Erwinia carotovora subsp. carotovora Pectic Enzymes: 
In Plant Gene Activation and Roles in Soft-Rot Pathogenesis

Z. Yang, C. L. Cramer, and G. H. Lacy

Laboratory for Molecular Biology of Plant Stress, Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0330 U.S.A.

Pectic enzyme expression in Erwinia carotovora subsp. carotovora was studied on potato tuber slices using a membrane-separated system. Accumulation of mRNAs was sequential for exo-pectate lyase (exo-PL), endo-PL, and endo-polynigalacturonase, respectively, and reached maxima by 6–12 hr. Induction kinetics in vitro with polygalacturonic acid differed from in planta kinetics. Accumulations of mRNA were correlated with rotting; under conditions incompatible with pathogenesis, induction was reduced, and exo-PL expression was delayed. Healed slices were resistant to rotting, but challenging E. c. subsp. carotovora were activated for transient endo-PL expression. This suggests that enzyme induction involves cell wall degradation products. Pretreatment of slices with Escherichia coli expressing E. c. subsp. carotovora exo-PL increased in planta accumulation of mRNAs for all three pectic enzymes in E. c. subsp. carotovora applied as a subsequent challenge, indicating that exo-PL reaction products activate other pectic enzyme genes. Endo-PL, expressed from E. coli, induced host accumulation of mRNAs for phenylalanine ammonia-lyase, a marker for plant defense-responses. These results support the hypothesis that pectic enzymes affect virulence by regulating pectic enzyme levels and affect host responses by inducing defense genes.

Erwinias produce diverse pectic enzymes including endo- and exo-pectate lyases (PLs), endo- and exo-polynigalacturonases (PGs), pectin lyase, pectin methylesterase, and oligogalacturonate lyase (reviewed in Collmer and Keen 1986 and in Kotoujansky 1987). The number of enzymes, increased by numerous isozymes, probably reflects the complex pectic structures in plant cell walls and the wide host range of the pathogen and suggests that the interactions of pectic enzymes with plant tissue are complicated. Different catalytic properties or substrate preferences and/or differential expression of these enzymes may contribute to this complexity (Collmer and Keen 1986; Kotoujansky 1987).

Expression of pectic enzymes is important in soft-rot pathogenesis caused by erwinias (Collmer et al. 1982; Collmer and Keen 1986). Zucker and his colleagues showed that nonpathogenic bacteria synthesized pectic enzymes constitutively, whereas their pathogenic bacteria was inducible (Zucker et al. 1972; Zucker and Hankin 1970). Since then, in vitro but not in planta regulation of pectic enzymes has been studied in soft-rot erwinias (Collmer et al. 1982; Reverchon and Robert-Baudouy 1987). In general, pectic enzyme synthesis is induced by isolated plant cell walls, enzyme substrates (pectate polymers and oligomers), and intermediates of pectate degradation pathways (Collmer and Bateman 1981,1982; Collmer et al. 1982) including saturated digalacturonate, unsaturated digalacturonate, 2-keto-3-deoxygluconate, 2,5-diketo-3-deoxygluconate, and 5-keto-4-deoxygluconate. In vitro studies show that pectic enzyme regulation is complex and characterized by multiple levels of genetic control (reviewed in Kotoujansky 1987), various sensitivities of one enzyme to different inducers (Condemine et al. 1986; Reverchon and Robert-Baudouy 1987), and various efficiencies for one inducer with different enzymes (Collmer et al. 1982).

In planta expression of pectic enzymes in pathogenesis has received very little attention. This is primarily due to difficulties in recovering sufficient quantities of bacteria from rotting tissue. For this reason, we developed a membrane-separated system to facilitate isolation of bacterial cells apart from soft-rotted potato (Solanum tuberosum L.) tubers for subsequent analysis of bacterial gene transcription (Yang et al. 1989). We showed that similar tissue maceration and induction of bacterial and plant gene expression occurs in both the membrane-separated and direct-contact interactions. Potato tubers are universally susceptible to soft rot albeit at different levels (Kelman et al. 1987); even the S. tuberosum-S. breviflin Phil. bacterial soft-rot resistant hybrids of Austin et al. (1988) develop rotting under some conditions. Therefore, in this paper, a compatible interaction (sensu Talboys et al. 1973) results in massive rotting that is not limited by host responses. An incompatible interaction occurs when either no rotting results or rotting is limited, presumably by general resistant responses of the host moderated by environmental conditions. Moisture is a major factor determining compatible (saturated moisture) versus incompatible (limited moisture) interactions (Yang et al. 1989).

Six or more pectolytic enzymes are produced in vitro (Roberts et al. 1986b) and at least two endo-pectate lyases (pls 9.5 and 10.5) are produced in planta (Yang et al. 1989) by Erwinia carotovora subsp. carotovora (Jones) Bergey et al. strain EC14. In this study, we utilized the membrane-separated system to examine in planta expression of E. c. subsp. carotovora genes encoding endo-PL (EC 4.2.2.2), exo-PL (EC 4.2.2.9), and endo-PG (EC 3.2.1.15), previously

Current address of Z. Yang: Department of Botany, University of Maryland, College Park, MD 20742.
Address all correspondence to G. H. Lacy.

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 1992.
shown to be associated with soft-rot pathogenicity (Roberts et al. 1986b). Specifically, we address: 1) Which genes are expressed in planta? 2) Is their induction associated with disease development? 3) Are these genes under coordinate regulation? 4) Is their in planta induction similar to in vitro induction? 5) Are they expressed under incompatible conditions? 6) What molecules are inducers in planta?

Reaction products of pectic enzymes activate plant defense responses (reviewed in Hahn et al. 1989 and Ryan 1987). Davis et al. (1984, 1986) show that E. c. subsp. carotovora endo-PL elicits accumulation of soybean (Glycine max (L.) Merr.) phytoalexins by releasing oligogalacturonides from host plant cell walls. In French bean (Phaseolus vulgaris (L.)), accumulation of phytoalexins elicited by challenge with Colletotrichum lindemuthianum (Sacc. & Magn.) Brioso & Cav. or fungal cell wall crude extracts is attributed to the activation of plant genes encoding biosynthetic enzymes (Cramer et al. 1985; Dixon et al. 1986). We have shown that inoculation with E. c. subsp. carotovora induces two plant defense-related genes, phenylalanine ammonia-lyase (PAL) and β,β,3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG) in potato tubers, which are involved in responses to soft rot (Yang et al. 1989, 1991). Pectic enzymes are important factors in soft-rot pathogenesis. We ask here if pectic enzymes are also involved in plant defense gene activation. Because Escherichia coli (Migula) Castellani & Chalmers does not induce potato tuber defense responses (Yang et al. 1989), E. c. strains containing E. c. subsp. carotovora genes cloned on plasmids were used to determine which pectic enzymes create reaction products that may induce defense responses.

**MATERIALS AND METHODS**

**Plasmids, bacterial strains, and plant materials.** Construction of plasmids pDR1 (encoding endo-PL) and pDR30 (encoding exo-PL and endo-PG) was described by Roberts et al. (1986a,b); plasmid pZY12 was constructed for this research by cloning a 1.9-kb BamHI-HincII fragment encoding only exo-PL from pDR30 into pUC118 (Fig. 1). E. c. subsp. carotovora strain EC14, E. coli strains DH5α, HB101, or HB101 or DH5α containing pDR1, pDR30, or pZY12 were maintained on plate count agar (Difco Laboratories, Detroit, MI) supplemented with ampicillin (30 μg/ml; Sigma, St. Louis, MO) when necessary. For plant inoculation, bacteria were cultured in tryptic soy broth (Difco) supplemented with appropriate antibiotics and incubated with orbital shaking (250 rpm) at 30° C. Following overnight growth, the bacterial suspension was diluted (1:100) in minimal medium supplemented with 1% (v/v) glycerol (Yang et al. 1989) and incubation with shaking was continued for 12 hr or until a turbidity of 1.0 at 550 nm was achieved. Potato tubers (*S. tuberosum* ‘Russet Burbank’) were purchased from a local market. Before inoculation, tubers were surface-disinfected and incubated at 30° C overnight as described previously (Yang et al. 1989).

**Inoculation and harvest of bacterial cells and plant tissues.** For analysis of in planta expression of bacterial genes, *E. c. subsp. carotovora* grown in minimal medium plus glycerol was inoculated (1.5×10⁷ colony-forming units [cfu] in 600 μl) onto polysulfone membranes (0.2-μm pore size, GA-8S, Gelman Sciences, Ann Arbor, MI) placed on cut surfaces of potato tuber slices under conditions compatible with compatible with soft-rot development as described for the membrane-separated system (Yang et al. 1989). To determine if pectic enzyme genes can be induced on wound-healed surfaces, tubers were sliced and incubated at 30° C for 3 days before inoculation. To test if *E. c. subsp. carotovora* exo-PL reaction products induce pectic enzymes, slices were preinoculated directly (without membranes) with *E. coli* or *E. coli* expressing exo-PL from plasmid pZY12. Preinoculated slices were incubated at 30° C under compatible conditions for 6 hr before inoculation with *E. c. subsp. carotovora* by using membranes. At various times after inoculation, bacteria were harvested from the membranes, frozen in liquid nitrogen, and stored at −75° C for subsequent RNA isolation.

To analyze induction of plant defense-related genes by pectic enzymes, tuber slices were inoculated directly with a 1.0-ml suspension of *E. coli* strain HB101 or *E. coli* containing pDR1 or pDR30 (10⁸ cfu). As a control for defense induction, 300 μl of *E. c. subsp. carotovora* in suspension (3×10⁷ cfu) was used to inoculate other slices. Slices were inoculated with 1.0 ml of minimal medium plus glycerol as a wound control. Six or 9 hr after inoculation, the top 1.0 mm of tuber tissue was sliced off, frozen immediately in liquid nitrogen, and stored at −75° C for subsequent RNA isolation.

**Induction of pectic enzymes by polygalacturonic acid.** *E. c. subsp. carotovora* cells grown in minimal medium

---

**Fig. 1.** Gene probes for endo-polygalacturonase (endoPG), and exo- and endo-pectate lyases (exoPL, endoPL) were derived from Erwinia carotovora subsp. carotovora DNA fragments, diagrammed in this figure, cloned in plasmids pDR30 and pDR1 (Roberts et al. 1986a,b). Endonuclease restriction sites (Av = Avai, Ba = BamHI, Bg = BglII, Ec = EcoRI, Hc = HincII, Hp = Hpal, Ps = PstI) are indicated. Activity of exoPL and endoPG is abolished by a Tn5 (Tn5) insertion. Because the genes may both extend into the BgII/BgII fragment, we used the 1.1-kb BamHI/BglII fragment for the exo-PL gene probe and the 0.7-kb BamHI/BglII fragment for the endo-PG gene probe. The 2.1-kb PstI/EcoRI fragment of pDR1 was used as probe for endo-PL; this fragment expresses the enzyme. The bar at the bottom indicates 1 kb.
plus glycerol (OD$_{550nm}$ = 1.0) were pelleted at 3,000 × g for 10 min at 4°C and resuspended in the same volume of minimal medium plus 0.8% polygalacturonic acid sodium salt (w/v; Sunkist). At various times of incubation at 30°C with orbital shaking (250 rpm), bacterial cells from 1.5 ml of culture were pelleted by centrifugation at 10,000 × g for 1.0 min at room temperature, frozen immediately in liquid nitrogen, and stored at −75°C for later RNA isolation.

RNA isolation and RNA:DNA hybridization. Bacterial and plant total RNAs were isolated as described previously (Yang et al. 1989). For RNA:DNA hybridization analyses, 10 µg of bacterial RNA was treated with RNase-free DNase I (Boehringer Mannheim Biochemicals, Indianapolis, IN), separated by electrophoresis on 1% agarose gels, transferred to a Nytran membrane (Schleicher and Schuell, Inc., Keene, NH), and hybridized to $^{32}$P-labeled DNA probes as described previously (Yang et al. 1989). To re-use the membrane, the hybridized probe DNA was stripped off by incubating the membrane in a 70% formamide and 6X SSPE (1.08 M sodium chloride, 60 mM monobasic sodium phosphate, and 6 mM EDTA at pH 7.4 [Sambrook et al. 1989]) at 65°C for 40 min. Each time the probe was stripped off, the membrane was autoradiographed for two days to assure that the probe was completely removed. As an internal control for constitutive expression of bacterial mRNA, the membranes were also probed with a 5.0-kb EcoRI fragment of pNO1001 encoding E. coli ribosomal proteins (S5, L18, L6, S8, S14, S17, L29, L16, and S3) (Cerretti et al. 1983). Probe preparation and labeling were as described (Yang et al. 1989). Following autoradiography, a semi-quantitative method for analyses of the films was selected; they were scanned with a densitometer, and mRNA levels were presented as percentage of maximum density for each autoradiogram. Sizes for bacterial mRNAs hybridizing to the gene probes were estimated using a 1-kb RNA molecular mass standards (0.16–1.77 kb and 0.24–9.5 kb RNA ladders, Bethesda Research Laboratories [BRL], Gaithersburg, MD). Using this method, the endo-PL probe was found in a previous study to hybridize to a 1.4-kb class of RNA (Yang et al. 1989).

PAL mRNA levels were determined by slot blot hybridization using a 1.8-kb PstI fragment of PAL cDNA from bean (Edwards et al. 1985). Five micrograms of total potato RNA was denatured by glyoxalation (Williams and Mason 1985) and blotted onto Nytran membrane in a Millipore slot apparatus (Millipore Corp., Bedford, MA). PAL DNA probe and hybridization conditions were described previously (Yang et al. 1989).

RESULTS

Characterization of gene probes. The origins of the gene probes are shown in Figure 1. Genes for endo-PL and exo-PL are related by nucleotide sequence analyses (L. S. Antonov and V. K. Stromberg, unpublished data), respectively, to genes mediating production of the Pelbc family of extracellular Erwinia enzymes and to the family of intracellular pectate lyases (described in Hinton et al. 1989). In this study, expression of exo-PL by the 1.9-kb BamHI-

HincII fragment of pDR30 cloned in pZY12 establishes that the gene encoding that enzyme resides in that fragment as suggested by Hinton et al. (1989). Because exo-PL and endo-PG activity is abolished by a Tn5 insertion or deletion of the central 0.5-kb BglII fragment, the genes encoding these enzymes may overlap, their promoters may overlap, or they may be expressed from an operon. Preliminary sequence analyses (V. K. Stromberg, unpublished data) suggest that the gene encoding endo-PG bears only slight nucleotide similarity to a PG from E. c. subsp. carotovora (Hinton et al. 1990). This may not be surprising because E. c. subsp. carotovora may also have multiple PGs as does the closely related soft-rot bacterium, E. c. subsp. atroseptica (George et al. 1991). The exo-PL and endo-PG probes hybridized to 1.8- and 1.3-kb mRNA, respectively, demonstrating that these genes are not encoded by a polycistronic message characteristic of a single operon. This observation was repeated once for each probe, and representative data are shown in Figure 2A and B. Both mRNAs accumulated to higher levels in cells recovered from tuber slices compared to cells grown in minimal medium plus glycerol (Fig. 2A,B). In a previous study (Yang et al. 1989), the exo-PL probe was found to hybridize to a 1.4-kb mRNA species.

Fig. 2. RNA:DNA hybridization analyses of exo-pectate lyase (PL), and endo-polygalacturonase (PG) mRNAs. Total RNA was isolated from Erwinia carotovora subsp. carotovora grown in minimal medium plus glycerol (1A,B) or inoculated on potato tuber slices in the membrane-separated system (see text) (2A,B). Ten micrograms of total RNA was separated by electrophoresis on a 1.2% agarose gel, transferred to and immobilized on a Nytran membrane, and hybridized to $^{32}$P-labeled exo-PL (A) and endo-PG (B) DNA probes. An RNA ladder (Bethesda Research Laboratories, Gaithersburg, MD) was used to determine molecular size (kb).
Kinetics of \textit{in planta} induction of three pectic enzyme mRNAs. Previously, we showed that mRNA levels and enzyme activity for \textit{E. c.} subsp. \textit{carotovora} endo-PL were induced \textit{in planta} in either membrane-separated or direct-contact interactions (Yang et al. 1989). Kinetics of endo-PL, exo-PL, and endo-PG mRNAs accumulations were studied during disease development utilizing the membrane-separated system. This experiment was repeated twice with similar results; representative data are presented in Figure 3A. Under compatible conditions, levels of endo-PL, exo-PL, and endo-PG mRNAs were induced within 3 hr after inoculation, and accumulations reached maxima between 6 and 12 hr and essentially returned to basal levels by 24 hr. For individual mRNAs, maximal accumulation occurred at different times after inoculation, suggesting that the three pectic enzymes appear sequentially \textit{in planta}. Exo-PL had the highest basal level of mRNA production at time zero and accumulated mRNA most rapidly, peaking at 6 hr. This was followed by endo-PL, which reached a peak at 9 hr. Accumulation of endo-PG was not significant until 6 hr and did not reach its maximum until 12 hr after inoculation, continuing with near maximal levels through 15 hr. Linear increase in tissue maceration occurred between 6 and 12 hr.

\textbf{In vitro} induction of pectic enzymes by polygalacturonic acid. To determine if \textit{in planta} expression of pectic enzymes is similar to or distinct from \textit{in vitro} expression, kinetics for \textit{in vitro} induction of endo-PL, exo-PL, and endo-PG mRNAs by polygalacturonic acid were examined. This experiment was repeated twice with similar results; representative data are shown in Figure 3B. In vitro induction of all three mRNAs was more rapid and more transient compared to \textit{in planta} induction. Both endo-PL and exo-PL mRNAs were induced to maxima during the early exponential increase of bacterial growth (within 60 min after adding inducer); their mRNA levels fell to basal levels by 4 hr. Levels of endo-PL mRNA increased by 60 min and reached peak levels within 4 hr during the late exponential growth of the bacterium. In vitro expression of endo-PG was induced simultaneously with exo-PL and before endo-PL, while \textit{in planta}, endo-PG induction was the latest to reach maximum accumulation of mRNAs among the three enzymes.

Expression of pectic enzyme mRNAs under incompatible conditions. Reduced moisture limits maceration (Yang et al. 1989). In Figure 4, we compared levels of expression of endo-PL, exo-PL, and endo-PG mRNAs under both high moisture (compatible) and low moisture (incompatible) conditions. All three mRNAs were induced under incompatible conditions as well as compatible conditions, but the mRNA accumulations were reduced markedly under incompatible conditions except for endo-PL. Under incompatible conditions, expression of exo-PL was delayed past 3 hr after inoculation, whereas accumulations for endo-PL and endo-PG mRNAs occurred at times similar to those observed under compatible conditions. Levels of endo-PL mRNA accumulation at 24 hr were higher in the incompatible compared to the compatible interactions; in the compatible interaction, endo-PL mRNA levels returned to basal levels by 24 hr. This experiment was repeated once; representative data are shown in Figure 4.

Wound-healed potato tuber tissues are resistant to \textit{E. c.} subsp. \textit{carotovora} (Tripathi and Gupta 1983). Because of its central role in soft rot (Collmer et al. 1982; Collmer and Keen 1986; Kotoujansky 1987), we wanted to learn if endo-PL might be induced by healed tissues as well as by wounded tissues. Analyses of the induction of pectic enzyme on tuber slices wound-healed for three days and completely resistant to maceration by \textit{E. c.} subsp. \textit{carotovora} showed that endo-PL mRNA was transiently induced to maximal levels at 4 hr after inoculation (data not shown).

\textbf{Induction of pectic enzyme genes by their own reaction products.} To determine if \textit{E. c.} subsp. \textit{carotovora} pectic enzyme reaction products are involved in the induction of pectic enzymes, tuber slices were preinoculated directly with \textit{E. coli} expressing the \textit{E. c.} subsp. \textit{carotovora} exo-PL gene from plasmid pZY12. For controls, \textit{E. coli} lacking the plasmids or containing only plasmid pUC118, the vector for cloned \textit{E. c.} subsp. \textit{carotovora} DNA, were used. Following incubation at 30° C for 6 hr, the preinoculated tuber slices were inoculated with \textit{E. c.} subsp. \textit{carotovora} using the membrane-separated system. Preinoculation with \textit{E. coli} expressing \textit{E. c.} subsp. \textit{carotovora} exo-PL resulted in significant increases in mRNA levels for exo-PL, endo-PL, and endo-PG over \textit{E. coli} alone. This experiment was repeated once; representative data are shown in Figure 5. These results indicate that exo-PL produces reaction products that activate expression of its own and other pectic enzyme genes during pathogenesis. Increases in endo-PG mRNA accumulation levels induced by exo-PL were most prominent among the three mRNAs examined, suggesting that exo-PL reaction products including unsaturated digalacturonic are efficient inducers of endo-PG. No maceration was caused by \textit{E. coli} alone or the expression of the \textit{E. c.} subsp. \textit{carotovora} genes in \textit{E. coli}; this result is similar to the observations of Roberts et al. (1986b).

\textbf{Activation of plant defense-related genes by pectic enzymes.} To determine if \textit{E. c.} subsp. \textit{carotovora} pectic enzymes are involved in the induction of plant defense responses, potato tuber slices were inoculated directly with \textit{E. coli} expressing \textit{E. c.} subsp. \textit{carotovora} pectic enzyme genes from plasmids. This experiment was repeated twice; representative data are shown in Figure 6. Inoculation with \textit{E. coli} alone did not increase PAL mRNA levels more than the wounding, which resulted from slicing the tuber. Plasmid pDR1 in \textit{E. coli}, encoding \textit{E. c.} subsp. \textit{carotovora} endo-PL, caused an increase in potato PAL mRNA which at 9 hr was similar in intensity to wild-type \textit{E. c.} subsp. \textit{carotovora} after 6 hr. At 6 hr (data not presented), the \textit{E. coli}/pDR1 response was not as strong as the \textit{E. c.} subsp. \textit{carotovora} response at the same time. Plasmid pDR30 in \textit{E. coli}, encoding endo-PG and exo-PL, did not induce accumulation of PAL mRNA.

\section*{DISCUSSION}

\textit{In planta} induction of pectic enzymes is sequential. Our studies, using a membrane-separated \textit{E. c.} subsp. \textit{carotovora}-potato interaction system, suggest that \textit{in planta}
Fig. 3. RNA:DNA hybridization analyses of the in planta and in vitro kinetics of transcriptional induction for exo-pectate lyase (PL; filled circles), endo-PL (open triangles) and endo-polygalacturonase (PG; filled triangles) mRNAs. A, in planta: bacterial RNAs were isolated from Erwinia carotovora subsp. carotovora cells induced under compatible conditions in the membrane-separated system (see text). Percentage of tuber tissue macerated (filled diamonds) was determined by weight loss after washing rotted tissue off tuber slices standardized for weight loss due to metabolic activities at various times following inoculation. B, in vitro: E. c. subsp. carotovora cells were grown in minimal medium (MM) plus glycerol to OD_{600} 1.0 and resuspended in MM plus polygalacturonic acid. At various times after resuspension, cells were harvested, and total RNA was isolated. E. c. subsp. carotovora populations (cfu; filled diamonds) were also determined at the harvest times. Following hybridization to a probe and autoradiography, that probe was stripped off both RNA blots (A and B), and the blots were reprobed separately with the other two probes. Autoradiograms (shown in the insets) were scanned by densitometer to provide semiquantitative estimates of the intensity of the DNA:RNA hybridizations. Relative mRNA accumulation levels were presented as percentage of maximum density.
accumulation of mRNAs for three pectic enzymes is sequential (exo-PL, endo-PL, and endo-PG) rather than simultaneous and that they are encoded on separate small transcripts rather than on polycistronically. This suggests that genes for pectic enzymes may be regulated separately rather than by a global mechanism. Accumulation profiles for these transcripts induced in *E. c. subsp. carotovora* during pathogenesis are distinct from accumulation profiles in *vitro*, indicating that *in planta* expression of pectic enzymes is different from *in vitro* expression. Sequential production of cell wall-degrading enzymes (pectic enzymes preceding cellulase) occurs in fungal pathogens (Cooper 1983). Several hypotheses for the biological significance for sequential induction are put forth. First, pathogenesis by *E. c. subsp. carotovora* may require that enzymes be expressed sequentially. Second, separate and sequential induction may allow a wide host-range pathogen such as *E. c. subsp. carotovora* to mobilize pectic enzyme expression to accommodate pathogenesis on a wide variety of hosts. Third, due to varying efficiencies of different inducers for pectic enzymes (Collmer et al. 1982; Reverchon and Robert-Baudouy 1987), sequential induction may imply cascade regulation (i.e., reaction products of first enzyme preferentially induce the next enzyme, and so on).

**Role of pectic enzyme expression in soft-rot pathogenesis.** Regulation of pectic enzymes probably plays an important role in soft-rot pathogenesis (Collmer et al. 1982; Collmer and Keen 1986). Our studies provide *in planta* evidence supporting this hypothesis. First, increases in gene transcription levels for the pectic enzymes examined were associated with increases in tissue maceration. Second, tissue maceration slows down as the expression of these genes decreases, presumably due to self-catabolite repression (Collmer and Bateman 1981). Third, induction of all three enzymes is reduced under conditions incompatible with soft rot compared to conditions compatible with rot. Other regulatory mechanisms are probably important in pathogenesis and remain to be examined. For instance, other research suggests that low oxygen levels effect changes in plant defense responses (Antonov et al. 1990; Rumeau et al. 1990; Vayda and Schaeffer 1988) but do not effect production of pectic enzymes by the pathogen (Maher and Kelman 1983).

**Pectic enzymes are involved in plant-bacterial communication.** Our data also provide background for speculation concerning the hypothesis that pectic enzyme reaction products may function in host defense responses as well as in pathogenesis (Cervone et al. 1989; Hahn et al. 1989). Significant levels of endo-PL mRNA were maintained through 24 hr under conditions incompatible with soft rot but not under conditions compatible with rot. This result suggests that endo-PL may be available to produce oligogalacturonides active as inducers of host defense responses and is consistent with our earlier finding that PAL mRNA

![Graph](image_url)

**Fig. 5. In planta accumulation of mRNAs for *Erwinia carotovora* subsp. *carotovora* pectic enzymes induced by tuber exposure to *E. c. subsp. carotovora*, *exo*-pectate lyase (PL), *endo*-polygalacturonase (PG) and *endo*-polygalacturonase (PG) in compatible and incompatible interactions. Total bacterial RNAs were isolated from *Erwinia carotovora* subsp. *carotovora* cells inoculated on potato tubers in the membrane-separated system (see text) under compatible conditions favoring rotting or under incompatible conditions favoring resistance to rot where moisture was limited (see text). Times 0 represents the minimal broth plus glycerol-grown cells used for inoculation. RNA:DNA hybridization analyses were performed as described in Figure 3.**
continues to increase through 12 hr after E. c. subsp. carotovora inoculation under incompatible conditions but not under compatible conditions (Yang et al. 1989). That PAL mRNA levels were higher for E. c. subsp. carotovora-inoculated tubers at 6 hr than at 9 hr may indicate that endo-PL is not solely responsible for the induction of the host response.

E. c. subsp. carotovora pectate lyase(s) releases oligosaccharide elicitors from plant cell walls that induce plant defense responses, including phytoalexin accumulation in soybean (Davis et al. 1984, 1986). In those studies, the possibility that the actual inducer is a minor contaminant in oligogalacturonide or enzyme preparations cannot be eliminated (Collmer and Keen 1986). In our study, E. coli does not induce a plant defense response, but E. coli expressing a cloned E. c. subsp. carotovora endo-PL does induce PAL mRNA. Therefore, our results confirm the capacity of pectate lyase to elicit, probably via oligosaccharides, a plant defense response involving activation of defense-related genes. This is consistent with Davis and Ausubel’s (1989) studies showing that crude preparations of E. c. subsp. carotovora endo-PL increased expression of a number of plant defense genes including PAL and 4-coumarate:coenzyme A ligase genes. Our results also suggest that endo-PL reaction products may be responsible at least in part for the increased PAL mRNA and enzyme activity levels induced by E. c. subsp. carotovora (Yang et al. 1989). Although complete digestion of pectate by endo-PL results in unsaturated trimers to hexamers (Roberts et al. 1986b), incomplete digestion would result in production of oligomers with eight to 12 galacturonic acid residues, large enough to act as plant defense elicitors (Davis et al. 1986). Expression of endo-PG and exo-PL from plasmid pDR30 did not induce PAL mRNA, although endo-PG from fungal pathogens triggers plant defense responses (Cervone et al. 1989; Collmer and Keen 1986). E. c. subsp. carotovora endo-PG expressed by E. coli may lack the ability to release active elicitor or may not release sufficient amounts of elicitors to induce defense response. Because small oligogalacturonides do not have elicitor activity (Davis et al. 1986; Hahn et al. 1989), exo-PL, which produces unsaturated dimers and trimers (Roberts et al. 1986b), is not expected to induce defense responses, but it may cleave elicitor-active large oligogalacturonides to nonelicitor-active small oligogalacturonides. This is consistent with our results showing that exo-PL mRNA accumulation was rapid under compatible conditions but was absent early in the interaction under incompatible conditions.

On the other hand, exo-PL may also generate pectic oligomers as inducers of pectic enzymes. Reaction intermediates are effective for inducing pectic enzymes in vitro; the same mechanism has been proposed for in planta induction (Collmer and Bateman 1981, 1982; Collmer et al. 1982; Collmer and Keen 1986; Stack et al. 1980). We found that on wound-healed tuber slices, endo-PL mRNA was induced transiently. Because wound-healed tuber slices were resistant to degradation by E. c. subsp. carotovora enzymes, this suggests that enzyme reaction products are required for continuing in planta induction. Transient accumulation of endo-PL mRNA is probably the result of constitutive inducers present in the wound-healed tuber slice or of trace amounts of plant cell wall fragments accessible to the pathogen. Our results indicate that exo-PL activates genes for production of endo-PG, endo-PL, and endo-PL presumably by releasing inducers. This may also explain why Erwinia chrysanthemi Burkholder et al. mutants deficient in oligogalacturonide lyase macerate plant tissue as well as the wild-type (Collmer and Keen 1986); E. chrysanthemi exo-PL expression could also produce inducers, thus, bypassing the need for oligogalacturonide lyase, which is thought to be required for generation of pectic enzyme inducers from pectic oligomers (Collmer and Bateman 1981).

Our results confirm that bacterial pectic enzymes are critical in plant-bacterial communication in erwinia soft rot. On one hand, enzymes such as exo-PL are involved in producing reaction intermediates as inducers to mediate their own induction. On the other hand, enzymes such as endo-PL activate plant defense responses by releasing oligogalacturonide elicitors from plant cell walls. This supports the hypothesis of Hahn et al. (1989) that bacterial

![Fig. 6. Activation of potato phenylalanine ammonia-lyase (PAL) gene by Erwinia carotovora subsp. carotovora pectic enzymes. Potato tubers were sliced (row 1, top) and inoculated with Escherichia coli HB101 (row 2), or HB101 containing plasmid pDR1 encoding E. c. subsp. carotovora endo-PL (row 3), or pDR30 encoding E. c. subsp. carotovora exo-PL and endo-PG (row 4) and incubated at 30° C for 9 hr. Tuber slices inoculated with E. c. subsp. carotovora (Ecc) were incubated for 6 hr (row 5) or 9 hr (row 6). Following incubation, the top 1 mm of tuber tissue was sliced off and used for RNA isolation. PAL mRNA levels were determined by RNA slot blot hybridization according to Yang et al. (1989).](image-url)
pectic enzymes have dual and apparently opposing functions: macerating plant tissues and triggering defense responses. Because elicitor activity depends on the number of galacturonic acid residues, the amount of elicitor may be determined by the efficiency of pectic decomposition (Hahn et al. 1989). Cervone et al. (1989) suggest that the incomplete digestion of pectate by endo-PL (optimal activity at pH 8–10) at the nonoptimal physiological pH of plant surfaces or tissues (pH 6 or lower) results in release of elicitor-active oligogalacturonides. However, their hypothesis did not consider the combined action of endo-PG (optimal activity pH ≤6) and endo-PL that, as indicated by our results, are both present at some periods during pathogenesis. Further, E. coli strains expressing high levels of a single E. chrysanthemi endo-PL successfully macerate plant tissues (Keen and Tamaki 1986), suggesting that high levels of endo-PL overcome plant defense responses. We propose that in planta expression of various pectic enzymes also plays an important role in shifting between the dual functions of pectic enzymes, thus affecting the outcome of soft-rot interactions (see Fig. 7). Studies using mutants that affect in planta expression of these enzymes would confirm this hypothesis. Furthermore, characterizations of in planta expression of other enzymes including various PL isoforms will provide important insight into the complex interaction of erwinia pectic enzymes with plants.

ACKNOWLEDGMENTS

We are grateful to Dr. D. R. Dean of Virginia Polytechnic Institute and State University (VPI & SU) for his generous gift of the E. coli ribosomal protein gene probe and to Mr. L. S. Antonov and Mrs. V. K. Stromberg for access to their unpublished sequence data on endo-PL, endo-PL, and endo-PG. We thank Dr. J. D. Eisenback for his assistance with the photographic work and Mrs. Stromberg for her critical review of this manuscript. Part of this work was supported by a USDA competitive grant (85-CRCR-1-1776) to G. H. Lacy.

LITERATURE CITED


