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The *Erwinia chrysanthemi pecT* Gene Regulates Pectinase Gene Expression

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A new type of *Erwinia chrysanthemi* mutant displaying a derepressed synthesis of pectate lyase was isolated. The gene mutated in these strains, *pecT*, encodes a 316-amino-acid protein with a size of 34,761 Da that belongs to the LysR family of transcriptional activators and presents 61% identity with the *E. coli* protein LrhA. PecT represses the expression of pectate lyase genes *pelC*, *pelD*, *pelE*, *pelL*, and *kdgC*, activates *pelB*, and has no effect on the expression of *pelA* or the pectin methylesterase genes *pemA* and *pemB*. PecT activates its own expression. The mechanism by which PecT regulates pectate lyase synthesis is independent of that of the two characterized regulators of pectate lyase genes, KdgR and PecS. In contrast to most of the members of the LysR family, *pecT* is not transcribed in a direction opposite that of a gene that it regulates. *pecT* mutants are mucoid when grown on minimal medium plates and flocculate when grown in liquid minimal medium, unless leucine or alanine is added to the medium. Thus, *pecT* may regulate other functions in the bacterium.

The synthesis and secretion of plant cell wall-degrading enzymes have been identified as one of the causes of the pathogenicity of *Erwinia chrysanthemi* (1). This plant pathogen is able to provoke the soft rot of a number of vegetable crops in the field or during storage. *E. chrysanthemi* 3937 has developed a set of enzymes to degrade pectin: two pectin methylesterases (encoded by the *pemA* and *pemB* genes) (21, 39), five major isoenzymes of pectate lyases (encoded by the *pelA*, *pelB*, *pelC*, *pelD*, and *pelE* genes), and at least four secondary pectate lyases. The gene of only one of them, *pelL*, has been cloned and studied (24). The action of these enzymes produces saturated and unsaturated oligogalacturonates that can be used as a carbon source and are metabolized by the products of the genes *ogl*, *kduI*, *kduD*, *kdgK*, and *kdgA* (11, 18, 33). The cellulolytic equipment of *E. chrysanthemi* is composed of the major, extracellular endoglucanase Z, the product of *celZ* (4), and of the periplasmic CelY protein, the product of *celY* (13).

The regulation of the *pel* genes has been studied in vitro and in planta (23). Numerous factors influence the expression of the *pel* genes: growth phase (17), temperature, nitrogen starvation, oxygen concentration, osmolarity, the presence of rapidly metabolizable sugars (16), iron concentration (12), and the presence of plant extracts (5) or pectin and pectin catabolism products (8). This complexity led us to suppose that there are several regulatory proteins controlling *pel* gene expression. In contrast, expression of the genes of the intracellular part of the pectin degradation pathway seems to be inducible only by pectin degradation products (8), and no evidence of induction of the *cel* genes has been found up to now.

The strategy used in our laboratory to study the regulation of the pectin degradation pathway has been to look for mutants synthesizing elevated levels of pectate lyases under uninduced conditions (17). With *E. chrysanthemi* 3937, this strategy allowed us to identify two regulatory loci: *kdgR* and the *pecS-pecM* locus. *kdgR* encodes a repressor that controls all the genes involved in pectinolysis: the *pel* and *pem* genes, the genes of the intracellular steps of the pathway, and the *out* genes that code for the proteins required for the secretion of pectate lyases and of the cellulase EGZ (7, 9, 34). A direct role of *kdgR* in the regulation of transcription has been proved for most of these genes: KdgR binds to the KdgR-box, a 17-bp sequence present in the regulatory region of controlled genes, preventing their transcription (28). The presence of 2-keto-3-deoxygluconate (KDG), a pectin catabolism product, prevents KdgR binding on the KdgR-box (27, 28). This fact accounts for the induction observed in the presence of pectin or pectin metabolism compounds. The *pecS-pecM* locus controls the *pel* genes, *pemA*, *celZ*, and the *outC* operon. A mutation in either of these two genes has the same phenotype, an elevated expression of the controlled genes. Moreover, *pecS* and *pecM* mutants synthesize a blue pigment. According to sequence data, it has been proposed that PecM could be an integral membrane sensor and that PecS could be a cytoplasmic regulatory protein (35). The signal to which these regulatory proteins respond is, for the moment, unknown.

Although the pectate lyase expression level is high in a double *kdgR-pecS* mutant, it remains inducible in the presence of polygalacturonate (PGA) (35). This suggests the existence of additional regulatory genes controlling *pel* gene expression. In this paper, we describe the characterization of *pecT*, a new gene regulating pectate lyase synthesis.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. Bacterial strains and plasmids used in this study are described in Table 1. *E. chrysanthemi* and *Escherichia coli* cells were grown at 30 and 37°C, respectively, in Luria broth medium or M63 minimal medium (25) supplemented with a carbon source (0.2%, except PGA and pectin [0.4%]) and, when required, with amino acids (40 μg/ml) and the following antibiotics at the concentrations indicated: ampicillin, 100 μg/ml; kanamycin, 50 μg/ml; and chloramphenicol, 20 μg/ml.

Genetics techniques. Transduction with phage φEC2 was done as described by Résibois et al. (32). Marker exchange recombinations were obtained after growth in a low-phosphate-concentration medium as described by Roeder and Collmer (36). Tn*5* mutagenesis was performed with plasmid pMO194. An overnight culture of *Pseudomonas aeruginosa* PAO5(pMO194) (0.2 ml) was mixed with 0.2 ml of a culture of strain A350 on an M63 agar plate. After 5 h at 30°C, cells were

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TABLE 1. Bacterial strains, plasmids, and phage used in this study

Strain	Genotype(s)	Source or reference
Strains		
E. chrysanthemi		
3937	Wild type	20
A266	*kdgK*::MuAp *lac*	18
A350	*lmrT*(Con) *lacZ2*	17
A691	*lmrT*(Con) *lacZ2 kduD*::MuAp *lac*	8
A837	*lmrT*(Con) *lacZ2 kdgR*	10
A1671	*lmrT*(Con) *lacZ2 kdgC*::MudI1734	11
A1787	*pelB*::*uidA*-Kan	16
A1789	*pemA*::*uidA*-Kan	16
A1798	*pelD*::*uidA*-Kan	16
A1828	*pelE*::*uidA*-Kan	16
A1831	*lmrT*(Con) *lacZ2 outT*::MudI1734	Laboratory collection
A1832	*lmrT*(Con) *lacZ2 outD*::MudI1734	Laboratory collection
A1854	*lmrT*(Con) *lacZ2 outB*::MudI1734	Laboratory collection
A1880	*pelC*::*uidA*-Kan	16
A1888	*pelA*::*uidA*-Kan	16
A1903	*lmrT*(Con) *lacZ2 outS*::*uidA*-Kan	7
A1956	*lmrT*(Con) *lacZ2 pecT*::Tn*5*	This work
A1988	*lmrT*(Con) *lacZ2 pelL*::*uidA*-Kan	24
A1995	*lmrT*(Con) *lacZ2 celZ*::*uidA*-Kan	S. Reverchon
A2011	*lmrT*(Con) *lacZ2 pecS*::*uidA*-Kan	35
A2148	*lmrT*(Con) *lacZ2 pecT*::*uidA*-Cm	This work
A2174	*lmrT*(Con) *lacZ2 pecT*::Cm	This work
A2182	*lmrT*(Con) *lacZ2 pecT*::*uidA*-Kan	This work
P. aeruginosa PAO5	*trp-54 rif-5 fon-1*	30
E. coli		
NM522	Δ(*lac-proAB*) *thi hsd-5 supE* (F′ *proAB*$^+$ *lacI*^{q} *lacZ* ΔM15)	Stratagene
HB101	F$^-$ *hsd-20 recA13 ara-14 proA2 leuB6 lacY1 galK2 xyl-5 mtl-1 rpsL20 supE44*	Laboratory collection
Plasmids		
pMO194	Cb Km Tc Tra *trfA*(Ts) IncP-1::Tn*5*	30
pBS	Ap^r *lacZ′*	Stratagene
pBSCm	Cm^r *lacZ′*	Stratagene
pT7-5	Ap^r	40
Phage ϕEC2	*E. chrysanthemi* generalized transducing phage	32

resuspended in 1 ml of M63 medium, and dilutions were spread on glucose-plus-kanamycin plates.

Enzyme assays. β-Glucuronidase assays were performed with toluenized cells grown to exponential phase with *p*-nitrophenyl-β-D-glucuronate as the substrate (29). β-Galactosidase assays were performed with toluenized cells grown to exponential phase with *o*-nitrophenyl-β-D-galactose as the substrate (25). Pectate lyases in culture supernatant or toluenized cells were assayed as described by Moran et al. (26). In situ pectate lyase activity was detected by flooding polygalacturonate-containing plates with a saturated solution of copper acetate. Degraded polygalacturonate formed a clear halo around the colonies.

Recombinant DNA techniques. Preparations of chromosome and plasmid DNA, restriction digestions, ligations, DNA electrophoresis, transformations, and electroporations were carried out as described by Sambrook et al. (37). Nucleotide sequence analysis was performed by the chain termination method with double-stranded DNA templates. Extension of primers was achieved with T7 DNA polymerase.

Analysis of plasmid-encoded proteins. Overexpression and labelling of plasmid-encoded proteins were obtained by using the T7 promoter-T7 polymerase system of Tabor and Richardson (40).

Nucleotide sequence accession number. The nucleotide sequence of the *pecT* gene will appear in the EMBL, GenBank, and DDJB databases under accession no. X85985.

RESULTS

Isolation of *pecT* mutants. Tn*5* mutagenesis of strain A350 was performed to isolate new types of mutants synthesizing pectate lyases constitutively. Of about 15,000 Kan^r clones, 6 were found to synthesize elevated levels of pectate lyases. The phenotypes of these mutants were analyzed to check if they corresponded to already identified regulatory mutants. Two of them produced a blue pigment and thus were assumed to be *pecS* or *pecM* mutants (35), and one mutant was able to grow

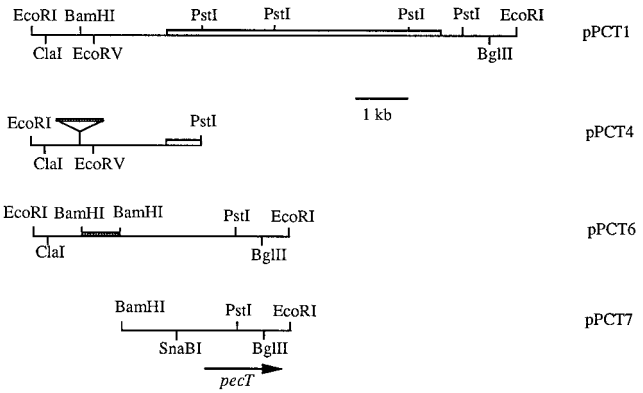

FIG. 1. Restriction maps of plasmids containing *pecT*. Open bars represent Tn*5* DNA; hatched bars represent Cm^r or Km^r cassette DNA.

TABLE 2. Pectate lyase synthesis and *pecT* expression under various growth and induction conditions[a]

Growth condition	Inducer	Wild-type pectate lyase activity	A2182(*pecT*::*uidA*)	
			Pectate lyase activity	β-Glucuronidase activity
Standard	None	0.02	0.47	2,020
	Galacturonate	0.60	1.45	1,600
	PGA	0.60	2.40	1,820
	Plant extract	0.03	0.61	2,000
	PGA + plant extract	2.90	5.30	1,820
37°C	None	0.02	0.15	2,080
	PGA	0.17	0.92	1,940
High osmolarity	None	0.01	0.02	2,270
	Galacturonate	0.08	0.12	1,600
Anaerobiosis	None	0.09	0.77	1,020
Low nitrogen	PGA	<0.01	0.24	375

[a] Standard conditions were obtained by cultivation in M63 medium containing 0.2% glycerol as the carbon source at 30°C with shaking at 200 rpm. Anaerobic cultures were realized by overlaying the culture medium with paraffin oil. Fumarate (2.5%) was added as an electron acceptor. High osmolarity was obtained by adding 0.3 M NaCl to M63 medium. Nitrogen starvation was performed in M63 medium deprived of $(NH_4)_2SO_4$ supplemented with arginine (200 μg/ml). β-Glucuronidase activity is expressed in nanomoles of *p*-nitrophenol formed per minute per milligram (dry weight) of bacteria. Pectate lyase activity is expressed in micromoles of unsaturated products formed per minute per milligram (dry weight) of bacteria. Each value is the average of at least three determinations.

on KDG as the sole carbon source and was therefore probably a *kdgR* mutant (10). To confirm that the three remaining mutants did not contain new alleles of *kdgR*, *pecS*, or *pecM*, a *kdgR*::Cm^r or a *pecS*::Cm^r mutation was transduced into these mutants. In none of these cases did the Cm^r marker cotransduce with the Km^r marker of Tn*5*. Thus, these three new mutants, A1956, A1982, and A1983, have a Tn*5* insertion in a previously uncharacterized gene regulating pectate lyase synthesis. The locus mutated in strain A1956 was named *pecT*, and this mutant was further characterized.

Cloning of *pecT* gene. To clone the mutated *pecT* locus, the chromosomal DNA of strain A1956 was digested with the enzyme *Eco*RI (which does not cut Tn*5* DNA) and ligated into plasmid pBSCm. Km^r clones were selected. Analysis of their plasmid content showed that they contained a 9-kb DNA fragment. A 5.7-kb portion of the insert is Tn*5* DNA, the remainder corresponding to chromosomal DNA adjacent to *pecT*. A rough restriction map of the insert of this plasmid, pPCT1, was constructed (Fig. 1). It contained a unique *Bam*HI site in the chromosomal DNA part, located 1.5 kb from the Tn*5* insertion site. After this fragment was subcloned to delete the part of the insert corresponding to Tn*5*, a cassette encoding a Cm^r gene was introduced into the *Bam*HI site (plasmid pPCT4) (Fig. 1). This construct was recombined into the chromosome of strain A350. The recombinant strain, A2129, had no detectable phenotype and expressed pectate lyases to the wild-type level. The Cm^r cassette cotransduced at 92% with the Tn*5* inserted in *pecT*, confirming their proximity. The cassette cotransduced at the same frequency with the Tn*5* inserted in strains A1982 and A1983, indicating that these strains are also *pecT* mutants. Thus, we have introduced a Cm^r marker close to the *pecT* gene. This made possible the cloning of the wild-type allele of *pecT* by selecting the nearby Cm^r marker. Since no new *Eco*RI site had been introduced with the Cm^r cassette, chromosomal DNA of strain A2129 was digested by *Eco*RI, and the fragments were ligated into *Eco*RI-linearized pBS. Cm^r clones were selected, and a restriction map of the plasmid content of one of them, pPCT6, was drawn (Fig. 1). A *Bam*HI deletion eliminated the Cm^r cassette and yielded plasmid pPCT7. To confirm that this plasmid contained the wild-type allele of *pecT*, *uidA*-Cm and *uidA*-Kan cassettes were introduced into the *Pst*I and *Bgl*II sites, respectively (Fig. 1). Both types of construct were recombined into the strain A350 chromosome. These recombinants, A2182 and A2148, exhibited a phenotype identical to that of strain A1956, indicating that the sites *Pst*I and *Bgl*II are located inside *pecT*. These insertions resulted in a β-glucuronidase-positive phenotype when the *uidA* reporter gene was transcribed in the *Bam*HI-*Eco*RI orientation, indicating that *pecT* is transcribed in this orientation.

Identification of genes controlled by *pecT*. The uninduced level of pectate lyase increased 20-fold in a *pecT* mutant (Table 2). To analyze the role of *pecT* in the regulation of individual *pel* genes, a *pecT*::Cm^r mutation was introduced into strains containing *pel-uidA* transcriptional fusions. *pelC*, *pelD*, *pelE*, and *pelL* levels of expression increased two- to fivefold in a *pecT* mutant (Table 3). In contrast, *pelA* expression was unchanged, and *pelB* expression decreased twofold (Table 3). The influence of *pecT* on the other genes of pectinolysis was also tested. Expression of the pectin methylesterase genes *pemA* and *pemB*, of the secretion genes *outS*, *outT*, *outB*, and *outC*, of the cellulase gene *celZ*, and of the genes of the intracellular part of the pectin degradation pathway (*kduD*, *kdgK*, and *kdgT*) was not notably modified in a *pecT* mutant (Table 3). However, *kdgC*, which codes for a protein of unknown function but exhibits homology with periplasmic pectate lyases, is expressed at a fivefold-higher level in a *pecT* mutant (Table 3).

Pectate lyase expression is regulated by many environmental conditions. We compared pectate lyase activities in the wild-type strain and in a *pecT* mutant under different induction

TABLE 3. Effect of *pecT* mutation on expression of various genes involved in pectin and cellulose degradation

Gene tested	Sp act	
	pecT$^+$	*pecT*
pemA	69	56
pemB	26	26
pelA	5.1	5.6
pelB	120	51
pelC	39	160
pelD	11	43
pelE	260	940
pelL	70	290
kdgC	22	110
outS	1,460	1,820
outB	800	740
outT	57	40
outC	280	230
kdgK	67	46
kdgT	2.5	2.6
kduD	6	5
kdgR	570	500
pecS	3,800	3,150

A *pecT* mutation was introduced into strains containing a fusion between the gene tested and the reporter genes *lacZ* (for *kdgC*, *kdgK*, *kdgT*, *kduD*, *outT*, and *kdgR*) or *uidA* (for *pemA*, *pemB*, *pelA*, *pelB*, *pelC*, *pelD*, *pelE*, *pelL*, *outS*, *outB*, *outC*, and *pecS*). Cultures were grown in M63 minimal medium plus glycerol. Specific activities of the reporter enzymes are expressed in nanomoles of *o*-nitrophenol (for β-galactosidase) or *p*-nitrophenol (for β-glucuronidase) liberated per minute per milligram (dry weight) of bacteria. Each value is the average of at least three determinations.

TABLE 4. Pectate lyase activity in various regulatory mutants[a]

Genetic background	Pectate lyase activity	
	Uninduced	Induced
Wild type	0.02	0.60
pecT	0.47	2.30
kdgR	0.41	1.90
pecS	0.34	4.40
pecT kdgR	3.70	4.70
pecT pecS	2.30	29.0
kdgR pecS	3.60	8.20
pecT kdgR pecS	17.3	23.0

[a] Cells were grown in M63 minimal medium plus glycerol. Polygalacturonate was added as an inducer. Pectate lyase activity is expressed as micromoles of unsaturated products liberated per minute per milligram (dry weight) of bacteria. Each value is the average of at least three determinations.

conditions to check whether there were changes in regulation in response to environmental parameters (Table 2). The regulation was conserved under the following conditions: induction by plant extract in the presence of PGA, decrease of pectate lyase synthesis at high temperatures and with nitrogen limitation, and increased pectate lyase synthesis under anaerobic conditions. Thus, PecT is not the protein responsible for regulation by these factors. At high osmolarity, there is only a small increase in pectate lyase synthesis in the *pecT* mutant (1.5- to 2-fold). The effect of *pecT* could be masked by the reduced synthesis of pectate lyase under this condition, or *pecT* could be involved in the regulation by osmolarity.

Independent regulation of pectate lyase synthesis by *pecT*, *pecS*, and *kdgR*. Double mutants were constructed to test whether *pecT* acts within a regulatory pathway with the already characterized regulatory genes *kdgR* and *pecS* or whether these three genes constitute independent regulatory circuits. Introduction of a *pecT* mutation did not modify expression of the *kdgR* and *pecS* genes (Table 3). The level of expression of *pecT* was unchanged in a *kdgR* or *pecS* background (data not shown). All the double mutants synthesized pectate lyases at a higher level than any of the single mutants (Table 4). Moreover, the triple *kdgR pecS pecT* mutant synthesized more pectate lyase under uninduced conditions than any of the double mutants. Additivity of the effects of these three mutations indicates that *kdgR*, *pecS*, and *pecT* regulate *pel* gene expression in three independent ways. It was interesting that pectate lyase activity remained inducible by PGA in the *kdgR pecS* and *pecT pecS* mutants, while only a low level of residual induction remains in the *kdgR pecT* mutant (Table 4). This suggests that *pecT* regulation could respond to the presence of PGA degradation products, as already shown for *kdgR*.

Regulation of *pecT*. Construction of a chromosomal *pecT*::*uidA* fusion allowed for the study of *pecT* regulation. *pecT* expression was not induced by PGA, galacturonate, or plant extract (Table 2). It was not sensitive to the catabolite repression exerted by glucose or by growth in a high-osmolarity medium. In contrast, *pecT* expression was reduced twofold in anaerobiosis and fivefold in a low-nitrogen medium (Table 2). *pecT* expression was unchanged in a *kdgR* or *pecS* mutant (data not shown). To test for possible autoregulation of *pecT*, plasmid pACYC184 bearing *pecT* was introduced into an *E. coli* strain containing a *pecT*::*uidA* fusion on a pBS plasmid. The presence of the wild-type copy of *pecT* increased the level of expression of the *pecT*::*uidA* fusion twofold (350 U versus 645 U). Thus, *pecT* appeared to be positively autoregulated.

Other phenotypes of *pecT* mutants. *pecT* mutants presented additional phenotypes: the bacteria flocculated when grown in liquid minimal medium, and the colonies were mucoid on minimal medium plates. The mucoidy increased when the plates contained the pectate lyase synthesis inducers galacturonate or PGA. This phenotype was not observed in rich medium. We tried to identify which compound, present in LB medium, prevented flocculation. Flocculation was no longer observed when Casamino Acids were added into minimal medium. Next, we tested individually the effect of the 20 amino acids and found that either leucine or alanine added at a concentration of 0.1 mg/ml prevented flocculation. The level of expression of the *pecT*::*uidA* fusion and the synthesis of pectate

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CATTACTATAGCTGTGAAGCTGGTTCCAGATAGCGATCGGAGCCAGAGCGATAAACAACATCATTGTTAA  140
TGACGTGTGATTAAAGTGTGATAGTGCTTTCGATTTCCGGCCCGATACTGAGAGCGAGCTCCATAAAACA  210
GCCTCTGGCGTCGGTAATTTATCCATCCTGATCGCATGACGAAGCATTAATCATGTTCAGACGGTGGTCG  280
AAAGGAATAATCTCCGAAGCAATTGACGTATTTTTTATACCTGATTCAAACGCTGAAACCGGTATCATAA  350
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CAAACGGAGAATCAATGATGCACGAAGTATGCGTTAAAAGTGAAAAATAAGAGATGTCGTATATTTTGAT  980
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TTTTGCCGTAACCGTTCGTCTGCGATACTGTCCGTCTTATATTGTGCGTCGGCCTTGCCGGGGCATCAAC 1260
                                                  M  T  N  T  S  R
ATCAACACCAGGTATGTTTGTTAAAAATTAAAATAGATAAGTGAAGAACCGTATGACAAATACAAGTCGC 1330
P  V  L  N  L  D  L  D  L  L  R  T  F  V  A  V  A  D  L  N  T  F  A  A
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  A  A  V  A  V  C  R  T  Q  S  A  V  S  Q  Q  M  Q  R  L  E  Q  L  I
CCGCCGCTGTGGCTGTGTGCAGAACACAGTCGGCAGTGAGCCAGCAGATGCAGCGTCTGGAACAGTTAAT 1470
  G  K  E  L  F  A  R  H  G  R  N  K  L  L  T  E  H  G  I  Q  F  L  G
TGGTAAGGAGCTGTTTGCCCGCCACGGGCGAAACAAGCTGCTGACCGAGCACGGCATCCAGTTTCTTGGG 1540
Y  A  R  K  I  L  Q  F  N  D  E  A  C  A  S  L  M  Y  S  D  I  Q  G  T
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  L  T  I  G  A  S  D  D  T  A  D  T  I  L  P  F  I  L  Q  R  V  T  N
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 V  F  P  K  L  S  I  A  V  S  I  K  R  S  A  E  M  T  D  M  L  Q  Q
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  E  M  M  S  P  E  L  R  V  L  G  E  E  E  G  L  P  K  L  P  D  T  R
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GGCACCCGTTCGCATTTGCTGCCGGTTAGCACCGGGACCGAAAGCGAGCTGCGGGAACCGCCGACAGATG 2240
  S  L  K  D  I  T
AATCATTAAAAGATATTACCTGAGTACGAGCGTAGCGGGGTGAAAGGCCCCGCTACGGCTTAGGTTTTCA 2310
                              >>>>>>>>>>       <<<<<<<<<<
TCCTTCAGTCAGTCTTTTCCATGTTGATTTTCCCACCACGGTTTGTCTGTTCACTTCTATACTTCCTTTG 2380
AACCCTCTTTGCCTAATACGCTGTTTGTGTTCTTTTTTTATTAAAACTGCATTTCTATTTTTTAAAGCC 2450
ATGTTGATCGAATGAAAAGGTTAATGGTTGAGCAATCGACGCAATTTTTTTACTAACATAAATGAAAAAA 2520
GTGTGCCCTGGATCAAAAAATACCCCTGATGGAACCTATGGGGGAACAGGAATTC              2575
```

FIG. 2. Sequence of 2,575-bp *Bam*HI-*Eco*RI DNA fragment containing *pecT*. The deduced amino acid sequence of PecT is indicated. Numbers on the right refer to the positions of the nucleotides. The putative Shine-Dalgarno sequence is underlined. The putative terminator is indicated by carets. The *pecT* nucleotide sequence will appear in the EMBL, GenBank, and DDJB nucleotide sequence databases under the accession no. X85985.

```
MTNTSRPVLNLDLDLLRTFVAVADLNTFAAAAVAVCRTQSAVSQQMQRLEQLIGKELFARHGRNKLLTEH  70
=  - ====-======================== ====================-==================
MISANRPIINLDLDLLRTFVAVADLNTFAAAAAAVCRTQSAVSQQMQRLEQLVGKELFARHGRNKLLTEH  70

GIQFLGYARKILQFNDEACASLMYSDIQGTLTIGASDDTADTILPFILQRVTNVFPKLSIAVSIKRSAEM 140
=== ======== =======-====-=--== =======---------=-=---- =-====-- = -== = =
GIQLLGYARKILRFNDEACSSLMFSNLQGVLTIGASDESADTILPFLLNRVSSVYPKLALDVRVKRNAYM 140

TDMLQQGKIDLVITTSNNDDLPHVLLRTSPTLWYCSADYQYQPGETVSLVVLDEPSPFRALALDQLTAAG 210
--===-   -==--==        - ====== ===-=-=  = == - ==-==-===== - =  =  =
AEMLESQEVDLMVTTHRPSAFKALNLRTSPTHWYCAAEYILQKGEPIPLVLLDDPSPFRDMVLATLNKAD 210

IPWKISYVASTLSAVRAAVKAGLGITVRSVEMMSPELRVLGEEEGLPKLPDTRYYLCRNPDHDNELTNAI 280
===---======= ==========-= = ======-=====-  -==== ==== = == -=  -====-- =
IPWRLAYVASTLPAVRAAVKAGLGVTARPVEMMSPDLRVLSGVDGLPPLPDTEYLLCYDPSSNNELAQVI 280

FSAIESGTRSHLLPVSTGTESELREPPTDESLKDIT                                    316
- =-==                 --
YQAMESYHNPCNTAQCLLRKGHIHC                                               305
```

FIG. 3. Alignment of PecT with *E. coli* LrhA protein. Double dashes indicate identical amino acids; single dashes indicate chemically equivalent amino acids.

lyases were both unchanged by the addition of leucine or alanine (data not shown).

Nucleotide sequence of *pecT*. The nucleotide sequence of the 2.5-kb *Bam*HI-*Eco*RI DNA insert of plasmid pPCT7 was determined (Fig. 2). This fragment contains only one open reading frame (ORF), from nucleotide 1313 to nucleotide 2260. This ORF overlaps the *Pst*I and *Bgl*II sites that were shown to be inside *pecT* by insertion mutagenesis. Moreover, the direction of transcription of this ORF corresponds to that determined by the construction of a gene fusion. Thus, this ORF corresponds to the *pecT* coding sequence. It encodes a 316-amino-acid protein with a calculated molecular mass of 34,761 Da. The ATG initiation codon is preceded by a putative ribosome binding site (GAAGAA) (Fig. 2). The ORF is followed by a putative stem loop structure (nucleotides 2271 to 2299) followed by a run of T's that could be a rho-independent terminator (ΔG, -22.1 kJ $\cdot$ mol^{-1}). The PecT sequence was compared with those contained in the National Biomedical Research Foundation data bank with the BLAST program. Homology was found with proteins of the LysR family of transcriptional regulators. The highest degree of homology was found with the *E. coli* LrhA protein (61% identity and 75% similarity), a protein of unknown function encoded by a gene close to the *nuo* operon (3) (Fig. 3). Genes of members of the LysR family are often transcribed in a direction opposite that of a gene which they regulate (38). It was not possible to detect a gene in the 1 kb of DNA sequenced upstream of *pecT*.

A 1.4-kb *Sna*BI-*Eco*RI DNA fragment containing the *pecT* coding sequence was introduced into plasmid pT7-5. The proteins encoded by this fragment were specifically labelled after induction of the T7 polymerase. One protein with a molecular mass of 30,000 Da was detected, which is in agreement with the size predicted for PecT by sequence data (data not shown).

DISCUSSION

In the search for new genes regulating pectinase synthesis, we have identified *pecT*, a gene coding for a protein of the LysR family of transcriptional regulators (LTR). More than 50 proteins have already been identified in this family (38). Although PecT shares some of the characteristics of the members of this family (size and highest degree of homology with other members of the family at the N-terminal part of the protein), it is atypical for some other aspects. LTRs are usually activators. Although it activates *pelB* expression, PecT represses the expression of five other genes (*pelCDEL* and *kdgC*), and its global effect on pectate lyase synthesis is that of a repressor. LTRs are usually negatively autoregulated. Together with a few other exceptions (MkaC and PhcA), PecT autoactivates its own expression. Furthermore, LTR-coding genes are usually transcribed in the direction opposite that of a regulated target gene. The 1-kb DNA sequence upstream of *pecT* does not seem to contain a gene, and *pecT* is not linked to the genes that it regulates. However, *oxyR*, *nac*, and *cysB* also lack a divergently transcribed regulated gene (38).

Alignment of the regulatory regions of genes controlled by *pecT* did not reveal a conserved region that could correspond to the PecT binding site. DNase I footprinting experiments with LTRs usually define long protected regions (45 bp). When several operators are recognized by the same LTR, alignment of these sequences usually shows very few conserved nucleotides (41). DNAse I footprinting experiments will be performed to determine the PecT binding sites and to see if they overlap the KdgR and the PecS binding sites. This will contribute to our understanding of how these three regulators cooperate to repress *pel* gene expression.

The homolog of PecT with the highest degree of similarity found among the LTRs was LrhA, an *E. coli* protein of unknown function (3). LrhA presents 95% similarity with PecT for the 120 N-terminal amino acids (Fig. 3). Since this part of the protein contains the helix-turn-helix DNA binding motif, we tested whether *lrhA* and *pecT* could be interchangeable. A plasmid bearing *lrhA* was unable to complement a *pecT* mutation or to activate *pecT*::*uidA* gene fusion expression (data not shown). Heterologous complementation by the two LTR proteins CatR and ClcR has been tested by Parsek et al. (31). These two proteins control two parallel catabolic pathways (catechol and 3-chlorocatechol, respectively) in *Pseudomonas putida*. Although ClcR was able to bind in vitro to the regulatory region of genes controlled by CatR and vice versa, *clcR* was not able to complement a *catR* mutation in vivo. An interchangeability between PecT and LrhA will be tested in vitro when the genes controlled by LrhA have been identified.

The genes regulated by *pecT* that have been identified up to now are only pectate lyase genes: *pelBCDEL* and *kdgC*, which encodes a protein that presents homology with periplasmic pectate lyases (11). The three regulation networks of *pecT*, *pecS*, and *kdgR* might initially seem redundant since all three control pectate lyase synthesis. However, the genes grouped in the *kdgR* regulon, which are expressed during pectin degradation, include pectin catabolism genes and are induced by pectin

degradation products. *pecS* regulates the synthesis and the secretion of extracellular enzymes. The PecS-inducing signal could be specific for the plant-pathogen interaction (2). The signal to which *pecT* responds is, for the moment, unknown, but this could also be a pectin degradation product. The multiplicity of these partially overlapping regulations might help the bacteria to adapt pectate lyase synthesis to particular environmental conditions.

In addition to its role in the regulation of pectinase expression, PecT might play a wider function in the cell. *pecT* mutants are mucoid on minimal medium plates, and the mucoidy increases when inducers of pectate lyase synthesis are present. The mucoidy usually results from an increased synthesis of exopolysaccharides (EPS). EPS production has been associated with the virulence of several pathogenic bacteria, such as *Erwinia stewartii* and *Erwinia amylovora* (22). The synthesis of EPS is coregulated with that of virulence factors (polygalacturonases A and B and a pectin methylesterase, etc.) in *Pseudomonas solanacearum* (15). Such coregulation may exist in *E. chrysanthemi* via *pecT*. A *pecT* mutant flocculates in minimal medium cultures. This phenotype was observed for *E. coli oxyR* mutants, a result of an absence of piliation (42). Flocculation was reverted by the addition of leucine. In *E. coli*, leucine is able to increase or decrease the expression of many operons via its interaction with Lrp, the leucine-responsive protein, which plays a key role in many metabolic pathways (6). In *E. coli*, Lrp is necessary for the expression of operons required for pilin biosynthesis (4). However, in that case, leucine has no inducing role. In *E. chrysanthemi*, the addition of leucine suppresses some of the phenotype due to the absence of PecT (flocculation). The relationship between pectate lyase synthesis, EPS synthesis, and a global regulator that could be Lrp, leads us to suggest that the number of genes required for the pathogenicity of *E. chrysanthemi* could be larger than previously supposed.

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