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Pectate Lyase PelI of *Erwinia chrysanthemi* 3937 Belongs to a New Family

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***Erwinia chrysanthemi* 3937 secretes five major isoenzymes of pectate lyases encoded by the *pelA*, *pelB*, *pelC*, *pelD*, and *pelE* genes and a set of secondary pectate lyases, two of which, *pelL* and *pelZ*, have been already identified. We cloned the *pelI* gene, encoding a ninth pectate lyase of *E. chrysanthemi* 3937. The *pelI* reading frame is 1,035 bases long, corresponding to a protein of 344 amino acids including a typical amino-terminal signal sequence of 19 amino acids. The purified mature PelI protein has an isoelectric point of about 9 and an apparent molecular mass of 34 kDa. PelI has a preference for partially methyl esterified pectin and presents an endo-cleaving activity with an alkaline pH optimum and an absolute requirement for Ca^{2+} ions. PelI is an extracellular protein secreted by the Out secretory pathway of *E. chrysanthemi*. The PelI protein is very active in the maceration of plant tissues. A *pelI* mutant displayed reduced pathogenicity on chicory leaves, but its virulence did not appear to be affected on potato tubers or *Saintpaulia ionantha* plants. The *pelI* gene constitutes an independent transcriptional unit. As shown for the other *pel* genes, the transcription of *pelI* is dependent on various environmental conditions. It is induced by pectic catabolic products and affected by growth phase, oxygen limitation, temperature, nitrogen starvation, and catabolite repression. Regulation of *pelI* expression appeared to be dependent on the three repressors of pectinase synthesis, KdgR, PecS, and PecT, and on the global activator of sugar catabolism, cyclic AMP receptor protein. A functional KdgR binding site was identified close to the putative *pelI* promoter. Analysis of the amino acid sequence of PelI revealed high homology with a pectate lyase from *Erwinia carotovora* subsp. *carotovora* (65% identity) and low homology with pectate lyases of the phytopathogenic fungus *Nectria haematococca* (*Fusarium solani*). This finding indicates that PelI belongs to pectate lyase class III. Using immunoblotting experiments, we detected PelI homologs in various strains of *E. chrysanthemi* and *E. carotovora* subsp. *carotovora* but not in *E. carotovora* subsp. *atroseptica*.**

The enterobacterium *Erwinia chrysanthemi* causes soft rot disease on various plants. The maceration process involves the depolymerization of pectin in plant cell walls. Pectin is a heteropolysaccharide with a backbone consisting of galacturonic acid, partially methylesterified and acetylesterified (45). Strains of *E. chrysanthemi* effect this depolymerization through the combination of enzymatic activities: pectin methyl esterase, pectin acetyl esterase, pectate lyase, pectin lyase, and polygalacturonase (4, 39). Pectate lyases appear to be the major pectinolytic enzymes produced by *E. chrysanthemi*, and they play an important role in the maceration of plant tissues (4). They cleave internal glycosidic bonds in polygalacturonic acid (PGA) or low-methylesterified pectins by β-elimination to yield oligomers that are 4,5-unsaturated at the nonreducing end. Pectate lyases exhibit reduced activity on a highly methylesterified or acetylesterified substrate, and thus their activity is facilitated by pectin methyl esterase and pectin acetyl esterase actions which remove the methoxyl and acetyl groups of the pectin to yield PGA (39, 43). In contrast, pectin lyase cleaves highly (98%) methylesterified pectin by β-elimination but is inactive on PGA. Polygalacturonases are distinguishable from the lyases by their hydrolytic reaction mechanism, and they generate saturated oligomers.

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In *E. chrysanthemi* 3937, five major endo-pectate lyases, PelA, PelB, PelC, PelD and PelE, were first identified by electrofocusing (6). On the bacterial chromosome, the five major *pel* genes are organized in two clusters, *pelB-C* and *pelA-E-D* (17). Moreover, in the mutant deleted for the five corresponding *pel* genes, a new set of pectate lyases was discovered (5, 22). Since their action is masked in the wild-type strain by that of the major isoenzymes, they are designated secondary pectate lyases. Despite their low activity, these enzymes could have an important role during the infection of plant tissues since some of them appeared to be plant inducible (22). In addition to the exo-pectate lyase PelX (7), two endo-pectate lyases, PelL and PelZ, have been identified (1, 25, 31). The *pelZ* gene is adjacent to the major *pelC* gene, while the *pelL* gene is situated in a separate cluster, adjacent to the *celZ* gene, encoding another cell wall-degrading enzyme, the cellulase EGZ. Most of the pectinases and the cellulase EGZ are secreted by *E. chrysanthemi* in the extracellular medium via the specific Out secretory pathway (36).

In *E. chrysanthemi*, the production of pectate lyases is sensitive to several environmental factors (17). Transcription of the *pel* genes is induced by pectin catabolic products, by late exponential growth phase, and under low-iron conditions, and it is repressed in conditions of nitrogen starvation, catabolite repression, and high temperature (18, 38). Other factors affect only the expression of a particular set of genes: elevation of osmolarity increases *pelE* expression, and *pelA*, *pelD*, and *pelE* expression is activated during oxygen limitation (18). The regulation of the *pel* genes is thus subjected to a variety of signals which are transduced via several regulatory systems, including

TABLE 1. Bacterial strains and plasmids used

Strain or plasmid	Description	Reference or origin
Erwinia chrysanthemi 3937 derivatives		
A350	*lacZ2*	19
A576	*lacZ2 arg-10 kdgK*	19
PGI6	*lacZ2 arg-10 kdgK pelI*::*lacZ* Km^r	19
A837	*lacZ2 kdgR*	Laboratory collection
A1524	*lacZ2 pecS*::Mu Cm	34
A2019	*lacZ2 arg-10 kdgK outD*::Mu Cm	Laboratory collection
A2174	*lacZ2 pecT*::Cm	41
A2507	*lacZ2 crp*::Cm	32
A2701	*lacZ2 pelI*::*uidA*	This work
A2733	*lacZ2 kdgR pelI*::*uidA*	This work
A2734	*lacZ2 pecS*::Mu Cm *pelI*::*uidA*	This work
A2735	*lacZ2 pecT*::Cm *pelI*::*uidA*	This work
A2736	*lacZ2 kdgK pelI*::*uidA*	This work
A2761	*lacZ2 crp*::Cm *pelI*::*uidA*	This work
A3003	*pelI*::*uidA*	This work
Other *Erwinia* strains		
B374	Wild-type *E. chrysanthemi*	Laboratory collection
EC16	Wild-type *E. chrysanthemi*	Laboratory collection
ENA49	Wild-type *E. chrysanthemi*	Laboratory collection
CU1	Wild-type *E. chrysanthemi*	Laboratory collection
CC3-2	Wild-type *E. carotovora* subsp. *carotovora*	Laboratory collection
CA36A	Wild-type *E. carotovora* subsp. *atroseptica*	Laboratory collection
SCRI1039	Wild-type *E. carotovora* subsp. *atroseptica*	Laboratory collection
SCRI193	Wild-type *E. carotovora* subsp. *carotovora*	Laboratory collection
Escherichia coli		
NM522	Δ(*lac-proAB*) Δ(*mcrB-hsdSM*)*5 supE thi* (F′ *proAB lacI*^{q} *lacZ*ΔM15)	Laboratory collection
BL21(DE3)	*E. coli* B, F$^-$ *dcm ompT hsdS gal* λ(DE3), T7 polymerase gene under the *lac*UV5 promoter	40
Plasmids		
pULB110	RP4::Mu3A, Ap^r Tc^r Km^s	44
pBR325	Ap^r Tc^r Cm^r	Laboratory collection
pBSAp	Bluescript KS+, Ap^r	Stratagene
pBSCm	Bluescript KS+, Cm^r	Stratagene
pT7-6	T7 ϕ10, Ap^r	42
R254T	pULB110 derivative, *pelI*::*lacZ*$^+$	This work
p365T	pBR325 derivative with a 12-kb *Bam*HI fragment from R254T, *pelI*::*lacZ*$^+$	This work
pT406	pBSAp derivative with a 4-kb *Bam*HI-*Hin*dIII fragment from p365T, Ap^r	This work
pE4	pT406 derivative with a *uidA*-Km insertion from pN406 in the *Eco*RI site	This work
RB39	pULB110 derivative, *pelI*$^+$ Km^r	This work
pB39	pUC18 derivative with a 15-kb *Bam*HI fragment from RB39, Km^r *pelI*$^+$	This work
pBTIS	pBSAp derivative with a 2.3-kb *Sal*I-*Eco*RI fragment from pB39, *pelI*$^+$	This work
pP23	pBTIS derivative with a *uidA*-Km insertion from pUIDK11 in the *Pst*I site, Ap^r Km^r *pelI*::*uidA*$^+$	This work
pTPL1	pT7-6 derivative with a 1.8-kb *Bgl*II-*Eco*RI fragment from pBTIS, Ap^r *pelI*$^+$	This work

the three repressors KdgR, PecS, and PecT and the global activator of sugar catabolism, cyclic AMP receptor protein (CRP) (32–34, 41).

To identify genes involved in pectin degradation in *E. chrysanthemi* 3937, we isolated mutations on the basis of their induction by pectic derivatives. Such mutations were obtained by selection of Mu-*lac* insertions generating polygalacturonate-inducible *lacZ* transcriptional fusions (19). About 25% of these fusions were assigned to an already identified gene of pectin catabolism. However, about 75% of them remained to be identified. Recently, analysis of two polygalactoronate-inducible mutations located in the *pelA-E-D* cluster allowed us to identify the *paeY* gene, encoding a type of enzyme never previously identified in bacteria, a pectin acetyl esterase (39). In this report, we present the characterization of another polygalacturonate-inducible gene, *pelI*, which encodes a novel pectate lyase, PelI.

MATERIALS AND METHODS

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

Media and growth conditions. Cells were grown in complete LB medium or in synthetic M63 medium (27). When required, the media were solidified with agar (15 g liter^{-1}). *E. chrysanthemi* and *Escherichia coli* cells were usually incubated at 30 and 37°C, respectively. Carbon sources were added at 2 g liter^{-1} except for polygalacturonate (grade II; Sigma Chemical Co.), which was added at 4 g liter^{-1}. Chrysanthemum extracts were prepared as described by Kelemu and Collmer (22) after heat treatment of plant leaves and stems. The media used to test the different physiological conditions have been previously described (18). When required, antibiotics were added at the following concentrations (micrograms per milliliter): kanamycin, 20; ampicillin, 50; chloramphenicol, 20; streptomycin, 100; and tetracycline, 10.

Matings and isolation of R-prime plasmids. Plasmid pULB110, a kanamycin-sensitive RP4::mini-Mu derivative (44), was used for chromosome mobilization. Matings between recipient and donor strains carrying this plasmid were performed by spreading 0.2-ml aliquots of overnight cultures of the strains on M63 medium plates and incubating them for 5 h at 30°C. Bacteria were resuspended in 1 ml of M63 medium and spread on the appropriate selective media. pULB110 can also generate R-prime derivatives containing an insert of bacterial DNA. To

isolate the R-prime plasmid bearing the MudI1681 insertion, *E. chrysanthemi* PGI6/pULB110 was mated with the *E. coli* HB101. Selection of kanamycin-resistant (Km^r) transconjugants was performed on LB medium plates supplemented with kanamycin and streptomycin.

Cell fractionation. The release of periplasmic proteins from *E. coli* cells was performed by osmotic shock (9) or, in the case of *E. chrysanthemi* cells, by spheroplast formation (37). For the release of overproduced protein, a freezing-thawing method was used (21).

Overproduction and purification of PelI. Overexpression of the *pelI* gene was obtained by using the T7 promoter/T7 RNA polymerase system (42). The *pelI* gene was subcloned into the pT7-6 expression vector under the T7 promoter (pTPL1). This plasmid was introduced in *E. coli* K38/pGP1.2, which contains the gene encoding T7 RNA polymerase downstream from the pλ promoter, which is under the control of the *c*I857 thermosensitive repressor. The plasmid-encoded proteins were labeled with [^{35}S]cysteine-methionine after thermal induction of the T7 polymerase (42).

To overproduce PelI, plasmid pTPL1 was introduced in *E. coli* BL21(DE3), which contains a chromosomal copy of the gene encoding T7 RNA polymerase under the control of the *lac*UV5 promoter (40). The BL21(DE3)/pTPL1 cells were grown at 30°C in LB supplemented with ampicillin (150 μg ml^{-1}). At an optical density at 600 nm of 0.8 to 1, the synthesis of T7 RNA polymerase was induced by addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and cells were grown for an additional 2 to 3 h.

Cells were harvested by centrifugation for 5 min at 5,000 × *g* at 4°C and then frozen at −80°C. The periplasmic fraction was extracted from cells by three cycles of freezing-thawing (21). Proteins were concentrated by 85% ammonium sulfate precipitation. The pellet was solubilized in 50 mM sodium phosphate buffer (pH 7)–5 mM EDTA–1.5 M ammonium sulfate and loaded onto a Phenyl-TSK gel column equilibrated and extensively washed with the same buffer and then with buffer containing 1 M ammonium sulfate. Upon application of 1 to 0.5 M ammonium sulfate linear gradient, the PelI protein was eluted at about 0.7 M ammonium sulfate concentration. The PelI fractions were then applied to a Superdex 200 (Pharmacia) gel filtration column. The fractions containing the pure PelI protein were pooled and concentrated in a Centricon 10 (Amicon).

Enzyme assays. Pectate lyase activity was determined by monitoring spectrophotometrically the formation of unsaturated products from PGA at 230 nm. Unless otherwise specified, the standard assay mixture consisted of 0.1 M Tris-HCl (pH 8.5), 0.1 mM $CaCl_2$, and 0.5 g of PGA per liter in a total volume of 1 ml. The appearance of products was monitored at 37°C over a period of 2 min. The molar extinction coefficient of unsaturated oligogalacturonides was assumed to be 5,200 (28). One unit of activity was defined as the amount of enzyme required to produce 1 μmol of unsaturated product per min. Activity on substrates presenting various degrees of methylation was determined by substituting 7, 22, 45, 60, 75, and 90% esterified citrus pectins (from Copenhagen Pectin) for polygalacturonate.

β-Glucuronidase was measured by monitoring the degradation of *p*-nitrophenyl-β-D-glucuronide into *p*-nitrophenol that absorbs at 405 nm (2). Specific activities are expressed as nanomoles of products liberated per minute per milligram (dry weight) of bacteria.

Analytical procedures. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed on slab gels (4% stacking gel and 12% separating gel), using the Mini-Protean II system (Bio-Rad). Proteins were stained with Coomassie blue G-250. Isoelectrofocusing was performed in a pH 3 to 10 gradient, using Pharmalytes. To detect pectate lyase activity, we used overlays with agarose gels containing PGA followed by staining with 0.05% ruthenium red (6).

The pectate lyase reaction products were analyzed by thin-layer chromatography (25). The chromatogram was developed with an *n*-butanol–water–acetic acid (5:3:2) mixture, and the products were visualized by treatment with a solution of phosphomolybdic acid.

Recombinant DNA techniques. Preparation of plasmid or chromosomal DNA, restriction digestions, ligations, DNA electrophoresis, alkaline blotting, and transformations were carried out as described by Sambrook et al. (37). For nucleotide sequence analysis, deletions were generated with restriction endonucleases. The chain termination technique was performed with double-stranded DNA templates, M13 primer or M13 reverse primer, [^{35}S]dATP, and T7 DNA polymerase (Pharmacia sequencing kit). Some sequences were determined by Genome Express SA (Grenoble, France). The resulting data were analyzed by using the Mac Molly program (SoftGene, Berlin, Germany).

Total cellular RNA was isolated by the frozen phenol method (26) from cells grown to early log phase in LB medium supplemented with galacturonate. Pectate lyase production was assayed to verify that the pectinase genes were correctly transcribed. After extraction, RNA integrity was determined by visualization on denaturing agarose gels in the presence of formaldehyde. For Northern blotting, about 50 μg of RNA was transferred from the gel to a nylon membrane (Hybond N+; Amersham). The DNA probe was labeled with [α-^{32}P]dCTP by using a random priming DNA labeling kit (Boehringer). Hybridization was performed at 42°C in the presence of 50% formamide, and filters were finally washed in 0.2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% SDS at 68°C. The same hybridization and washing conditions were used after Southern blotting with *Eco*RI-digested chromosomal DNA extracted from various *Erwinia* strains.

Construction of the *pelI*::*uidA* fusion. Insertion of a *uidA*-Km cassette in the correct orientation generates transcriptional fusion (2). The *uidA*-Km cassette was liberated by *Pst*I digestion of pUIDK11 and inserted into the *Pst*I site of plasmid pBTIS. In one of the recombinant plasmids, pP23, *uidA* is oriented in the same transcriptional direction as *pelI*, giving rise to a *pelI*::*uidA* fusion. Plasmid pP23 was introduced into *E. chrysanthemi* cells by electroporation. The *pelI* mutation was then integrated into the *E. chrysanthemi* chromosome by marker exchange recombination after successive cultures in low-phosphate medium in the presence of the appropriate antibiotic (35).

Maceration of plant tissue. Small cubes (3-mm sides) were cut from commercial Bindge potato tubers. They were placed into 0.1 M Tris-HCl (pH 8)–0.5 mM $CaCl_2$, and from 0.01 to 1 U of pectate lyase was added per ml. Samples were incubated at 30°C and examined after 1, 2, 3 and 4 h. All tests were performed three times and compared with repeated buffer control tests. The degree of tissue maceration was estimated by determining the ease with which the tissue could be pulled apart with a spatula. The maceration index was scored on a scale of 0 (no maceration) to 3 (complete tissue disintegration).

Pathogenicity test. *Saintpaulia ionantha* potted plants were infected as previously described (10). Thirty plants were inoculated, after wounding of a leaf, with 50 μl of a bacterial suspension (10^8 bacteria) of the *pelI* mutant. Thirty other plants were inoculated in the same conditions with the wild-type strain 3937. Results of infections were scored daily for 10 days and classified as (i) no symptoms, (ii) local necrosis limited to the leaf, and (iii) systemic infection of the plant. Potato tubers were inoculated as previously described (24). Sterile pipette tips containing 5 μl of bacterial suspension (10^7 bacteria) were inserted into the tuber parenchyma to a depth of 10 mm. Thirty tubers were inoculated with each strain and incubated in a dew chamber. Every day for 3 days, tubers were sliced vertically through the inoculation point, and the weight of decayed tissue was taken as the characteristic of disease severity. Chicory leaves were slightly wounded prior to inoculation. Twenty leaves were infected for each strain, using 10^6 bacteria per inoculation site. After incubation in a dew chamber for 24 h, the length of rotted tissue was measured to estimate the disease severity. Pathogenicity tests were repeated in at least two independent experiments.

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper will appear in the EMBL, GenBank, and DDBJ nucleotide sequence databases under accession no. Y13340.

RESULTS

Isolation of the *pelI* gene. We cloned the previously unidentified fusion of strain PGI6 by isolation of an R-prime plasmid selected for the Km^r marker of the MudI1681 insertion. Plasmid R254T contained, in addition to the Mu insertion, a fragment of adjacent chromosomal DNA of about 25 kb. To localize more precisely the position of the Mu insertion, we subcloned a smaller 12-kb *Bam*HI DNA fragment into pBR325, which conferred the Lac^+ phenotype (pT365). Restriction analysis confirmed that this fragment contains the S end of the MudI1681 phage, including the *lacZ* gene (Fig. 1). We subcloned the 4-kb *Bam*HI-*Hin*dIII fragment situated at the junction of the Mu insertion (Fig. 1). Determination of the nucleotide sequence of this junction and comparison with sequences present in databases revealed that the Mu insertion is situated in a region presenting about 60% identity with the coding sequence of pectate lyase gene *pel3* (or *pelB*) of *Erwinia carotovora* (14, 23). No pectate lyase of this family has been previously identified in *E. chrysanthemi.*

To isolate the wild-type *pelI* gene, we first inserted a *uidA*-Km cassette into the *Eco*RI site situated 3 kb upstream from the Mu insertion (Fig. 1). After recombination of this insertion into the 3937 chromosome, we selected an R-prime plasmid bearing the *uidA*-Km insertion and adjacent chromosomal DNA. Subcloning into pUC18 enable us to isolate a *Bam*HI fragment of 15 kb conferring the Km^r phenotype (Fig. 1). Deletion analysis and subcloning experiments led to the isolation of the 2.3-kb *Sal*I-*Eco*RI fragment of plasmid pBTIS exhibiting pectate lyase activity (Fig. 1).

Nucleotide sequence of the *pelI* region. The nucleotide sequence of a 2,316-bp *Sal*I-*Eco*RI fragment was determined. Sequence analysis revealed the presence of a unique complete open reading frame (ORF) which began with an ATG codon at position 892, separated by five nucleotides of a potential ribosome-binding site, AAGGAG, and ended with TGA at posi-

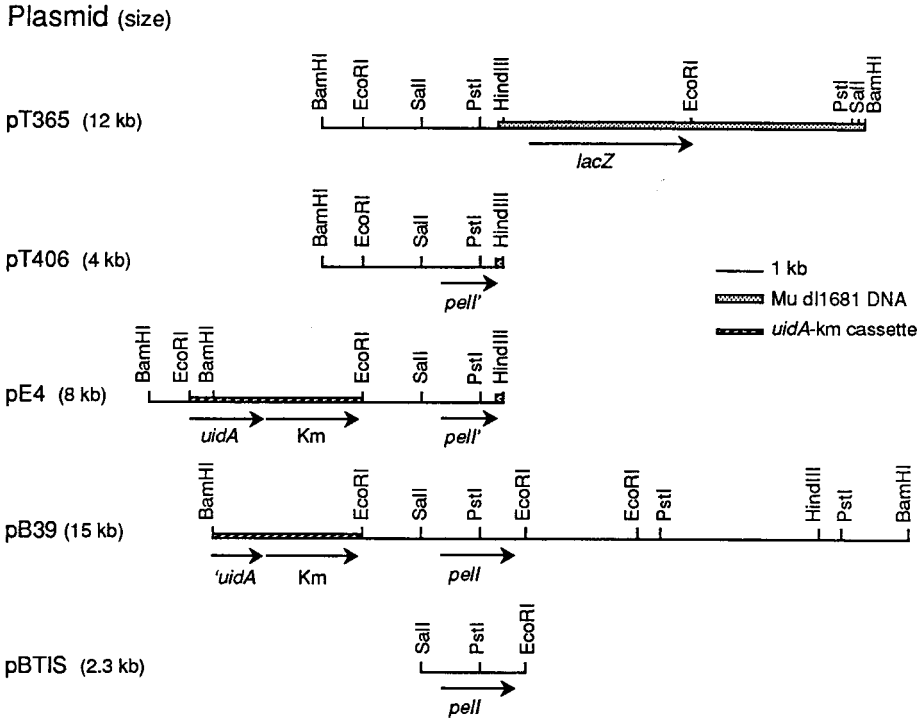

FIG. 1. Physical map of the *Erwinia chrysanthemi* 3937 *pelI* region. The positions of the different genes are indicated by arrows. The thin line corresponds to *E. chrysanthemi* chromosomal DNA.

tion 1924. The Mu insertion of the mutant PGI6 is situated at the end of this ORF (position 1821). The *pelI* gene of strain 3937 encodes a 344-amino-acid protein with a molecular mass of 36,856 Da, including a typical amino-terminal signal sequence with a potential cleavage site between two alanine residues at positions 19 and 20 of the protein sequence. The PelI mature protein contains 325 amino acids and has a calculated molecular mass of 34,817 Da and a calculated pI of 7.9. The deduced amino acid sequence of PelI presents 67% identity with PelB (or Pel-3) of *E. carotovora* (Fig. 2) (14, 23). The extensive similarity between PelB and Pel-3 (93% identity) and their similar gene organizations indicate that they are identical enzymes isolated from two strains of *E. carotovora*. Lower homology was observed between PelI and the four pectate lyases of the phytopathogenic fungus *Nectria haematococca* (*Fusarium solani* f. sp. *pisi*) (11–13) (Fig. 2).

The *pelI* gene is preceded by a potential promoter with the following homology to the classical sigma 70 consensus: 5 nucleotides of 6 (TTTACA) for the −35 region at position 658 and 3 nucleotides of 6 (TAAATA) for the −10 region at position 681, with a spacing of 17 nucleotides. Since the *pelI* transcription is induced in the presence of PGA, we looked for a potential KdgR-binding site in the promoter region. The KdgR box corresponds to two imperfect inverted repeats of nine nucleotides (17). A potential KdgR box, TAAAAAACA GATCTTTGTC (residues matching with the consensus are underlined), is centered 19 nucleotides upstream from the putative *pelI* promoter. However, this sequence differs from the consensus by the absence of a T residue (at position 13) which is strictly conserved in all operators that interact in vitro with the purified KdgR protein (29). To confirm the functionality of this potential operator, we carried out gel retardation experiments using the 0.44-kb *Eco*RV-*Apa*LI DNA fragment containing the promoter region of the *pelI* gene. A single band of DNA-protein complex was observed (Fig. 3), demonstrating the presence of a functional KdgR-binding site in this DNA region. A potential CRP-binding site partially overlaps the KdgR-binding site (ACAGATCTTTGTCACA; residues matching with the CRP consensus are underlined). This finding suggests that despite a good assigned −35 box, the *pelI* transcription might be activated by CRP.

Centered 69 nucleotides after the *pelI* translational stop is a GC-rich inverted repeat followed by a run of T residues, typical of a Rho-independent transcription terminator (AAGAC CGGGC-5 nucleotides-GCCCGGTCTTTTTATTT; calculated free energy of formation = −74 kJ mol^{-1}). All of the sequenced *pel* genes of *E. chrysanthemi* have a stop codon followed by a sequence similar to those of Rho-independent terminators, and this structure was shown to be an efficient terminator in the case of *pelB* (20). The *pelI* gene is preceded and followed by partial potential ORFs transcribed on the other DNA strand. DNA homology searching revealed significant homology (20 to 29% identity) between the product of the ORF situated on the 3′ *pelI* side and the C-terminal end of glycerol dehydrogenases from various bacteria. In contrast, no homology was detected between the polypeptide encoded by the ORF situated on the 5′ *pelI* side and the proteins present in databases. To identify the *pelI* transcripts, we performed Northern blot analysis using a 0.77-kb *Asp*I probe. RNAs were extracted from a *kdgK* mutant grown under inducing conditions to obtain a high level of transcription. The *pelI* probe strongly hybridized with a 1.3-kb mRNA (data not shown). This size is in agreement with those deduced from the nucleotide sequence for a monocistronic *pelI* mRNA (1,310 nucleotides).

Analysis of *pelI* expression. A *pelI*::*uidA* transcriptional fusion was constructed by inserting an *uidA*-Km cassette (2) in the *Pst*I site located in the *pelI* ORF (Fig. 1). After recombi-

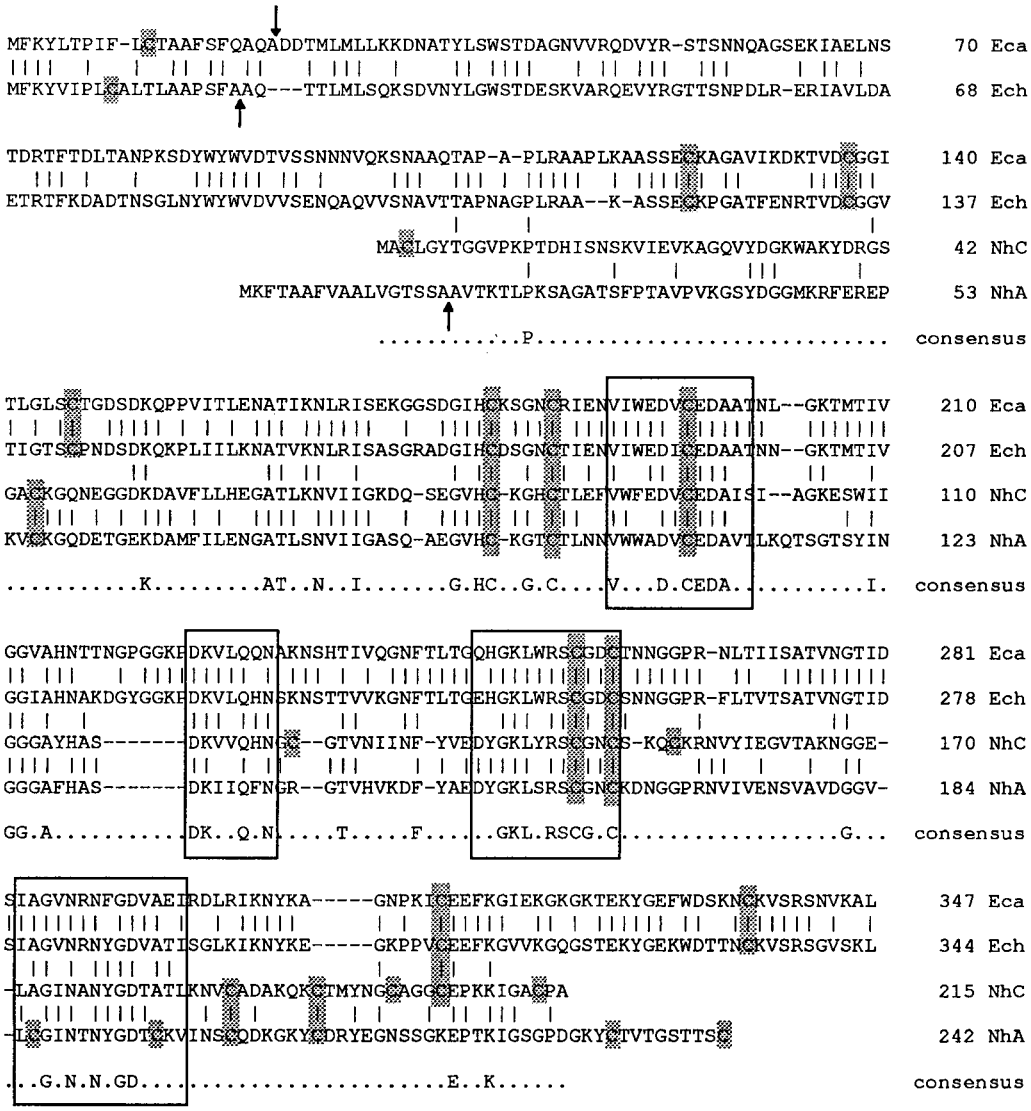

FIG. 2. Comparison of amino acid sequences of PelI and different pectate lyases. Ech, PelI of *E. chrysanthemi*; Eca, PelB of *E. carotovora* (14); NhA and NhC, PelA and PelC, respectively, of *N. haematococca* (11, 12). Vertical lines indicate identical residues; arrows indicate putative cleavage sites of the signal sequences. The most highly conserved regions are boxed. The grey zones indicate the cysteine residues.

nation into the *E. chrysanthemi* chromosome, expression of the *pelI* fusion was monitored during bacterial growth. Expression of the *pelI*::*uidA* fusion clearly increased when the cells entered the late exponential growth phase and was coincident with the production of major pectate lyases (data not shown).

Expression of the *pelI*::*uidA* fusion under various growth conditions is presented in Table 2. In noninducing conditions, *pelI* showed a significant basal level of expression. Its transcription was stimulated about sixfold in the presence of either PGA or chrysanthemum extracts. In the presence of a readily utilizable carbon source, such as glucose, a decrease in *pelI* transcription was observed. Nitrogen starvation also inhibited *pelI* expression. Decreasing the temperature to 25°C weakly affected *pelI* expression, while increasing the temperature to 37°C repressed its transcription. Oxygen limitation led to a significant increase in *pelI* expression, either in the absence or in the presence of inducer. An increase in the medium osmolarity had no significant effect on the *pelI* transcription. We assayed the *pelI*::*uidA* expression in rotted tissues obtained after infection of potato tubers. The *pelI* fusion appeared to be highly expressed in these infected tissues (Table 2).

The *pelI*::*uidA* fusion was transduced into strains containing mutations affecting pectate lyase production (Table 3). Pectate lyase activity, mainly corresponding to the major isoenzymes

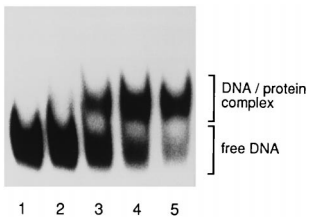

FIG. 3. Analysis of KdgR binding to the *pelI* promoter region. The 0.44-kb *Eco*RV-*Apa*LI fragment corresponding to the 5′ untranslated end of *pelI* was end labeled and purified. Mobility shift assay was performed either without (lane 1) or with 2, 4, 10, and 20 nM purified KdgR protein (lanes 2 to 5, respectively).

TABLE 2. Expression of the *pelI*::*uidA* fusion in various conditions

Carbon source(s)	Growth condition[a]	β-Glucuronidase sp act[b] (nmol of product liberated/min/mg [dry wt] of bacteria)
Glycerol		55
Glycerol + PGA		326
Glycerol + plant extract		345
Glucose		23
Glycerol	25°C	51
Glycerol + PGA	25°C	275
Glycerol	37°C	37
Glycerol + PGA	37°C	98
Glycerol	Oxygen limitation	145
Glycerol + PGA	Oxygen limitation	930
Glycerol	Nitrogen starvation	12
Glycerol + PGA	Nitrogen starvation	28
Galacturonate		149
Galacturonate	High osmolarity	150
	Infected potato tubers	1,561

[a] Cultures of strain A2701 (*pelI*::*uidA*) were grown in M63 minimal medium to late log phase, in the presence or absence of the inducing compound (PGA or galacturonate) and, unless stated otherwise, at 30°C. The specific media used for the different physiological conditions are described in the text. Assays were also performed on rotted tissues obtained after 48 h of infection of potato tubers. In this case, the number of bacteria was determined by dilution plating and the corresponding bacterial dry weight was calculated.

[b] Reflects expression of the *pelI*::*uidA* fusion. The results reported are the average of at least three independent experiments; the standard deviation was, in each case, less than 15%.

(PelA, PelB, PelC, PelD, and PelE), strongly increased in the *kdgR*, *pecS*, and *pecT* mutants in the absence of PGA, due to inactivation of the one of the repressors of their transcription. *pelI*::*uidA* expression increased about sixfold in the presence of the *kdgR* mutation and only twofold in *pecS* and *pecT* mutants. Thus, both *pecS* and *pecT* are involved in *pelI* regulation, but KdgR plays a major role. In the *kdgK* mutant, the presence of PGA results in accumulation of the intracellular inducer, KDG, and most of the pectinolytic genes showed a very high induction ratio. While the major *pel* genes were strongly induced, *pelI*::*uidA* expression increased only sixfold in the *kdgK* mutant, as observed in the wild-type background (Table 3). The fact that KDG accumulation does not increase *pelI* transcription suggests that full induction of *pelI* occurs at low KDG levels. Both expression of the *pelI* fusion and pectate lyase activity were very low in the *crp* mutant, confirming the role played by CRP in the activation of *pelI* transcription.

Chromosomal localization of the *pelI* gene. The *pelI* locus was localized by using the Km^r marker of the *pelI*::*uidA* fusion. Chromosomal mobilization mediated by plasmid pULB110 was used for conjugation with various polyauxotrophic recipients. The Km^r marker cotransferred at 54% with *ade-377* and at 4% with *pan*. Thus, *pelI* appeared to be located in the vicinity of the *pelA-E-D* locus encoding three major pectate lyases (Fig. 4). However, *pelI* is not very close to this cluster, since no cotransduction could be observed between *pelI* and *pelA* or *pelE.*

Identification of the PelI protein. Analysis by SDS-PAGE of extracts from *E. coli* K38/pGP1.2/pTPL1 revealed the presence of a protein of about 34 kDa, mainly located in the periplasmic space of *E. coli* (Fig. 5A). This molecular mass is in accordance with that deduced from the nucleotide sequence of the *pelI* gene. Isoelectric focusing followed by specific staining of pectinolytic activity indicated that the PelI isoelectric point is about 9 (Fig. 5B).

The PelI protein was purified from *E. coli* BL21(DE3)/pTPL1 (Fig. 5C) and used to obtain polyclonal antibodies. After SDS-PAGE and immunoblotting, a strong signal corresponding to a 34-kDa protein and a weak signal corresponding to a 48-kDa protein were observed in total extracts of *E. chrysanthemi* A350 (Fig. 6B). The signal corresponding to a 34-kDa protein was absent in extracts of the *pelI* mutant A2701 (Fig. 6B), confirming that this protein corresponds to the *pelI* product. Immunoblotting experiments were performed to study the PelI localization in *E. chrysanthemi* and to test the involvement of the Out system in the PelI secretion. The 34-kDa protein was present in the supernatant and absent in the cells of the *kdgK* mutant A576 (Fig. 6A). In contrast, analysis of the *out* mutant A2019 revealed no PelI in the supernatant, but PelI was present in the cell extract (Fig. 6A). Thus, PelI is an extracellular pectate lyase of *E. chrysanthemi* secreted by the Out machinery. To test whether PelI secretion is species specific, we introduced plasmid pBTIS in *E. carotovora* subsp. *carotovora* SCRI193 and in *E. chrysanthemi* 3937. PelI produced from this multicopy plasmid was entirely retained in *E. carotovora*

TABLE 3. Expression of the *pelI*::*uidA* fusion in various mutants

Strain (main genotype)	Growth condition[a]	Sp act[b]	
		Pectate lyase (μmol of product liberated/min/mg [dry wt] of bacteria)	β-Glucuronidase (nmol of product liberated/min/mg of [dry wt] bacteria)
A2701 (*pelI*::*uidA*)	Glycerol	0.04	55
	Glycerol + PGA	3.02	326
A2736 (*kdgK pelI*::*uidA*)	Glycerol	0.04	49
	Glycerol + PGA	50.73	317
A2733 (*kdgR pelI*::*uidA*)	Glycerol	1.46	341
	Glycerol + PGA	4.79	294
A2734 (*pecS pelI*::*uidA*)	Glycerol	0.33	95
	Glycerol + PGA	7.82	225
A2735 (*pecT pelI*::*uidA*)	Glycerol	0.94	112
	Glycerol + PGA	6.88	290
A2761 (*crp pelI*::*uidA*)	Glucose	0.01	9
	Glucose + PGA	0.06	9

[a] Cultures were grown in M63 minimal medium to late log phase, in the presence or absence of PGA.

[b] β-Glucuronidase specific activity reflects the expression of the *pelI*::*uidA* fusion; pectate lyase specific activity corresponds to the action of the major isoenzymes. The results reported are the average of at least three independent experiments; the standard deviation was, in each case, less than 10% for β-glucuronidase assays and less than 20% for pectate lyase assays.

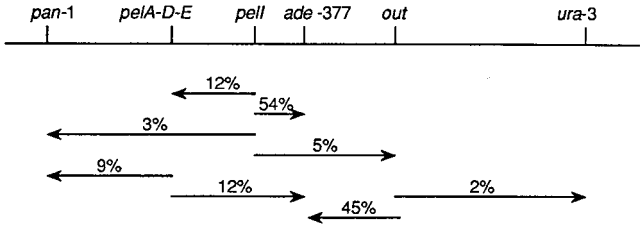

FIG. 4. Location of the *pelI* gene on the genetic map of *E. chrysanthemi* 3937. Localization was performed by chromosomal mobilization using plasmid pULB110. Percentages of cotransfer between two markers are indicated. Arrowheads point to the unselected marker.

cells, whereas it was correctly secreted by *E. chrysanthemi* (data not shown).

The Mu insertion of the PGI6 mutant, which is situated 103 bp upstream of the *pelI* stop codon, leads to formation of a stable hybrid protein with the same apparent molecular mass as PelI (34 kDa) which is secreted by *E. chrysanthemi* (Fig. 6A). However, this protein displayed no pectate lyase activity. Thus, the replacement of the 34 C-terminal amino acids does not modify the secretability of the hybrid protein from *E. chrysanthemi* cells but eliminates its activity (Fig. 6A).

Occurrence of PelI homologs in various *Erwinia* species. To detect PelI homologs in other *Erwinia* strains, we used both immunoblotting and DNA hybridization. After separation by SDS-PAGE, total extracts from cultures grown in LB supplemented with galacturonate were analyzed by immunoblotting with PelI antibodies. Depending on the five analyzed *E. chrysanthemi* strains, we observed one, two, or even three bands specifically interacting with the PelI antibodies (Fig. 6B). In *E. chrysanthemi* ENA49, only a 30-kDa protein was detected. In *E. chrysanthemi* B374, a 48-kDa protein was detected in addition to the 34-kDa protein, while in *E. chrysanthemi* EC16, the 48-kDa protein was detected in addition to the 30-kDa protein. In *E. chrysanthemi* CU1, the three bands corresponding to 30, 34, and 48 kDa were observed. In the two *E. carotovora* subsp. *carotovora* strains CC3-2 and SCRI193, a 28-kDa protein and a 27-kDa protein, respectively, showed a specific interaction with the PelI antibodies (Fig. 6B). In contrast, no interaction was detected with the two *E. carotovora* subsp. *atroseptica* strains CA36A and SCRI1039.

Southern hybridizations were performed with the labeled 0.77-kb *Asp*I fragment included in the *pelI* ORF as the probe and *Eco*RI-digested chromosomal DNAs of the *Erwinia* strains. Under stringent conditions, *pelI* homologs were detected in the five tested *E. chrysanthemi* strains, 3937, B374, ENA49, CU1, and EC16. In each case, a single *Eco*RI fragment hybridized with the *pelI* probe (data not shown). In contrast, no hybridizing signals could be detected in the two *E. carotovora* subsp. *carotovora* strains CC3-2 and SCRI193 or in the two *E. carotovora* subsp. *atroseptica* strains CA36A and SCRI1039.

Characterization of PelI activity. The characterized pectate lyases have a basic pH optimum and an absolute requirement for Ca^{2+} ions. We analyzed the biochemical properties of PelI, purified from BL21(DE3)/pTPL1 (Fig. 5C). PelI presented good activity between pH 8.8 and 9.7, with an optimum at pH 9.2. PelI required a high Ca^{2+} concentration for optimal activity (optimum at 0.6 mM in a 0.3 to 1 mM range) and was completely inhibited by the addition of 10 mM EDTA. The PelI enzyme showed maximal activity on pectin with a degree of methylesterification from 22 to 45% but presented a lower activity on PGA and no activity on highly methylesterified pectin (more than 75%). The initial velocity for the reaction

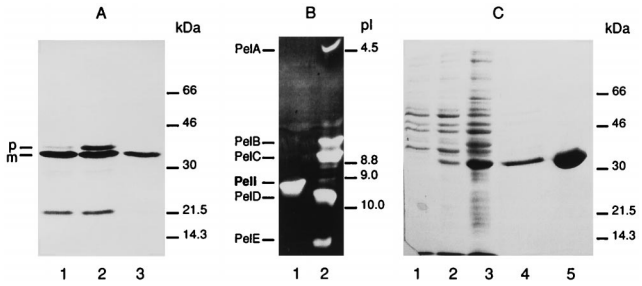

FIG. 5. Cellular localization and purification of the PelI protein in *E. coli*. (A) Subcellular fractionation of the *E. coli* K38/pGP1.2/pTPL1 cells, labeled with [^{35}S]cysteine-methionine. The proteins were separated by SDS-PAGE, and the gels were autoradiographed. Lanes: 1, whole-cell extract; 2, osmotically shocked cell fraction; 3, periplasmic fraction. The positions of the molecular weight standards and of the PelI precursor (p) and mature (m) forms are indicated. (B) Electrofocusing was followed by specific detection of pectate lyase activity. Lanes: 1, purified PelI; 2, supernatant of *E. chrysanthemi* 3937. Positions of the five major pectate lyases (PelA to PelE), of PelI, and of the pI standards are indicated. (C) Overproduction and purification of PelI. The proteins were separated by SDS-PAGE, and the gels were stained with Coomassie blue G-250. Lanes: 1 and 2, cell lysates of *E. coli* BL21(DE3)/pTPL1 before induction (lane 1) and after induction with IPTG (lane 2); 3, periplasmic extract from induced cells; 4, PelI eluted from a Phenyl-TSK gel column; 5, 5 μg of purified PelI protein. Size markers are indicated.

was determined at different PGA concentrations in the presence of 0.6 mM Ca^2. Apparent K_m and V_{max} values for PelI were 0.12 ± 0.03 g liter^{-1} and 230 ± 20 μmol min^{-1} mg^{-1}, respectively. At 50°C, the half-time of PelI thermoinactivation was 15 min in the presence of 0.6 mM Ca^{2+} and only 5 min in the presence of 50 μM EDTA, indicating that Ca^{2+} interacts with the enzyme, increasing its thermostability.

Thin-layer chromatography was used to characterize the PelI reaction end products. PelI catalyzes the formation of multiple products, including unsaturated dimers, trimers, and longer oligomers (Fig. 7). Total digestion gave a majority of dimers, while partial digestion gave a mixture of longer oligomers. This pattern is very similar to that observed for PelD, while PelB gives a majority of trimers as end products (Fig. 7).

Role of *pelI* in the pathogenicity of *E. chrysanthemi*. We compared the ability of PelI to macerate plant tissues with that

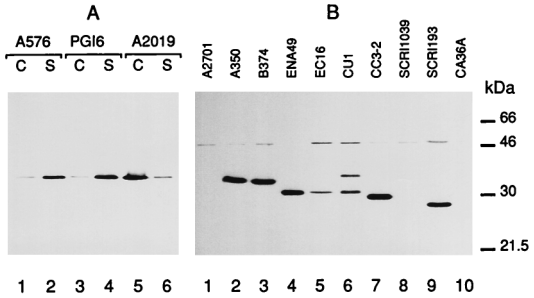

FIG. 6. Secretion of PelI and identification of PelI homologs in various *Erwinia* strains. (A) PelI cellular localization in *E. chrysanthemi*. Lanes: 1 and 2, cell extracts (c) and culture supernatants (s) of the parental strain A576; 3 and 4, PGI6; 5 and 6, A2019 (*out* mutant). The proteins were separated by SDS-PAGE and analyzed by Western immunoblotting with PelI antibodies. (B) Occurrence of PelI homologs in various *Erwinia* strains. Total extracts of cultures grown in LB supplemented with galacturonate were separated by SDS-PAGE and revealed by Western immunoblotting with PelI antibodies. Extracts are from the *pelI* mutant A2701 (lane 1) and the parental strain A350 (lane 2), the *E. chrysanthemi* wild-type strains B374 (lane 3), ENA49 (lane 4), EC16 (lane 5), and CU1 (lane 6), *E. carotovora* subsp. *carotovora* CC3-2 (lane 7) and SCRI193 (lane 9), and *E. carotovora* subsp. *atroseptica* SCRI1039 (lane 8) and CA36A (lane 10). Positions of molecular mass standards are indicated.

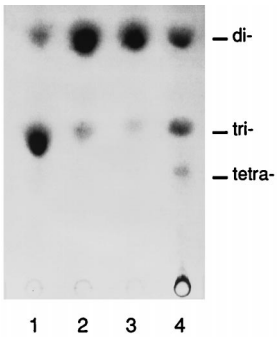

FIG. 7. Separation of the PelI reaction products by thin-layer chromatography. The reaction mixtures contained 0.1 M Tris-HCl (pH 8.5), 0.2 mM $CaCl_2$, 1.5 mg of PGA per ml, and 1 U of pectate lyase activity per ml. Incubation was performed at 30°C for 12 h with PelB (lane 1), PelD (lane 2), and PelI (lane 3) or for 4 h with PelI (lane 4); 5 μl of each sample was applied to chromatogram sheets. The positions of unsaturated di-, tri-, and tetramers are indicated.

of the two major isoenzymes PelC and PelD, by incubating serial dilutions of purified enzymes with potato cubes (Fig. 8). PelI and PelD, at levels less than 0.1 U ml^{-1}, showed evidence of maceration within 1 h. PelC was less active in tissue maceration, since the addition of 0.1 U ml^{-1} caused tissue softening only after 4 h.

We also compared the maceration provoked by the *pelI* mutant A3003 and the wild-type strain on chicory leaves and potato tubers. On chicory leaves, the length of rotted tissues measured 24 h after inoculation appeared reduced when the *pelI* mutant was compared to the 3937 strain (27 ± 8 and 46 ± 12 mm, respectively). No significant reduction was observed on potato tubers 1, 2, or 3 days after infection (data not shown). We also compared the pathogenic behavior of the *E. chrysanthemi pelI* mutant on potted plants of *S. ionantha* with that of the wild-type strain. After infection of 30 plants with strains 3937 and A3003, we monitored the appearance of symptoms during 10 days. We observed no significant difference in the progress of the disease between the *pelI* mutant and the wild-type strain (data not shown). In the first days, bacteria invaded the plant vascular system and maceration spread through the leaf blade and petiole. From days 4 to 10, maceration progressed in other leaves and the plants developed a systemic response. The soft rot disease developed in 90 and 96% of the plants infected with the *pelI* mutant and the 3937 strain, respectively.

DISCUSSION

This paper reports the molecular cloning and characterization of the gene encoding a novel pectate lyase isoenzyme of *E. chrysanthemi*, PelI. Eight pectate lyases (PelA, PelB, PelC, PelD, PelE, PelL, PelZ and PelX) have already been identified in *E. chrysanthemi* 3937 (17, 31). No homology was detected between PelI and these eight pectate lyases, indicating that PelI belongs to a new family. However, PelI homologs were identified in another soft-rotting *Erwinia*, *E. carotovora* subsp. *carotovora*, and in a phytopathogenic fungus, *N. haematococca* (*Fusarium solani* f. sp. *pisi*). Such homologies demonstrate the existence of a new family of pectate lyases existing in several phytopathogenic organisms. Among the four Pels of *N. haematococca*, PelA, PelB, PelC, and PelD, the identity ranges from 51 to 65%, and some of them (PelA and PelB) present typical fungal leader peptides (11–13). Alignment of PelI of *E. chrysanthemi* with PelB of *E. carotovora* and PelA and PelC of *N. haematococca* revealed some conserved regions (Fig. 2). The rudimentary consensus sequence is made up of 42 exact matches among the four proteins. Four regions show blocks of conserved residues without deletion/insertion: region I, vwwe DvCEDAat; region II, DKvlQhN; region III, dyGKlxRsCGnC; and region IV, laGvNxNYGDvati (capital letters represent residues conserved in six sequences [PelI of *E. chrysanthemi*, PelB of *E. carotovora*, and PelA, PelB, PelC, and PelD of *N. haematococca*]; lowercase letters represent residues conserved in more than 50% of the six sequences). Such consensus sequences might qualify as signatures of pectate lyases of this new class.

As proposed by other authors (5a), the classification of pectate lyases has to be reconsidered. Class I corresponds to the pectate lyase superfamily containing several well-described bacterial and plant proteins (15) and includes PelA, PelB, PelC, PelD, and PelE of *E. chrysanthemi*. Class II corresponds to the periplasmic pectate lyases of *E. carotovora* (16) and includes KdgC of *E. chrysanthemi* (8). Three novel families have been recently described: class III, containing PelB/Pel-3 of

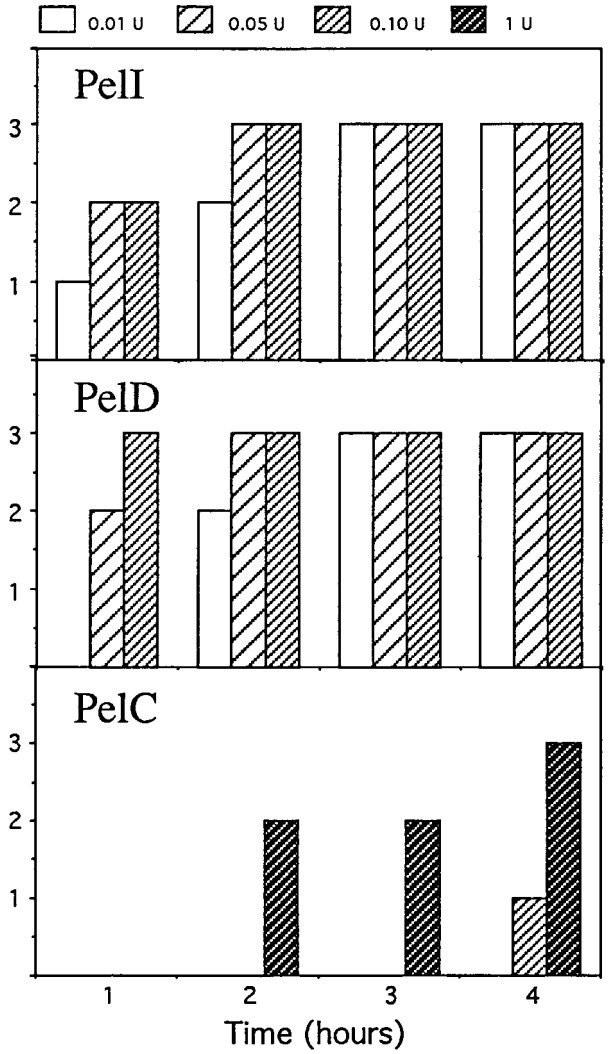

FIG. 8. Maceration of potato tuber tissue by different *E. chrysanthemi* Pels. Purified enzymes were prepared from *E. coli* clones overproducing one isoenzyme; 0.01 to 1 U of PelI, PelD, or PelC was incubated with 3-mm potato cubes. For each test, the degree of tissue maceration, or the reduction in cellular cohesion, was scored from 0 (no maceration) to 3 (complete tissue softening) after different time periods.

E. carotovora, the four Pels of *N. haematococca*, and PelI of *E. chrysanthemi*; class IV, which includes PelL and PelX of *E. chrysanthemi* (1, 25); and class V, containing only PelZ of *E. chrysanthemi* (31).

The signatures proposed for class III pectate lyases (Fig. 2) are clearly different from that described for class I. The recent identification of new members of class III in the *hrp* cluster of two pathogenic bacteria *Erwinia amylovora* (5a) and *Pseudomonas syringae* (7a) indicates that proteins of class III are probably distributed in a wide range of microorganisms. Moreover, analysis of five different *E. chrysanthemi* strains revealed one to three bands specifically interacting with the PelI antibodies (Fig. 7). The detection of only one signal in each *E. chrysanthemi* strain by DNA hybridization indicates that, if they exist, the additional genes have only a low homology with *pelI*. Immunoblotting indicated that the presence of PelI homologs is a feature common to strains of *E. chrysanthemi* and *E. carotovora* subsp. *carotovora* but not to *E. carotovora* subsp. *atroseptica*.

An unusual feature in the amino acid composition of the mature PelI protein is the presence of 10 cysteine residues. All of the pectate lyases of class III appear to be cysteine rich (12 to 14 cysteines in Pels of *N. haematococca*). The 10 cysteine residues of PelI are conserved in PelB/Pel-3 of *E. carotovora*, and five of them are also conserved in the *N. haematococca* Pels (Fig. 2). Other *E. chrysanthemi* pectate lyases contain few cysteine residues: one in PelZ, two in PelA, PelD, and PelE, and four in PelB, PelC, and PelL. In the major *E. chrysanthemi* pectate lyases, cysteine residues are involved in disulfide bonds (46). If several of the PelI cysteines are involved in disulfide bond formation, this implies a very rigid structure of the protein. Structural analysis of two class I pectate lyases, PelC and PelE (46, 47), demonstrated that these proteins fold in an unusual motif of parallel β strands coiled in a large helix stabilized by a stack of asparagine residues. The absence of homology between primary sequences suggests a different structure for class III pectate lyases. X-ray crystallography of PelI would now be useful to gain more insights into the tertiary structure of the class III enzymes.

Digestion of PGA by PelI yielded products with an absorbance peak at 232 nm, characteristic of unsaturated oligogalacturonides. Evidence that PelI is an endo-cleaving lyase arises from the fact that it catalyzes the formation of multiple products, with a majority of dimers, as observed with PelD or PelE (Fig. 7). In contrast, PelI resembles more closely the *E. chrysanthemi* pectate lyases PelB, PelC, PelI, and PelZ in its preference for low-methylesterified pectin as the substrate (25, 30, 43). Its very alkaline optimum pH is similar to that observed for PelB and PelC (43). The optimal Ca^{2+} concentration required for PelI activity, 0.6 mM, is significantly higher than the concentration required for the other *E. chrysanthemi* pectate lyases (0.1 to 0.2 mM) (25, 30, 43). Analysis of purified PelI protein indicated that its specific activity is about 10-fold higher than that of the secondary isoenzymes PelL and PelZ but about 15-fold lower than that of the most active isoenzymes, PelE and PelB. However, the PelI protein appeared able to macerate plant tissue very efficiently, as observed for PelD (Fig. 8). Early investigations using *E. chrysanthemi* EC16 revealed differences among the isoenzymes in their capacity to macerate plant tissues (3). In *E. chrysanthemi* 3937, the isoenzymes PelD, PelE, and PelI are the most effective in causing maceration. PelB, PelC, PelL, and PelZ are also capable of maceration, while PelA is unable to efficiently macerate plant tissue (25, 31).

PelI appeared to be another protein secreted by *E. chrysanthemi* via the Out system. The presence of a candidate signal sequence at the N-terminal end of the PelI protein is consistent with the isolation of the mature protein from the periplasmic fraction of *E. coli* containing the *pelI* gene (Fig. 5A). In *E. chrysanthemi*, PelI is detected in the culture supernatants of the wild-type strain but is retained in the cells of the *out* mutant (Fig. 6A). The C-terminal 34 amino acids of PelI appeared not to be important for PelI stability and secretion, whereas they are necessary for the enzymatic activity. To our knowledge, this is the first description of a modification of a secreted protein which does not affect its secretability by the Out machinery.

As shown for the other *pel* genes (17), the expression of *pelI* is controlled by several environmental stimuli (Table 2). *pelI* transcription is induced by pectin catabolic products, in late exponential growth phase, and in conditions of oxygen limitation. In contrast, *pelI* transcription is repressed in the presence of glucose, in conditions of nitrogen starvation, and at high temperatures. For the other *pel* genes, induction by pectic derivatives is at least partially mediated by the *kdgR* gene product (29). The KdgR repressor binds to a specific DNA sequence found in the vicinity of the promoters of the controlled genes. The *pelI* regulatory region contains a sequence showing homology with the consensus determined for the KdgR binding site (17). This sequence differs from the consensus by the absence of a T residue which is strictly conserved in all operators that interact in vitro with the KdgR protein. However, we confirmed the functionality of the *pelI* operator by gel retardation experiments using the purified KdgR protein (Fig. 3). A sequence matching the binding site of CRP, the global activator of sugar catabolism, overlaps this operator. Analysis of the *pelI*::*uidA* fusion demonstrated that the *pelI* expression is activated by CRP but is also regulated by the two repressors PecS and PecT. Thus, *pelI* expression appeared to be strictly controlled by all of the proteins involved in pectinolysis regulation. Preliminary analysis of the *pelI* transcription after infection of potato tubers indicated a high expression in planta. Thus, PelI can be considered a plant-inducible isoenzyme. The high induction observed in planta may result either from a constituent(s) specific to the plant cell wall or from a general physiochemical parameter(s) linked to the plant tissue. While *pelI* appeared not to be of primary importance for *E. chrysanthemi* pathogenicity on potato tubers or *Saintpaulia* plants, the *pelI* mutant produced less maceration of chicory leaves. Thus, PelI could be involved in the specificity of the bacterium toward some plant hosts. Such possible specificity was previously described for other isoenzymes (5, 31), and this could be a good explanation for the diversity of pectate lyases produced by *E. chrysanthemi.*

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