirmed by the behavior of mutants ERH208 and ERH214, which are respectively affected in *uxaA* and *uxaB* and in *uxaC* and *uxaB* genes. The former was induced by Tn*9* and might be either a Tn*9* insertion in *uxaB* polar on *uxaA* or a Tn*9*-induced deletion covering *uxaB* and *uxaA*. ERH214 is a spontaneous mutant which might be a small deletion covering the *uxaC* and *uxaB* genes but leaving expression of *uxaA* unaffected.

If the gene order actually is *exuT-uxaC-uxaB-uxaA*, the mutant ERH221, which is *exuT uxaA*, should be the result of a multiple event. ERH221 was induced by Tn*9* which, as mentioned earlier, seems to generate deletions in its vicinity at very high frequencies (see above). The *exuT uxaA* mutation might be the result of a more complex rearrangement induced by Tn*9*; for instance, the transposon could have inserted in one of these two genes and provoked an inversion of the chromosomal fragment located between the two genes, breaking the continuity of the second mutated gene. IS*1*-induced inversions of adjacent DNA have been reported previously (3). This hypothesis is currently being tested.

Several of the Gar$^-$ Gur$^-$ mutants we isolated, some of which were spontaneous and some of which were induced by Tn*9*, were affected in the four genes of the *exu-uxa* cluster. These mutations do not seem to be due to the inactivation of a gene, the product of which would be necessary for the expression of the genes of the cluster; indeed, neither R′ *exuT-uxaC-uxaB* plasmids nor R′ *uxaC-uxaB-uxaA* plasmids complemented these mutations, although a very small R′ *exuT-uxaC-uxaB-uxaA* plasmid which carried only 7 kilobases (kb) of chromosomal DNA did (Table 2). Since the former plasmids carry overlapping parts of this 7 Kb DNA fragment, the putative "activator" gene present in this little fragment would also be carried by one of the two other types of R′ plasmids. The most likely explanation is that these mutations also result from deletions in the *exu-uxa* region. Deletions would therefore seem to occur very frequently in that region. Such an high proportion of deletions is not unusual. In a very accurate study of mutations occurring in the *lacI* gene of *E. coli*, Farabaugh et al. showed that 14% of spontaneous mutations are deletions (5).

kdgK and *kdgA* genes, as well as the *exuT-uxaC-uxaB-uxaA* cluster, have been localized on the B374 chromosome. *kdgA* mapped between the *trp* and *his* markers, *kdgK* was very close to the *ile* marker, and the *exu-uxa* cluster mapped near the *thy* marker (see Fig. 2). So far, not enough genetic markers are available in B374 to allow mapping by cotransduction. It is therefore difficult to establish a correlation between the frequencies of cotransfer by RP4::mini-Mu and the frequencies of transduction. We have two sets of data which allow a very preliminary correlation between the frequency of cotransfer of two markers and their physical distance. In *E. coli*, *thr* and *leu* are 1.5 min away from each other (i.e., ~67 kb) and they are cotransferred by pULB113 at ~45%. In B374, we have isolated an R-prime plasmid which carries the *trp-kdgA* region of the chromosome and carries 80 kb of chromosomal DNA. Therefore *trp* and *kdgA* are at most 80 kb apart, and they are cotransfered at 60% by pULB113. We attempted to isolate R-prime plasmids carrying the *ile-kdgK* region of the B374 chromosome but did not succeed. Recent results suggest that it is most probably due to the presence in that region of an unidentified factor which is lethal for most *E. coli* strains (van Gijsegem et al., submitted for publication). We definitely need additional results to be able to establish the function which correlates the frequency of cotransfer of two markers by pULB113 with their physical distance.

In *E. coli*, *kdgK* lies at min 78, (i.e., 7 min away from *ile*), *exuT uxaC uxaA* lies at min 67 (i.e., at 6 min away from *thy*), and *uxaB*, which is not linked to the previous cluster, lies at min 52. *kdgA* is localized between *his* and *trp* as in *Erwinia* spp. although the distance between *his* and *trp* in *E. coli* K-12 is much longer (16 min of the chromosome) than in B374. It seems that for the genes involved in GA degradation, the maps of *E. coli* and *E. chrysanthemi* are not really correlated. Finally, the fact that *kdgK* mutants do not grow with PGA as sole carbon source indicates that all the metabolites produced by PGA degradation are finally transformed into KDG.

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