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Nucleotide Sequence of a Pectate Lyase Structural Gene, *pel1* of *Erwinia carotovora* subsp. *carotovora* Strain 71 and Structural Relationship of *pel1* with Other *pel* Genes of *Erwinia* Species

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Of the various exoproteins secreted by *Erwinia carotovora* subsp. *carotovora* strain 71, Pel1 is the major pectate lyase species with tissue macerating activity. Nucleotide sequencing of a 2.2-kb *pel1*$^+$ DNA segment revealed a 1,122 base pair open reading frame which could encode pre-Pel1 of 374 amino acid residues. A signal peptide of 22 amino acid residues is present within the NH_2-terminal region of pre-Pel1. Transcription of *pel1* was initiated at the guanine residue 111 base pairs upstream of the start codon. Consensus sequences for the binding of KdgR, a negative regulatory factor known to control some of the *E. chrysanthemi* pectinases, flank the promoter of *pel1*. Although *pel1* belongs to the *pelBC* family, it is more closely related to the *pel* genes of *E. carotovora* than to the *pelBC* genes of *E. chrysanthemi*.

Erwinia carotovora subsp. *carotovora* elicits postharvest decay (= soft rot) in a wide variety of plant products. The production of pectolytic enzymes, especially pectate lyases (Pel species), by the pathogen is the most critical factor in the development of the disease. All *E. c.* subsp. *carotovora* strains and most other soft rot *Erwinia* spp. produce a number of Pel species that are readily distinguished by their isoelectric points (pIs) (Barras *et al.* 1994; Collmer and Keen 1986). *E. c.* subsp. *carotovora* strain 71 (Ecc71), the model organism in our laboratory, produces at least five Pel species: The three most basic Pel species (Pel1, Pel2, and Pel3) are secreted outside the bacterial cell, whereas Pel4 and Pel5 are predominantly localized in the periplasm (Willis *et al.* 1987; Zink and Chatterjee 1985). Previous studies have shown that Pel1 is the major pectate lyase species of Ecc71. This particular Pel species has attracted considerable attention as a model for studies of regulation, secretion, and three-dimensional structure (Chatterjee *et al.* 1993; A. Collmer and M. Lindeberg, personal communication; F. Jurnak, personal communication). To facilitate these studies, we report here the structural characteristics of *pel1*, which encodes this Pel species.

The nucleotide sequence of the 2.2-kb DNA segment, previously shown to elicit Pel1 production (Chatterjee *et al.* 1993), revealed a 1,122-bp open reading frame corresponding to *pel1* which could encode a polypeptide of 374 amino acid residues (Fig. 1). A potential ribosome binding site (RBS) precedes this ORF. Transcription of *pel1* is initiated at the guanine residue 111-bp upstream of the ATG start codon as determined by primer extension analysis (Fig. 2). The promoter sequence of *pel1* contains a perfectly matched –35 box but a poorly matched –10 box corresponding to the consensus motifs of the *E. coli* σ^{70} promoter sequence (Fig. 1). Two putative KdgR boxes flank the promoter sequence of *pel1* (Fig. 1). KdgR is a repressor known to affect the expression of various pectinases and pectate catabolic genes in *E. chrysanthemi* (Nasser *et al.* 1994). However, the significance of these sequences in the expression of *pel1* as well as *pel-3*, *peh-1*, *aepA*, and the other *pel* genes of *E. carotovora* (Hinton *et al.* 1989; Liu *et al.* 1993, 1994; Murata *et al.* 1994) is not yet known.

The deduced protein product of *pel1* possesses a molecular mass of 40.7 kDa and isoelectric point of 9.96. A signal peptide of 22 amino acid residues is present in the NH_2-terminal region of pre-Pel1 (Fig. 1). The mature Pel1 was purified from the spent culture of an Ecc71 derivative, and the amino acid sequence of the NH_2-terminal end was determined by the Protein Core Facility, University of Missouri, Columbia, MO. The sequence of the first 22 amino acid residues of extracellular Pel1 is identical with the predicted sequence of pre-Pel1 polypeptide beginning with the 23rd alanine residue (Fig. 1). The predicted mature Pel1 protein has a molecular mass of 38.5 kDa and a pI of 10.0. These values match well with the values derived from SDS-PAGE and IEF analysis of the purified Pel1 (data not shown). These results suggested that the signal peptide of Pel1 was cleaved between the two alanine residues at positions 22 and 23 during the secretion of pre-Pel1.

Previous analyses have placed the *pel* genes of *Erwinia* into three families based on amino acid homology and cellular locations of their products. Two distinct families of *pel* genes, *pelBC* and *pelADE*, that encode extracellular Pel species with very limited amino acid homology, were delineated from *E. chrysanthemi* strain EC16 (Tamaki *et al.* 1988). While the genes for extracellular Pels of *E. chrysanthemi* strain 3937 (Hugouvieux-Cotte-Pattat and Robert-Baudouy 1992; Van Gijsegem 1989) belong to these two *pel* families, the genes for extracellular Pel species of *E. c.* subsp. *carotovora* strain SCRI193 (Hinton *et al.* 1989) belong solely to the *pelBC*

family. The third family includes the periplasmic PL153 of *E. carotovora* strain EC153 (Trollinger *et al.* 1989), PLb of *E. c.* subsp. *carotovora* strain SCRI193 (Hinton *et al.* 1989), and PLY of *Yersinia pseudotuberculosis* ICPB3821 (Manulis *et al.* 1988). These periplasmic proteins are similar to each other but are not homologous to the extracellular Pel species. The dendrogram (Fig. 3) shows the relationship between the Ecc71 Pel1 protein and various Pel proteins. The *pel* genes sort into four clusters (Fig. 3): Cluster 1 contains the *pelBC* family; cluster 2 represents the *pelADE* family; cluster 3 includes the periplasmic *pel* family; and cluster 4 contains the Ecc 71 Pel3, encoded by the recently discovered *pel3* gene (Liu *et al.* 1994). The Ecc 71 Pel1 belongs to the *pelBC* family in cluster 1 (Fig. 3). Within the *pelBC* family, two subgroups may exist: one subgroup consisting of the *pel* genes of *E. carotovora* subspecies and the other subgroup containing the *pelBC* genes of *E. chrysanthemi.* None of the *E. carotovora pel* genes appears to belong to the *pelADE* family.

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CTCGAGTGCATTTATTATCTACTAAAAAAGTAACCTTATGATTTACCGTTACTTTAAAGG    60
    KdgR  Box
AAAATTATTTTCTATAAAAAATAAAACCATCCAATCATCAGTATTACAAAATGTTTCATC   120
                                -35                -10        ★
CGCAATACATTTAACATTTCACCCTTGAACTGATCTTATTTTTTGACCACACTCCCCTTG   180
                          KdgR  Box
GTTTTTCACCAAAATTGAAATTCATTTTTGTTGAAAAATTTACACTTGTTACATCGGGCA   240
                                                RBS
TAGGGATCGATAAATGCCCATGAAAATTCTATTCCAAGGAGACAGTGATAATGAAATACC   300
                                                      M  K  Y  L
TATTGCCTACGGCAGCCGCTGGATTGCTATTACTCGCGGCTCAACCCGCAATGGCCGCAA   360
  L  P  T  A  A  A  G  L  L  L  L  A  A  Q  P  A  M  A↓ A  N
ATACGGGCGGCTATGCCACTACGGATGGTGGAGAAGTGTCCGGTGCCGTGAAAAAAACGG   420
  T  G  G  Y  A  T  T  D  G  G  E  V  S  G  A  V  K  K  T  A
CACGTTCCATGAAAGAAATTGTGGATATATTGAAGCCGCGCAAGTGGATTCAAAAGGCAA   480
  R  S  M  K  E  I  V  D  I  L  K  P  R  K  W  I  Q  K  A  R
GAAAGTCAAAGGCGGTGCCTTACCCGCTCATCATCACCTATAGCGGTAATGAAGACTCAT   540
  K  S  K  A  V  P  Y  P  L  I  I  T  Y  S  G  N  E  D  S  L
TAATCAAAGCGGCTGAAAAGAATATCTGCGGCCAGTGGAGTAAAGACGCACGCGGCGTAC   600
  I  K  A  A  E  K  N  I  C  G  Q  W  S  K  D  A  R  G  V  Q
AAATCAAAGAGTTCACCAAAGGCATTACTATCCAGGGCACCAATGGCTCATCCGCCAACT   660
  I  K  E  F  T  K  G  I  T  I  Q  G  T  N  G  S  S  A  N  F
TCGGTGTCTGGATTGTGAACTCTTCTAATGTCGTGGTACGTAACATGCGCTTTGGCTATA   720
  G  V  W  I  V  N  S  S  N  V  V  V  R  N  M  R  F  G  Y  M
TGCCGGGCGGCGCGCAAGACGGCGATGCCATTCGTATCGATAACTCCCCGAACGTCTGGA   780
  P  G  G  A  Q  D  G  D  A  I  R  I  D  N  S  P  N  V  W  I
TCGACCACAACGAAATCTTTGCCAAGAACTTTGAGTGTAAGGGCACGCCAGACAATGACA   840
  D  H  N  E  I  F  A  K  N  F  E  C  K  G  T  P  D  N  D  T
CCACCTTTGAATCGGCTGTCGATATCAAAAAAGGGTCAACTAACGTCACGGTATCCTACA   900
  T  F  E  S  A  V  D  I  K  K  G  S  T  N  V  T  V  S  Y  N
ATTATATTCATGGTATCAAGAAAGTCGGCCTGAGCGGCGCAACGAATACGGATACGGGCC   960
  Y  I  H  G  I  K  K  V  G  L  S  G  A  T  N  T  D  T  G  R
GTAACCTGACTTACCATCACAATATTTATAGCGATGTTAACTCACGCCTGCCGCTGCAAC  1020
  N  L  T  Y  H  H  N  I  Y  S  D  V  N  S  R  L  P  L  Q  R
GTGGTGGTCTGGTTCACGCGTACAACAACCTGTATGACGGCATCACCGGTTCAGGCTTTA  1080
  G  G  L  V  H  A  Y  N  N  L  Y  D  G  I  T  G  S  G  F  N
ACGTGCGTCAGAAAGGGATCGCACTGATTGAAAGCAACTGGTTCGAGAATGCGCTCAACC  1140
  V  R  Q  K  G  I  A  L  I  E  S  N  W  F  E  N  A  L  N  P
CAGTGACAGCACGTAACGACAGCTCAAACTTTGGTACCTGGGAGCTGCGTAACAACAACA  1200
  V  T  A  R  N  D  S  S  N  F  G  T  W  E  L  R  N  N  N  I
TCACGAAACCGGCAGACTTCTCCAAATACAAAATCACCTGGGGCAAGCCTTCCTCTCCTC  1260
  T  K  P  A  D  F  S  K  Y  K  I  T  W  G  K  P  S  S  P  H
ACATCAATGCGGATGACTGGAAGAGCACCGGTAAGTTCCCTGCCGTCTCCTATAAGTACA  1320
  I  N  A  D  D  W  K  S  T  G  K  F  P  A  V  S  Y  K  Y  T
CTCCAGTTTCTGCACAGTGCGTGAAGGATAAACTGGCAAACTATGCTGGCGTCGGTAAAA  1380
  P  V  S  A  Q  C  V  K  D  K  L  A  N  Y  A  G  V  G  K  N
ACCTGGCAGTACTGACAGCAGCTAACTGCAAATAAACGCGGTCAGGCTTTCTCCGTCGTC  1440
  L  A  V  L  T  A  A  N  C  K  •
GCAAGACAGGAAGCAATAAGTCTGAATATCCCGCGCCGTGACTCTTCATACAGAAGCATG  1500

GTTCTACCAAGCGAGCTC                                             1518
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Fig. 1. Nucleotide sequence of a 1,518-base pair region of *pel1* and the flanking DNA. The deduced amino acid sequence of *pel1* is also given. The transcriptional start site is indicated by a star. The putative ribosome binding site (RBS), the –35 and the –10 region of the potential σ^{70}-like promoter are single-underlined. The putative KdgR binding sites are shown in shadow. The signal peptide is highlighted in bold, and the signal peptide cleavage site is indicated by the vertical arrow. The double-underlined sequence of first 22 amino acid residues of Pel1 was determined using purified Pel1. The nucleotide sequence of *pel1* has been deposited into the GenBank database under accession number L32171.

In contrast to the diversity among the extracellular Pel species, the periplasmic Pels appear to have been conserved during evolution, as previously noted by Hinton *et al.* (1989). The idea that some of the *pel* genes may have undergone species-specific evolution is supported by the following observations. Thus far, members of the *pelADE* family have been found only in *E. chrysanthemi* strains and the periplasmic *pel* family in *E. carotovora* subspecies and *Yersinia*, but not in *E. chrysanthemi*. Furthermore, as stated above, although *E. carotovora pel* genes fall within the *pelBC* family, they are more closely related to each other than to the *E. chrysanthemi pel* genes of this family.

Despite a limited overall homology between PelBC and PelADE, Tamaki *et al.* (1988) found two regions of strong homology between the members of these two families of *E. chrysanthemi* strain EC16. Subsequently, both regions were found to be spatially located around the putative Ca^{2+} binding site in PelC (Yoder *et al.* 1993). These regions were believed to be important in Pel structure and catalytic activity. A comparison of 12 extracellular Pels of *E. carotovora* and *E. chrysanthemi* belonging to the *pelBC* family revealed similarity throughout the whole length of the proteins (Hinton *et al.* 1989). In our computer analysis, amino acid sequence alignment of Ecc71 Pel1 with other extracellular Pel species showed that Pel1 is very similar to the members of PelBC family, not only in the regions mentioned above, but also in the entire sequence of the polypeptide. In light of the similarities in sequence and catalytic activity, we suggest that Pel1 could also possess a conformation of parallel β helix motif similar to *E. chrysanthemi* (EC16) PelC, which is the prototype model (Yoder *et al.* 1993). This prediction is currently being tested (F. Jurnak, personal communication). We should note that *pel1* and the other *Erwinia pel* genes share little (< 20%) homology with *pnlA* of *E. c.* subsp. *carotovora* 71 (Chatterjee *et al.* 1991) and *pnl* of *E. carototova* strain Er (Ohnishi *et al.* 1991) that encode pectin lyases. These findings indicate that species of Pel and Pnl, which differ in catalytic properties and regulation (Barras *et al.* 1994), are structurally different as well.

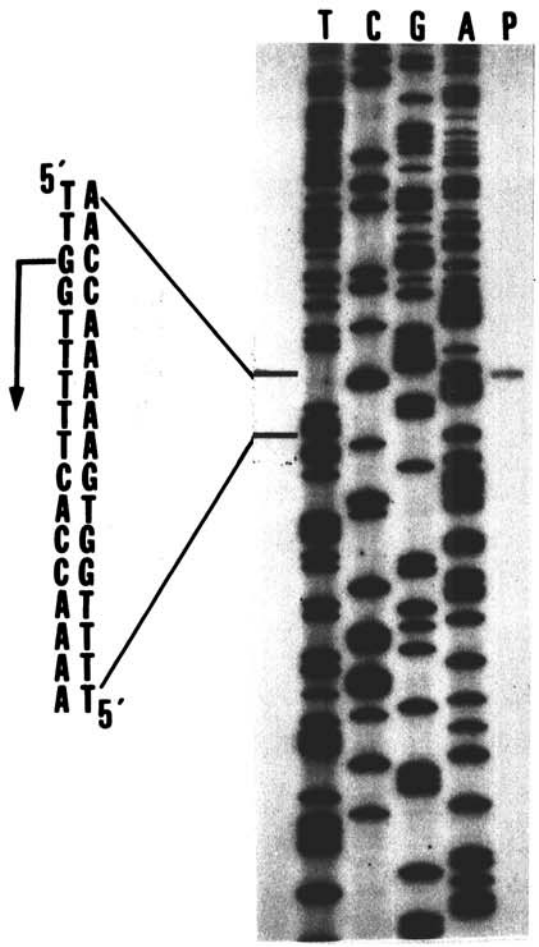

Fig. 2. Primer extension assay of the 5′ end of *pel1* transcript from *Erwinia carotovora* subsp. *carotovora* 71. The portions of sequence pertinent to the transcriptional start site are shown. The guanine residue in lane P is identified as the *in vivo* initiation site of *pel1* mRNA.

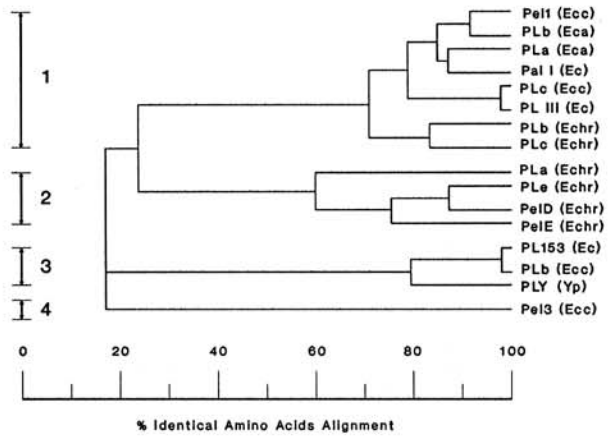

Fig. 3. The relationship between Ecc71 Pel1 and various Pel proteins of *Erwinia carotovora* (Ec), *E. carotovora* subsp. *carotovora* (*E. c.* subsp. *carotovora*), *E. carotovora* subsp. *atroseptica* (Eca), *E. chrysanthemi* (Echr), and *Yersinia pseudotuberculosis* (Yp). The Pel species include Pel1 of *E. c.* subsp. *carotovora* strain 71, PLa and PLb of Eca strain EC (Lei *et al.* 1987, 1988), PalI of Ec strain Er (Ito *et al.* 1988), PLc of *E. c.* subsp. *carotovora* strain SCRI193 (Hinton *et al.* 1989), PLIII of Ec strain Er (Yoshida *et al.* 1991b), PLa, PLb, PLc and PLe of Echr strain EC16 (Keen and Tamaki 1986; Tamaki *et al.* 1988), PelD and PelE of Echr strain 3937 (Reverchon *et al.* 1989; Van Gijsegem 1989), PL153 of Ec strain Ec153 (Trollinger *et al.* 1989), PLb of *E. c.* subsp. *carotovora* strain SCRI193 (Hinton *et al.* 1989), PLY of Yp strain ICPB3821 (Manulis *et al.* 1988), and Pel3 of *E. c.* subsp. *carotovora* strain 71 (Liu *et al.* 1994). See text for the relationship between the four Pel clusters. This analysis was conducted using the CLUSTAL program in PC/GENE package (IntelliGenetics, Inc., CA).

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PAL I     (Ec)      1 MKYLLPSAALGLLLAARGPTDNG ? A  23
PLc       (Ecc)     1 MKYLLPSAAAGLLLLAAQPTMA  ? A  23
PL III    (Ec)      1 MKYLLPSAAAGLLLLAAQPTMA  ↓ A  23
PLb       (Eca)     1 MKYLLPTAAAGLLLLAAQPAMA  ↓ A  23
PLa       (Eca)     1 MKYLLPSAAAGLLLLAAQPAMA  ↓ A  23
                      ****** ************ **
Pel1      (Ecc71)   1 MKYLLPTAAAGLLLLAAQPAMA  ↓ A  23
                      ** *     *****   *  *
PLb       (Echr)    1 MKSLITPIAAGLLLAFSQYSLA  ↓ A  23
PLc       (Echr)    1 MKSLITPITAGLLLALSQPLLA  ? A  23
```

Fig. 4. Alignment of the signal peptide sequence of *E. c.* subsp. *carotovora*71 Pel1 with those of other *Erwinia carotovora* (Ec), *E. carotovora* subsp. *carotovora* [*E. c.* subsp. *c.*(Ecc)], *E. carotovora* subsp. *atroseptica* (Eca), and *E. chrysanthemi* (Echr) extracellular Pel species in the *pelBC* family. Asterisks indicate identical amino acid residues. The numbers refer to the amino acid positions starting from the NH_2-terminal end. Arrows represent known cleavage sites, whereas ? indicates a putative cleavage site. Identical amino acid residues in the signal peptides of *E. carotovora* and *E. chrysanthemi* Pel species are shaded.

The analysis of signal peptides of Ecc71 Pel1, *E. carotovora* PL III (Yoshida *et al.* 1991a), *E. c.* subsp. *atrosceptica* PLa, and PLb (Lei *et al.* 1987 1988), *E. chrysanthemi* PLb (Keen and Tamaki 1986) and corresponding regions of other extracellular Pel species of the PelBC family revealed that these peptides share very high homology in sequence, size and the putative cleavage sites (Fig. 4). Sequences of the signal peptide of Pel1 and those of *E. carotovora* Pel species are more similar to each other than to the sequences of signal peptides of PelBC of *E. chrysanthemi* with the exception of the signal peptide of PAL1 (Fig. 4). These observations provide additional support for the view that some of the *pel* genes may have undergone species-specific evolution.

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