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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†

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Our previous genetic analysis (J. W. Willis, J. K. Engwall, and A. K. Chatterjee, Phytopathology 77:1199–1205, 1987) had revealed a tight linkage between *pel-3* (*pel*, pectate lyase gene) and *peh-1* (*peh*, polygalacturonase gene) within the chromosome of *Erwinia carotovora* subsp. *carotovora* 71. Nucleotide sequencing, transcript assays, and expression of enzymatic activities in *Escherichia coli* have now confirmed that a 3,500-bp segment contains the open reading frames (ORFs) for Pel-3 and Peh-1. The 1,041-bp *pel-3* ORF and the 1,206-bp *peh-1* ORF are separated by a 579-bp sequence. The genes are transcribed divergently from their own promoters. In *E. coli* and *E. carotovora* subsp. *carotovora* 71, *peh-1* is better expressed than *pel-3*. However, plant signals activate the expression of both the genes in *E. carotovora* subsp. *carotovora*. A consensus integration host factor (IHF)-binding sequence upstream of *pel-3* appears physiologically significant, since *pel-3* promoter activity is higher in an *E. coli* IHF$^+$ strain than in an IHF$^-$ strain. While *peh-1* has extensive homology with plant and bacterial *peh* genes, *pel-3* appears not to have significant homology with the *pel* genes belonging to the *pelBC*, *pelADE*, or periplasmic *pel* families. Pel-3 also is unusual in that it is predicted to contain an ATP- and GTP-binding site motif A (P-loop) not found in the other Pels.

Most soft-rot *Erwinia* species including *Erwinia carotovora* subsp. *carotovora* produce plant cell wall-degrading enzymes such as pectate lyase (Pel), polygalacturonase (Peh), cellulase (Cel) and protease (Prt) (7, 10). The degree of virulence depends largely upon the levels of these enzymes produced by the bacteria. Another typical feature is the production of multiple forms of Pels that are differentiated by their isoelectric points (pIs) (31, 34, 47). In *Erwinia chrysanthemi*, the *pel* genes are clustered (1, 41, 45): one cluster constitutes the *pelBC* family and the other cluster constitutes the *pelADE* family. However, each *pel* gene of these families is individually transcribed.

In *E. carotovora* subsp. *carotovora* strains, there also is evidence for clustering of the pectolytic enzyme genes (see, for example, references 20, 35, and 47), although the organization of the *E. carotovora* subsp. *carotovora pel* genes has not been studied as thoroughly as that of the *E. chrysanthemi pel* genes. *E. carotovora* subsp. *carotovora* 71, the strain most studied in our laboratory, produces at least three extracellular Pel species, a pectin lyase, an endo-Peh, and possibly also an exo-Peh, and the genes for most of these enzymes have been cloned (5, 24, 47, 50). Willis et al. (47) found a tight linkage between *pel-3* and *peh-1*. However, the lack of a polar effect of Tn*5* insertions suggested that these genes were not components of an operon. Moreover, our subsequent studies of the cloned DNA in *E. coli* revealed a differential expression of the genes in that the *pel-3* gene was expressed rather poorly compared with *peh-1* (3). To better understand the organization and expression of the *pel-3* and *peh-1* genes of *E. carotovora* subsp. *carotovora* 71, we have determined the nucleotide sequence of the 3,500-bp DNA segment that encodes the enzymes and partially characterized the DNA segment that controls *pel-3* expression. We report here that (i) *pel-3* belongs to a new Pel family, (ii) the genes are divergently transcribed from their own promoters, (iii) the consensus integration host factor (IHF) sequence present in front of the *pel-3* gene affects its expression, and (iv) the expression of both *pel-3* and *peh-1* is activated by plant components.

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MATERIALS AND METHODS

Bacterial strains and plasmids. Bacterial strains and plasmids are described in Table 1. The strains carrying drug markers were maintained on Luria-Bertani (LB) agar containing appropriate antibiotics. *E. carotovora* subsp. *carotovora* 71 and its derivatives were maintained on yeast extract-glucose-calcium carbonate (YGC) agar.

Media. The compositions of LB medium, minimal salts medium, polygalacturonate-yeast extract agar, salts-yeast extract-glycerol (SYG) medium, SYG plus celery extract medium, and YGC agar are described in our previous publications (1, 29). When required, media were supplemented with antibiotics at the indicated concentrations (micrograms per milliliter) as follows: ampicillin, 50 (1,000 for *E. carotovora* subsp. *carotovora* 71); kanamycin, 50; spectinomycin, 50; streptomycin, 100; and tetracycline, 10. Media were solidified by the addition of Difco agar (1.5%) (Difco, Detroit, Mich.).

Preparation of samples for enzyme assays and assay conditions. Cultural conditions, preparation of culture supernatants and cell extracts, and assay conditions for Peh were previously described (8, 29). The standard Pel assay (6, 51) was modified by increasing $CaCl_2$ concentration from 750 nM to 2.4 mM and raising the pH from 8.5 to 9.5; these modifications were necessary since under standard assay conditions Pel-3 activity remained suboptimal. β-Galactosidase activity was assayed according to Miller (25).

IEF and in situ activity assays. Ultrathin-layer isoelectric focusing (IEF) and localization of bands with pectolytic activity were carried out essentially as described by Willis et al. (47).

Recombinant DNA techniques. Standard procedures were

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristic(s)[a]	Reference or source
E. carotovora subsp. *carotovora*		
71	Wild type	50
AC5006	Lac$^-$ derivative of 71	28
E. coli		
DH5α	ϕ80*lacZ* ΔM15 Δ(*lacZYA-argF*) *U169 hsdR17 recA1 endA1 thi-1*	BRL[b]
HB101	*proA2 lacY1 hsdS20* (r^-_B m^-_B) *recA56 rpsL20*	Laboratory collection
MC4100	IHF$^+$	Laboratory collection
HP4110	IHF$^-$ derivative of MC4100	49
Plasmids		
pBluescript II SK$^+$	Ap^r	Stratagene
pMN481	Promoter-probing vector; Ap^r	26
pUT-mini-Tn5Km	Source of Km^r cassette	12
pAKC213	*peh-1*$^+$ and *pel-3*$^+$ of *E. carotovora* subsp. *carotovora* 71	47
pAKC673	*pel-1*$^+$ of *E. carotovora* subsp. *carotovora* 71; 4-kb *Eco*RI-*Pst*I *pel-1*$^+$ DNA fragment cloned in pUC18	Laboratory collection
pAKC761	*pel-3*$^+$ Ap^r; 1.35-kb *Hin*dIII-*Eco*RI *pel-3*$^+$ DNA fragment from pAKC213, cloned into pSK$^+$ (Fig. 1)	This study
pAKC781	*peh-1*$^+$ Ap^r; 2.5-kb *Acc*I-*Sst*I *peh-1*$^+$ DNA fragment from pAKC213, cloned into pSK$^+$ (Fig. 1)	This study
pAKC783	*pel-1*$^+$ of *E. carotovora* subsp. *carotovora* 71, Ap^r; 1.86-kb *Eco*RI-*Xho*I *pel-1*$^+$ DNA fragment from pAKC673, cloned in pSK$^+$	This study
pAKC788	*pel-3-lacZ* Ap^r; 295-bp upstream region of *pel-3*, cloned into pMN481	This study
pAKC789	*peh-1-lacZ* Ap^r; 284-bp upstream region of *peh-1*, cloned into pMN481	This study
pAKC788Km	*pel-3-lacZ*, Km^r; same as pAKC788, except for Km^r	This study
pAKC789Km	*peh-1-lacZ*, Km^r; same as pAKC789, except for Km^r	This study
pMN481Km	Km^r; Ap^r inactivated by inserting Km^r cassette from pUT-mini-Tn5Km	This study

[a] Ap, ampicillin; Km, kanamycin; Sp, spectinomycin; Sm, streptomycin; Tc, tetracycline.
[b] BRL, Bethesda Research Laboratories.

used in the isolation of plasmid and chromosomal DNA, transformation, electroporation, restriction digests, gel electrophoresis, electroelution of DNA fragments, and DNA ligation (39). Restriction and modifying enzymes were obtained from Promega Biotec (Madison, Wis.) and U.S. Biochemicals (Cleveland, Ohio). The random primer system of U.S. Biochemicals was used for labeling DNA.

Nucleotide sequence. Unidirectional 5′ to 3′ deletions within the internal fragments of pAKC761 and pAKC781 (Fig. 1 and Table 1) were made by using the Erase-a-Base system (Promega Biotec). Derivatives containing overlapping deletions differing in size by approximately 0.2 kb were used for sequence analysis. Single-stranded sequence templates of these plasmids, obtained by standard procedures (39), were used in sequencing reactions with the Sequenase kit of U.S. Biochemicals.

Computer methods. The nucleotide sequence and deduced protein sequences were analyzed with the PC/GENE package (Intelligenetics, Inc., Mountain View, Calif.). Alignment of amino acid sequences was performed with the CLUSTAL program, the functional domain of protein was analyzed with the PROSITE program, and the protein homology search was carried out with the SwissProt database.

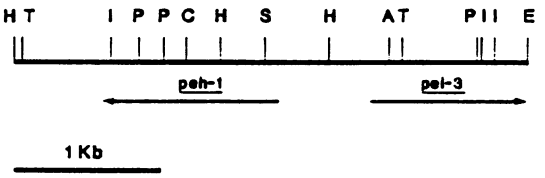

FIG. 1. Restriction map of the cloned DNA fragment containing *pel-3* and *peh-1*. The 3.5-kb *Eco*RI-*Hin*dIII DNA fragment was subcloned from pAKC213 (48). The arrows indicate the locations and directions of transcription of the *pel-3* and *peh-1* coding regions. A, *Acc*I; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; I, *Hin*cII; P, *Pvu*I; S, *Sca*I; T, *Sst*I.

Transcript assay. *E. carotovora* subsp. *carotovora* strains were grown in SYG to a Klett unit value of ca. 250 as described in Liu et al. (23). Standard procedures were used for RNA isolation and Northern (RNA) blot hybridization (5, 39).

RNA samples (20 μg) and a size marker RNA ladder (GIBCO BRL, Gaithersburg, Md.) were electrophoresed in a formaldehyde-agarose gel and transferred to a Biotrans (ICN, Irvine, Calif.) nylon membrane. The 362-bp *Acc*I-*Hin*dIII fragment and the 743-bp *Hin*dIII fragment corresponding to the 5′ regions of *pel-3* and *peh-1* (Fig. 1), respectively, were labeled with [^{32}P]dCTP and used as probes. After hybridization, the blots were washed under stringent conditions: 30 min in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate (SDS) at room temperature and then two sessions of 30 min in 1× SSC–0.1% SDS at 68°C followed by 10 min in 0.5× SSC–0.1% SDS at 68°C.

Determination of the transcriptional start sites. RNA samples used in Northern analysis were also utilized in primer extension assays (5). The ^{32}P-labeled oligonucleotide primers were annealed to the RNA samples; for *pel-3*, the oligonucleotide primer 5′ ATGGTGTCATCAGCCTGAG 3′, corresponding to the base positions 1,910 to 1,892 within the complementary strand, was used, and for *peh-1*, the oligonucleotide primer 5′ ACTCGCTTGCCTGATTG 3′, corresponding to base positions 1,232 to 1,248, was used (Fig. 2). The extension products were run in an 8% acrylamide–urea sequencing gel in parallel with a DNA sequencing ladder, generated with strands complementary to *pel-3* mRNA or *peh-1* mRNA and the primers described above.

PCR amplification and construction of *lacZ* fusion. We performed PCR to amplify the sequence separating *pel-3* and

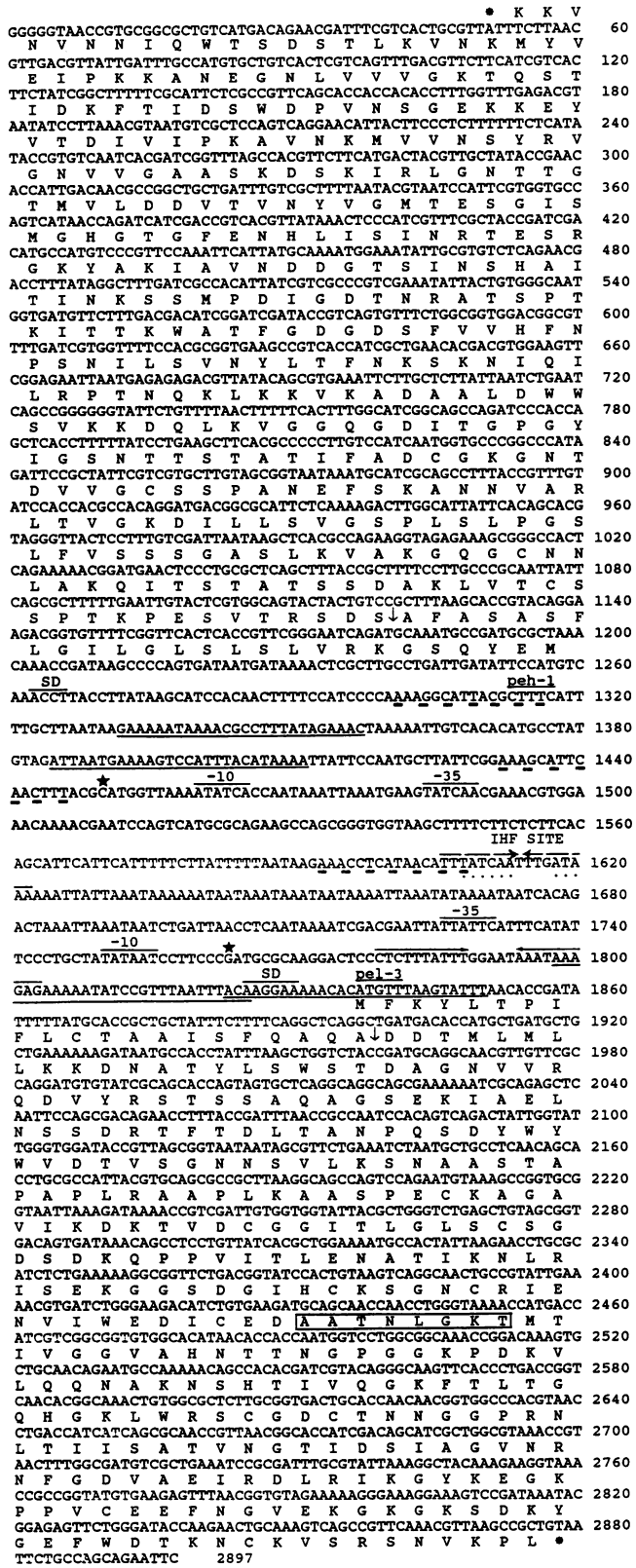

FIG. 2. The nucleotide and deduced amino acid sequences of *pel-3* and *peh-1* of *E. carotovora* subsp. *carotovora* 71. Numbers on the right refer to the positions of nucleotides. The putative Shine-Dalgarno (SD) sequence and sequences similar to the −10 and −35 consensus sequences are shown. Stars (★) represent the transcriptional start sites of *pel-3* and *peh-1* genes. An inverted repeat between 1,606 and 1,622 bp is indicated by broken arrows. The potential IHF-binding site located in the inverted repeat is indicated by dots. The putative KdgR-binding sites are underlined. The other three putative KdgR boxes discussed in the text are indicated by broken lines. The putative hairpin structure in the *pel-3* leader sequence is indicated by solid horizontal double arrows. The ATP- and GTP-binding site motif A of Pel-3 is boxed. The perpendicular arrows indicate the potential signal peptide cleavage sites of the deduced Pel-3 and Peh-1 polypeptides.

peh-1 (Fig. 2) from the linear pAKC213 DNA with the same oligonucleotide primers as those used for primer extension (see above). The reactions were carried out in a programmable heating block (Lauda Refrigerating RM6 Circulator; Brinkman, Westbury, N.Y.), with 15 rounds of temperature cycling: 94°C for 1 min, 55°C for 2 min, and 72°C for 3 min. The reaction mixture contained 100 pmol of each primer, 100 ng of denatured template, and 5 U of *Taq* polymerase (Promega) in a total volume of 100 μl. The reaction buffer recommended by the manufacturer was used. After a final 5-min incubation at 72°C, the PCR products were separated in a low-melting-temperature agarose gel, and the desired product was excised and purified. The amplified DNA fragment was digested with *Hin*dIII to produce two fragments corresponding to the *pel-3* and *peh-1* regulatory regions, respectively. These fragments were treated with Klenow fragment and then ligated into the *Eco*RV site of pBluescript II SK$^+$. After confirming the fidelity of amplification by sequence analysis, the two segments containing the *pel-3* or *peh-1* promoters were obtained by digestion with appropriate endonucleases and ligated in frame with the *lacZ* reporter gene of the vector, pMN481 (26) (Table 1).

Nucleotide sequence accession number. The nucleotide sequence of *pel-3* and *peh-1* of *E. carotovora* subsp. *carotovora* 71 has been entered into the Genetic Sequence Data Bank (GenBank) under the accession number L32172.

RESULTS AND DISCUSSION

While pAKC213 specifies Pel-3 with a pI of 8.0 and Peh-1 with a pI of 9.94, the subclones derived from pAKC213 produce either the Pel or the Peh species (Fig. 3). The 3.5-kb *Hin*dIII-*Eco*RI fragment (Fig. 1) was sequenced, and the data revealed the presence of two open reading frames separated by a DNA segment of 579 bp (Fig. 2). The *pel-3* gene corresponds to the 1,041-bp open reading frame; it is predicted to encode a pro-Pel-3 of 347 amino acid residues having a molecular mass of 37,179 Da and a pI of 8.08. The first 21 amino acid residues at the N-terminal end (Fig. 2) are predicted to constitute the signal sequence (13). From the deduced amino acid sequence, the mature Pel-3 polypeptide is predicted to have a pI of 7.96, which matches with the result of IEF analysis (Fig. 3). An unexpected observation was the lack of homology of the deduced amino acid sequence of Pel-3 with any Pel proteins or with other proteins whose sequences are available in various sequence data bases.

The Pels of *E. carotovora* subsp. *carotovora* are either extracellular or located within the periplasm (6, 18, 43). The genes encoding the extracellular Pels of *E. carotovora* subsp. *carotovora*, *E. chrysanthemi*, and *E. carotovora* subsp. *atrosep-tica* (18) constitute the gene families *pelBC* and *pelADE*. In addition to overall amino acid sequence similarity, all extracellular Pels have two well-known regions of strong homology spatially located around the putative Ca^{2+}-binding site (46). These two consensus regions have also been found in Pel-1, Pel-2, and Pel-X of *E. carotovora* subsp. *carotovora* 71 (22),

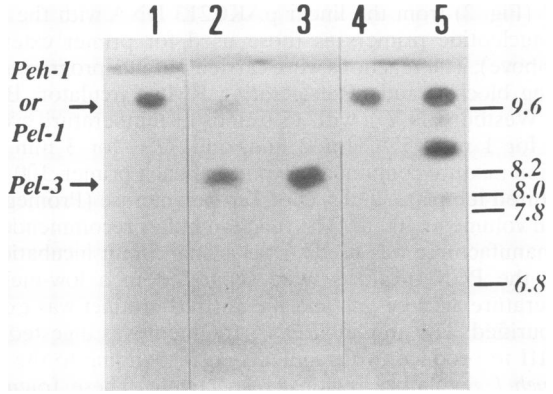

FIG. 3. Pel and Peh profiles of *E. coli* clones detected by IEF followed by activity gel overlays compared with the profile of *E. carotovora* subsp. *carotovora* 71. *E. coli* clones were grown in LB medium supplemented with appropriate antibiotics, and their periplasmic fractions were prepared according to Murata et al. (28). *E. carotovora* subsp. *carotovora* 71 was grown in SYG supplemented with celery extract, and the culture supernatant was prepared as described elsewhere (28). Samples containing 0.5 to 1.0 U of activity were applied to prefocused acrylamide gels, and the gels were focused to equilibrium. To visualize the bands containing pectolytic activity, precast substrate gels (0.25% pectate, 1% agarose in Pel or Peh assay buffer) were placed directly on the focused acrylamide gel and developed as described by Willis et al. (47). Lanes: 1, HB101/pAKC781 periplasmic fraction developed for Peh activity in standard Peh buffer; 2, HB101/pAKC213 periplasmic fraction developed for Pel activity in modified Pel buffer; 3, HB101/pAKC761 periplasmic fraction developed for Pel activity in modified Pel buffer; 4, HB101/pAKC783 periplasmic fraction developed for Pel activity in standard Pel buffer; 5, *E. carotovora* subsp. *carotovora* 71 culture supernatant developed for Pel activity in standard Pel buffer. The numbers at the right refer to the isoelectric points (pIs) of IEF standards (Bio-Rad, Inc.). Pel-1 of pI ca. 9.96 (4) was used as a control. Refer to Table 1 for the characteristics of bacterial strains and plasmids.

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Ecc71 Peh-1   M----EYQSGKRVLSLSLGLIGLFSASAFASDSRTVSEPKTPSSCTVLKA   46
Ecc Peh       M----EYQSGKRVLSLSLGLIGLFSASAFASDSRTVSEPKAPSSCTVLKA   46
Ec Peh        MRFDMEYQSGKRVLSLSLGLIGLFSASAWASDSRTVSEPKTPSSCTTLKA   50
Ecc PehA      M----EYQSGKRVLSLSLGLIGLFSASAWASDSRTVSEPKTPSSCTTLKA   46
              *    **********************.**********.****** ***

Ecc71 Peh-1   DSSTATSTIQKALNNCGQGKAVKLSAGSSSVFLSGPLSLPSGVSLLIDKG   96
Ecc Peh       DSSTATSTIQKALNNCGQGKAVKLSAGSSSVFLSGPLSLPSGVSLLIDKG   96
Ec Peh        DSSTATSTIQKALNNCDQGKAVRLSAGSTSVFLSGPLSLPSGVSLLIDKG  100
Ecc PehA      DSSTATSTIQKALNNSDQGKAVRLSAGSTSVFLSGPLSLPSGVSLLIDKG   96
              ***************  *****.*****.**********************

Ecc71 Peh-1   VT-----------------------LRAVNNAKSFENAPSSCGVVDTNGK  123
Ecc Peh       VT-----------------------LRAVNNAKSFENAPSSCGVVDTNGK  123
Ec Peh        VT----------------------LRAVNNAKSFENEPSSCGVVDKNGK  127
Ecc PehA      VTSVFLSGPLSLPSGVSLLIDKGVTLRAVNNAKSFENAPSSCGVVDKNGK  146
              **                      ************ ******** ***

Ecc71 Peh-1   GCDAFITATSTTNSGIYGPGTIDGQGGVKLQDKKVSWWDLAADAKVKKLK  173
Ecc Peh       GCDAFITATSTTNSGIYGPGTIDGQGGVKLQDKKVSWWDLAADAKVKKLK  173
Ec Peh        GCDAFITAVSTTNSGIYGPGTIDGQGGVKLQDKKVSWWELAADAKVKKLK  177
Ecc PehA      GSDAFITAVSTTNSGIYGPGTIDGQGGVKLQDKKVSWWELAADAKVKKLK  196
              * ****** ******************************.***********

Ecc71 Peh-1   QNTPRLIQINKSKNFTLYNVSLINSPNFHVVFSDGDGFTAWKTTIKTPST  223
Ecc Peh       QNTPRLIQINKSKNFTLYNVSLINSPNFHVVFSDGDGFTAWKTTIKTPST  223
Ec Peh        QNTPRLIQINKSKNFTLYNVSLINSPNFHVVFSDGDGFTAWKTTIKTPST  227
Ecc PehA      QNTPRLIQINKSKNFTLYNVSLINSPNFHVVFSDGDGFTAWKTTIKTPST  246
              **************************************************

Ecc71 Peh-1   ARNTDGIDPMSSKNITIAHSNISTGDDNVAIKAYKGRSETRNISILHNEF  273
Ecc Peh       ARNTDGIDPMSSKNITIAHSNISTGDDNVAIKAYKGRSETRNISILHNEF  273
Ec Peh        ARNTDGIDPMSSKNITIAYSNIATGDDNVAIKAIKGRAETRNISILHNDF  277
Ecc PehA      ARNTDGIDPMSSKNITIAYSNIATGDDNVAIKAYKGRAETRNISILHNDF  296
              ****************** ***.********** ***.**********.*

Ecc71 Peh-1   GTGHGMSIGSETMGVYNVTVDDLVMTGTTNGLRIKSDKSAAGVVNGVRYS  323
Ecc Peh       GTGHGMSIGSETMGVYNVTVDDLIMTGTTNGLRIKSDKSAAGVVNGVRYS  323
Ec Peh        GTGHGMSIGSETMGVYNVTVDDLKMNGTTNGLRIKSDKSAAGVVNGVRYS  327
Ecc PehA      GTGHGMSIGSETMGVYNVTVDDLKMNGTTNGLRIKSDKSAAGVVNGVRYS  346
              ********************** * ************************

Ecc71 Peh-1   NVVMKNVAKPIVIDTVYEKKEGSNVPDWSDITFKDITSQTKGVVVLNGEN  373
Ecc Peh       NVVMKNVAKPIVIDTVYEKKEGSNVPDWSDITFKDITSQTKGVVVLNGEN  373
Ec Peh        NVVMKNVAKPIVIDTVYEKKEGSNVPDWSDITFKDVTSETKGVVVLNGEN  377
Ecc PehA      NVVMKNVAKPIVIDTVYEKKEGSNVPDWSDITFKDVTSETKGVVVLNGEN  396
              ************************************.** ***********

Ecc71 Peh-1   AKKPIEVTMKNVKLTSDSTWQINNVNLNK                      402
Ecc Peh       AKKPIEVTMKNVKLTSDSTWQIKNVTVKK                      402
Ec Peh        AKKPIEVTMKNVKLTSDSTWQIKNVNVKK                      406
Ecc PehA      AKKPIELTMKNVKLTSDSTWQIKNVNVKK                      425
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FIG. 4. Alignment of Peh-1 of *Erwinia carotovora* subsp. *carotovora* 71 (Ecc71 Peh-1) with Peh of *E. carotovora* subsp. *carotovora* SCRI193 (Ecc Peh) (17), Peh of *E. carotovora* Ec (Ec Peh) (21), and PehA of *E. carotovora* subsp. *carotovora* SCC3193 (Ecc PehA) (38). Identical amino acids are marked with asterisks, and chemically similar amino acids are marked with dots. Similar amino acids are grouped as A, S, and T; N and Q; D and E; I, L, M, and V; H, K, and R; and F, W, and Y. Numbers at the right correspond to the amino acid positions in each protein.

fungal pectin lyases (16), plant pollen, tubulins, and style proteins (2, 9, 32, 37, 48). However, these sequences were not found in the predicted *pel-3* product. Moreover, Pel-3 does not have homology with periplasmic Pels known to have very high homology with each other (18). The presence of 10 cysteine residues in mature Pel-3 is also unusual, since extracellular Pels have been found to contain no more than 2 to 4 cysteine residues and periplasmic Pels do not contain cysteine (18). These findings suggest that Pel-3 has a structure different from that of the other Pels. Further support for this hypothesis comes from the prediction that the mature Pel-3 contains an ATP- and GTP-binding site motif A (P-loop) (40) at 178 to 185 amino acid residues (AATNLGKT; Fig. 2). This sequence or other sequences corresponding to P-loop structures have not been found in the other Pels, though it is suggested that extracellular Pels have some homology with the putative guanine nucleotide-binding sequence present in plant tubulins (46).

The coding region of *peh-1* is 1,206 bp, sufficient to encode a pro-Peh-1 42.6-kDa polypeptide of 402 amino acid residues (Fig. 2). The predicted pI of 9.94 for Peh-1 matches quite well with the pI observed in the IEF gel (Fig. 3). The features of the 26 amino acid residues at the N-terminal end are typical of prokaryotic signal peptides (13). Peh-1 of *E. carotovora* subsp. *carotovora* 71 has very strong homology with Peh proteins of other *E. carotovora* strains (Fig. 4) (17, 21, 38). Moreover, like the Peh of SCRI193 and SCC3193, Peh-1 of *E. carotovora* subsp. *carotovora* 71 also is similar to tomato Peh (17, 21, 38). There are three possible start codons (ATG at 1,257 bp, GTG at 1,284 bp, and ATG at 1,308 bp) in frame and putative Shine-Dalgarno sequences preceding the ATG codon at 1,257 bp and the GTG codon at 1,284 bp. The utilization of these start codons would produce signal peptides of 26, 35, or 43 amino acid residues. While we do not know which of these start codons is actually being utilized for Peh-1 synthesis in *E. carotovora* subsp. *carotovora* 71, considering the optimal size of signal peptides and the consensus sequence of amino acid residues at the N-terminal ends of the other Pehs we postulate that the ATG codon at 1,257 bp is most likely used as the start codon.

In order to delineate the promoter regions of *pel-3* and *peh-1*, we identified their transcriptional start sites by primer extension analysis (5). The transcriptional start site of *pel-3* is located at the guanine residue at the 74-bp position relative to the presumed translational start site (Fig. 5A). Transcription of *peh-1* was initiated at the guanine residue 193 bases upstream of the putative translational start site (Fig. 5B). From these start sites, the genes are divergently transcribed.

While there is a −10 sequence (-TATAAT-) preceding the transcriptional start site of *pel-3* (Fig. 2), a rather poorly matched −35 region (-TTATTC-) was found. In contrast,

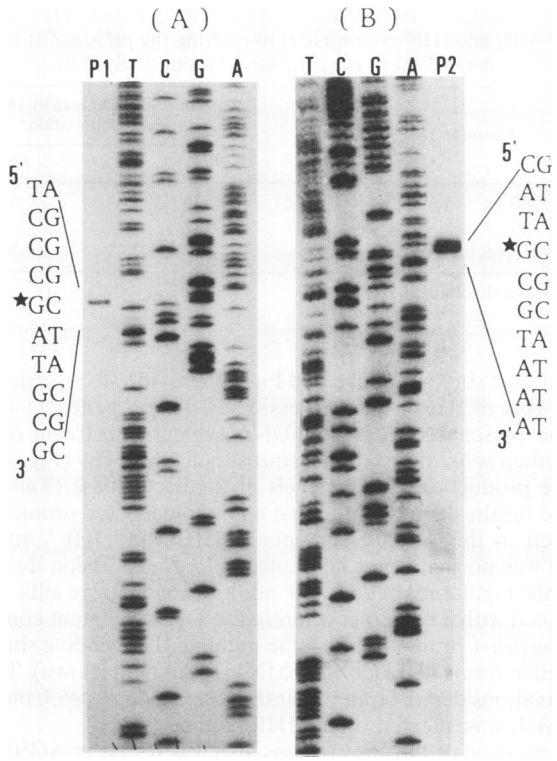

FIG. 5. Primer extension assay of the *pel-3* and *peh-1* transcripts. Total RNA was isolated from *E. carotovora* subsp. *carotovora* 71 grown in SYG medium to a Klett unit value of ca. 250. ^{32}P-end-labeled oligonucleotide primer corresponding to *pel-3* (base positions 1892 to 1910; Fig. 2) or to *peh-1* (base positions 1232 to 1248; Fig. 2) were annealed to 20 μg of RNA and extended by using deoxynucleotide triphosphates and reverse transcriptase. The extension products of *pel-3* (lane P1 in panel A) and *peh-1* (lane P2 in panel B) were electrophoresed in parallel with sequence ladders produced by using the identical primers and strands of either *pel-3* or *peh-1* as templates. The portions of the sequence pertinent to the transcriptional start sites are shown. Stars indicate the in vivo initiation points corresponding to the extension products.

consensus −35 and −10 sequences were found in front of the *peh-1* transcriptional start site (Fig. 2). Assuming that the −10 sequence of *pel-3* and the −35 and −10 regions of *peh-1* were utilized, we suggest that the putative promoters of *pel-3* and *peh-1* are separated by about 220 bp. These results and the expression studies described below exclude the possibility of utilization of a divergent promoter in the expression of *pel-3* and *peh-1*.

The existence of an extremely AT-rich sequence between the putative promoter elements of *pel-3* and *peh-1* is remarkable. The average AT content of the 5′ noncoding region of *pel-3* and *peh-1* is 72%, whereas the AT content of the noncoding region of the *E. chrysanthemi pel* genes is reported to range from 58 to 70% (19). Moreover, certain segments of the *pel-3* and *peh-1* noncoding region have even higher AT content. For example, the 159-bp sequence from 1,572 bp to the −35 box (1,730 bp) of *pel-3* is 84% AT-rich, while the 103-bp DNA segment downstream of 1,572 bp is 92% AT-rich (Fig. 2). The latter AT-rich sequence is closer to the *pel-3* promoter than to the *peh-1* promoter. The significance of these sequences in gene expression is not known. However, extrapolating from observations in the other systems (19, 27), we envision these sequences as affecting gene expression by DNA bending or DNA looping.

A palindromic sequence in this AT-rich region (from 1,604 bp to 1,622 bp) contains a near perfect IHF-binding sequence, in that 12 of 13 nucleotides of *E. carotovora* subsp. *carotovora* 71 DNA are identical to the consensus IHF sequence (42). Recent evidence with diverse bacteria indicates that IHF, as a global regulator, influences several cellular processes, including lambda site-specific recombination and plasmid maintenance and transfer as well as expression of genes for various metabolic processes (14, 49). IHF is also known to induce DNA bending near its target site (36) to create a higher-ordered structure, such as DNA looping, DNA bending, or both, thereby facilitating protein-protein or protein-DNA interactions. The presence of an AT-rich IHF-binding sequence taken along with the poor expression of *pel-3* in IHF$^-$ *E. coli* (see below) strongly suggests that an IHF-like factor in *Erwinia* species is involved in the regulation of Pel-3 production.

A potential hairpin loop structure is located at 33 to 58 bp preceding the putative translational start site of *pel-3* (Fig. 2). We consider this structure important in regulation because it has a symmetry that is typical of operator sites. Overlapping this structure, there are two potential KdgR boxes (33) that cover the SD sequence and extend into the 5′ end of the *pel-3* coding region (Fig. 2). KdgR is a repressor which binds to a 25-bp imperfect palindromic sequence found in various *E. chrysanthemi* genes including *pel*, *peh*, and *out* as well as others involved in the intracellular catabolism of pectate (11, 30, 33). The presence of a putative operator and two overlapping KdgR-binding sites below the transcriptional start site implies that these sequences function in a cooperative manner to effectively suppress *pel-3* expression. Similarly, the two additional putative KdgR boxes in the 5′ untranslated region (Fig. 2) may affect the expression of *peh-1*. Aside from these four KdgR-binding sites, three other sequences, distributed throughout the regulatory region of *pel-3* and *peh-1*, show some similarity to the KdgR box at the core sequences [AAAAAATG*AAA*C(N_{1-4})TG*TTT*CATTTTTT] indicated by underlining; however, the space between the core sequences is about 9 to 10 bp as opposed to the 4- to 7-bp space found in the general consensus sequence as shown above (see also Fig. 2 and reference 19). Recent findings with the *E. chrysanthemi* EC16 *pelE* promoter have raised the possibility that some sequences not highly homologous to the KdgR box may interact with a repressor unrelated to KdgR (15, 44). It would be of interest to ascertain whether a repressor functions in *E. carotovora* subsp. *carotovora* 71 by interacting with some of these regulatory sequences.

To compare the expression of *pel-3* and *peh-1* in *E. carotovora* subsp. *carotovora* 71, we performed Northern blot analysis. The results (Fig. 6) show that the *pel-3* and *peh-1* transcripts were 1,350 and 1,490 bases, respectively; these values match quite well with the transcript sizes predicted from the nucleotide sequence (Fig. 2). The levels of *pel-3* transcript were much lower than the levels of *peh-1* transcript. By extrapolating from these observations, we suggest that the low level of Pel-3 activity in *E. carotovora* subsp. *carotovora* 71 cultures could be attributed to low-level transcription of the *pel-3* gene. The data presented below for *pel3-lacZ* fusions support this view.

To delineate the regions within the intervening 579-bp DNA sequence affecting *pel-3* and *peh-1* expression, we amplified by PCR the sequences upstream of these genes and cloned them in front of *'lacZ* within the vector pMN481 (26). The constructs (Fig. 7) were transformed into the IHF$^+$ *E. coli* strain MC4100 and the IHF$^-$ *E. coli* strain HP4110 (49). Bacterial cultures were assayed for β-galactosidase activity. pAKC788

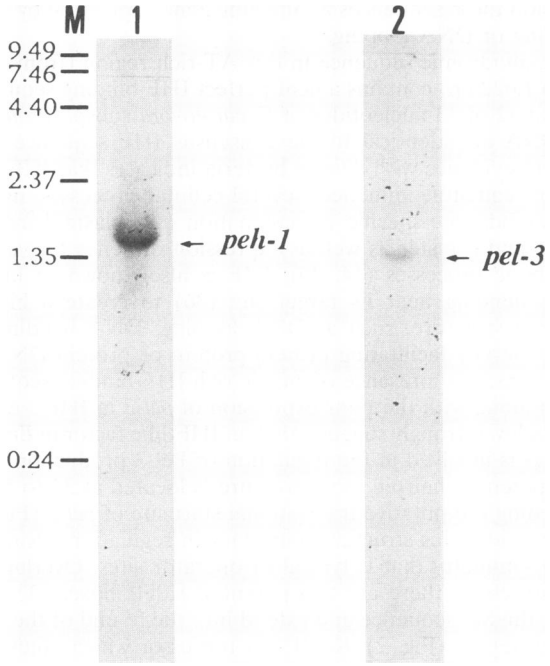

FIG. 6. Analysis of *pel-3* and *peh-1* transcripts by Northern blot hybridization. Total RNA was isolated from *E. carotovora* subsp. *carotovora* 71 grown in SYG medium to a Klett unit value of ca. 250, electrophoresed, transferred to Biotrans, and hybridized to ca. 10^6 cpm of *pel-3* probe or *peh-1* probe per ml in a total volume of 10 ml. Lanes 1 and 2, containing 20 μg of RNA, were hybridized to the *peh-1* probe and the *pel-3* probe, respectively. Lane M refers to the 0.24 to 9.49 kb RNA ladder (Bethesda Research Laboratories, Inc.). The arrows mark the *pel-3* transcript of 1,350 bases and the *peh-1* transcript of 1,490 bases.

exhibited the predicted *pel-3* promoter activity, as indicated by *lacZ* expression in *E. coli*. More significantly, in this construct *lacZ* expression was influenced by IHF since the levels were six- to sevenfold higher in the IHF^+ strain than in the IHF^- strain (Table 2). These findings, taken along with the presence of an IHF-binding sequence upstream of the *pel-3* transcriptional start site (see above and Fig. 2), provide strong evidence for a role of IHF in the expression of this *pel* gene.

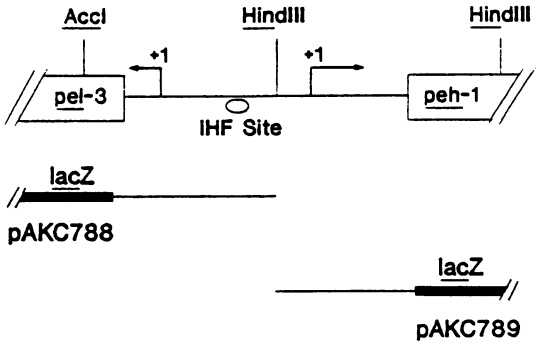

FIG. 7. Schematic representation of the *pel-3* and *peh-1* regulatory regions fused to the promoterless *lacZ* of pMN481 (26) (Table 1), producing the plasmids pAKC788 and pAKC789. The entire intervening sequence containing the 5′ ends of the *pel-3* and *peh-1* coding regions (Fig. 2) was amplified by PCR. Fragments derived from this DNA were cloned such that the promoter and upstream regions of either *pel-3* or *peh-1* were in frame with *lacZ*. Translational start sites are indicated by +1, and the directions of transcription are indicated by arrows.

TABLE 2. Levels of β-galactosidase activity in *E. coli* IHF^+ strain MC4100 and IHF^- strain HP4110 carrying the *pel3-lacZ* fusion (pAKC788) or the *peh1-lacZ* fusion (pAKC789)

Plasmid	β-Galactosidase activity (Miller units) with strain:	
	HP4110	MC4100
pAKC788	36	244
pAKC789	562	770
pMN481 (vector control)	ND[a]	ND

[a] ND, not detected.

The presence of a 284-bp DNA segment upstream of the presumed *peh-1* translational start site in pAKC789 (Fig. 7) led to the production of high levels of β-galactosidase (Table 2). These results demonstrated that a functional *peh-1* promoter is located in this DNA fragment. In IHF^- and IHF^+ strains, there was no significant difference in *lacZ* expression directed by this regulatory region. In addition, similar results were obtained with a construct wherein the DNA fragment containing the *peh-1* promoter along the putative IHF-binding site was placed in frame with *'lacZ* in pMN481 (data not shown). These observations demonstrated that the expression of *pel-3*, but not of *peh-1*, was affected by the IHF-binding site.

Expression of the *lacZ* fusions also was tested in AC5006, a $LacZ^-$ derivative of wild-type *E. carotovora* subsp. *carotovora* 71, to determine the activities of the promoters in a bacterium in which they normally function. Since *E. carotovora* subsp. *carotovora* 71 and its derivatives are ampicillin resistant, the antibiotic marker in these constructs was changed to kanamycin by inserting a kanamycin cassette from pUT-mini-Tn5Km (12) into the β-lactamase-coding region within pMN481. The results shown in Table 3 indicate that the basal levels of β-galactosidase with *pel3-lacZ* or *peh1-lacZ* constructs were markedly higher than the levels in the IHF^+ *E. coli* strain MC4100 (Table 2). Since this response in the *E. carotovora* subsp. *carotovora* strain was not due to the copy number of the plasmids (data not shown), we postulate that activators of these genes that are absent in *E. coli* exist in *Erwinia* species and are responsible for the higher basal level of promoter activity. Indeed, the occurrence of activator (*aep*) genes that stimulate exoenzyme production in *E. carotovora* subsp. *carotovora* 71 (23) is consistent with this hypothesis. In the *E. carotovora* subsp. *carotovora* 71 derivatives, we also found that β-galactosidase levels were threefold higher with the *pel3-lacZ* fusion and twofold higher with the *peh1-lacZ* fusion in SYG medium containing celery extract than in SYG medium alone

TABLE 3. β-Galactosidase activity in AC5006[a] carrying the *pel3-lacZ* fusion (pAKC788Km) or the *peh1-lacZ* fusion (pAKC789Km)

Plasmid	β-Galactosidase activity (Miller units) in indicated medium	
	SYG	SYG + CE[b]
pAKC788Km	5,162	19,821
pAKC789Km	8,610	16,506
pMN481Km (vector control)	ND[c]	ND

[a] A $LacZ^-$ derivative of *E. carotovora* subsp. *carotovora* 71 (Table 1).
[b] CE, celery extract.
[c] ND, not detected.

(Table 3). We conclude that the DNA segments upstream of *pel-3* and *peh-1* cloned in front of *lacZ* contained sequences that allowed induction by plant signal(s).

Collectively these findings allow the following conclusions and predictions. (i) *pel-3* and *peh-1* are transcribed from their own promoters. (ii) *peh-1* expression is much higher than *pel-3* expression. (iii) A positive factor, such as IHF, may be required for the activation of *pel-3* promoter; however, *peh-1* expression appears not to depend upon IHF. (iv) Plant signals activate the expression of *pel-3* and *peh-1*.

In summary, we have shown that the tightly linked *pel-3* and *peh-1* genes are expressed differently, and we have delineated some of the *cis*-acting sequences that may play a role. Our findings for the first time implicate IHF in the expression of a gene for a pectolytic enzyme. However, these findings with *E. coli* need to be extended to determine whether IHF-like proteins and the cognate gene(s) occur in these *Erwinia* strains and if they control other genes required for pathogenicity. Another significant observation was the detection of a novel Pel species. Purification of Pel-3, histochemical localization of the enzyme in infected tissues, and the determination of biochemical characteristics of the enzyme are in progress to elucidate the kinetic properties of the novel Pel species and the role it plays in pathogenicity of *E. carotovora* subsp. *carotovora* 71.

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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. **Budelier, K. A., A. G. Smith, and C. S. Gasser.** 1990. Regulation of a stylar transmitting tissue-specific gene in wild-type and transgenic tomato and tobacco. Mol. Gen. Genet. **224:**183–192.
3. **Chatterjee, A.** Unpublished data.
4. **Chatterjee, A., Y. Liu, H. Murata, T. Souissi, and A. K. Chatterjee.** 1992. Physiological and genetic regulation of a pectate lyase structural gene, *pel-1* of *Erwinia carotovora* subsp. *carotovora* strain 71, p. 241–251. *In* E. W. Nester and D. P. S. Verma (ed.), Advances in molecular genetics of plant-microbe interactions. Kluwer Academic Publishers, Dordrecht, The Netherlands.
5. **Chatterjee, A., J. L. McEvoy, J. P. Chambost, F. Blasco, and A. K. Chatterjee.** 1991. Nucleotide sequence and molecular characterization of *pnlA*, the structural gene for damage-inducible pectin lyase of *Erwinia carotovora* subsp. *carotovora* 71. J. Bacteriol. **173:**1765–1769.
6. **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.
7. **Chatterjee, A. K., J. L. McEvoy, H. Murata, and A. Collmer.** 1991. Regulation of the production of pectinases and other extracellular enzymes in the soft-rotting *Erwinia* spp., p. 45–58. *In* S. S. Patil, S. Ouchi, D. Mills, and C. Vance (ed.), Molecular strategies of pathogens and host plants. Springer-Verlag, New York.
8. **Chatterjee, A. K., K. K. Thurn, and D. J. Tyrell.** 1985. Isolation and characterization of Tn*5* insertion mutants of *Erwinia chrysanthemi* that are deficient in polygalacturonate catabolic enzymes oligogalacturonate lyase and 3-deoxy-D-glycero-2,5-hexodiulosonate dehydrogenase. J. Bacteriol. **162:**708–714.
9. **Chauvaux, S., P. Beguin, J.-P. Aubert, K. M. Bhat, L. A. Gow, T. M. Wood, and A. Bairoch.** 1990. Calcium-binding affinity and calcium-enhanced activity of *Clostridium thermocellum* endoglucanase D. Biochem. J. **265:**261–265.
10. **Collmer, A., and N. T. Keen.** 1986. The role of pectic enzymes in plant pathogenesis. Annu. Rev. Phytopathol. **24:**383–409.
11. **Condemine, G., C. Dorel, N. Hugouvieux-Cotte-Pattat, and J. Robert-Baudouy.** 1992. Some of the *out* genes involved in the secretion of pectate lyases in *Erwinia chrysanthemi* are regulated by *kdgR*. Mol. Microbiol. **6:**3199–3211.
12. **de Lorenzo, V., M. Herrero, U. Jakubzik, and K. N. Timmis.** 1990. Mini-Tn*5* transposon derivatives for insertion mutagenesis, promoter probing, and chromosomal insertion of cloned DNA in gram-negative eubacteria. J. Bacteriol. **172:**6568–6572.
13. **Duffaud, G. D., S. K. Lehnhardt, P. E. March, and M. Inouye.** 1985. Structure and function of the signal peptide. Curr. Top. Membr. Transp. **24:**65–104.
14. **Friedman, D. I.** 1988. Integration host factor: a protein for all reasons. Cell **55:**545–554.
15. **Gold, S., S. Nishio, S. Tsuyumu, and N. T. Keen.** 1992. Analysis of the *pelE* promoter in *Erwinia chrysanthemi* EC16. Mol. Plant-Microbe Interact. **5:**170–178.
16. **Gysler, C., J. A. M. Harmsen, H. C. M. Kester, J. Visser, and J. Heim.** 1990. Isolation and structure of the pectin lyase D-encoding gene from *Aspergillus niger*. Gene **89:**101–108.
17. **Hinton, J. C. D., D. R. Gill, D. Lalo, G. S. Plastow, and G. P. C. Salmond.** 1990. Sequence of the *peh* gene of *Erwinia carotovora*: homology between *Erwinia* and plant enzymes. Mol. Microbiol. **4:**1029–1036.
18. **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.
19. **Hugouvieux-Cotte-Pattat, N., and J. Robert-Baudouy.** 1992. Analysis of the regulation of the *pelBC* genes in *Erwinia chrysanthemi* 3937. Mol. Microbiol. **6:**2363–2376.
20. **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.
21. **Lei, S. P., H. C. Lin, S. S. Wang, P. Higaki, and G. Wilcox.** 1992. Characterization of the *Erwinia carotovora peh* gene and its product polygalacturonase. Gene **117:**119–124.
22. **Liu, Y., and A. K. Chatterjee.** Unpublished data.
23. **Liu, Y., H. Murata, A. Chatterjee, and A. K. Chatterjee.** 1993. Characterization of a novel regulatory gene *aepA* that controls extracellular enzyme production in the phytopathogenic bacterium *Erwinia carotovora* subsp. *carotovora*. Mol. Plant-Microbe Interact. **6:**299–308.
24. **McEvoy, J. L., H. Murata, and A. K. Chatterjee.** 1990. Molecular cloning and characterization of an *Erwinia carotovora* subsp. *carotovora* pectin lyase gene that responds to DNA-damaging agents. J. Bacteriol. **172:**3284–3289.
25. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Minton, N. P.** 1984. Improved plasmid vectors for the isolation of translational *lac* gene fusions. Gene **31:**269–273.
27. **Montgomery, J., V. Pollard, J. Deikman, and R. L. Fischer.** 1993. Positive and negative regulatory regions control the spatial distribution of polygalacturonase transcription in tomato fruit pericarp. Plant Cell **5:**1049–1062.
28. **Murata, H., M. Fons, A. Chatterjee, A. Collmer, and A. K. Chatterjee.** 1990. Characterization of transposon insertion Out$^-$ mutants of *Erwinia carotovora* subsp. *carotovora* defective in enzyme export and of a DNA segment that complements *out* mutations in *E. carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica*, and *Erwinia chrysanthemi*. J. Bacteriol. **172:**2970–2978.
29. **Murata, H., J. L. McEvoy, A. Chatterjee, A. Collmer, and A. K. Chatterjee.** 1991. Molecular cloning of an *aepA* gene that activates production of extracellular pectolytic, cellulolytic, and proteolytic enzymes in *Erwinia carotovora* subsp. *carotovora*. Mol. Plant-Microbe Interact. **4:**239–246.
30. **Nasser, W., S. Reverchon, and J. Robert-Baudouy.** 1992. Purification and functional characterization of the KdgR protein, a major repressor of pectinolysis genes of *Erwinia chrysanthemi*. Mol. Microbiol. **6:**257–265.

31. **Plastow, G. S., P. M. Border, J. C. D. Hinton, and G. P. C. Salmond.** 1986. Molecular cloning of pectinase genes from *Erwinia carotovora* subsp. *carotovora* (strain SCRI193). Symbiosis **2:**115–122.
32. **Rafnar, T., I. J. Griffith, M.-C. Kuo, J. F. Bond, B. L. Rogers, and D. G. Klapper.** 1991. Cloning of *Amb* a *I* (Antigen E), the major allergen family of short ragweed pollen. J. Biol. Chem. **266:**1229–1236.
33. **Reverchon, S., Y. Huang, C. Bourson, and J. Robert-Baudouy.** 1989. Nucleotide sequence of the *Erwinia chrysanthemi ogl* and *pelE* genes, negatively regulated by the KdgR gene product. Gene **85:**125–134.
34. **Ried, J. L., and A. Collmer.** 1985. Activity strain for rapid characterization of pectic enzymes in isoelectric focusing and sodium dodecyl sulfate-polyacrylamide gels. Appl. Environ. Microbiol. **50:**615–622.
35. **Roberts, D. P., P. M. Berman, C. Allen, V. K. Stromberg, G. H. Lacy, and M. S. Mount.** 1986. Requirement for two or more *Erwinia carotovora* subsp. *carotovora* pectolytic gene products for maceration of potato tuber tissue by *Escherichia coli*. J. Bacteriol. **167:**279–284.
36. **Robertson, C. A., and H. A. Nash.** 1988. Bending of the bacteriophage λ attachment site by *Escherichia coli* integration host factor. J. Biol. Chem. **263:**3554–3557.
37. **Rogers, H. J., A. Harvery, and D. M. Lonsdale.** 1992. Isolation and characterization of a tobacco gene with homology to pectate lyase which is specifically expressed during microsporogenesis. Plant Mol. Biol. **20:**493–502.
38. **Saarilahti, H. T., P. Heino, R. Pakkanen, N. Kalkkinen, I. Palva, and E. T. Palva.** 1990. Structural analysis of the *pehA* gene and characterization of its protein product, endopolygalacturonase, of *Erwinia carotovora* subspecies *carotovora*. Mol. Microbiol. **4:**1037–1044.
39. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
40. **Saraste, M., P. R. Sibbald, and A. Wittinghofer.** 1990. The P-loop—a common motif in ATP- and GTP-binding proteins. Trends Biochem. Sci. **15:**430–434.
41. **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.
42. **Timothy, R. H., E. Santero, S. Porter, and S. Kustu.** 1990. The integration host factor stimulates interaction of RNA polymerase with NIFA, the transcriptional activator for nitrogen fixation operons. Cell **63:**11–22.
43. **Trollinger, D., S. Berry, W. Belser, and N. T. Keen.** 1989. Cloning and characterization of a pectate lyase gene from *Erwinia carotovora* EC153. Mol. Plant-Microbe Interact. **2:**17–25.
44. **Tsuyumu, S., Miura, and S. Nishio.** 1991. Distinct induction of pectinases as a factor determining host specificity of soft-rotting *Erwinia*, p. 31–34. *In* S. S. Patil, S. Ouchi, D. Mills, and C. Vance (ed.), Molecular strategies of pathogens and host plants. Springer-Verlag, New York.
45. **Van Gijsegem, F.** 1989. Relationship between the *pel* genes of the *pelADE* cluster of *Erwinia chrysanthemi* strain B374. Mol. Microbiol. **3:**1415–1424.
46. **Yoder, M. D., N. T. Keen, and F. Jurnak.** 1993. New domain motif: the structure of pectate lyase C, a secreted plant virulence factor. Science **260:**1503–1507.
47. **Willis, J. W., J. K. Engwall, and A. K. Chatterjee.** 1987. Cloning of genes for *Erwinia carotovora* subsp. *carotovora* pectolytic enzymes and further characterization of the polygalacturonases. Phytopathology **77:**1199–1205.
48. **Wing, R. A., J. Yamaguchi, S. K. Larabell, V. M. Ursin, and S. McCormick.** 1989. Molecular and genetic characterization of two pollen-expressed genes that have sequence similarity to pectate lyases of the plant pathogen *Erwinia*. Plant Mol. Biol. **14:**17–28.
49. **Wu, Y., and P. Datta.** 1992. Integration host factor is required for positive regulation of the *tdc* operon of *Escherichia coli*. J. Bacteriol. **174:**233–240.
50. **Zink, R. T., and A. K. Chatterjee.** 1985. Cloning and expression in *Escherichia coli* of pectinase genes of *Erwinia carotovora* subsp. *carotovora*. Appl. Environ. Microbiol. **49:**714–717.
51. **Zink, R. T., J. K. Engwall, J. L. McEvoy, and A. K. Chatterjee.** 1985. *recA* is required in the induction of pectin lyase and carotovoricin in *Erwinia carotovora* subsp. *carotovora*. J. Bacteriol. **164:**390–396.