The Plant Cell, Vol. 1, 275–284, March 1989

Regulation of the *Aspergillus nidulans* Pectate Lyase Gene (*pelA*)

Ralph A. Dean[a] and William E. Timberlake[a,b,1]

[a]Department of Plant Pathology, University of Georgia, Athens, Georgia 30602
[b]Department of Genetics, University of Georgia, Athens, Georgia 30602

Aspergillus nidulans **pectate lyase was purified from culture filtrates. The enzyme catalyzed a random eliminative cleavage reaction, had an apparent molecular weight of 40,000, and a pI of 4.2. Pectate lyase antisera were produced and used to identify pectate lyase clones in a cDNA expression library. Thirteen of 14 clones identified immunologically cross-hybridized. The identity of the single-copy pectate lyase gene, which we designated *pelA*, was confirmed in two ways. First, several cDNA clones expressed pectate lyase activity in *Escherichia coli*. Second, targeted mutation of the gene in *A. nidulans* resulted in complete loss of enzyme activity. *pelA* encodes a 1,300-nucleotide mRNA that was present in cells grown with polygalacturonic acid as carbon source but absent from cells grown with glucose or acetate as carbon source. Thus, pectate lyase expression is regulated at the level of mRNA accumulation.**

INTRODUCTION

Degradation of pectin, a major component of the primary plant cell wall and middle lamella, has long been proposed to be a requirement for penetration and colonization of plant tissues by pathogens. Pectin-degrading enzymes such as polygalacturonase and pectate lyase are often the first extracellular degradative enzymes produced during infection (Jones et al., 1972; Mankarios and Friend, 1980). In addition to their primary degradative activities, these enzymes make cell walls more susceptible to further breakdown by other enzymes (Bauer et al., 1973, Keegstra et al., 1973). Pectolytic enzymes isolated from pathogenic organisms have been shown to cause tissue maceration and host cell death (Bateman and Basham, 1976; Cooper, 1983; Keon et al., 1987). Moreover, development of disease symptoms correlates with enzyme levels in infected tissues (Wijesundera et al., 1984; Holz and Knox-Davies, 1985; Marcus et al., 1986). Although this type of circumstantial evidence indicates that pectolytic enzymes are pathogenic determinants, their proposed importance in at least some plant-pathogen interactions is controversial (Puhalla and Howell, 1975; Howell, 1976).

Application of molecular genetic techniques to bacterial pathogens has permitted direct evaluation of the role of pectolytic enzymes in disease development. The soft-rotting bacterium *Erwinia chrysanthemi* produces a number of pectolytic enzymes. Strain EC16 produces four pectate lyase isozymes, designated PLa, b, c, and e (Collmer and Keen, 1986; Collmer, 1987). The corresponding genes, when expressed in *Escherichia coli*, confer on the organism the ability to cause at least limited maceration of potato tubers (Keen and Tamaki, 1986). Reid and Collmer (1988) demonstrated that deletion of all four genes from *E. chrysanthemi* EC16 resulted in a significant reduction in virulence. Thus, pectate lyase is an important virulence factor for *E. chrysanthemi* and pectate lyase genes can confer pathogenicity on other species.

Even though fungi are a considerably more diverse and economically important group of plant pathogens than bacteria, the role of pectin-degrading enzymes in fungal pathogenesis remains unclear (Keon et al., 1987). It is likely that elucidation of the mechanisms controlling expression of pectolytic enzymes in fungi will increase our understanding of fungal pathogenesis. However, the sophisticated molecular genetic techniques one would like to bring to bear on the problem are only now being developed for phytopathogenic fungi (Turgeon et al., 1985; Parsons et al., 1987; Rodriguez and Yoder, 1987).

We demonstrate in the accompanying paper that *Aspergillus nidulans* (=*Emericella nidulans*), a saprophytic Ascomycete, produces significant amounts of polygalacturonase and pectate lyase in culture and has limited phytopathogenic ability (Dean and Timberlake, 1989). Expression of these enzymes is induced by substrate and repressed by preferred carbon sources, as is the case with other fungi (e.g. Cooper and Wood, 1975; Cooper 1983). One possible explanation for the low virulence of *A. nidulans* is that pectolytic enzyme production is completely repressed by endogenous sugars present in wounded

[1] To whom correspondence should be addressed.

plant tissues. Catabolite repression has been reported as a mechanism of disease resistance in some plant-pathogen interactions (Horton and Keen, 1966; Patil and Dimond, 1968; Holz and Knox-Davies, 1986a, 1986b). As a first step toward understanding the mechanisms regulating pectolytic enzyme production and altering patterns of pectolytic gene expression during plant infection, we have isolated and characterized the pectate lyase gene (*pelA*) from *A. nidulans*. We show here that the gene is unique in the genome, contains at least two introns, and codes for 1300-nucleotide poly(A)$^+$ mRNA. *pelA* expression is highly regulated at the level of mRNA accumulation.

RESULTS

Pectate Lyase Purification and Antibody Production

The strategy we adopted to isolate the *A. nidulans* pectate lyase (PL) gene was to purify the enzyme, raise antibodies, and then use the antibodies to screen a cDNA expression library. PL was purified from culture filtrates of *A. nidulans* grown for 3 days with polygalacturonic acid as sole carbon source (Dean and Timberlake, 1989). Filtrates were passed over a DEAE-Sephadex column and enzyme was eluted with a linear NaCl concentration gradient. A major peak of activity eluted at ~250 mM NaCl, whereas a minor peak of activity eluted at ~400 mM NaCl (Figure 1A). The major activity peak was fractionated by chromatography on Bio-Gel P-100 (Figure 1B) and fractions were analyzed by SDS-PAGE (Figure 1C). PL activities in the fractions directly correlated with the abundance of a 40-kD protein. A sample of the most active fraction from the Bio-Gel P-100 column was subjected to isoelectric focusing under nondenaturing conditions. A single protein band (pI=4.2) had PL activity and, after elution, a single protein of M_r 40,000 was observed on SDS-PAGE gels (data not shown). These results strongly suggested that the 40-kD protein shown in Figure 1C was PL.

The partially purified PL was used to determine the mechanism of transelimination by comparison of the fraction of Na^+ polygalacturonic acid glycosidic bonds cleaved with the reduction in solution viscosity. Cleavage of only 0.9% of glycosidic bonds led to a 50% reduction in viscosity (data not shown), indicating that *A. nidulans* PL makes random, internal cleavages in the polymeric substrate.

Antibodies were raised against the putative PL protein. Figure 2 shows that the PL antiserum reacted strongly with the 40-kD protein and weakly with a few other lower molecular weight bands. Preimmune serum did not react with *A. nidulans* proteins.

Isolation and Analysis of PL cDNA Clones

A bacteriophage λ cDNA library was constructed by using poly(A)$^+$ RNA from cells grown with polygalacturonic acid as sole carbon source (see "Methods"). Fourteen putative PL clones were identified in this collection by screening

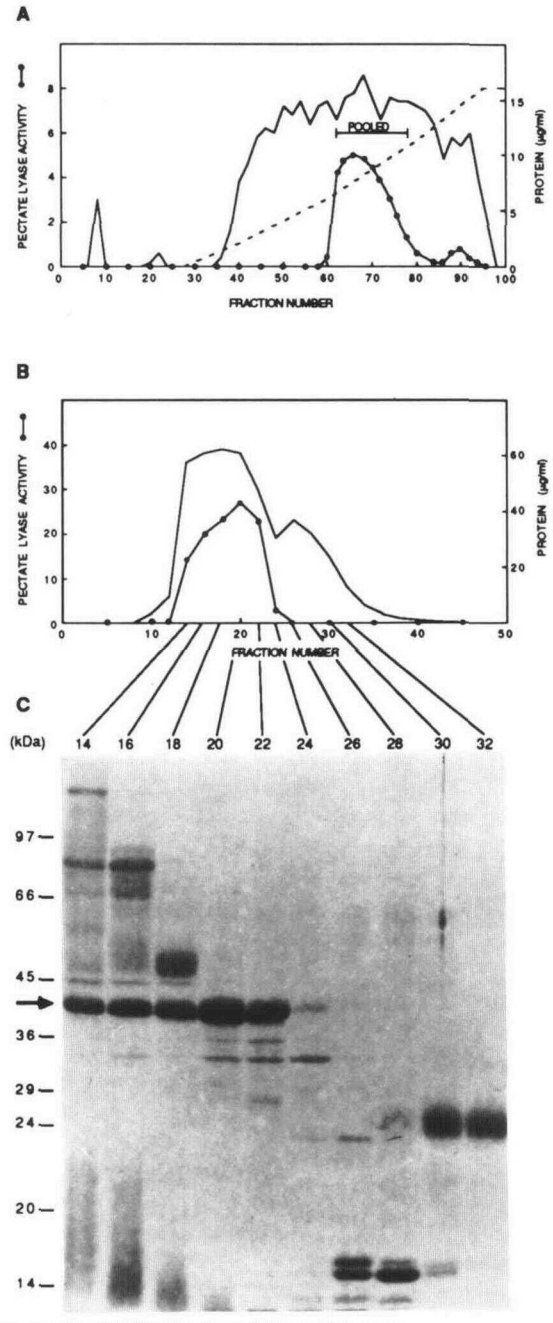

Figure 1. Partial Purification of Pectate Lyase.

(A) A concentrated culture filtrate was applied to a DEAE-Sephadex G50 column and the column was eluted with a 0.0 to 0.5 M NaCl gradient (– – –). Protein concentrations in the fractions are shown (——). Enzyme activity (●—●) was determined by the TBA assay (see "Methods") and fractions containing PL activity were pooled as indicated.

(B) The pooled fractions from **(A)** were concentrated and fractionated on a Bio-Gel P-100 column.

(C) Equal volumes of the indicated fractions were subjected to denaturing polyacrylamide gel electrophoresis and gels were stained with Coomassie Brilliant Blue R-250. The arrow in **(C)** indicates the position of the proposed PL band.

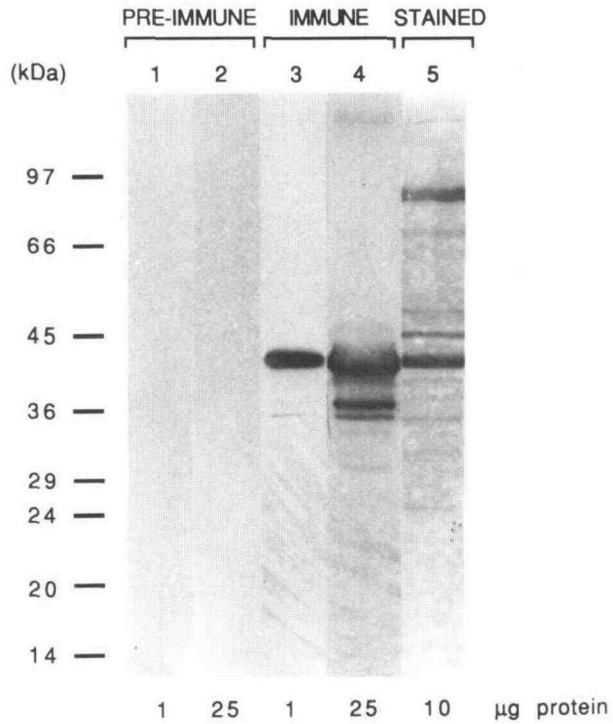

Figure 2. Protein Gel Blots Probed with PL Antiserum.

The indicated amounts of extracellular protein were fractionated by polyacrylamide gel electrophoresis and transferred to nitrocellulose, and membranes were incubated with either preimmune or anti-PL rabbit serum, as indicated. Bound IgG was detected by reaction with horseradish peroxidase-conjugated goat anti-rabbit IgG. Lane 5 shows a gel lane containing 10 µg of protein stained with Coomassie Brilliant Blue R-250.

with PL antiserum. The clones were rescued as Bluescript plasmids and digested with EcoRI, and a gel blot was hybridized with radiolabeled pRD091 DNA. Figure 3 shows that 13 of the 14 cDNA inserts (0.5 to 1.1 kb) cross-hybridized. The plasmid with the largest insert (pRD171) did not cross-hybridize, had a restriction map unrelated to the other clones, and was not studied further.

Table 1 shows that 4 of the 14 clones produced PL activity in *E. coli*. Activity was confined to cells harboring plasmids with ≥1 kb inserts, except for pRD171, which produced no activity. When gel blots of *A. nidulans* DNA digested with one of several restriction enzymes were hybridized with pRD091 (1.0 M NaCl, 68°C) and washed at low stringency (0.3 M NaCl, 21°C), single bands were detected, indicating that the gene is present once in the genome (data not shown).

Isolation of the *A. nidulans* PL Gene

pRD091 was restriction mapped, as seen in Figure 4, and used as a hybridization probe to identify the corresponding clone in an *A. nidulans* cosmid bank. One positive clone, pL30C5, was obtained by screening 3000 cosmids. The region containing the putative PL gene was subcloned into pUC18 as a 5.5-kb BamHI fragment to yield pRD301. A restriction map of this region is given in Figure 5A. To establish formally that pRD301 contained the PL gene (which we designate *pelA*) and to determine what proportion of PL activity produced in culture was due to expression of the gene, we inactivated it by site-directed mutagenesis (Miller et al., 1985). The polarity and approximate position of the *pelA* transcription unit shown in Figure 5A was determined from the restriction map of pRD091. Plasmid pRD303 was constructed by replacing a 1.4-kb SalI fragment containing the 3′ half of *pelA* with a fragment containing the *A. nidulans trpCΔB* gene (Figure 5A; see Yelton et al., 1985). The 8.3-kb BamHI fragment was isolated from the plasmid and used to transform a *trpC*⁻ *A. nidulans* strain (FGSC237) to tryptophan prototrophy. DNA was isolated from 20 transformants and digested

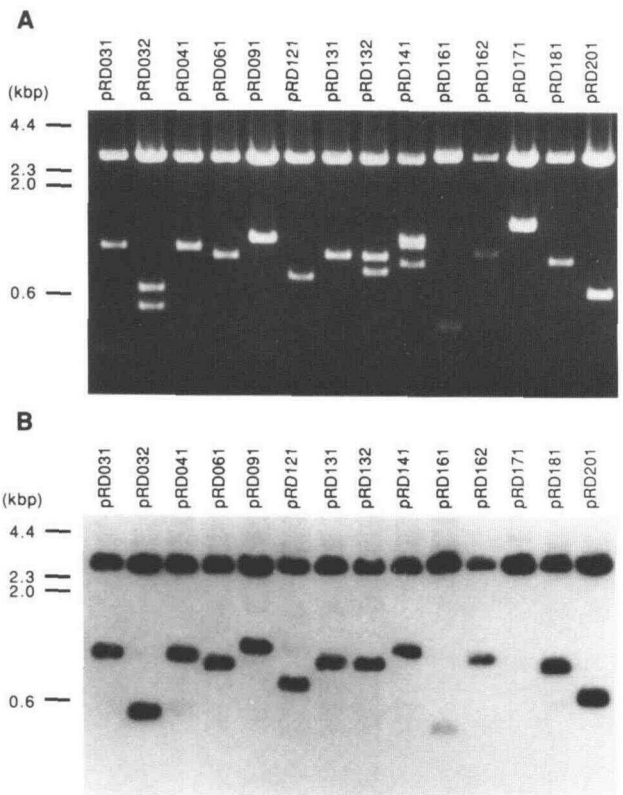

Figure 3. Hybridization Analysis of PL cDNA Clones.

(A) Bluescript plasmids containing cDNA inserts encoding PL cross-reacting material were digested with EcoRI, fractionated by electrophoresis in 1% agarose gels, and stained with ethidium bromide.

(B) Gel blots were hybridized with radiolabeled pRD091 DNA. Only the pRD171 insert failed to hybridize with the probe.

Table 1. Pectate Lyase Activity Expressed by cDNA Clones in *E. coli*

Clone	Pectate Lyase Activity[a]	Length of cDNA Insert
		bp
pRD031	39.5	1000
pRD032	0.0	550
pRD041	885.3	1000
pRD061	0.0	900
pRD091	9.6	1100
pRD121	0.0	700
pRD131	0.0	900
pRD132	0.0	900
pRD141	556.1	1050
pRD161	0.0	500
pRD162	0.0	950
pRD181	0.0	850
pRD201	0.0	600
pRD171	0.0	1350

[a] Pectate lyase activity = $\Delta A_{550\ nm}$/hr/μg of protein of the cell-free extract as determined by the TBA assay.

with BamHI, and gel blots were hybridized with diagnostic probes. DNA from 12 transformants, represented by TRD016 in Figure 5, B to D, produced hybridization patterns consistent with replacement of the wild-type *pelA* gene with the *pelA::trpCΔB* fragment (Figure 5). TRD016 lacked the 1.4-kb SalI fragment that had been deleted in pRD303 (Figure 5B). PL cDNA hybridized with a 5.5-kb fragment in FGSC237 and with the expected 8.3-kb fragment in TRD016 (Figure 5C). A *trpC* probe hybridized with the resident *trpC*$^-$ allele in the recipient and transformant and with the 8.3-kb BamHI fragment in the transformant (Figure 5D). The differences in the relative intensities of the signals from the *pelA::trpCΔB* and the *trpC*$^-$ fragments may have resulted from unequal blot transfer or from tandem reiteration of the inserted fragment. In any event, no intact copies of *pelA* were present in TRD016, as demonstrated in Figure 5, B and C.

Three transformants were tested for their ability to produce PL. TRD016 and TRD008 have an inactivated *pelA* gene, whereas TRD014 does not. All strains grew equally well, even though no PL was detected in the filtrates of TRD008 or TRD016, as opposed to the wild-type levels produced by TRD014 (Table 2). Polygalacturonase production was unaffected by *pelA* inactivation. These results show that *pelA* encodes *A. nidulans* PL and that PL is not essential for growth on polygalacturonic acid.

pelA Contains Two Introns

The restriction maps of *pelA* cDNA and genomic clones had minor inconsistencies. For example, several restriction fragments from pRD911 (the pRD091 cDNA insert recloned in pUC13) were slightly smaller than equivalent fragments from the genomic clone pRD301, as shown in Figure 6A. Similar results were obtained with different cDNA clones, indicating that they were not the result of a cDNA cloning artifact. Our interpretation of the data is presented in Figure 6B. The results suggest that there are at least two small (<100 bp) introns in *pelA*, one to the left and one to the right of the EcoRV site near the middle of the gene.

Regulation of *pelA* mRNA Levels

We have shown that *A. nidulans* PL activity is induced by polygalacturonic acid and is subject to catabolite repression, but catabolite repression is not relieved by the *creA204* mutation (Dean and Timberlake, 1989). We determined *pelA* mRNA levels in the wild-type and a *creA204* strain to determine how they correlated with enzyme levels. RNA was isolated from cells after transferring them from medium containing glucose as carbon source to medium containing polygalacturonic acid or polygalacturonic acid plus glucose as carbon source, and gel blots were hybridized with radiolabeled pRD091. Results are shown in Figure 7A. No *pelA* mRNA was detected in cells grown with glucose (or acetate) as sole carbon source. By contrast, a 1300-nucleotide transcript accumulated when cells were transferred to medium containing polygalacturonic acid as sole carbon source or polygalacturonic acid plus glucose. The *creA204* strain produced very high levels of *pelA* mRNA when grown with polygalacturonic acid as sole carbon source. Both strains produced *pelA* mRNA with the mixed carbon source, but only after glucose had been expended (data not shown; see Dean and Timberlake, 1989). Accumulation of PL activity in the growth medium (Figure 7B) was correlated with mRNA levels. However, *pelA* mRNA often became detectable before PL activity appeared in the medium and transcript levels in the *creA204* strain grown with polygalacturonic acid were disproportionately high. Genomic clone pRD301 hybridized

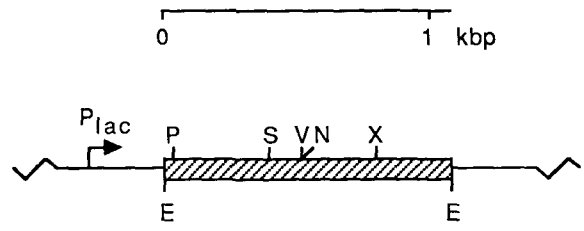

Figure 4. Partial Restriction Map of pRD091.

Abbreviations used are B, BamHI; E, EcoRI; V, EcoRV; N, NcoI; S, SalI; X, XhoI; *Plac*, *lacZ* promoter.

only with *pelA* mRNA, indicating that it is not tightly linked to other genes.

DISCUSSION

Pectin-degrading enzymes are produced by many pathogens during invasion of plant tissues (Cooper, 1983; Keon et al., 1987). Compelling genetic evidence for the role of these enzymes in pathogenesis has recently been obtained for disease caused by a soft-rotting *Erwinia* species (Collmer, 1987; Reid and Collmer, 1988). Similar evidence is not available for fungal pathogens because the genes encoding pectolytic enzymes have not previously been cloned and used to alter patterns of enzyme production. We showed that the saprophytic Ascomycete *A. nidulans* produces high levels of polygalacturonase and pectate lyase (PL) in culture and exhibits limited phytopathogenic potential (Dean and Timberlake, 1989). Pectin-degrading enzymes that cleave internally, as does *A. nidulans* PL, are considered to be most important in pathogenesis because they are effective at macerating plant tissues (Cooper, 1983; Collmer, 1987). We have taken advantage of the *A. nidulans* molecular genetic system to clone and then inactivate the PL structural gene *pelA*, opening new avenues for investigating the role of PL in fungal pathogenesis.

Unlike the *Erwinia pel* genes, *A. nidulans pelA* is physically and functionally unique in the genome. Two PL activities were separable by DEAE-Sephadex chromatography, but both were lost following disruption of *pelA*. Therefore, it is likely that these activities are allomorphic. The existence of only one or a few copies of genes simplifies molecular genetic analysis of their functions.

Growth on polygalacturonic acid and polygalacturonase activity was unaffected by *pelA* disruption (Table 2). We have shown that polygalacturonase alone is sufficient for growth of *A. nidulans* on polygalacturonic acid at low pH in high buffered medium (Dean and Timberlake, 1989). In moderately buffered medium, polygalacturonase accumulation precedes that of PL. PL only accumulates when the pH of the medium increases and becomes conducive to PL activity. Therefore, it is not surprising that, under the growth conditions employed in this study, inactivation of *pelA* did not seriously affect growth of the fungus on polygalacturonic acid. We have not determined the effect of *pelA* inactivation on growth at elevated pH. We predict that simultaneous disruption of the genes encoding polygalacturonase and PL will lead to an inability to utilize polygalacturonic acid as carbon source and perhaps decreased pathogenicity.

The *pelA* gene appears to contain at least two short introns (Figure 6), although the exact locations and sizes of the introns are unknown. Genes with multiple small introns appear to be common in *A. nidulans* (e.g. Oakley et al., 1987; May et al., 1987) and other filamentous fungi (Ballance, 1986).

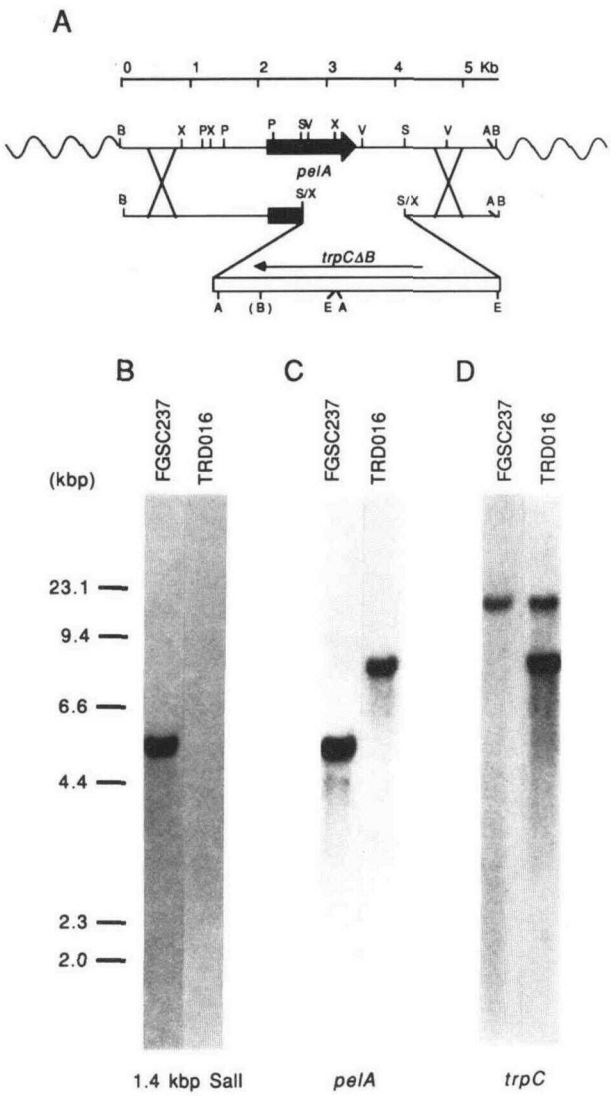

Figure 5. Disruption of *pelA*.

(A) Depiction of chromosomal (upper line) integration of the 8.3-kb BamHI fragment from pRD303 (lower line), in which the 3′ end of *pelA* has been replaced by a restriction fragment containing *trpCΔB*. Restriction sites are as in the legend to Figure 4 except A=XbaI.

(B) to **(D)** Gel blot analysis of DNA from FGSC237 and a *pelA*::*trpCΔB* transformant. DNA samples were digested with BamHI and fractionated in 0.8% agarose gels. Gel blots were hybridized with a radiolabeled 1.4-kb SalI fragment containing the 3′ end of *pelA* **(B)**; the 1.1-kb EcoRI insert from cDNA clone pRD091 **(C)**, or pTA11 containing the *trpC* gene **(D)**.

pelA codes for a 1300-nucleotide poly(A)$^+$ mRNA that accumulates in the presence of inducer (polygalacturonic

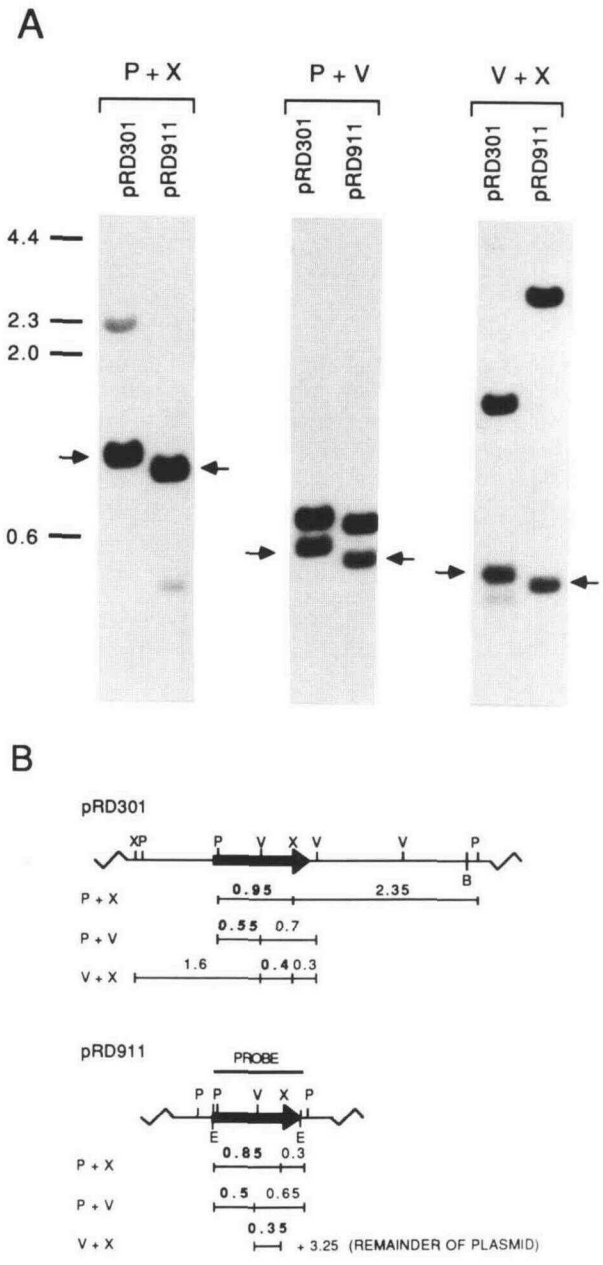

Figure 6. Restriction Analysis of *pelA* cDNA and Genomic Clones.

(A) DNA of pRD301, containing a genomic *pelA* insert, and pRD911, containing a cDNA insert, was digested with PstI and XhoI (P+X), PstI and EcoRV (P+V) or EcoRV and XhoI (V+X) and fractionated by electrophoresis in 1% agarose gels. Gel blots were hybridized with the 1.1-kb EcoRI fragment from cDNA clone pRD091. pRD911 contained the same insert as pRD091, but cloned into pUC13.

(B) Schematic summary of the data shown in **(A)**. Numbers in bold type represent internal gene and cDNA fragments; corresponding bands are indicated by arrows in **(A)**.

acid). The size of the mRNA is consistent with the M_r of the protein. No mRNA or pectate lyase activity was detectable when the organism was grown in medium containing only preferred carbon sources such as glucose or

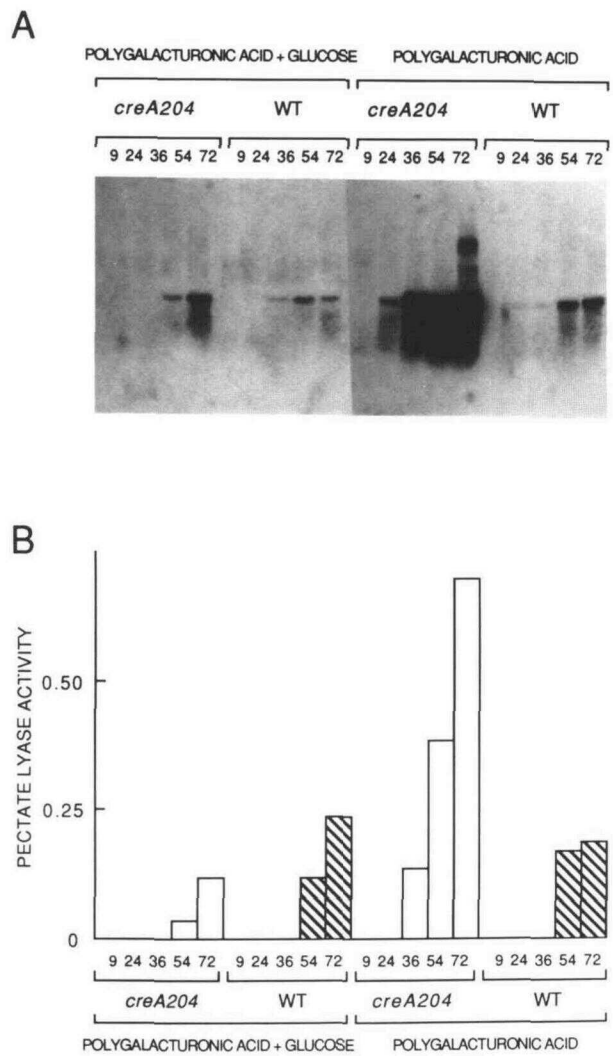

Figure 7. Regulation of *pelA* Transcript Levels in Wild-Type and *creA204* Strains.

(A) Total RNA was isolated from a relevant wild-type strain (RD502; WT) and a *creA204* strain (RD514). The cells were first germinated for 14 hr in glucose-containing medium, transferred to medium containing polygalacturonic acid alone or polygalacturonic acid plus glucose, and incubated for the times (hr) indicated. RNA samples were fractionated by electrophoresis in formaldehyde-agarose gels and blots were hybridized with radiolabeled cDNA clone pRD091. Equal loading of the samples was confirmed by staining duplicate gels with ethidium bromide (data not shown). **(B)** PL activity was assayed in the dialyzed culture filtrates (TBA assay).

Table 2. Polygalacturonase and Pectate Lyase Activities Produced by *A. nidulans pelA::trpCΔB* Transformants

	Pectate Lyase Activity[a]		Polygalacturonase Activity[b]	
Strain	77 hr	99 hr	77 hr	99 hr
TRD008	0.00	0.00	0.48	0.25
TRD014	0.47	1.03	0.51	0.30
TRD016	0.00	0.00	0.53	0.38

[a] Pectate lyase activity = $\Delta A_{550\ nm}$/hr/ml of dialyzed culture filtrate as determined by the TBA assay.
[b] Polygalacturonase activity = $\Delta A_{509\ nm}$/hr/ml of dialyzed culture filtrate as determined by the TBA assay.

acetate. In medium containing both polygalacturonic acid and glucose, *pelA* mRNA began to accumulate only after glucose was depleted from the medium. Thus, *pelA* mRNA accumulation, like PL production, is carbon catabolite repressed. Interestingly, the *creA204* mutation resulted in overproduction of *pelA* mRNA by cells grown in medium containing polygalacturonic acid as sole carbon source, but had little or no effect on *pelA* mRNA levels in cells grown with glucose alone or glucose plus polygalacturonic acid as carbon source. If *creA* actually encodes a repressor of transcription, it should now be possible to construct *pelA* alleles that are not subject to catabolite repression. The fact that *pelA* mRNA and PL enzyme levels were not strictly correlated indicates that posttranscriptional control mechanisms play a role in regulation of extracellular enzyme levels.

The availability of *A. nidulans pelA* clones makes a number of experimental strategies for studying the effect of PL enzyme production on pathogenicity possible. For example, genetically marked co-isogenic $pelA^+$ and $pelA^-$ strains can be co-inoculated into plant tissues to test their relative aggressiveness (Dean and Timberlake, 1989). Similarly, the gene can be placed under the control of constitutive or inducible promoters (Adams et al., 1988) and the effects of its altered expression on pathogenicity can be examined. The availability of *A. nidulans pelA* should also facilitate isolation of homologous genes from pathogenic fungi. Many fungal genes having similar functions have been cloned by use of heterologous hybridization probes. Alternatively, now that $pelA^-$ *A. nidulans* strains exist, genes encoding pectate-degrading enzymes from other ascomycetous fungi may be isolable by their expression in *A. nidulans*. The "pda" phytoalexin detoxification gene from the pea pathogen *Nectria haematococca* was isolated by using this approach, proving its feasibility (Weltring et al., 1988). Obtaining genes encoding pectolytic enzymes from other fungi will make it easier to determine their roles in pathogenicity and virulence. In addition, comparisons of gene structure and regulation may provide information on the evolution of plant pathogenesis.

METHODS

Strains, Media, and Growth Conditions

A. nidulans strains are described in the accompanying paper (Dean and Timberlake, 1989). TRD008 and TRD016 (*pabaA1, yA2; pelA::trpCΔB*) and TRD014 (*pabaA1, yA2*) were obtained by transformation of FGSC237 with pRD303. Standard *A. nidulans* genetic techniques were used (Pontecorvo et al., 1953; Clutterbuck, 1974; Yelton et al., 1984). For determination of PL activities and purification of the enzyme, minimal medium containing 1% polygalacturonic acid and appropriate supplements was inoculated to a density of 1×10^5 conidia/ml and shaken at 300 rpm, 37°C. For studies of regulation of *pelA* expression, minimal medium containing 1% glucose and appropriate supplements was inoculated to a density of 1×10^6 conidia/ml and shaken at 300 rpm, 37°C, for 14 hr. Cells were harvested by filtration through Mira-Cloth (Behring Diagnostics), washed with minimal medium, divided, resuspended in minimal medium containing 1% Na^+ acetate, 1% glucose, 1% Na^+ polygalacturonic acid, or 1% glucose plus 1% Na^+ polygalacturonic acid, and incubated as above. Samples were taken at intervals for RNA isolation and PL activity determination.

Pectolytic Enzyme Assays

The thiobarbituric acid (TBA; Preiss and Ashwell, 1963) and Nelson-Somogyi reducing group (Nelson, 1944; Somogyi, 1952) assays were used to determine polygalacturonase and PL activities as described in the accompanying paper (Dean and Timberlake, 1989). PL activity was also measured by following loss of viscosity of polygalacturonic acid solutions and by spectrophotometric determination of concentrations of Δ4,5- unsaturated bonds (E_{235}=4600 M^{-1} cm^{-1}; Marcus et al., 1986).

Purification of Pectate Lyase

Procedures were carried out at 4°C. Four 1-l *A. nidulans* cultures were filtered through Whatman No. 1. $NH_4(SO_4)_2$ was added to the filtrate to 20% (w/v), precipitated material was removed by centrifugation and the supernatant fluid was concentrated to about 100 ml by ultrafiltration. This was dialyzed overnight against 2×1 l of 100 mM Tris-HCl, pH 8.0, and applied to a 2.2×25 cm DEAE Sephadex G-50 column equilibrated with 100 mM Tris-HCl, pH 8.0. The column was washed with 100 mM Tris-HCl and eluted at 1 ml/min with a 400-ml linear gradient of 0 to 500 mM NaCl in 100 mM Tris-HCl, pH 8.0. Fractions containing PL activity were combined, dialyzed overnight against deionized water, and lyophilized. The samples were redissolved in 50 mM Na^+ phosphate buffer, pH 7.0, and applied to a 1.5×15 cm Bio-Gel P-100 column. The column was developed with the same buffer. Samples of each fraction were subjected to electrophoresis in denaturing 10% polyacrylamide gels according to Laemmli (1970) and stained with Coomassie Brilliant Blue R-250. For non-denaturing isoelectric focusing, 4 to 6 pH range ampholites (Haake-Buchler Instruments) were used in 3 mm, 5% acrylamide tube gels. NaOH (0.1 M) and H_3PO_4 (0.1 M) were used as catholyte and anolyte, respectively. Gels were sliced into 0.5-cm segments that were eluted overnight in deionized water. The pH of the solutions was measured and PL activity was determined. Some gels were

directly subjected to SDS-PAGE as described by O'Farrell (1975). Protein concentrations were determined by the Bradford (1976) assay.

Immunological Techniques

Antibodies were produced by using a modification of the Knudsen (1985) method. Partially purified PL (0.5 to 1.0 mg of a 0.2 to 0.3 M NaCl DEAE Sephadex fraction) was subjected to electrophoresis in denaturing 10% acrylamide gels and electrophoretically transferred to nitrocellulose (Towbin et al., 1979). Proteins were visualized by staining with 0.3% Ponceau S. Following destaining with Tris-buffered saline (50 mM Tris-HCl, 200 mM NaCl, pH 7.4), the PL band was excised and the protein was dissolved in dimethyl sulfoxide. An equal volume of Freund's complete adjuvant was added and the mixture was emulsified and injected subcutaneously into New Zealand White rabbits. Two booster injections were given at 2-week intervals with similar amounts of protein dissolved in Freund's incomplete adjuvant. Nitrocellulose blots of protein gels were blocked with 5% nonfat dry milk suspended in TBS. After incubation with primary antiserum (1:250), antibody-antigen complexes were visualized by using horseradish peroxidase-conjugated goat anti-rabbit IgG as recommended by the supplier (Bio-Rad).

Nucleic Acid Isolation and Analysis

Poly(A)$^+$ RNA for construction of a cDNA library was isolated from mycelium grown for 60 hr in medium containing polygalacturonic acid as sole carbon source as described (Timberlake, 1986). Total RNA for time course studies was isolated as described (Adams et al., 1988). DNA was isolated as described (Yelton et al., 1984), except that cells were first lyophilized and then pulverized in microcentrifuge tubes. RNA and DNA were fractionated in denaturing and non-denaturing agarose cells, respectively. Nucleic acids were transferred to nylon membranes (Hybond-N, Amersham Corp.) and hybridized with radiolabeled DNAs as recommended by the membrane supplier. pSal*argB* (K. Miller and W.E. Timberlake, unpublished results) was used as an *argB*-specific probe. pTA11, containing a 4.2-kb XhoI fragment from the *A. nidulans trpC* region cloned into pIC19H (Marsh et al., 1984) was used as a *trpC*-specific probe (Miller et al., 1985; T. Adams and W. E. Timberlake, unpublished results). Other probes are described in "Results."

Construction and Screening of the cDNA Library

Double-stranded cDNA was synthesized by using a modification of the Gubler and Hoffman (1983) procedure. Moloney murine leukemia virus reverse transcriptase was used for first-strand synthesis and DNA polymerase I and RNase H were used for second-strand synthesis, essentially as described by D'Alessio et al. (1987). After addition of EcoRI linkers, cDNA was ligated with bacteriophage λ ZAP (Stratagene) arms and packaged in vitro with Gigapak-Plus (Stratagene) following the supplier's suggestions. The resultant library contained 1.2×10^6 recombinants. Approximately 10^5 plaque-forming units were screened by using PL antiserum (Huynh et al., 1985; Ausubel et al., 1987). λ ZAP cDNA clones were plated on BB4 *E. coli* cells and grown for 6 hr at 37°C. Nitrocellulose filters, previously soaked in 10 mM isopropyl-β-D-thiogalactopyranoside and air-dried, were placed on the developing plaques for 14 hr. Immunodetection procedures were as for gel blots, as described above. Recombinant phages were subjected to two rounds of plaque purification prior to induced excision and rescue as Bluescript phagemids (Stratagene; Short et al., 1988).

PL Activity Produced by cDNA Clones

XL1-Blue *E. coli* cells (Stratagene), harboring Bluescript phagemids with PL cDNA inserts, were grown in LB medium at 28°C for 16 hr. Isopropyl-β-D-thiogalactopyranoside was added to 10 mM and the cells were incubated for 2 hr. Cells were washed three times by centrifugation with 200 mM Tris-HCl, pH 8.8, and disrupted in the same buffer by sonication. Cell debris was removed by microcentrifugation and the supernatant fluid was used to assay for PL activity.

Construction of a *pelA::trpCΔB* Plasmid

pRD303, containing the *trpCΔB* disruption of *pelA*, was constructed by using standard procedures (Maniatis et al., 1982; Ausubel et al., 1987). *trpCΔB* is a functional allele in which the single BamHI site in the *trpC* locus has been eliminated by in vitro mutagenesis (Yelton et al., 1985). The PL cDNA clone pRD091 was used to isolate pL30C5 from an ordered Lorist2 (Gibson et al., 1987) cosmid library containing *A. nidulans* nuclear DNA inserts (R. Lynch, T. Adams, and W.E. Timberlake, unpublished results). A 5.5-kb BamHI fragment containing the *pelA* gene was subcloned into a chloramphenicol-resistant derivative of pUC13 (pUC13cmr; obtained from T. Schmidhauser and D. Helinski, University of California, San Diego) to create pRD301. The SalI site in the polylinker of the pUC13cmr plasmid used was destroyed by end filling catalyzed by Klenow DNA polymerase I. The *pelA* 3′ (1.4-kb) SalI fragment was replaced with the 4.2-kb XhoI fragment containing the *trpCΔB* gene from pAIT-2 (Miller et al., 1985).

ACKNOWLEDGMENTS

We thank our colleagues in the laboratory for their critical reviews of the manuscript and Michael Hynes for providing the *creA204* strain. This research was supported by grants to W.E.T. from the U.S. Environmental Protection Agency (with O. Yoder and H. Van Etten), U.S. Department of Agriculture/Central Research Grants Office, and National Institutes of Health.

Received January 12, 1989.

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