JOURNAL OF BACTERIOLOGY, Aug. 1995, p. 4553–4556 Vol. 177, No. 15
0021-9193/95/$04.00+0

Use of Tn*5*tac1 To Clone a *pel* Gene Encoding a Highly Alkaline, Asparagine-Rich Pectate Lyase Isozyme from an *Erwinia chrysanthemi* EC16 Mutant with Deletions Affecting the Major Pectate Lyase Isozymes

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Received 20 March 1995/Accepted 22 May 1995

***Erwinia chrysanthemi* mutant CUCPB5047, Δ(*pelA pelE*) Δ(*pelB pelC*)::28bp Δ(*pelX*) Δ4bp *pehX*::ΩCm^r, was constructed, mutated with Tn*5*tac1, and screened for isopropyl-β-D-thiogalactopyranoside-dependent pectate lyase (Pel) production. A Km^r *Sac*I fragment from the hyperexpressing Pel$^+$ mutant CUCPB5066 was cloned into *Escherichia coli* and sequenced. The gene identified, *pelL*, encodes a novel, asparagine-rich, highly alkaline enzyme that is similar in primary structure to PelX and in enzymological properties to PelE.**

The enterobacterium *Erwinia chrysanthemi* causes diseases involving maceration and killing of parenchymatous tissues in many plants. The bacterium also produces and secretes a variety of plant cell wall-degrading enzymes, including cellulases, xylanases, and a remarkably diverse array of pectic enzymes (2). These include exo-poly-α-D-galacturonosidase (PehX); exopolygalacturonate lyase (PelX); and most importantly for tissue maceration, four or five (depending on the strain) isozymes of pectate lyase (PelABCDE), an enzyme that cleaves internal α-1,4 galacturonosyl linkages in plant cell walls and middle lamellae by β elimination (2, 6).

E. chrysanthemi EC16 produces four Pel isozymes when grown in media containing pectate (a spontaneous deletion affects *pelD*) (25). They range in pI from 4.6 (PelA) to 9.8 (PelE). PelE is the most effective in killing and macerating plant tissues, and the maceration capacity of an EC16 *pelE* mutant is reduced by 50% (1, 11, 18). However, a mutant with directed deletions affecting *pelABCE* retains a residual capacity to macerate plant tissues (20). A strain 3937 *pelABCDE* mutant also retains residual maceration activity, and the differential effects on virulence in different hosts of various *pel* mutations in this strain suggest that Pel isozyme multiplicity is a factor in the wide host range of *E. chrysanthemi* (3).

Direct evidence that *E. chrysanthemi* EC16 produces a second set of plant-inducible Pel isozymes was found by culturing CUCPB5012, a Δ(*pelA pelE*) Δ(*pelB pelC*)::28bp Δ(*pelX*) Δ4bp *pehX*::Tn*phoA* mutant, in media containing chrysanthemum soluble extracts or cell walls (12). Extracellular fluids from these plant-induced cultures possessed macerating activity and several novel Pel isozymes, as determined by activity-stained isoelectric focusing gel analysis.

Isolation of a Tn*5*tac1-induced derivative of *E. chrysanthemi* CUCPB5047 (Δ*pelABCE*) that hyperexpresses PelL. Libraries of CUCPB5012 DNA in pBluescript II, pTTQ18 (22), and a derivative of pOU61 (13) were constructed in *Escherichia coli* DH5α (21) but never yielded colonies with a stable pectolytic phenotype in pectate semisolid agar medium (23), which suggested that the residual *pel* genes are toxic when expressed in heterologous bacteria. Tn*5*tac1 offered the possibility of identifying such genes by positive function in their native background. Tn*5*tac1 carries *lacI*^{q} and an outreading P_{tac} promoter that permits isopropyl-β-D-thiogalactopyranoside (IPTG)-dependent expression of downstream genes (5, 7). To use Tn*5*tac1 in *E. chrysanthemi*, an appropriate delivery plasmid and recipient strain had to be constructed, because standard *E. coli* plasmid vectors replicate in *E. chrysanthemi* and, like Tn*5*tac1, CUCPB5012 is kanamycin resistant.

A Tn*5*tac1 delivery vector was constructed by transposition of Tn*5*tac1 from λ::Tn*5*tac1 into *E. coli* SY327 λ *pir* carrying pGP704 (16), which contains mob_{RP4} and ori_{R6K}. Km^r plasmids were transformed into S17-1 λ *pir* and screened for their ability to deliver Tn*5*tac1. The resulting suicide plasmid, pCPP2997, replicates only in *E. coli* strains that carry pir_{R6K} and can be conjugated into *E. chrysanthemi* by S17-1 λ *pir*. A Km^s equivalent of *E. chrysanthemi* CUCPB5012 was constructed by marker exchange of *pehX*::ΩCm^r into CUCPB5010, the Δ(*pelA pelE*) Δ(*pelB pelC*)::28bp Δ(*pelX*) Δ4bp strain from which CUCPB5012 had been derived (12). The resulting strain, CUCPB5047, was mutagenized by mating with *E. coli* S17-1 λ *pir*(pCPP2997), followed by selection for transconjugants with Tn*5*tac1 (Km^r) and CUCPB5047 (Cm^r) antibiotic markers. The frequency of Km^r Cm^r *E. chrysanthemi* mutants was 1×10^{-5} per donor. One in 5,000 Km^r mutants produced blue-pigmented colonies on IPTG-containing media, indicative of the expression of normally cryptic genes for indigoidine production (24). The bacterial strains and plasmids used in this study are described in Table 1.

Km^r *E. chrysanthemi* colonies were transferred with toothpicks to pectate semisolid agar medium containing IPTG. Approximately 1 in 1,000 was able to cause pitting. Activity-stained isoelectric focusing gels revealed that all of the 10 colonies with a pectolytic phenotype produced the same band of activity with a highly alkaline pI, which was visualized in substrate overlays buffered to detect Pel activity (19) (Fig. 1). One of these mutants was designated CUCPB5066 and further characterized. *E. chrysanthemi* CUCPB5066 produced substantial pitting in pectate semisolid agar medium supplemented with IPTG and lacking plant extracts (Fig. 1). In the absence of IPTG, the pitting and blue-pigmented colony phenotypes were reduced but not abolished, indicating that Tn*5*tac1-directed expression is not tightly regulated in *E. chrysanthemi* (data not shown). A preliminary report by Lojkowska et al. (15a) de-

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TABLE 1. Bacterial strains and plasmids used in this study

Designation	Relevant characteristic	Reference or source
E. coli		
SY327 λ *pir*	Δ(*lac pro*) *argE*(Am) *rif nalA recA56* λ pir_{R6K}	16
S17-1 λ *pir*	RP4-2-Tc::Mu Kan::Tn*7* λ pir_{R6K} *pro hsdR recA*	K. N. Timmis
NM522	Δ(*lac-proAB*) Δ(*mcrB-hsdSM*)*5* (r_K^- m_K^-) (F′ *proAB lacI*^{q}*Z*ΔM15)	Stratagene (La Jolla, Calif.)
E. chrysanthemi		
CUCPB5012	Δ(*pelA pelE*) Δ(*pelB pelC*)::28bp Δ(*pelX*)Δ4bp *pehX*::Tn*phoA*	12
CUCPB5010	Δ(*pelA pelE*) Δ(*pelB pelC*)::28bp Δ(*pelX*)Δ4bp	4
CUCPB5047	Δ(*pelA pelE*) Δ(*pelB pelC*)::28bp Δ(*pelX*)Δ4bp *pehX*::ΩCm^r	This work
CUCPB5066	Tn*5*tac1 mutant of CUCPB5047; IPTG-inducible Pel$^+$	This work
Plasmids and phage		
pUC18	Ap^r	28
pBluescript II	Ap^r	Stratagene
pTTQ18	Multicopy expression vector; *lacI*^{q}	22
pOU61	Amplifiable low-copy-number expression vector	13
pGP704	ori_{R6K} mob_{RP4} Ap^r	16
pCPP2997	pGP704::Tn*5*tac1	This work
pWSK29	Low copy number; Ap^r	27
pCPP2291	22-kb *Sac*I fragment from *E. chrysanthemi* CUCPB5066 in pWSK29; Km^r Pel$^+$; *pelL* expressed from P_{tac}, yielding moderate level of Pel activity	This work
pCPP2292	16-kb *Cla*I-*Sac*I fragment from pCPP2291 in pWSK29; *pelL* expressed from P_{tac}, yielding moderate level of Pel activity	This work
pCPP2293	2.6-kb *Bam*HI fragment from pCPP2292 in pUC18; contains *pelL* and part of *celZ*; *pelL* in opposite orientation to vector P_{tac}; low levels of Pel produced	This work
pCPP2294	1.1-kb *Eco*RV-*Bam*HI fragment from pCPP2293 in pBluescript II SK(+); contains 3′ portions of *pelL* and *celZ*	This work
pCPP2295	1.0-kb *Sal*I-*Bam*HI fragment from pCPP2293 in pBluescript II SK(+); contains ′*pelL* and ′celZ	This work
pCPP2296	1.5-kb *Bam*HI-*Eco*RV fragment from pCPP2293 in pBluescript II KS(−); contains *pelL*′	This work
pPEL748	pINIII^{113} A-2 derivative hyperexpressing *pelE*	11
λ::Tn*5*tac1	Tn*5* with outward-facing P_{tac}	5

scribed the cloning of *pelL* from *E. chrysanthemi* 3937. Because the product of the 3937 gene, which apparently is not toxic to *E. coli*, shares salient properties with the isozyme produced by CUCPB5066, the latter protein was also designated PelL.

Molecular cloning of a fragment of the *E. chrysanthemi* CUCPB5066 genome carrying Tn*5*tac1 and *pelL*. To identify the gene downstream of P_{tac} that was responsible for PelL production in CUCPB5066, we ligated *Sac*I fragments of CUC PB5066 DNA into pWSK29 (27) and selected *E. coli* NM522 transformants on M9 minimal medium supplemented with 1.0% (wt/vol) Casamino Acids, 0.2% (wt/vol) glucose, kanamycin (50 μg/ml), and ampicillin (100 μg/ml). *Sac*I does not cut within Tn*5*tac1. The low-copy-number vector and minimal medium were chosen to minimize any *pelL* expression. Southern blots of *Sac*I-digested DNA probed with Tn*5*tac1 confirmed that the clones carried the same fragment that was mutated in CUCPB5066. One transformant, carrying a plasmid designated pCPP2291, was further analyzed by growth on M9-Casamino Acids-glucose agar; suspension at an optical density at 600 nm of 0.5 in broth containing M9-Casamino Acids, 0.2% (wt/vol) glycerol, and 1 mM IPTG; by further incubation for 4 h; and then by an A_{232} assay of culture supernatant and sonicated cell extracts. The latter fraction of *E. coli* NM522 (pCPP2291) caused an increase in the A_{232} of reaction mixtures containing polygalacturonic acid, demonstrating that it possessed Pel activity (19). Activity-stained isoelectric focusing gels indicated that IPTG-induced CUCPB5066 and *E. coli* NM522(pCPP2291) both produced PelL, which aligned with the most alkaline of the Pel isozymes produced by CUCPB 5047 (data not shown). NM522(pCPP2291) cultures grown

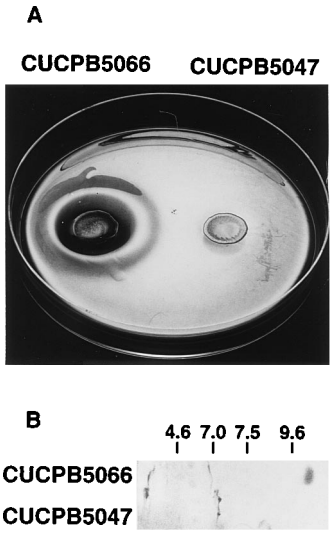

FIG. 1. Pitting of IPTG-amended pectate semisolid agar and production of a highly alkaline Pel isozyme by *E. chrysanthemi* Tn*5*tac1 mutant CUCPB5066. (A) Standard pectate semisolid agar medium was amended with 1 mM IPTG but no plant extracts (23). The PelABCEX$^-$ PehX$^-$ *E. chrysanthemi* mutant, CUCPB 5047, did not produce detectable pectolytic activity on this medium, whereas its Tn*5*tac1-mutagenized derivative, CUCPB5066, did. (B) Culture supernatants from CUCPB5047 and CUCPB5066 grown in King's Medium B amended with 1 mM IPTG were analyzed on an activity-stained isoelectric focusing gel for the production of Pel isozymes (19). The locations of pI markers (Bio-Rad, Richmond, Calif.) in an adjacent lane are indicated.

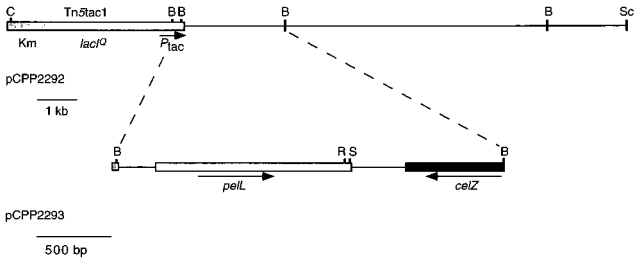

FIG. 2. Restriction map of subclones carrying *pelL*. pCPP2992 carries a *Cla*I-*Sac*I fragment of the *Sac*I insert in pCPP2291 that was initially cloned from *E. chrysanthemi* CUCPB5066. Restriction sites shown in pCPP2993 are those that were used to generate sequencing templates (pCPP2294, pCPP2295, and pCPP2296). Restriction enzymes: C, *Cla*I; B, *Bam*HI; R, *Eco*RV; S, *Sal*I; Sc, *Sac*I.

from low densities under conditions in which P_{tac} was not completely repressed (with glycerol rather than glucose, with yeast extract, or when induced by IPTG) lost Pel activity, suggesting that expression of *pelL* was toxic.

Sequence analysis of *pelL*. The Pel activity of subclones derived from various *Bam*HI, *Eco*RV, and *Sal*I restriction digestions suggested that *pelL* was just downstream of P_{tac} (Fig. 2). DNA sequencing (both strands) was performed with an Automated DNA Sequencer, model 373A (Applied Biosystems, Foster City, Calif.), in the Cornell Biotechnology Center, using a combination of M13 primers and custom primers complementary to P_{tac} and to newly obtained *pelL* sequences. Double-stranded DNA sequencing templates were prepared with Plasmid Mini Kits (Qiagen, Chatsworth, Calif.), and sequences were assembled with the SeqMan program of DNAStar (Madison, Wis.).

The *pelL* open reading frame encoded a predicted protein of 425 amino acids with an N-terminal signal peptide of 25 amino acids (Fig. 3). The signal peptide possessed the typical features of a positively charged amino acid near the N terminus, a hydrophobic core, and a cleavage site between adjacent alanine residues in the sequence VLAAD (26). The predicted mature protein has a molecular weight of 42,849 and an unusually high asparagine content of 11.8%. A putative ribosome-binding site, consisting of GAGG, was located 7 bp upstream of the translation initiation codon. A putative sigma 70 promoter was identified just downstream of a potential catabolite activator protein-binding site, but no KdgR-binding site could be discerned upstream of the *pelL* open reading frame (17). The low level of PelL production by *E. coli* DH5α (pCPP2293) suggests that this region can direct *pelL* transcription (data not shown). A rho-independent terminator was found downstream of *pelL*. Sequencing of the DNA beyond the *pelL* terminator revealed a convergently oriented open reading frame that was virtually identical to *celZ* of *E. chrysanthemi* 3937 (9) (Fig. 2).

BLAST searches of the GenBank, EMBL, and SwissProt databases revealed the *E. chrysanthemi* EC16 PelX as the only protein with significant similarity (4). Similarities between PelL, PelX, PelE (11), and Pel-3 (an isozyme from *Erwinia carotovora* EC71 that is unlike any known Pel proteins [15]) were further investigated with the Genetics Computer Group Bestfit program (8). PelL was 29% identical and 49% similar to the C-terminal 500 amino acids of the 697-amino-acid mature PelX protein but showed no significant similarity to any of the other proteins. Previous alignment analyses of the extracellular Pel proteins of *E. chrysanthemi* and *E. carotovora* (which did not include PelX) had revealed several consensus sequences

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1    ATTTAAAACAGCCTCTGTTCAAGGACTGTCGAAGGAGTGCGACTTTCTTCCAATGTAATT   60
          -35          -10                                -----
61   TTACCTCGACAGCTTGGTTGATTAAATCTGCCAGATTGACTCACGCTCATCATCAATGTT  120
121  GCTATTGCTGTGAGGTCGGTTGAGTCGCCGCTTATTTTTCTGGCGACATTTCCCGGCGTG  180
181  CGCATCTTGGGGCCGATGGATAGATAAATTTTAGAATATCATCAGGTAAGAGATGAAATA  240
                                                    M  K  Y
241  TTTAAATTGTTTTATCAGTACCGGGTTGCGACGCTTTTTCTTAGTCAATAGTACATCTGT  300
     L  N  C  F  I  S  T  G  L  A  A  F  F  L  V  N  S  T  S  V
301  TCTGGCTGCGGATTGCAGTTCGGATCTTACCAGCGGCATCAGCACTAAACGTATTTATTA  360
     L  A  A  D  C  S  S  D  L  T  S  G  I  S  T  K  R  I  Y  Y
361  CGTTGCGCCGAATGGCAGCAGCAGCAATAATGGGAATAGTTTTAATTCTCCAATGAGTTT  420
     V  A  P  N  G  S  S  S  N  N  G  N  S  F  N  S  P  M  S  F
421  CACTGCGGCTATGGCAGCAGCTAACGCTGGAGAAGTGATTCTGCTTAAGCCAGGAACATA  480
     T  A  A  M  A  A  A  N  P  G  E  L  I  L  L  K  P  G  T  Y
481  TACTATTCCTTATACGCAGGGTAAAGGGAATACCATAACCTTTAATAAATCGGGGAAAGA  540
     T  I  P  Y  T  Q  G  K  G  N  T  I  T  F  N  K  S  G  K  E
541  GGGCTCGCCGATTTATGTGCGCGCAGCGAATTGCGCTCGTCGCAGTATTTGATTTTTCATT  600
     G  S  P  I  Y  V  A  A  A  N  C  G  R  A  V  F  D  F  S  F
601  CCCGGACAGTCAATGGGTTCAGGGGTCATACGGTTTCTATGTGACCGGGGGATTATTGGTA  660
     P  D  S  Q  W  V  Q  A  S  Y  G  F  Y  V  T  G  D  Y  W  Y
661  TTTTAAAGGCATTGAAGTCACTCGTGCCCGGCTATCAGGGAGCCTATGTGACCGCTAGCCA  720
     F  K  G  I  E  V  T  R  A  G  Y  Q  G  A  Y  V  T  G  S  H
721  TAATACCTTTGAGAATACCGCGTTTCACCATAACCGAAACACTGGTCTTGAAATCAATAA  780
     N  T  F  E  N  T  A  F  H  H  N  R  N  T  G  L  E  I  N  N
781  CGGTGGTTCATATAACACGGTAATTAACTCCGATGCTTATCCTAATTATGATCCTAAAAA  840
     G  G  S  Y  N  T  V  I  N  S  D  A  Y  R  N  Y  D  P  K  K
841  GAATGGCAGTATGGCTGATGGCTTCGGGCCGAAACAAAAACAAGGCCCAGGTAACCGCTT  900
     N  G  S  M  A  D  G  F  G  P  K  Q  K  Q  G  P  G  N  R  F
901  TGGCGGATGCCCGGCATGGGAAAACTCTGATGACGGTTTTGACTTATTTGACAGTCGACA  960
     G  G  C  R  A  W  E  N  S  D  D  G  F  D  L  F  D  S  P  Q
961  GAAAGTCGTAATTGAGAATAGTTGGGCTTTTCGCAATGGTATCAACTATTGGAGTGATAG 1020
     K  V  V  I  E  N  S  W  A  F  R  N  G  I  N  Y  W  S  D  S
1021 TTCGTTCGCAGGTAACGAAATGGGTTTAAATTAGGGGGGAATCAACGAGTTGGTAACCA 1080
     S  F  A  G  N  G  N  G  F  K  L  G  G  N  Q  A  V  G  N  H
1081 CAGAATTACACGGTCCGTTGCATTTGGGAATGTGAGTAAGGGCTTTGATCAGAATAATAA 1140
     R  I  T  R  S  V  A  F  G  N  V  S  K  G  F  D  Q  N  N  N
1141 TGCCGGTGGTGTCACAGTAATAAATAACACGTCATACAAGAATGGTATTAACTATCGCTT 1200
     A  G  G  V  T  V  I  N  N  T  S  Y  K  N  G  I  N  Y  G  F
1201 CGGCAGTAATGTGAAATCAGGTCAGAAGCACTACTTCCGTAATAACGTTTCTCTGTCCGG 1260
     G  S  N  V  K  S  G  Q  K  H  Y  F  R  N  N  V  S  L  S  G
1261 CTCGGCGACTGTTAATAATGCAGATGCGAAGTCTAACTCATGGGACACCGGCCCTGTGGC 1320
     S  A  T  V  N  N  A  D  A  K  S  N  S  W  D  T  G  P  V  A
1321 TTCCGCATCTGATTTTGTCAGCCTGGATACCTCACTGGCTACGATATCACGTGATAATGA 1380
     S  A  S  D  F  V  S  L  D  T  S  L  A  T  I  S  R  D  N  D
1381 CGGGACATTGCCTGAAACTGCGCTGTTTCGCTTGTCGACTAATTCAAAGTTGATTAATGC 1440
     G  T  L  P  E  T  A  L  F  R  L  S  T  N  S  K  L  I  N  A
1441 CGGTACGAAGGAGAGCAATATCAGTTATTCAGGTAGTGCGCCGGATTTAGGTGCGTTTGA 1500
     G  T  K  E  S  N  I  S  Y  S  G  S  A  P  D  L  G  A  F  E
                                          <---------
1501 GCGTAATTAAGTCATCAGATATTATGCATCAGGGCTGCAATATGCAGCCCTGTTTTATGT 1560
     R  N  *
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FIG. 3. Nucleotide sequence of *pelL* and deduced amino acid sequence of its product. The sequence begins with the Tn5tac1 junction. A putative catabolite activator protein-binding site is marked with dashed overlines and is located just upstream of the presumed promoter. The shading denotes a sequence that is present (12 of 14 nucleotides) upstream of *E. chrysanthemi* EC16 *pelX* (4). The putative ribosome-binding site and PelL signal peptide are indicated with double and single underlines, respectively. The arrows are above the rho-independent transcription terminator. The stop codon is indicated by the asterisk.

(10, 25). To determine whether any of these sequences were present in PelL, we identified their location in the PelE sequence and then examined the corresponding PelL sequence, as aligned by Bestfit, for similarity. None of these sequences appeared to be conserved in PelL. It thus appears that PelL is not a member of the superfamily of Pel proteins that are prominent in pectate-grown cultures of *E. chrysanthemi* and *E. carotovora*.

Comparison of the properties of PelL and PelE. PelE was chosen for comparison with PelL because, of the pectate-inducible Pel isozymes, PelE has the highest pI, it is the most important in maceration, its enzymological properties and tertiary structure have been characterized, and preliminary viscometric assays indicated that PelL, like PelE but unlike PelX, was an endo-cleaving enzyme (11, 14, 18). The isozymes were obtained in 20 mM Tris-HCl, pH 8.0, from sonicated extracts of *E. coli* cells carrying pPEL748 (*pelE*$^+$) or pCPP2292 (*pelL*$^+$) and, thus, were devoid of any contaminating pectolytic activities. The pH optimum of both isozymes was 8.5 to 9.0, and as shown in Table 2, both isozymes had the same relative activity on different pectic substrates and were affected similarly by the

TABLE 2. Relative activities of PelL and PelE on different pectic substrates[a]

Substrate[b]	PelL	PelE
Polygalacturonate	0.55	0.59
Polygalacturonate + 1.5 mM $CaCl_2$	1.26	0.84
Polygalacturonate + 10 mM EDTA	0.02	0.00
Pectin (31% methoxylated) + 1.5 mM $CaCl_2$	1.60	1.66
Pectin (68% methoxylated) + 1.5 mM $CaCl_2$	0.54	0.66
Pectin (93% methoxylated) + 1.5 mM $CaCl_2$	0.05	0.04
Pectin (31% methoxylated) + 10 mM EDTA	0.00	0.00

[a] Activity is defined as micromoles of unsaturated product released per minute per milliliter of enzyme sample, as determined by increasing A_{232} values of reaction mixtures containing 0.1% (wt/vol) substrate, 100 mM Tris-HCl (pH 8.5), the indicated amendments, and ≤5% (vol/vol) enzyme sample.

[b] Pectic substrates were all obtained from Sigma.

addition of $CaCl_2$ or EDTA. Both isozymes had an absolute requirement for calcium and were inactive on highly methoxylated pectin. This is characteristic of pectate lyases, but interestingly, both isozymes were more active on 31% methoxylated pectin than on polygalacturonate. Viscometric assays revealed that the two isozymes reduced the viscosity of polygalacturonate at the same rapid rate characteristic of endo-cleaving enzymes, and tactile assessments of potato tuber discs (0.7 by 10 mm) incubated in diminishing Pel concentrations indicated that the isozymes macerated at approximately the same rate (data not shown). Thus, despite the lack of any similarity in their primary sequences, PelL and PelE appear to have the same salient enzymological and biological properties. We are now constructing reporter transposon insertions in these two genes to further explore their relative levels of expression and their roles in pathogenesis.

Nucleotide sequence accession number. The nucleotide sequence of *pelL* has been assigned GenBank accession number L42248.

We thank Douglas E. Berg for λ::Tn*5*tac1, Noel T. Keen for pPEL748, Kenneth N. Timmis for S17-1 λ *pir*, Meng Fang Wu for construction of *E. chrysanthemi* CUCPB5047, Cathy Zumoff for assistance with enzyme assays, and Kent Loeffler for photography.

This work was supported by NRI Competitive Grants Program/USDA awards 91-37303-6321 and 94-37303-0734.

REFERENCES

1. **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.
2. **Barras, F., F. Van Gijsegem, and A. Chatterjee.** 1994. Extracellular enzymes and pathogenesis of soft-rot *Erwinia*. Annu. Rev. Phytopathol. **32:**201–234.
3. **Beaulieu, C., M. Boccara, and F. Van Gijsegem.** 1993. Pathogenic behavior of pectinase-defective *Erwinia chrysanthemi* mutants on different plants. Mol. Plant Microbe Interact. **6:**197–202.
4. **Brooks, A. D., S. Y. He, S. Gold, N. T. Keen, A. Collmer, and S. W. Hutcheson.** 1990. Molecular cloning of the structural gene for exopolygalacturonate lyase from *Erwinia chrysanthemi* EC16 and characterization of the enzyme product. J. Bacteriol. **172:**6950–6958.
5. **Chow, W. Y., and D. E. Berg.** 1988. Tn*5*tac1, a derivative of transposon Tn*5* that generates conditional mutations. Proc. Natl. Acad. Sci. USA **85:**6468–6472.
6. **Collmer, A., and N. T. Keen.** 1986. The role of pectic enzymes in plant pathogenesis. Annu. Rev. Phytopathol. **24:**383–409.
7. **Cookson, B. T., D. E. Berg, and W. E. Goldman.** 1990. Mutagenesis of *Bordetella pertussis* with transposon Tn*5*tac1: conditional expression of virulence-associated genes. J. Bacteriol. **172:**1681–1687.
8. **Devereaux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Gene **12:**387–395.
9. **Guiseppi, A., B. Cami, J. L. Aymeric, G. Ball, and N. Creuzet.** 1988. Homology between endoglucanase Z of *Erwinia chrysanthemi* and endoglucanase of *Bacillus subtilis* and alkalophilic *Bacillus*. Mol. Microbiol. **2:**159–164.
10. **Hinton, J. C. D., J. M. Sidebotham, D. R. Gill, and G. P. C. Salmond.** 1989. Extracellular and periplasmic isoenzymes of pectate lyase from *Erwinia carotovora* subspecies *carotovora* belong to different gene families. Mol. Microbiol. **3:**1785–1795.
11. **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.
12. **Kelemu, S., and A. Collmer.** 1993. *Erwinia chrysanthemi* EC16 produces a second set of plant-inducible pectate lyase isozymes. Appl. Environ. Microbiol. **59:**1756–1761.
13. **Larsen, J. E. L., K. Gerdes, J. Light, and S. Molin.** 1984. Low-copy-number plasmid-cloning vectors amplifiable by derepression of an inserted foreign promoter. Gene **28:**45–54.
14. **Lietzke, S. E., M. D. Yoder, N. T. Keen, and F. Jurnak.** 1994. The three-dimensional structure of pectate lyase E, a plant virulence factor from *Erwinia chrysanthemi*. Plant Physiol. **106:**849–862.
15. **Liu, Y., A. Chatterjee, and A. K. Chatterjee.** 1994. Nucleotide sequence and expression of a novel pectate lyase gene (*pel-3*) and a closely linked endopolygalacturonase gene (*peh-1*) of *Erwinia carotovora* subsp. *carotovora* 71. Appl. Environ. Microbiol. **60:**2545–2552.

15a. **Lojkowska, E., M. Boccara, J. Robert-Baudouy, and N. Hugouvieux-Cotte-Pattat.** 1994. The *pelL* gene encodes a secondary pectate lyase of *Erwinia chrysanthemi*, abstr. 211, p. 67. *In* Abstracts of the Seventh International Symposium on Molecular Plant-Microbe Interactions 1994. International Society for Molecular Plant-Microbe Interactions.

16. **Miller, V. L., and J. J. Mekalanos.** 1988. A novel suicide vector and its use in construction of insertion mutations: osmoregulation of outer membrane proteins and virulence determinants in *Vibrio cholerae* requires *toxR*. J. Bacteriol. **170:**2575–2583.
17. **Nasser, W., S. Reverchon, G. Condemine, and J. Robert-Baudouy.** 1994. Specific interactions of *Erwinia chrysanthemi* KdgR repressor with different operators of genes involved in pectinolysis. J. Mol. Biol. **236:**427–440.
18. **Payne, J. H., C. Schoedel, N. T. Keen, and A. Collmer.** 1987. Multiplication and virulence in plant tissues of *Escherichia coli* clones producing pectate lyase isozymes PLb and PLe at high levels and of an *Erwinia chrysanthemi* mutant deficient in PLe. Appl. Environ. Microbiol. **53:**2315–2320.
19. **Ried, J. L., and A. Collmer.** 1985. An activity stain for the rapid characterization of pectic enzymes in isoelectric focusing and sodium dodecyl sulfate polyacrylamide gels. Appl. Environ. Microbiol. **50:**615–622.
20. **Ried, J. L., and A. Collmer.** 1988. Construction and characterization of an *Erwinia chrysanthemi* mutant with directed deletions in all of the pectate lyase structural genes. Mol. Plant Microbe Interact. **1:**32–38.
21. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Stark, M. J. R.** 1987. Multicopy expression vectors carrying the *lac* repressor gene for regulated high-level expression of genes in *Escherichia coli*. Gene **51:**255–267.
23. **Starr, M. P., A. K. Chatterjee, P. B. Starr, and G. E. Buchanan.** 1977. Enzymatic degradation of polygalacturonic acid by *Yersinia* and *Klebsiella* species in relation to clinical laboratory procedures. J. Clin. Microbiol. **6:**379–386.
24. **Starr, M. P., G. Cosens, and H.-J. Knackmuss.** 1966. Formation of the blue pigment indigoidine by phytopathogenic *Erwinia*. Appl. Microbiol. **14:**870–872.
25. **Tamaki, S. J., S. Gold, M. Robeson, S. Manulis, and N. T. Keen.** 1988. Structure and organization of the *pel* genes from *Erwinia chrysanthemi* EC16. J. Bacteriol. **170:**3468–3478.
26. **von Heijne, G.** 1986. A new method for predicting signal sequence cleavage sites. Nucleic Acids Res. **14:**4683–4690.
27. **Wang, R. F., and S. R. Kushner.** 1991. Construction of versatile low-copy-number vectors for cloning, sequencing and gene expression in Escherichia coli. Gene **100:**195–199.
28. **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.