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Molecular Cloning and Sequencing of a Pectate Lyase Gene from *Yersinia pseudotuberculosis*

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A pectate lyase gene (*pelY*) from *Yersinia pseudotuberculosis* was cloned in *Escherichia coli* DH-5α. The gene was expressed in either orientation in pUC plasmids, indicating that the insert DNA carried a *Y. pseudotuberculosis* promoter which functioned in *E. coli*. However, when cloned in the orientation which placed the coding region downstream of the vector *lac* promoter, expression of *pelY* was nine times higher than it was in the opposite orientation and the growth of *E. coli* cells was inhibited. Nucleotide sequence analysis of the *pelY* gene disclosed an open reading frame of 1,623 base pairs (PLY). The peptide sequence at the amino-terminal end of the protein contains a typical signal peptide sequence, consistent with the observation that the mature PLY protein accumulated largely in the periplasmic space of *E. coli*. The pI of PLY produced in *E. coli* cells was 4.5, and its activity was inhibited 90% or more by EDTA. The enzyme macerated cucumber tissue about 1,000 times less efficiently than did PLe from *Erwinia chrysanthemi* EC16. The *pelY* gene has no sequence similarity to the *pel* genes thus far sequenced from *Erwinia* spp.

Pectate lyase (PL) is one of the most important enzymes involved in the maceration of plant tissues by soft-rotting *Erwinia* strains (5, 6, 11). A consistent feature of these bacteria is the occurrence of multigene families encoding functionally similar PLs. Several of these *pel* genes have been cloned and sequenced from strains of *E. chrysanthemi* (2, 9, 12; S. Tamaki, S. Gold, M. Robeson, S. Manulis, and N. T. Keen, submitted for publication) and *E. carotovora* (14, 15), and marker exchange mutagenesis experiments have shown that several individual *pel* genes are required for high pathogenicity (11). Surprisingly, PLs are also produced by other, nonphytopathogenic enterobacteria, such as members of the genera *Klebsiella* and *Yersinia* (1, 4, 22). Since it would be of interest to compare the structure, organization, and regulation of *pel* genes in these organisms with those of *Erwinia* spp., we undertook the cloning and characterization of a *pel* gene(s) from *Yersinia pseudotuberculosis* (4). We report here that there is no detectable homology between the single detected *Yersinia pelY* gene and any of the families of clustered *pel* genes thus far sequenced from *Erwinia* spp.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and the plasmids used and constructed in this work are given in Table 1.

Media and culture conditions. Bacterial strains were grown and maintained on Luria medium containing ampicillin at a concentration of 50 μg/ml (broth) or 100 μg/ml (solid medium). *Y. pseudotuberculosis* was grown at 30°C, and *Escherichia coli* strains were grown at 37°C. Cultures of *E. coli* for enzyme assays were grown at 28 to 30°C.

PL assays. PL activity in culture fluids or in periplasmic fractions was determined by monitoring the A_{232} as described previously (9). The activity of PLY on pectin was assayed at 235 nm with 1% citrus pectin (P9135; Sigma Chemical Co.) instead of polygalacturonic acid (P3889; Sigma). Purified PLe (10) from *E. chrysanthemi* EC16 was used as a control. The effect of Ca^{2+} on PL activity was determined with reaction mixtures lacking $CaCl_2$ or with 3 or 0.5 mM EDTA added instead of $CaCl_2$.

The screening of *E. coli* clones for PL production was done on YC agar plates (9) containing 100 μg of ampicillin per ml. The cells were incubated at 30°C for 24 h and were then lysed by exposing the plates to chloroform vapor for 20 min. The plates were then transferred to 30°C for 2 h. Positive PL clones were detected by the overlay method of Ried and Collmer (18) or by flooding the plates with 1 M $CaCl_2$ (9).

Standard DNA techniques. Restriction enzyme digestions, ligation conditions, preparation of competent cells, transformation procedures, and gel electrophoresis are described by Maniatis et al. (16) or Keen et al. (9). Large-scale isolation of plasmid DNA was done by the alkaline lysis procedure (16). Plasmid constructs were checked by miniboil plasmid extractions and restriction with the appropriate enzymes (7, 9). For subcloning and plasmid constructions, the desired DNA fragments were recovered from low-melting-point agarose gels by the method of Crouse et al. (7) or by electroelution from ultrapure agarose (Bio-Rad Laboratories) with 0.3 mM sodium acetate (pH 8.0) at 220 V.

Total-DNA isolation and construction of genomic libraries. *Y. pseudotuberculosis* chromosomal DNA was isolated as described previously (9). The chromosomal DNA was digested to completion with either *Eco*RI or *Bam*HI and was ligated with pUC19 cut with *Eco*RI or *Bam*HI, respectively. The ligated DNA was transformed into *E. coli* DH-5α, and transformants were selected on LB-ampicillin plates. From each library, 3,500 colonies were screened for PL production on polygalacturonic agar plates (9).

Subcloning. Plasmid DNA from a PL-positive clone (pPELY11) was further subcloned. The DNA was partially digested with *Sau*3A, and 5-kilobase (kb) fragments were purified from an agarose gel by electroelution. These fragments were ligated into the *Bam*HI site of plasmid pUC119, which had been dephosphorylated with calf intestine alkaline phosphatase.

DNA sequence analysis. Exonuclease III deletions were generated from either end of the insert fragment of pPELY14

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TABLE 1. Bacterial strains, bacteriophage, and plasmids used

Strain, plasmid, or phage	Description	Source or reference
E. coli		
DH-5α	F^- *lacZ*ΔM15 *endA1 recA1 hsdR17 supE44 thi-1 gyrA relA1* λ^-	Bethesda Research Laboratories, Inc.
MV1193	Δ(*lac-proAB*) *thi supE44* Δ(*srl-recA*)*306*::Tn*10* (Tet^r) (F′ *traD36 proAB lacZ*ΔM15)	20
Y. pseudotuberculosis ICPB 3821		A. Chatterjee (4)
Phage M13K07		20
Plasmids		
pUC19		23
pUC119 and pUC118		20
pPELY11	20-kb *Eco*RI fragment from *Y. pseudotuberculosis* DNA cloned in pUC19; PL positive	This report
pPELY12	4.5-kb *Xba*I-*Sac*I fragment from pPELY11 cloned in pUC19; PL positive	This report
pPELY14	3.6-kb fragment from partial *Sau*3A digest of pPELY11 cloned in pUC119 in the orientation opposite to that of the vector *lac* promoter; moderately PL positive	This report
pPELY15	3.6-kb fragment from pPELY14 cloned in pUC118 (downstream of the *lac* promoter); strongly PL positive	This report

and pPELY15 by the method of Henikoff (8). Deletions from the 5′ and 3′ ends of the gene were done in plasmids pPELY14 and pPELY15, respectively. After religation, the deletion plasmids were transformed into *E. coli* DH-5α. Appropriate deletions were assayed on YC plates for PL activity and transformed into *E. coli* MV1193. These bacteria were transfected with λM13K07, and templates of single-stranded plasmid DNA were isolated (20).

The DNA sequences of overlapping deletions were determined by the dideoxy chain termination method (19). All data were confirmed by sequencing both strands and were analyzed by the computer program of Pustell and Kafatos (17).

Characterization of the PL produced by pPELY15 clone. The isoelectric point (pI) of the PLY protein was determined by preparative isoelectric focusing (9). *E. coli* DH-5α cells containing pPELY15 were grown in 30 ml of L broth with 50 μg of ampicillin per ml for 16 h at 28°C. The periplasmic fraction was prepared as described previously (9). This fraction was dialyzed against 5 mM Tris hydrochloride (pH 8.0) and purified on an LKB 8100 preparative isoelectric focusing column with Bio-Rad 3-10 or 3-5 Ampholines. The runs were done at 650 V for 48 h at 4°C. Fractions (2.5 ml) were collected from the column and assayed for pH, PL activity, and protein concentration. Protein was determined by the method of Bradford (3) with bovine serum albumin as a standard.

Molecular weight was determined on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gels by the method of Laemmli (13). The electrophoresis was done on whole-cell proteins of *E. coli* DH-5α containing desired plasmids. Cultures were grown for 16 h at 28°C on 5 ml of L broth with 50 μg of ampicillin per ml. Isopropyl-β-D-thiogalactopyranoside (IPTG) at 1 mM was added at the time of culture initiation. Portions (1.5 ml) of these cultures were centrifuged, and the cells (ca. 12 mg) were washed once with 1.5 ml of 0.01 M Tris hydrochloride (pH 7.5). The pellets were then suspended in 100 μl of water, an equal volume of 2× sample buffer (13) was added, and the samples were boiled for 5 min. The gels were run at 160 V for 5 h, with phosphorylase *b* (97,400 daltons [Da]), bovine serum albumin (66,200 Da), ovalbumin (42,700 Da), carbonic anhydrase (31,000 Da), and soybean trypsin inhibitor (21,500 Da) as molecular mass standards (Bio-Rad). The gels were stained with Coomassie brilliant blue R250.

Plant tissue maceration. Maceration was determined by incubating five thin cucumber mesocarp slices (ca. 0.5 by 1 cm, 0.1 to 1 mm thick) with various dilutions of periplasmic fractions or purified PL. The assays were done in a total volume of 1 ml of 0.02 M Tris hydrochloride (pH 8.5) at 30°C for 1 to 5 h. Loss of tissue cohesiveness was tested with a spatula. The maceration index was scored on a scale of 0 to 5, where 0 indicated no maceration and 5 denoted complete tissue softening and disintegration. Controls were enzymes boiled for 5 min or buffer alone.

RESULTS

Cloning of the *pelY* gene. Two libraries of *Y. pseudotuberculosis* were constructed in pUC19 and screened in *E. coli* DH-5α. Two PL-positive clones were recovered from the *Eco*RI library, but none from the *Bam*HI library. Only one of the two positive clones gave consistent responses on pectate agar plates, and it was therefore selected for further study. When plasmid DNA was isolated and transformed into *E. coli* DH-5α, all of the resultant transformants were pectolytic. Restriction of this plasmid (pPELY11) with *Eco*RI showed the presence of plasmid pUC19 and a single insert fragment of about 20 kb (Fig. 1). A 4.5-kb *Xba*I-*Sac*I fragment subcloned in pUC19 (pPELY12) produced about the same level of PL activity in DH-5α as did pPELY11. No activity was detected when the *Sac*I-*Eco*RI or *Eco*RI-*Xba*I fragment was similarly subcloned (Fig. 1).

Subcloning. pPELY11 was partially digested with *Sau*3A, and ca. 5-kb fragments were subcloned into the *Bam*HI site of pUC119. Of 350 transformants screened, 11 were PL positive. The purified plasmids isolated from these clones were restricted with *Bam*HI and *Hin*dIII. All of the PL-positive plasmids had a common 500-base-pair *Bam*HI-*Hin*dIII fragment (Fig. 1). This data, as well as results from the construction of pPELY12, indicated that the 20-kb

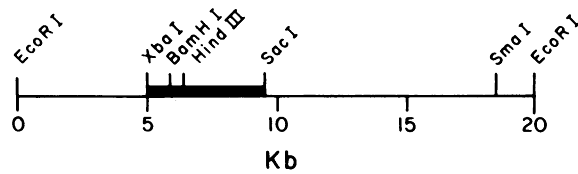

FIG. 1. Restriction map of a ca. 20-kb *Eco*RI fragment of *Y. pseudotuberculosis* DNA. The fragment was cloned into the *Eco*RI site of pUC19, and the resultant construct was designated pPELY11. The thick line between the *Xba*I and *Sac*I sites represents the sequence which encodes PL activity.

*Eco*RI insert of pPELY11 contained only one *pel* gene. The PL-positive plasmid with the smallest *Sau*3A insert was named pPELY14 and used for further study.

To determine the orientation of the *pel* gene, pPELY14 was cut with *Eco*RI and *Pst*I and the insert was subcloned into pUC118 restricted with the same enzymes. The resultant construct was designated pPELY15. All transformants carrying pPELY15 produced higher levels of PL than did those with pPELY14 (Table 2). In addition, IPTG increased expression of the gene only in pPELY15 (Table 2). These results suggested that the 3.6-kb insert of pPELY15 carries a *Y. pseudotuberculosis* promoter sequence which is functional in *E. coli* and that the *pelY* coding region in pPELY15 is oriented downstream from the vector *lac* promoter.

E. coli cells carrying pPELY15 formed smaller colonies than did cells with pPELY14, suggesting that overproduction of the *Yersinia* PL exerted a toxic effect on *E. coli* cells. This could also explain the relatively low number of PL-positive clones recovered from the *Eco*RI library (2 of 3,500) since clones in which the *pelY* gene was oriented downstream from the vector *lac* promoter probably did not grow well and were missed.

Characterization of the PL produced by the clones. The results presented in Table 2 demonstrate that more than 90% of the PL activity produced by *E. coli* cells carrying pPELY15 was secreted into the periplasm. Electrofocusing of periplasmic fractions from *E. coli* cells carrying pPELY11 or pPELY15 (Fig. 2) disclosed only one detectable PL, with a pI of 4.5. This is consistent with the production of the protein by *Y. pseudotuberculosis* (4). SDS-gel electrophoresis of whole-cell proteins from *E. coli* cells carrying pPELY15 (Fig. 3) showed an intense band at ca. 55,000 Da. This band was also predominant in the periplasmic fraction but was absent in cells carrying only pUC118. Since EDTA completely inhibits the activity of PLs produced by *Erwinia* spp. (6), we examined its effect on PLY activity. The

TABLE 2. Production of PL by *E. coli* DH-5α cells containing pPELY14 or pPELY15[a]

Plasmid	IPTG	PL activity (U/g of cells) in fraction		
		Periplasmic	Extracellular	Intracellular[b]
pPELY14	−	28	ND[c]	1.1
	+	23	ND	1.0
pPELY15	−	249	3.6	14.0
	+	388	4.1	19.6

[a] Cultures were grown for 16 h in 15 ml of L broth with 50 μg of ampicillin per ml. IPTG at 1 mM was added at the time of initiation of growth. The cells were centrifuged, and the supernatant was dialyzed. The periplasmic fractions were prepared as described in the text.

[b] The pelleted spheroplasts were lysed with 5 ml of 0.01 M Tris hydrochloride (pH 8.0), centrifuged, and assayed for PL activity.

[c] ND, No activity was detected.

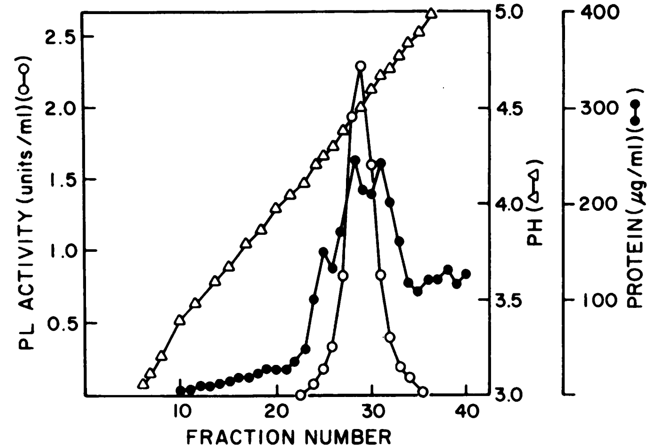

FIG. 2. Preparative isoelectric focusing of the periplasmic fraction from *E. coli* DH-5α cells containing pPELY15. The run conditions and PL assays are described in the text.

omission of Ca^{2+} from the reaction mixture caused a 43% inhibition of the PL activity. The addition of 0.5 or 3 mM EDTA further reduced the activity by 90 and 92%, respectively. PLY was 1.9 times more active on pectate than on

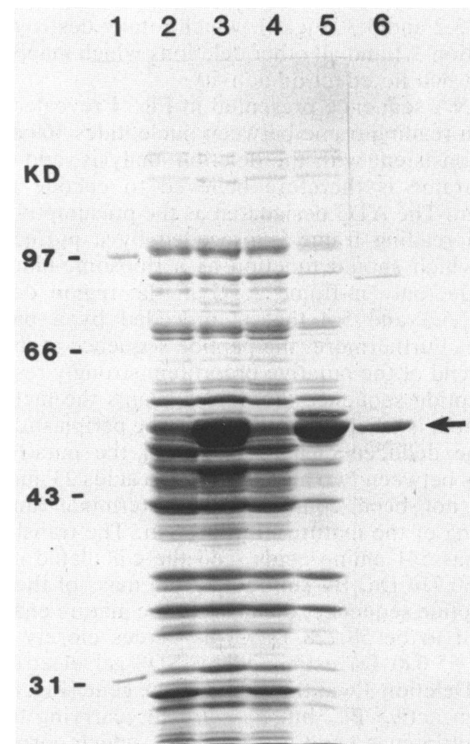

FIG. 3. SDS-gel electrophoresis of DH-5α cells containing pUC118 or pPELY15 grown with or without IPTG. The samples were prepared as described in the text, and 10 μl was applied to each lane. Lanes: 1, standards; 2, pUC118 without IPTG; 3, pPELY15 without IPTG; 4, pUC118 with IPTG; 5, pPELY15 with IPTG; 6, periplasmic fraction from pPELY15 plus IPTG, concentrated 20×. The arrow denotes the presumed mature PLY. Some of the standard protein bands are weakly visible since the photograph was underexposed to resolve the mature enzyme. The protein bands in lane 5, except the PLY band, are weaker than in the other lanes because the pellet with overexpressed pPELY15 had 30 to 40% less fresh weight. KD, Kilodaltons.

pectin, whereas PLe was 3.5 times more active. Thus, PLY differs considerably from the *Erwinia* PLs in enzymatic properties.

Plant tissue maceration. To determine the ability of PLY to macerate, 10 U of the enzyme was incubated with cucumber slices. PLe (0.1 U) from *E. chrysanthemi* EC16 was used as a positive control. After 1 h, the maceration indices for PLY and PLe were 0 and 3, respectively, and after 5 h they were 3 and 5. The results of several experiments with various concentrations of PLY and PLe indicated that PLY is less than 0.001 as efficient as the PLe protein in plant maceration.

DNA sequence of the *pelY* gene. To map the endpoints of the *pelY* gene more precisely and to generate templates for DNA sequence determination, exonuclease III deletions were generated from either end of the DNA fragment in pPELY14 and pPELY15. The positions of some of the relevant deletions are shown in Fig. 4. Deletion 5.2 was presumed to occur in the 5′ noncoding end of the gene since it had no effect on PL activity. Deletions 5.3 and 5.4 totally destroyed activity and thus appeared to occur in or near coding regions. These two deletions were then recloned in pUC118 (downstream of the *lac* promoter), and cells were grown with IPTG. Deletion 5.4 was completely inactive, whereas deletion 5.3 exhibited low pL activity in the pectate plate assay and the spectrophotometric assay (4.5 U/g of cells, compared with ca. 400 U/g for deletion 5.2).

Deletions which defined the 3′ end of the *pelY* gene included 3.2 and 3.3 (Fig. 4), which totally destroyed activity. Deletion 3.1 and all other deletions which mapped to the right of it had no effect on activity.

The DNA sequence presented in Fig. 4 revealed a single long open reading frame between nucleotides 369 and 1991. This is consistent with the deletion analysis, and the open reading frame is therefore believed to encode the PLY preprotein. The ATG designated as the presumptive start of the open reading frame is preceded by a purine-rich sequence which should function as a ribosome-binding site. This is the only in-frame ATG in the region defined by deletions 5.3 and 5.4 that is preceded by a purine-rich sequence. Furthermore, the peptide sequence at the amino-terminal end of the putative preprotein strongly resembles a signal peptide sequence (21) and explains the fact that the PLY protein is efficiently secreted to the periplasm of *E. coli* cells. The deduced cleavage point of the putative signal peptide is between two alanines (amino acids 23 and 24), but this has not been confirmed by N-terminal amino acid sequencing of the mature PLY protein. The translated preprotein has 541 amino acids, and the calculated molecular mass is 60,716 Da. By subtracting the mass of the putative signal peptide sequence, the mass of the mature enzyme was calculated to be 58,228 Da. This agrees closely with the value of 55,000 Da estimated by SDS-gel electrophoresis (Fig. 3). Deletion 3.6 at the 3′ end of the gene (Fig. 4) did not lead to an active PL, but *E. coli* cells carrying this DNA yielded an intense band at 43,000 Da, which corresponded well with the calculated molecular mass (44,550 Da) of the truncated protein (data not shown). Possible transcriptional initiation signals were found between deletions 5.2 (PL positive) and 5.3 (PL negative). These sequences are positioned at bases 238 to 243 and 257 to 261 (Fig. 4). No sequence which could function as a catabolite activator protein-binding site was found. This is consistent with the study of Chatterjee et al. (4), in which repression of PLY production was not observed in *Y. pseudotuberculosis*. In the 3′ end downstream of the translational stop of *pelY* (positions 2028 and 2044) there are sequences which may form stem-loop structures and thus may function as terminators of transcription.

DISCUSSION

The soft-rot erwinias produce four or more PL isozymes which are encoded by a corresponding number of clustered *pel* genes (6, 11). Although we cannot rule out the possibility of other *pel* genes that were not cloned, we were able to select clones from *Y. pseudotuberculosis* which contained only the *pelY* gene. Deletion analyses of the insert DNA in pPELY11 suggested that only one *pel* gene was present in this clone, unlike results with *E. chrysanthemi* (11). One or possibly two PLs have also been reported for *Yersinia enterocolitica* and *Klebsiella oxytoca* (1). It appears, therefore, that the *pel* gene organization is less complex in these organisms than in *Erwinia* spp.

Most PL activity occurred in the periplasmic fraction when the cloned *pelY* gene was expressed in *E. coli* (Table 2). This is consistent with the occurrence of a putative signal peptide sequence in the gene product (Fig. 4) and agrees with the previous results of Chatterjee et al. (4).

The *Yersinia* PL exhibited several differences from the *Erwinia* PLs. The molecular mass of the mature PLY was 58,228 Da, which is considerably higher than the ca. 39-kDa mass observed for the mature PLs of *Erwinia* spp. (6, 11). Our data for the *Yersinia* PL are in agreement with the value of 55 kDa previously reported for the PL of *Y. enterocolitica* (1). Another difference from the *Erwinia* PLs is that the *Yersinia* enzyme was 1.9 times less active on pectin than on pectate, whereas the *E. chrysanthemi* PLe was 3.5 times less active. Similar to the *Erwinia* PLs, the *Yersinia* enzyme is calcium dependent. However, whereas EDTA completely abolishes activity of the *Erwinia* enzymes, a maximum inhibition of ca. 90% was observed with the *Yersinia* enzyme. Our results also conflict with those of Bagley et al. (1), who reported that PLs from *K. oxytoca* and *Y. enterocolitica* do not require divalent cations for activity.

The acidic pI value for PLY is similar to those reported for the PLs of *K. oxytoca* and *Y. enterocolitica* (1). All of these enzymes are inefficient macerators of plant tissue, similar to PLa from *E. chrysanthemi*, which also has a low isoelectric point (2). Although it is possible that the low pI values of these PLs are solely responsible for their low maceration efficiency, other factors may also be involved, since efficient macerating enzymes with low pI values have been described for other organisms (6).

Computer searching disclosed no homology between the amino acid sequence of PLY and those of the protein products of sequenced *Erwinia pel* genes. Thus, these genes appear to have evolved from different lineages. Since three distinct families of *pel* genes have thus far been recovered from *Erwinia* spp. (11; Tamaki et al., submitted; D. and N. T. Keen, Trollinger, unpublished data), at least three families of *pel* genes exist in the family *Enterobacteriaceae*. It is not clear why such catalytically similar proteins evolved independently. Perhaps this reflects different functions in nature. For instance, it has been speculated (1, 4) that the PLs of *Yersinia* and *Klebsiella* spp. might be advantageous to their survival as saprophytes. Chatterjee et al. (4) also suggested that the PLs of *Yersinia* and *Klebsiella* spp. might have strictly catabolic functions related to bacterial nutrition, whereas the *Erwinia* enzymes might also have cytolytic and other specific functions in plant hosts. This possibility is supported by our findings that *pelY* is structurally distinct from the *Erwinia pel* genes, does not occur as a multigene

```
10 20 30 40 50 60
TT AAA TTA ACG TAC AAG TCG GAT AAG AAT TCG TCT GCT TAT ATC GCA CTC GGT AAT GTT TCT

70 80 90 100 110 120
GGT ACC AAA AAT ACC GAT GAG CCC CAA ACA GGT TAT CCC GTA CCG GTA AAA TAT ACC TTC

130 Xba I 140 150 160 170 180
TAA TTT ATA TCT AGA CCC AAA ATA ATT TCA GTC GCA GGA ACG CTG CTT GCT CCG CCC TCG

190 5.2↓ 200 210 220 230 240
AAG ACG CAG CCC GCT TGA TCG CCC GAG TCA GCA CCG CAG CCA ACG CAC ATG CAG CTT GAA

250 260 270 280 290 300
GTA TGA CCG GAA CCC TAT TCC GTT CCC ATG AGA TTA CCG GCA ACG GCA AAT AAG AAC AGT

310 320 330 340 350 360
GAA ATA ATC CCC ATC CCC GCT TCG GTC ACT GCT CAC GAT AAC GAT TTC TCG CAG GTA AGA

5.3↓ 370 380 390 400 410 420
TAA CCG ATG AAA AAA AGA GCA TTA TTG TTG AGT ATG TCA GTG CTG GCA ATG CTG TAT ATC
        Met Lys Lys Arg Ala Leu Leu Leu Ser Met Ser Val Leu Ala Met Leu Tyr Ile

430 440 450 460 470 480
CCC GCC GGG CAA GCC GCA GAG ATA GAC CGT CTC ACC GTA GTT AAG CAG TAT GTC GAT AAT
Pro Ala Gly Gln Ala↓Ala Glu Ile Asp Arg Leu Thr Val Val Lys Gln Tyr Val Asp Asn

490 500 510 520 5.4↓ 530 540
GTA CTG AAC AAA GCA TCA GAT ACT TAT CAC GGT GAT AAA CCT AGC CCA TTG CTG GCT GAT
Val Leu Asn Lys Ala Ser Asp Thr Tyr His Gly Asp Lys Pro Ser Pro Leu Leu Ala Asp

550 560 570 580 590 600
GGT GTT GAC CCC CGA ACC GGG CAG CAA ATG GAG TGG ATA TTC CCT GAT GGC CGT CGT GCT
Gly Val Asp Pro Arg Thr Gly Gln Gln Met Glu Trp Ile Phe Pro Asp Gly Arg Arg Ala

610 620 630 640 650 660
GTG CTA TCC AAC TTC TCT GCA CAA CAA AAT CTG ATG CGG GTT ATG AGT GGG CTG AGT GAA
Val Leu Ser Asn Phe Ser Ala Gln Gln Asn Leu Met Arg Val Met Ser Gly Leu Ser Glu

670 680 690 700 EcoR V 710 720
CTT AGC GGT GAT CCG CAG TAC CAA AAG CGT GCT GAA GAT ATC GTG CGT TAC CAT TTC CAA
Leu Ser Gly Asp Pro Gln Tyr Gln Lys Arg Ala Glu Asp Ile Val Arg Tyr His Phe Gln

730 740 750 760 770 780
AAT TAT CAA GAT AAT AGC GGC TTG CTC TAC TGG GGA GGC CAC CGT TTT GTT GAT CTG AAA
Asn Tyr Gln Asp Asn Ser Gly Leu Leu Tyr Trp Gly Gly His Arg Phe Val Asp Leu Lys

790 800 810 820 830 840
ACC TTG CAG CCG GAA GGC CCC AGC GAA AAA GAG AAA GTT CAT GAG CTG AAA AAT GCT TAT
Thr Leu Gln Pro Glu Gly Pro Ser Glu Lys Glu Lys Val His Glu Leu Lys Asn Ala Tyr

850 860 870 880 890 900
CCC TAT TAT GAC CTG ATG TTC AGT GTT GAT AGC GAC GCC ACC ACC CGT TTT ATT CGC GGT
Pro Tyr Tyr Asp Leu Met Phe Ser Val Asp Ser Asp Ala Thr Thr Arg Phe Ile Arg Gly

910 920 930 BamH I 940 Nae I 950 960
TTC TGG AAT GCT CAT GTG TAT GAT TGG CGG ATC CTT GAA ACC AGC CGG CAC GGT GAG TAC
Phe Trp Asn Ala His Val Tyr Asp Trp Arg Ile Leu Glu Thr Ser Arg His Gly Glu Tyr

970 980 990 1000 1010 1020
GGT AAG CCA ATG GGG GCA TTG TGG GAA AGT ACG TTT GAG CAA CAA CCT CCT TTC TTT GCG
Gly Lys Pro Met Gly Ala Leu Trp Glu Ser Thr Phe Glu Gln Gln Pro Pro Phe Phe Ala

1030 1040 1050 1060 1070 1080
ACC AAA GGG CTC AGT TTC CTT AAC GCC GGT AAT GAC CTG ATC TAT TCC GCC TCC CTG CTG
Thr Lys Gly Leu Ser Phe Leu Asn Ala Gly Asn Asp Leu Ile Tyr Ser Ala Ser Leu Leu

1090 1100 1110 1120 1130 Bcl I 1140
TAT AAA TAT CAG CAA GAT CAG GGC GCA TTA GTC TGG GCA AAA CGT TTG GCT GAT CAA TAT
Tyr Lys Tyr Gln Gln Asp Gln Gly Ala Leu Val Trp Ala Lys Arg Leu Ala Asp Gln Tyr

1150 1160 1170 1180 1190 1200
GTG TTG CCG CGA GAT GCC AAG ACT GGC CTC GGT GTC TAT CAG TTC ACT CAG GCA CTG AAA
Val Leu Pro Arg Asp Ala Lys Thr Gly Leu Gly Val Tyr Gln Phe Thr Gln Ala Leu Lys

1210 1220 1230 1240 1250 1260
CGT GAA GAG CCA ACA GAT GAT GCG GAT ACC CAT TCC AAG TTT GGC GAC CGT GCA CAG CGG
Arg Glu Glu Pro Thr Asp Asp Ala Asp Thr His Ser Lys Phe Gly Asp Arg Ala Gln Arg

1270 Ava I 1280 1290 1300 1310 1320
CAG TTC GGA CCC GAG TTC GGC CCA ACG GCG CTT GAA GGC AAT ATG ATG CTC AAA GGC CGC
Gln Phe Gly Pro Glu Phe Gly Pro Thr Ala Leu Glu Gly Asn Met Met Leu Lys Gly Arg

1330 1340 1350 1360 1370 1380
ACC AGT ACC CTT TAT TCT GAA AAC GCC TTG ATG CAA CTG CAA CTG GGT AAG GAT TTA GGC
Thr Ser Thr Leu Tyr Ser Glu Asn Ala Leu Met Gln Leu Gln Leu Gly Lys Asp Leu Gly

1390 1400 1410 1420 Hind III 1430 1440
GGC CAA GGT GAC GAT TTA CTG AAA TGG ACC GTG GAT GGC CTG AAA GCT TTC GCC AAA TAC
Gly Gln Gly Asp Asp Leu Leu Lys Trp Thr Val Asp Gly Leu Lys Ala Phe Ala Lys Tyr

1450 1460 1470 1480 1490 1500
GGC TAC AAC GAG CAG GAC AAT ACG TTC CGC CCG ATG ATC GCT AAT GGT CAG GAT TTA TCG
Gly Tyr Asn Glu Gln Asp Asn Thr Phe Arg Pro Met Ile Ala Asn Gly Gln Asp Leu Ser

1510 1520 1530 1540 1550 1560
AAT TAC ACT CTG CCA CGT GAT GGT TAT TAC GGC AAA AAA GGA TCG GTA CTC AAG CCT TAT
Asn Tyr Thr Leu Pro Arg Asp Gly Tyr Tyr Gly Lys Lys Gly Ser Val Leu Lys Pro Tyr

1570 1580 1590 1600 1610 3.6↓ 1620
AAG GCG GGT AAC GAG TTT CTG ATT TCC TAT GCG CGT GCT TAT GCT GTC GAT AAT GAC CCC
Lys Ala Gly Asn Glu Phe Leu Ile Ser Tyr Ala Arg Ala Tyr Ala Val Asp Asn Asp Pro

1630 1640 1650 1660 1670 EcoR V 1680
TTA CTG TGG AAG GTG GCT CGC GGT ATT GCC AGC GAT CAG GGA TTG GGC GAT ATC GGC AGT
Leu Leu Trp Lys Val Ala Arg Gly Ile Ala Ser Asp Gln Gly Leu Gly Asp Ile Gly Ser

1690 1700 1710 1720 1730 1740
GCG CCA GGC AAA GAG ATG AAG GTC AAA CTG GAT ACC ACC AAT AGC GAT CCG TAT GCG CTG
Ala Pro Gly Lys Glu Met Lys Val Lys Leu Asp Thr Thr Asn Ser Asp Pro Tyr Ala Leu

1750 1760 1770 3.5↓ 1780 1790 1800
TTT GCT TTG CTG GAC CTC TAC AAT GCC AGC CAG GTC GCT GAG TAC CGG TCT TTG GCG GAG
Phe Ala Leu Leu Asp Leu Tyr Asn Ala Ser Gln Val Ala Glu Tyr Arg Ser Leu Ala Glu

1810 1820 3.4↓ Mlu I 1830 1840 1850 1860
AAA GTT GCC GAT AAC ATC ATC AAA ACG CGT TAT ATC GAC GGT TTC TTT ATG GCT TCA CCG
Lys Val Ala Asp Asn Ile Ile Lys Thr Arg Tyr Ile Asp Gly Phe Phe Met Ala Ser Pro

1870 1880 1890 1900 3.3↓ 1910 1920
GAT CGG CAA TAT GCC GAT GTG GAT GCT ATC GAG CCT TAT GCT TTG TTG GCA TTG GAA GCA
Asp Arg Gln Tyr Ala Asp Val Asp Ala Ile Glu Pro Tyr Ala Leu Leu Ala Leu Glu Ala

1930 1940 1950 1960 3.2↓ 1970 1980
TCA TTG CGT AAC AAA CCA CAA GCG GTT GCC CCT TTC CTT AAT GGG GCT GGT TTT ACC GAA
Ser Leu Arg Asn Lys Pro Gln Ala Val Ala Pro Phe Leu Asn Gly Ala Gly Phe Thr Glu

1990 2000 2010 2020 2030 2040
GTG CTT ACC TGA TCG CAG ACG GTT CAG CCC GAA TTT CTA CCC GTG ACA ATG ACC TGT TCT
Val Leu Thr —

2050 2060 2070 2080 2090 2100
TGC TCA ATG TCG GTG AGA CGT TGC AGC CGA ATG GCC GTA AAT AGC CCC GAC AGC AGC CAT

BstX I 2110 2120 2130 2140 2150 2160
CCG ATT GGC CCC TGC TCG TAA AAG TGC CCG GCT GCT GCT CCC CAG AGT CCG TTA CCC TCT

2170 2180 2190 2200 2210 2220
GCC ACT TTT CCC CAC CGG TTA TTG CAG CCC AAT AAA AAG GAC ACT GAG ATG AAA AAA CAA

2230 2240 2250 2260 3.1↓ 2270
ATT TTA GCG ACG TTG CTA CTG GCC GCT CTG TTT TGT GTA CCG GTT TA
```

FIG. 4. Nucleotide sequence determined for the *pelY* gene. The predicted amino acid sequence of the preprotein is shown, and selected restriction sites are noted. The positions of selected exonuclease III deletions referred to in the text are indicated with arrows and numbers. For deletions at the 5′ end of the sequence, DNA to the left of the arrow was deleted. For deletions at the 3′ end, DNA to the right of the arrow was deleted. The presumed leader peptide is underlined, and the possible cleavage site is indicated by an arrow. The putative ribosome-binding site and other possible signal sequences discussed in the text are underlined.

family, and encodes a PL which inefficiently macerates plant tissue.

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LITERATURE CITED

1. **Bagley, S. T., and M. P. Starr.** 1979. Characterization of intracellular polygalacturonic acid trans-eliminase from *Klebsiella oxytoca*, *Yersinia enterocolitica*, and *Erwinia chrysanthemi*. Curr. Microbiol. **2**:381–386.
2. **Barras, F., K. K. Thurn, and A. K. Chatterjee.** 1987. Resolution of four pectate lyase structural genes of *Erwinia chrysanthemi* (EC16) and characterization of the enzymes produced in *Escherichia coli*. Mol. Gen. Genet. **209**:319–325.
3. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
4. **Chatterjee, A. K., G. E. Buchanan, M. K. Behrens, and M. P. Starr.** 1979. Synthesis and excretion of polygalacturonic acid trans-eliminase in *Erwinia*, *Yersinia* and *Klebsiella* species. Can. J. Microbiol. **25**:94–102.
5. **Chatterjee, A. K., and M. P. Starr.** 1980. Genetics of *Erwinia* spp. Annu. Rev. Microbiol. **34**:645–676.
6. **Collmer, A., and N. T. Keen.** 1986. The role of pectic enzymes in plant pathogenesis. Annu. Rev. Phytopathol. **24**:383–409.
7. **Crouse, G. F., A. Frischauf, and H. Lehrach.** 1983. An integrated and simplified approach to cloning into plasmids and single-stranded phages. Methods Enzymol. **101**:78–89.
8. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28**:351–359.
9. **Keen, N. T., D. Dahlbeck, B. Staskawicz, and W. Belser.** 1984. Molecular cloning of pectate lyase genes from *Erwinia chrysanthemi* and their expression in *Escherichia coli*. J. Bacteriol. **159**:825–831.
10. **Keen, N. T., and S. Tamaki.** 1986. Structure of two pectate lyase genes from *Erwinia chrysanthemi* EC16 and their high-level expression in *Escherichia coli*. J. Bacteriol. **168**:595–606.
11. **Kotoujansky, A.** 1987. Molecular genetics of pathogenesis by soft-rot Erwinias. Annu. Rev. Phytopathol. **25**:405–430.
12. **Kotoujansky, A., A. Diolez, M. Boccara, Y. Bertheau, T. Andro, and A. Coleno.** 1985. Molecular cloning of *Erwinia chrysanthemi* pectinase and cellulase structural genes. EMBO J. **4**:781–785.
13. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
14. **Lei, S.-P., H.-C. Lin, L. Heffernan, and G. Wilcox.** 1985. Cloning of the pectate lyase genes from *Erwinia carotovora* and their expression in *Escherichia coli*. Gene **35**:63–70.
15. **Lei, S.-P., H.-C. Lin, S.-S. Wang, J. Callaway, and G. Wilcox.** 1987. Characterization of the *Erwinia carotovora pelB* gene and its product pectate lyase. J. Bacteriol. **169**:4379–4383.
16. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Pustell, J., and F. C. Kafatos.** 1984. A convenient and adaptable package of computer programs for DNA and protein sequence management, analysis and homology determination. Nucleic Acids Res. **12**:643–655.
18. **Ried, J. L., and A. Collmer.** 1985. Activity stain for rapid characterization of pectic enzymes in isoelectric focusing and sodium dodecyl sulfate-polyacrylamide gels. Appl. Environ. Microbiol. **50**:615–622.
19. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
20. **Vieira, J., and J. Messing.** 1987. Production of single-stranded plasmid DNA. Methods Enzymol. **153**:3–11.
21. **von Heijne, G.** 1985. Signal sequences: the limits of variations. J. Mol. Biol. **184**:99–105.
22. **Walker, M. J., and J. M. Pemberton.** 1987. Construction of a transposon containing a gene for polygalacturonate trans-eliminase from *Klebsiella oxytoca*. Arch. Microbiol. **146**:390–395.
23. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.