

## System for Simultaneous Study of Bacterial and Plant Gene Expression in Soft Rot of Potato

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An experimental system using potato tuber slices and *Erwinia carotovora* subsp. *carotovora* was developed to study simultaneous *in planta* regulation of bacterial and plant genes in soft rot interactions. This system involves inoculation of tuber slices with *E. c.* subsp. *carotovora* separated from host tissue by a biologically inert polysulfone membrane. This arrangement gives reproducible development of tissue maceration and permits rapid and quantitative isolation of bacteria free of plant cells and debris and isolation of bacteria-free plant cells. Similar tissue maceration and plant and bacterial gene induction occurred in membrane-separated versus direct-contact interactions. Northern hybridization showed increases in *E. c.* subsp. *carotovora* pectate

lyase (PL) mRNA levels 4 hr after inoculation with maximum levels at 9 hr. PL activity, monitored by isoelectric-focused activity overlays, was induced within 2 hr and increased through 24 hr and was consistent with mRNA transcription and maceration patterns. Host defense responses were monitored by phenylalanine ammonia-lyase (PAL) activity and mRNA levels. Tuber mRNAs (2.4 to 2.6 kb) hybridized with bean PAL cDNA sequences. Induction of PAL enzyme activity and mRNA levels was greater and more rapid in tuber slices inoculated with *E. c.* subsp. *carotovora* compared with noninoculated slices suggesting that *E. c.* subsp. *carotovora* triggers active host defense responses superimposed upon wound responses.

*Erwinia* soft rots are destructive to numerous crop plants in storage and in the field. These bacteria produce diverse plant cell-degrading enzymes (CDEs) involved in soft rot pathogenesis (Collmer and Keen 1986; Kotoujansky 1987). These enzymes include pectate lyases (PLs), polygalacturonases, pectin lyases, cellulases, phospholipases, and proteases. Research has focused on molecular and genetic characterization of genes encoding these enzymes from several soft-rotting *Erwinia* species (Collmer and Keen 1986; Roberts *et al.* 1986a; Allen *et al.* 1987; Kotoujansky 1987). PLs, encoded by *pel* genes, are among the best studied and most important CDEs in soft rot (Collmer and Keen 1986; Kotoujansky 1987).

Studies using *pel* genes cloned in plasmids in *Escherichia coli* (Miguela) Castellani & Chalmers and PL nonproducing *Erwinia* mutants have generated conflicting results on the role of PL in pathogenicity. *E. coli* strains containing *pelE* or *pelC* from *Erwinia chrysanthemi* Burkholder *et al.* produce tissue maceration suggesting that a single PL is sufficient to cause disease (Collmer and Keen 1986; Keen and Tamaki 1986). In *Erwinia carotovora* subsp. *carotovora* (Jones) Bergey *et al.* strain EC14, however, Roberts *et al.* (1986b) showed that more than one cloned pectic enzyme is required for maceration. Pathogenicity tests of specific *E. chrysanthemi* *Pel*<sup>-</sup> mutants generated by insertion-directed mutagenesis indicate that some PL isozymes are more important in pathogenesis than others (M. Boccara, A. Diolet, M. Rouve, and A. Kotoujansky. Role of the different pectate lyase isozymes in the virulence of *Erwinia chrysanthemi*. Presented at the Fourth International Symposium of Molecular Genetics of Plant-Microbe Interactions, Acapulco, Mexico, May 15-20, 1988.) and that deletion of all secreted PLs eliminates most but not all maceration ability (Ried and Collmer 1988).

Apparently, regulation of pectic enzymes plays a role in soft rot pathogenicity (Collmer *et al.* 1982; Kotoujansky 1987; Zucker and Hankin 1970). Knowledge of *in planta* enzyme regulation would aid in understanding the significance of PLs as well as other CDEs in soft rot pathogenesis. Unfortunately, little is known about *in planta* regulation of CDEs although *in vitro* regulation of pectic enzymes is under investigation (Reverchon and Robert-Baudouy 1987; Zucker *et al.* 1972). Our ignorance in this area is due largely to lack of techniques for *in planta* studies. Gene fusion using *lacZ* or *lux* genes as reporter genes has been used to study gene regulation in phytopathogenic bacteria (Reverchon and Robert-Baudouy 1987; J. Shaw and C. Kado, molecular genetics of plant-microbe interactions *in vivo*, presented at the Fallen Leaf Lake Conference on the Genus *Erwinia*, Fallen Leaf Lake, CA., U.S.A., September 17-20, 1987). Isolation of enzymes from rotting plant tissues and enzyme-specific antibodies have also been used (Quantick *et al.* 1983; Thurn *et al.* 1987). However, none of the techniques has been successfully applied to quantitative studies of *in planta* CDE regulation. A major obstacle to studying soft rot interactions is that the bacterial populations are intermixed with macerated tissue, rendering it difficult to recover sufficient number of bacteria or their pathogenicity components *in planta* to conduct quantitative analyses.

Due to the complexity of most plant-pathogen interactions, an experimental system allowing simultaneous analysis of both pathogen and host responses is clearly advantageous to understanding key molecular events. Although significant progress has been made in identifying pathogenicity-related factors in *erwiniae*, the capacity of plants to respond to these pathogens has received less attention. This is due in part to the common view that soft rot *erwiniae* are only opportunistic or weak pathogens or even saprophytes due to their tendency to attack stressed tissues (Pérombelon and Kelman 1980) and thus show less

potential for contributing to our understanding of plant resistance mechanisms.

Several lines of evidence, however, suggest that potato is capable of mobilizing active defense responses to erwiniae. Accumulation of antibacterial phenolics and sesquiterpenoid phytoalexins is correlated with potato resistance to soft rot under high oxygen levels or low temperatures (Ghanekar *et al.* 1984; Lyon 1984). Recently, Austin *et al.* (1988) transferred resistance to soft rot from wild, non-tuber-bearing *Solanum brevidens* Phil. into *S. tuberosum* L. through somatic hybridization. Tubers from these hybrids and subsequently from sexual progeny also expressed resistance. To monitor the expression of active defense responses in our potato (*S. tuberosum*) tuber system, we followed the induction of phenylalanine ammonia-lyase (PAL). PAL catalyzes the first committed step in phenylpropanoid biosynthesis leading to production of phenolics and lignin, compounds important in resistance to soft rot (Tripathi *et al.* 1976) and to fungal pathogens in a variety of plant species (Collinge and Slusarenko 1987; Dixon *et al.* 1986). In potato leaves, PAL mRNA and enzyme activities are induced rapidly in response to *Phytophthora infestans* deBary (Fritzscheier *et al.* 1987). PAL is also induced by wounding, but mRNA induction kinetics are distinct for wound- versus pathogen-induction (Lawton and Lamb 1987). The plant-pathogen system described here allows reproducible harvests of sufficient amounts of responding plant tissue, free of bacteria, for detailed kinetic analysis of PAL induction at the enzyme and mRNA levels.

In testing our *E. c.* subsp. *carotovora*-tuber system, we obtained answers to the following questions:

- 1) Is soft rot pathogenesis similar if the *E. c.* subsp. *carotovora* inoculum is or is not separated from the tuber by a biologically inert membrane?
- 2) Can we observe *in planta* induction of bacterial pathogenicity-related genes?
- 3) Can we observe *in planta* induction of genes related to plant defense?

## MATERIALS AND METHODS

### Bacterial strains, potato tubers, and their preparation.

*E. c.* subsp. *carotovora* strain EC14 was cultured in minimal medium supplemented with 1% (v/v) glycerol (Zucker and Hankin 1970) in an orbital shaker (250 rpm at 30° C for 12 hr) until a turbidity of 1.0 to 1.3 at OD<sub>550 nm</sub> was reached. This suspension served as the inoculum source. Tubers (*S. tuberosum* cv. Russet Burbank) purchased from local markets were scrubbed in household dish detergent with a soft brush, rinsed in deionized water, air-dried, and incubated overnight at 30° C. Before inoculation, they were soaked in 0.5% sodium hypochlorite for 60 sec, rinsed several times with deionized water, and air-dried in a laminar flow hood at room temperature (22° to 26° C).

**Inoculation and harvest of bacterial cells and plant tissues.** Potato tubers were cut into 1-cm-thick slices and placed in petri dishes on sterile Whatman No. 1 filter paper (Whatman, Maidstone, England) wetted with 3 ml of sterile water. For "membrane-separated" interactions, polysulfone membranes (GA-8S, 0.2 µm pore size, Gelman Sciences, Ann Arbor, MI) cut to the size of tuber slices were laid over the tuber slices. Each membrane was saturated with

1 ml of sterile deionized water and spread with 600 µl of the *E. c.* subsp. *carotovora* inoculum. Dishes containing inoculated tuber slices were supported on plastic rods above a layer of water in closed plastic chambers, incubated at 30° C, and aerated and humidified by bubbling air through the water (70 to 90 cc/min). For "direct-contact" interactions, 600 µl of bacterial suspension was spread directly on tuber slices in petri dishes and incubated as described above. These conditions resulted in compatible interactions with tissue maceration in the membrane-separated interaction equivalent to that in the direct-contact interaction. To obtain an incompatible interaction (negligible maceration), a slight modification of the incubation conditions was made in the membrane-separated interaction. The lid of the incubation chamber was left ajar to facilitate evaporation; 1 ml of sterile water was added to the membrane every 2 hr to prevent membrane drying. As wound response controls, tuber slices with or without membranes were spread with sterile minimal medium plus 1% glycerol instead of the bacterial suspension and incubated as described above.

At various times after inoculation, bacteria were washed off the membrane or the surface of the inoculated tuber slices with RNA isolation solution (50 mM glucose, 10 mM EDTA, and 25 mM Tris HCl, pH 8.0). Two to six membranes or tuber slices were collected for each time point, depending upon the magnitude ( $1.5 \times 10^7$  to  $3.2 \times 10^9$  colony forming units [cfu] per membrane) of bacterial growth on the membrane or tuber slice. The bacteria were pelleted by centrifugation at  $10,000 \times g$  for 60 sec and frozen at -75° C for later RNA isolation.

Plant tissue was also harvested at various times following inoculation. Macerated tissue was washed away using a stream of deionized water and the top 1 mm of intact tuber tissue was sliced off, frozen in liquid nitrogen, and stored at -75° C for RNA isolation.

**RNA isolation.** Total bacterial RNA was isolated by a modified procedure of Krol *et al.* (1982); frozen bacterial cells were resuspended in 1.2 ml of RNA isolation buffer and lysed by adding 120 µl of lysis buffer (0.5 M Tris HCl, 0.2 M EDTA, and 10% sodium dodecyl sulfate [SDS], pH 8.0). Lysates were extracted immediately with 1.32 ml phenol mixture (phenol:cresol:8-hydroxyquinoline; 1000:140:0.4, w/w). RNA was precipitated with 0.1 volume of 3 M sodium acetate and 2.5 volumes of ethanol at -20° C overnight. Precipitate was collected by centrifugation at  $13,000 \times g$  at 4° C for 15 min and washed with 70% ethanol. RNA pellets were dried *in vacuo* and resuspended in 10 to 30 µl of water. RNA concentration was estimated by absorbance at 260 nm. DNA contamination was removed by incubating 20 µg of RNA with 10 units of RNase-free DNase I (Bethesda Research Laboratories [BRL], Gaithersburg, MD) for 30 min at 37° C. RNA treated with DNase I was used immediately for northern blot analysis as described below.

Total plant RNA was isolated from 5 g of potato tissue homogenized directly in a phenol:Tris buffer (pH 9.0) emulsion as described by Haffner *et al.* (1978). The final RNA concentration was determined by absorbance at 260 nm. Where noted, poly(A)<sup>+</sup> RNA was isolated from total potato RNA according to Kingston (1987) using at least two cycles of oligo-dT cellulose adsorption (Boehringer Mannheim, Indianapolis, IN).

**Northern hybridization analysis.** To monitor changes in bacterial mRNA levels, 20  $\mu\text{g}$  of bacterial RNA treated with DNase I from each treatment or time point was denatured by glyoxylation (Williams and Mason 1985), separated on 1% agarose gels in 10 mM  $\text{NaH}_2\text{PO}_4$  buffer, and blotted onto nylon membranes (Nytran, Schleicher and Schuell, Inc., Keene, NH) by capillary transfer. Following transfer, the membranes were baked (2 hr at 80° C) and prehybridized overnight at 42° C in prehybridization buffer (80% formamide, 5 $\times$  Denhardt's solution, 5 $\times$  SSC, 10 mM  $\text{NaH}_2\text{PO}_4$ , 1% SDS, 5 mM EDTA, and 100  $\mu\text{g}/\text{ml}$  of denatured sheared salmon sperm DNA [Sigma, St. Louis, MO]). Hybridization was carried out for 48 hr in the same solution with the addition of formamide to a final concentration of 80% and denatured  $^{32}\text{P}$ -labeled *E. c. subsp. carotovora* PL DNA sequences. The PL probe was prepared from a 2.1-kilobase (kb) *Pst*I-*Eco*RI fragment from pDR1 (Roberts *et al.* 1986a) by random-primer labeling according to the supplier's protocol (Random Primer Kit, BRL). Following hybridization, membranes were washed in 0.1 $\times$  SSC at 65° C for 60 min and exposed to film.

To monitor induction of potato PAL mRNA levels, 20  $\mu\text{g}$  of total RNA or 2  $\mu\text{g}$  of poly(A)<sup>+</sup> RNA was glyoxylated and used for northern transfer as described above. Prehybridization and hybridization conditions and times were identical to those used for bacterial RNAs, with the exception that hybridization was carried out in 50% formamide and  $^{32}\text{P}$ -labeled PAL cDNA sequences were used. The  $^{32}\text{P}$ -labeled PAL probe was generated by random-primer labeling from a 1.8-kb *Pst*I fragment of pPAL5 (Edwards *et al.* 1985) containing PAL cDNA sequences from bean (*Phaseolus vulgaris* L.). Final wash conditions were 0.2 $\times$  SSC at 50° C for 60 min prior to autoradiography.

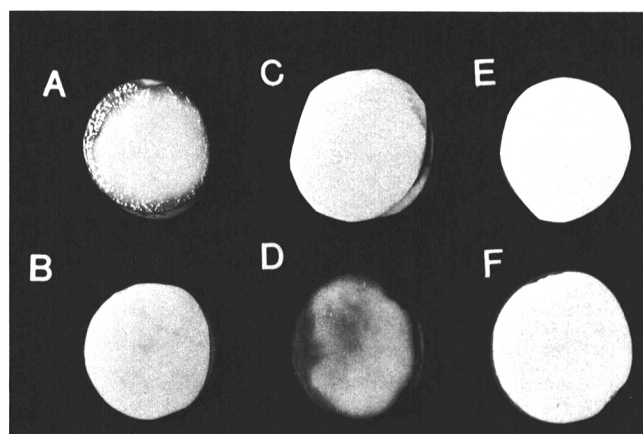
**Isoelectric focusing and PL activity overlay.** Isoelectric focusing and PL activity overlay of *E. c. subsp. carotovora* extracellular enzymes were performed as described by Ried and Collmer (1985). Samples consisted of supernatant (that is, extracellular components) collected from 10<sup>6</sup> bacterial cells washed from inoculated tuber slices or polysulfone membranes for each time point. Following incubation at 30° C for 30 min, the overlay gel was stained with 0.05% ruthenium red. The sizes of clear zones revealing relative amounts of enzyme activity were compared visually.

## RESULTS

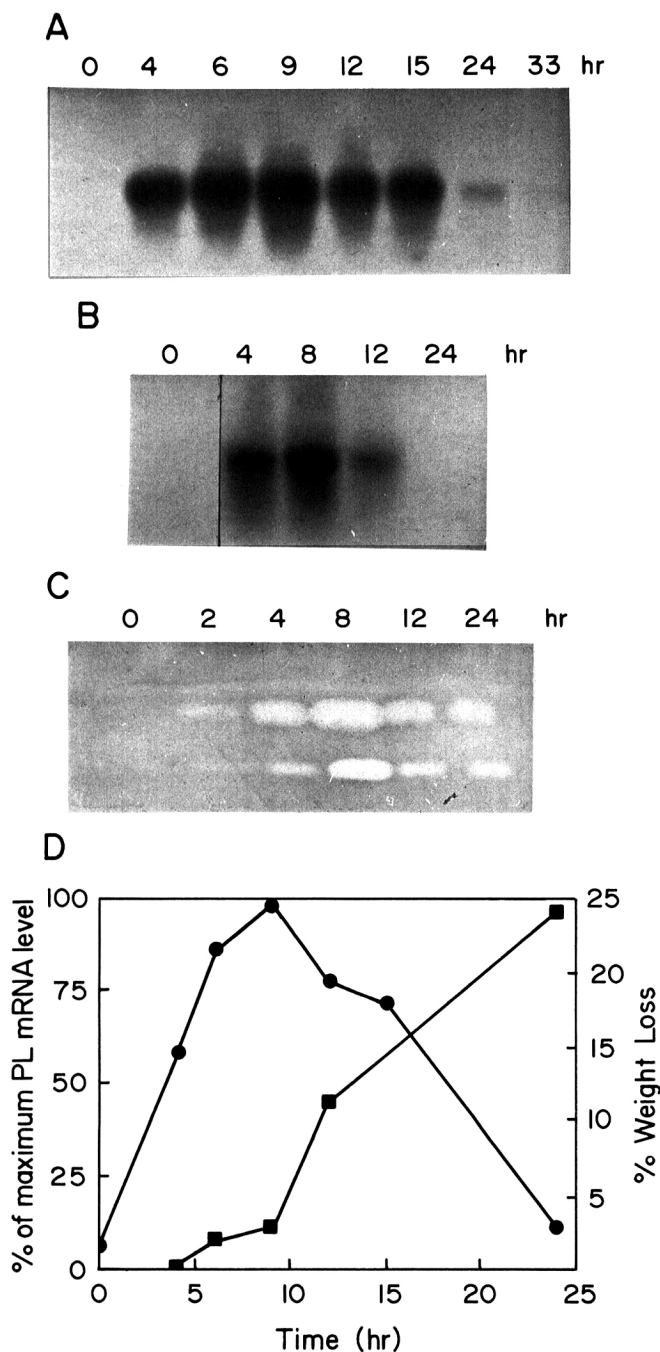
**Soft rot pathogenesis in membrane-separated interactions.** Soft rot pathogenesis was compared using two systems: potato tuber slices were inoculated with an *E. c. subsp. carotovora* suspension directly (direct-contact interaction) or indirectly by using an inert polysulfone membrane to separate the tuber from the bacteria (membrane-separated interaction). When membrane moisture was maintained, the membrane-separated interaction produced rotting equivalent to the direct-contact interaction (Fig. 1). On inoculated membranes, bacterial populations increased from  $1.5 \times 10^7$  cfu per membrane at the time of inoculation to  $3.2 \times 10^9$  cfu per membrane after 24 hr incubation. Initial tissue maceration was visible 6 hr after inoculation and substantial rotting was evident by 24 hr (Fig. 2D). The degree of maceration

can be affected by varying chamber humidity or membrane moisture (data not shown). Similar bacterial suspensions, spread on membranes placed on agar plates (plate count agar, Difco, Detroit, MI), showed substantial growth after 24 hr incubation at 30° C but did not contaminate the agar; no growth occurred on the agar even with continued incubation for 48 hr after the membrane was removed. Thus, tissue maceration in membrane-separated interactions is due to diffusion of *E. c. subsp. carotovora* pathogenicity factors and not to inoculation of bacterial cells through the membrane. No tissue maceration occurred on control tuber slices inoculated with minimal medium instead of bacterial suspension. Further, minimal medium, spent *E. c. subsp. carotovora* culture filtrates, or cultures of *E. coli* strain HB101 did not induce a defense response of tuber slices in addition to the wound response (data not shown).

**In planta regulation of bacterial pathogenicity-related genes.** Expression of PL was used as a marker for bacterial response under compatible conditions. Total *E. c. subsp. carotovora* RNA was isolated from cells recovered from inoculated membranes or tuber slices and used for northern blot hybridization. The immobilized RNA was hybridized with the labeled 2.1-kb *Pst*I-*Eco*RI fragment encoding endo-pectate lyase (PL) cloned from *E. c. subsp. carotovora* in pDR1. A single mRNA class of approximately 1.4 kb hybridized to the probe (Fig. 2A). Hybridization using a fragment internal to the PL encoding sequences (350-bp *Hinc*II-*Hinc*II fragment) gave equivalent results (data not shown). At time 0, a weak signal was observed only after prolonged autoradiography and represents the basal level of PL expression that had been observed previously (Collmer *et al.* 1982). A much stronger signal was evident within 4 hr after inoculation. Levels of mRNA reached a maximum at 8 to 9 hr and decreased to basal levels by 24 hr. The induction patterns were similar when bacteria were in direct contact with tuber tissues (Fig. 2B) or when the bacteria and host were separated by a polysulfone membrane (Fig. 2A). Kinetics of PL mRNA accumulation



**Fig. 1.** Comparison of soft rot pathogenesis models. Rotting of potato tuber slices was tested by inoculating tuber slices directly with 600  $\mu\text{l}$  of *Erwinia carotovora* subsp. *carotovora* suspension or by physical separation of inoculum from the tuber with a polysulfone membrane. Observations were made 24 hr after inoculation. **A**, Direct-contact interaction. **B**, Tuber slice inoculated with minimal medium plus glycerol. **C**, Membrane-separated interaction. **D**, Membrane removed from C 24 hr after inoculation. **E**, Membrane inoculated with minimal medium plus glycerol. **F**, Membrane removed from E at 24 hr.



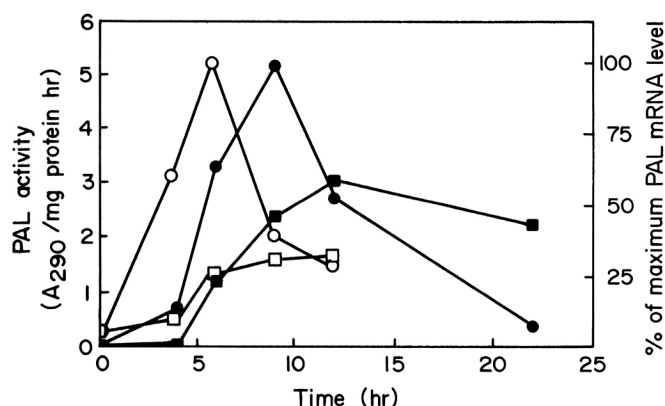
**Fig. 2.** *In planta* kinetics of pectate lyase (PL) mRNA induction in the membrane-separated interaction (A) or in the direct-contact interaction (B). Bacteria were harvested at the times indicated after tuber slice inoculation with *Erwinia carotovora* subsp. *carotovora*. PL mRNA was detected by northern blot hybridization to the  $^{32}$ P-labeled 2.1-kb fragment of pDR1 encoding an *E. c.* subsp. *carotovora* PL (see text). C, *In planta* PL activity in the membrane-separated interaction. At various times after inoculation, bacteria were washed off the membrane, viable cells estimated by plate count, and extracellular supernatant focused isoelectrically (IEF) on thin-layer acrylamide gels supported on plastic (0.35 mm, pH 3 to 11). Supernatant collected from  $10^6$  cells for each time point was applied to a polyacrylamide IEF gel, and PL activities were revealed by agarose polygalacturonate gel overlay stained with ruthenium red (see text). Two major PLs were induced with pIs 10.5 (upper) and 9.5 (lower), respectively. D, Correlation of PL mRNA levels and tissue maceration. Percent maximum mRNA levels were determined densitometrically from autoradiographs of northern blot shown in A (filled circles). Percent tuber tissue macerated was determined by weight loss after washing rotted tissue off tuber slices standardized for weight at various times after inoculation (filled squares).

was correlated with tissue maceration (Fig. 2D).

To establish that the PL mRNA induction observed in our system was due to the interaction of *E. c.* subsp. *carotovora* and potato, two experiments were performed. In the first, PL mRNA levels were analyzed from *E. c.* subsp. *carotovora* grown continuously in minimal medium plus 1% glycerol. A very small increase in PL mRNA levels was observed at the onset of the stationary growth phase, similar to the early stationary growth phase peak of PL activity reported for *E. chrysanthemi* (Collmer and Bateman 1982). In the second experiment, *E. c.* subsp. *carotovora* cultures placed on moistened polysulfone membranes in the absence of tuber slices showed a similar early stationary (or 2 to 4 hr post-inoculation) increase in PL mRNA levels. However, *in planta* induction of PL mRNA levels in the presence of tuber slices exceeded the peak levels seen in these two experiments by greater than 20-fold (data not shown).

*In planta* induction of PL enzyme activity was also observed during membrane-separated compatible interactions. Two PLs (pIs 9.5 and 10.5) were induced as early as 2 hr after tuber inoculation (Fig. 2C). The peak of the induction was at 8 hr, which is consistent with expression of PL mRNA. Induction of the pI 10.5 PL was stronger and more rapid than that of the pI 9.5 PL.

**PAL as a marker for host defense responses.** Analysis of PAL and enzyme activity and mRNA levels was used to determine whether *E. c.* subsp. *carotovora* triggers a defense response in potato tubers incubated under compatible conditions. PAL enzyme activity was examined in potato tissues wounded by slicing or wounded and challenged with *E. c.* subsp. *carotovora* (Fig. 3). PAL activity was induced within 4 hr of *E. c.* subsp. *carotovora* inoculation, reached a maximum at 9 hr, and dropped to



**Fig. 3.** Effects of wounding and *Erwinia carotovora* subsp. *carotovora* inoculation on phenylalanine ammonia-lyase (PAL) enzyme activity and mRNA levels. Plant tissues were harvested at various times after wounding (by slicing) or *E. c.* subsp. *carotovora* inoculation (direct contact, compatible conditions). PAL activity (*E. c.* subsp. *carotovora*, filled circles; wounding, filled squares) was determined as described by Lawton *et al.* (1983). PAL activity is defined as units of absorbance at 290 nm per hour per milligram of protein of tissue homogenate. To determine levels of potato PAL mRNA induction (*E. c.* subsp. *carotovora*, open circles; wounding, open squares), 5  $\mu$ g of total RNA was immobilized to a Nytran membrane using Millipore slot blot and hybridized to  $^{32}$ P-labeled bean PAL cDNA. Relative mRNA levels were measured by densitometry following autoradiography.



the basal level by 24 hr. Wounding alone also induced PAL activity, but the induction was later and less pronounced compared to *E. c. subsp. carotovora* induction.

Total RNA was isolated from potato tubers wounded or challenged directly with *E. c. subsp. carotovora*. Northern hybridization analysis detected an inducible species of poly(A)<sup>+</sup> RNA of 2.4 to 2.6 kb that cross-hybridized to bean pPAL5 cDNA sequences (Fig. 4). PAL mRNA levels were induced by all treatments but were greater and accumulated more rapidly in response to *E. c. subsp. carotovora* inoculation compared to wounding (Fig. 3 and 5). Direct inoculation induced transient mRNA accumulation with maximum at 6 hr; wounding resulted

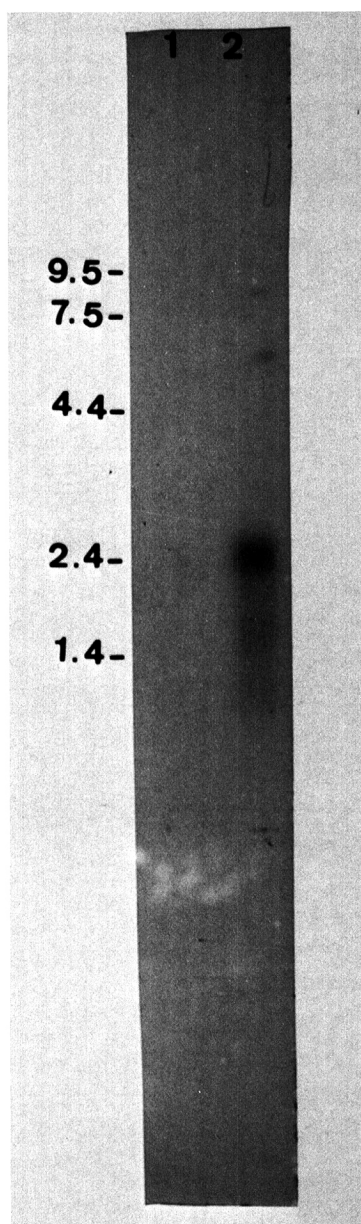
in slow increases in PAL mRNA through 12 hr.

**PAL induction in membrane-separated system.** We reasoned that PAL may show greater induction in an incompatible interaction, so membrane-separated plant responses were monitored under reduced moisture conditions. Total RNA was isolated from tuber slices inoculated with bacteria separated from the tuber by polysulfone membranes. Northern hybridizations showed increases in PAL mRNA levels within 4 hr and continued accumulation through 12 hr (Fig. 5, lanes 8 through 10). In this experiment, tissue maceration was negligible after 12 hr. PAL activity was consistent with the mRNA induction and was not induced by the membrane alone (data not shown).

## DISCUSSION

We have developed a membrane-separated system for studying molecular interactions of potato and *E. c. subsp. carotovora* in soft rot pathogenesis. We are able to obtain comparable compatible (maceration) or incompatible (no maceration) interactions by manipulating environmental conditions. We have established *in planta* induction kinetics for a gene related to bacterial pathogenicity (PL) and a gene related to plant defense (PAL). The membrane facilitates harvest of bacterial cells and plant tissues but clearly does not interfere with maceration or any pathogen- or host-derived signals required for molecular interactions and gene expression.

Results using this system allow a number of conclusions to be drawn concerning molecular events in soft rot pathogenesis. First, *in planta* regulation of *E. c. subsp.*



**Fig. 4.** Hybridization of potato poly(A)<sup>+</sup> RNA to bean phenylalanine ammonia-lyase (PAL) cDNA sequences. Two micrograms of total poly(A)<sup>+</sup> RNA from untreated tuber tissue (lane 1) or tuber tissue 6 hr after direct inoculation with *Erwinia carotovora* subsp. *carotovora* (lane 2) was used for northern blotting. The hybridization probe was <sup>32</sup>P-labeled bean pPAL5 insert sequences. Molecular size determinations utilized a single-stranded RNA ladder (Bethesda Research Laboratories).



**Fig. 5.** Northern hybridization of potato phenylalanine ammonia-lyase (PAL) mRNAs induced by wounding and inoculation with *Erwinia carotovora* subsp. *carotovora*. Potato tuber slices were untreated (lane 1), treated with minimal medium plus glycerol as a wound control (lanes 2-4), inoculated directly with *E. c. subsp. carotovora* (lanes 5-7), or inoculated with *E. c. subsp. carotovora* separated by a polysulfone membrane (lanes 8-10) for the times indicated. Twenty micrograms of total RNA was immobilized on northern blots and hybridized to <sup>32</sup>P-labeled bean pPAL5 cDNA sequences. The RNA in lane 4(\*) was apparently degraded; subsequent analysis of the RNA in lanes 2-4 shows that PAL mRNA levels at 12 hr were slightly greater than those seen at 9 hr.

*carotovora* PLs showed that two major PLs were induced by potato tuber tissues. Induction of these enzymes is correlated closely with tissue maceration, supporting the role of these enzymes in soft rot pathogenesis. The kinetics of the extracellular PL activities is consistent with that of mRNA induction, suggesting that expression of *pel* genes in *E. c.* subsp. *carotovora* is not subject to translational or secretional regulation, at least for the gene encoding the pI 9.5 isozyme.

Second, *E. c.* subsp. *carotovora* elicits a host defense response in potato distinct from a wound response. PAL mRNA and enzyme activity were elevated in response to *E. c.* subsp. *carotovora* in both compatible and incompatible interactions. Active host response is a common feature of plant-microbe interactions involving more specialized pathogens and is characterized by a rapid, transient activation of defense-related genes including PAL (Collinge and Slusarenko 1987; Cramer *et al.* 1985, 1986; Dixon *et al.* 1986; Lawton *et al.* 1983). Our results with potato and *E. c.* subsp. *carotovora* show that a similar response occurs in soft rot, although *E. c.* subsp. *carotovora* is often assumed to be a weak pathogen or a saprophyte on compromised hosts. Comparison of PAL induction kinetics in *E. c.* subsp. *carotovora* interactions versus wounding clearly demonstrates that *E. c.* subsp. *carotovora* triggers a host response more rapid and intense than the wound response. That PAL mRNA accumulation induced by *E. c.* subsp. *carotovora* also results when a membrane is placed between the tuber slice and the bacterium suggests that the inducer is diffusible or is produced by the interaction of diffusible CDEs on the host plant. It has been shown that oligomers released from soybean (*Glycine max* (L.) Merr.) cell wall polymers by *Erwinia* enzymes are able to trigger plant defense responses (Ryan 1987).

Soft rot *Erwinia*-plant interactions in nature may involve delicate interregulation of their gene products or metabolic products. The fate of soft rot pathogenesis might be determined by the rate of synthesis of bacterial CDEs and the rate of activation of plant defense genes. The system described here allows reproducible production of pathogenesis and simultaneous isolation of bacterial and plant components generated during host-pathogen interactions without cross-contamination of plant tissues and bacterial cells. This system will be useful for answering questions such as:

- 1) Which enzymes are induced *in planta*?
- 2) Are the kinetics of the induction correlated with soft rot development?
- 3) Is there sequential or simultaneous induction among various genes related to bacterial pathogenicity and plant defense?
- 4) What environmental factors influence regulation of host and pathogen responses?
- 5) What host and bacterial factors are required for triggering compatible or incompatible interactions?
- 6) Do different host plants elicit differential regulation of bacterial pathogenicity factors?

In combination with genetic manipulation such as site-specific mutagenesis, the membrane-separated system will provide valuable insight into the significance of *in planta* regulation of CDEs in *Erwinia* soft rot pathogenesis and will be a powerful tool for discovering components of the interaction that trigger plant defense responses and

expression of bacterial pathogenicity genes.

Now that resistant potato germ plasm is available (Austin *et al.* 1988), arrangements have been made to use our system to compare molecular responses in compatible and incompatible interactions under similar environmental conditions (J. Helgeson, University of Wisconsin, Madison, personal communication with G. H. L.).

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