JOURNAL OF BACTERIOLOGY, June 1990, p. 3284–3289
0021-9193/90/063284-06$02.00/0

Vol. 172, No. 6

Molecular Cloning and Characterization of an *Erwinia carotovora* subsp. *carotovora* Pectin Lyase Gene That Responds to DNA-Damaging Agents†

J. L. McEVOY, H. MURATA, AND A. K. CHATTERJEE*
Department of Plant Pathology, University of Missouri, Columbia, Missouri, 65211

Received 2 January 1990/Accepted 20 March 1990

***recA*-mediated production of pectin lyase (PNL) and the bacteriocin carotovoricin occurs in *Erwinia carotovora* subsp. *carotovora* 71 when this organism is subjected to agents that damage or inhibit the synthesis of DNA. The structural gene *pnlA* was isolated from a strain 71 cosmid gene library following mobilization of the cosmids into a moderate PNL producer, strain 193. The cosmid complemented *pnl*::Tn*5* but not *ctv*::Tn*5* mutations. A constitutive level of PNL activity was detected in RecA$^+$ and RecA$^-$ *Escherichia coli* strains carrying the *pnlA*$^+$ gene on the high-copy-number plasmid pBluescript SK$^-$. Mappings of Mu dI1734 (Km *lac'ZYA*) insertions in *pnlA* and unidirectional deletion analyses allowed localization of the gene to approximately 1.4 kilobases of DNA. A typical *pnlA-lacZ* transcriptional fusion was inducible in a RecA$^+$ but not a RecA$^-$ derivative of strain 71. In contrast, the *pnlA-lacZ* fusion was not inducible in a RecA$^+$ *E. coli* strain. DNA sequences homologous to *pnlA* were detected in *E. carotovora* subsp. *carotovora* and *E. carotovora* subsp. *atroseptica* strains and in one of four *Erwinia rhapontici* strains but not in *Erwinia chrysanthemi*.**

Pectin lyase (PNL) is one of several tissue-macerating enzymes produced by the soft-rot *Erwinia* species (4, 5). PNL activity increases in many strains of soft-rot *Erwinia* following exposure to one of a number of DNA-damaging agents (9–11, 18, 19). Moreover, many plants contain DNA-damaging agents (1) that are believed to be inducers of PNL (20).

In *Erwinia carotovora* subsp. *carotovora* 71, PNL activity increased more than 100-fold in the presence of 500 ng of mitomycin C (MC) per ml (24). Cellular lysis and a bacteriocin, carotovoricin, were coinduced with PNL. To explain the coinduction of several phenotypes by DNA-damaging agents, Tsuyumu and Chatterjee (19) proposed that *pnl*, *ctv*, and temperate bacteriophages of the soft-rot erwiniae are induced by a mechanism similar to that of the SOS system of *Escherichia coli*. In the SOS system, DNA damage activates the product of *recA* gene, which cleaves LexA, the repressor of a number of unlinked genes (21, 22). In strain 71, it has been shown that a LexA-repressible SOS-like system exists (14) and that *recA* is required for PNL and carotovoricin production and for cellular lysis (24). The similarities between the SOS system of *E. coli* and the PNL and carotovoricin production in strain 71 notwithstanding, the *E. coli* LexA appeared not to affect the basal levels of PNL and carotovoricin production (14).

In this communication we report our studies on the isolation and localization of the PNL structural gene, *pnlA*; the construction of *pnlA-lacZ* transcriptional fusions; the *recA*-mediated transcriptional activation of *pnlA-lacZ*; and the occurrence of *pnlA* homologs in various species of soft-rot *Erwinia*. (A preliminary account of some of this work has been published elsewhere [13].)

* Corresponding author.
† Contribution no. 10,999 from the Missouri Agricultural Experiment Station.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture media. *E. coli* strains and plasmids used in this study are described in Table 1. The wild-type *Erwinia* strains used in the hybridization studies were obtained from S. H. DeBoer (*E. carotovora* subsp. *carotovora* 71 and 193; *E. carotovora* subsp. *atroseptica* 5, 12, 31, and 198), G. P. C. Salmond (SCRI193), the International Collection of Plant Pathogenic Bacteria (formerly at University of California, Davis; late M. P. Starr, curator) (*Erwinia rhapontici* 1, 102, 109, and 112), and our laboratory collection (*E. chrysanthemi* EC16 and EC183 [19]). AC5117 is a *recA*::Tn*5* derivative (24) and AC5122 is a *pnlA*::Tn*5* mutant (13) of strain 71. Bacterial strains were maintained on L agar (3) containing antibiotics (ampicillin, 50 μg/ml; gentamicin, 10 μg/ml; kanamycin, 50 μg/ml; spectinomycin, 50 μg/ml; or streptomycin, 100 μg/ml) when appropriate. Minimal medium was as previously described (3).

Induction of cultures for PNL activity. Cultures were grown in L broth at 28°C with shaking. During early log phase (A_{600} of 0.4 to 0.6), MC was added to a final concentration of 0.5 μg/ml unless otherwise stated. Cultures were harvested by centrifugation (12,100 × *g*, 5 min, 4°C) after 8 h or at the time specified. Supernatants were assayed for PNL activity without any further treatment. The cells were suspended in 1/10 of the original volume of 10 mM Tris hydrochloride (pH 7.0) and subjected to two 30-s pulses from a sonicator (Braunsonic 1510) at 60 W. These cell extracts were then centrifuged, and the supernatants were assayed for PNL activity.

Enzymatic assays. Quantitative PNL assays were performed as previously described (19) except that the reaction mixture was supplemented with 50 mM NaCl, which was found to stimulate PNL activity (unpublished data). The reaction mixture contained sample, 0.4% citrus pectin (Sigma Chemical Co., St. Louis, Mo.), 0.4 mM EDTA, 50 mM NaCl, and 22 mM Tris hydrochloride (pH 8.0). One unit of

TABLE 1. *E. coli* strains and plasmids

Strain or plasmid	Relevant characteristics	Source or reference
Strains		
DH5α	φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)*U169 recA1 thi-1*	Bethesda Research Laboratories
HB101	*pro leu thi recA* Str^r	24
MC4100	*araD139* Δ(*lacIPOZYA*) U169 *recA*$^+$ *thi* Str^r	2
M8820	Δ(*proAB-argF-lacIPOZYA*) *recA*$^+$ Str^r	2
POI1734	Mu dI1734 *ara*::(Mu cts) Δ(*proAB-argF-lacIPOZYA*) Km^r Str^r	2
Plasmids		
pRK2013	Mob$^+$ Tra$^+$ Km^r	6
pSF6	Mob$^+$ Spc^r Str^r	16
pBluescript SK$^-$ and SK$^+$	Ap^r	Stratagene
pAKC270	Mob$^+$ Pnl$^+$ Spc^r Str^r	pSF6 containing approx 40 kb of strain 71 genomic DNA; this study
pAKC271	Mob$^+$ Pnl$^+$ Spc^r Str^r	pSF6 containing approx 40 kb of strain 71 genomic DNA; this study
pAKC272	Mob$^+$ Pnl$^+$ Spc^r Str^r	*Eco*RI deletion derivative of pAKC270 harboring 4.5 kb of strain 71 DNA; this study
pAKC277	Mob$^+$ *pnlA-lacZ* Spc^r Str^r Km^r	Mu dI1734 *kan-lac* insertion in *pnlA* of pAKC272; this study
pAKC278	Pnl$^+$ Ap^r	3.4-kb *Eco*RI fragment of pAKC272 cloned into pBluescript SK$^-$; this study
pAKC279	Pnl$^+$ Ap^r	3.4-kb *Eco*RI fragment of pAKC272 cloned into pBluescript SK$^+$; this study
pAKC285	Pnl$^+$ Ap^r	*Exo*III-*Exo*VII deletion of pAKC279; this study
pAKC286	Pnl$^-$ Ap^r	*Exo*III-*Exo*VII deletion of pAKC279; this study
pAKC287	Pnl$^+$ Ap^r	*Exo*III-*Exo*VII deletion of pAKC278; this study
pAKC288	Pnl$^-$ Ap^r	*Exo*III-*Exo*VII deletion of pAKC278; this study

PNL is defined as the amount of enzyme that produces an increase in A_{235} of 1.0/min at 30°C. PNL-specific activity is defined as units of activity per milligram of culture protein. Total protein of bacterial cultures was determined with a protein reagent (BCA; Pierce Chemical Co., Rockford, Ill.) β-Galactosidase assays were performed by the method of Miller (15).

PNL agar plate assay. Cells were patched to L agar containing 500 ng of MC per ml and incubated at 28°C for 4 h. Each plate was then overlaid with an agarose-pectin mixture (5 ml) consisting of 1% citrus pectin, 0.4 mM EDTA, 100 μg of thimerosal per ml, 22 mM Tris hydrochloride (pH 8.0), and 0.7% agarose. Following an additional 15 h of incubation at 28°C, the plates were flooded with a 0.05% solution of ruthenium red. Colonies which had produced PNL were surrounded by a dark halo.

DNA techniques. Total DNA was isolated as previously described (17). The procedures for the isolation of plasmid DNA, electrophoresis, restriction digestion, ligation, nick translation, and hybridization have been described by Maniatis et al. (12). Alkaline blotting of DNA from agarose to nylon membranes was performed by the method of Whitford and DiCioccio (23).

Cloning of *pnlA*. A cosmid gene library was constructed by ligating *Sau*3A partially digested chromosomal DNA from strain 71 into the *Bam*HI site of pSF6 (13, 16); the DNA were packaged in lambda particles and used to transduce *E. coli* HB101. The bank was then mobilized into the moderate PNL-producing *E. carotovora* subsp. *carotovora* strain, 193, by using the transfer functions of pRK2013 (6). Transconjugants were selected on minimal glucose agar containing spectinomycin and streptomycin. Since hyperproduction of PNL in strain 193 could not be detected over the background activity of the agar plate assay, transconjugants were screened for a change in the PNL phenotype by using the quantitative PNL assay described above.

Subcloning and deletion analysis of *pnlA*. Cosmid pAKC270 was partially digested with *Eco*RI, religated with T4 ligase per the instructions of the manufacturer (Promega Corp., Madison, Wis.), and transformed into HB101. Transformants carrying strain 71 insert DNA were then mated with the *pnl*::Tn*5* strain AC5122, and the transconjugants were screened for their Pnl phenotype. One resulting Pnl$^+$ plasmid, pAKC272, containing 4.5 kilobases (kb) of strain 71 DNA, was selected for subsequent analysis.

A 3.4-kb *Eco*RI restriction fragment of pAKC272 was isolated from low-melting-point agarose (SeaKem) and ligated to the *Eco*RI site of pBluescript SK$^-$ (Stratagene, La Jolla, Calif.). *E. coli* DH5α cells were then transformed with the ligation mix, and white ampicillin-resistant transformants were selected on Luria agar containing ampicillin and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside. Plasmids from transformants were analyzed by restriction analysis for the presence of insert DNA. Plasmids containing *E. carotovora* subsp. *carotovora* DNA were used in the transformation of the Pnl$^-$ strain AC5122. Pnl phenotypes of the constructs were determined by using the PNL agar plate assay.

Further localization of the *pnl* gene involved the construction of 5′-to-3′ unidirectional deletions within the 3.4-kb *Eco*RI fragment in pBluescript SK$^+$ and SK$^-$ vectors. This was performed essentially as described by Henikoff (7), except that *Exo*VII was substituted for S1 nuclease and used per the instructions of the manufacturer (Bethesda Research Laboratories, Inc., Gaithersburg, Md.).

Construction of *pnlA-lacZ* fusions. *pnlA-lacZ* operon fusions were constructed with the mini-Mu–*lac* element, Mu dI1734, previously described by Castilho et al. (2). Briefly,

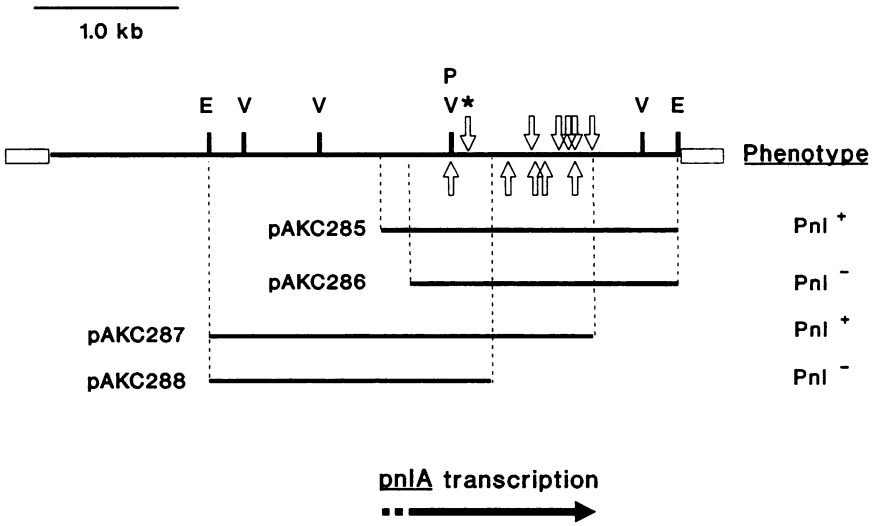

FIG. 1. Restriction endonuclease map of plasmid pAKC272 and schematic representation of insertions and deletions in *pnl* DNA. Insert DNA of pAKC272 (approximately 4.5 kb) is represented by a solid black line. The open boxes represent adjacent pSF6 vector DNA. Mu dI1734 insertions inactivating *pnlA* are represented by the open arrows. Arrows above the line depict insertions in the orientation such that β-galactosidase is induced by MC. Arrows below the line depict insertions in the opposite orientation such that β-galactosidase is not inducible by MC. The location and direction of transcription of *pnlA* are indicated by the bold arrow at the bottom of the figure. Abbreviations: E, *Eco*RI; V, *Eco*RV; P, *Pvu*II; *, *pnlA-lacZ* insertion (on plasmid pAKC277) used in subsequent studies. The 3.4-kb *Eco*RI fragment of insert DNA is not cleaved by *Bam*HI, *Bgl*II, *Cla*I, *Hin*dIII, *Pst*I, *Pvu*I, or *Sal*I.

the *pnlA*-carrying plasmid pAKC272 was transformed into an *E. coli* strain that was lysogenic for Mu *c*ts and carried the mini-Mu–*lac* element Mu dI1734. After heat induction, the Mu mixed lysate containing mini-Mu plasmid cointegrates was used to infect a Mu c^+ $RecA^+$ *E. coli* strain, M8820, and clones that exhibited resistance to kanamycin and spectinomycin were selected. Individual plasmids were then transferred to the *pnlA*::Tn*5* strain AC5122 by triparental matings. These constructs were screened for PNL production by using the PNL agar plate assay. Those that were Pnl^- were then screened for the induction of β-galactosidase production by MC. *Hin*dIII, which cleaves pAKC272 once (within the vector) and Mu dI1734 twice (0.1 kb from the 5′ end of the *lacZ* transcriptional unit and 1.0 kb from the 3′ end), was used in determining the location and orientation of the insertions.

RESULTS AND DISCUSSION

Cloning and localization of *pnlA*. Initial attempts to clone the *pnl* gene(s) of *E. carotovora* subsp. *carotovora* 71 entailed the construction of gene libraries in cosmid vector pHC79 (8) or pSF6 (16) and the screening of *E. coli* clones for the Pnl^+ phenotype. We failed to obtain a Pnl^+ *E. coli* by using either $RecA^+$ or $RecA^-$ strains. Since the *E. coli* $recA^+$ DNA is as effective as the *E. carotovora* subsp. *carotovora* $recA^+$ DNA in mediating PNL production (24), our inability to obtain a Pnl^+ clone led us to consider that the function of $recA^+$ may not be sufficient for detectable expression of the PNL structural gene in *E. coli*. In view of this possibility, we utilized strain 193, which appears to have the full complement of the PNL machinery, except that the system is less efficient in producing PNL than is strain 71; in the presence of 0.5 μg of MC per ml, strain 193 produced 24 U of PNL activity per mg of protein, while strain 71 produced 87 U. We expected that the strain 193 background would allow the detection of PNL hyperproduction because of a gene dosage effect or the alleviation of potential problems of gene expression that may occur in heterologous *E. coli* cells.

By mobilization of a portion of a strain 71 cosmid (pSF6) library into strain 193, 2 transconjugants of ca. 200 screened were found to hyperproduce PNL. The cognate cosmids (pAKC270 and pAKC271) appeared, by restriction analysis, to contain virtually identical insert DNA. Thus only pAKC270 was used in subsequent studies. pAKC270 (containing about 40 kb of strain 71 DNA) and an *Eco*RI deletion derivative of this plasmid, pAKC272 (harboring 4.5 kb of insert DNA) (Fig. 1), not only hyperproduced PNL in strain 193 but also restored PNL production in a *pnlA*::Tn*5* mutant of strain 71 (Table 2). In analogous experiments, pAKC270 and pAKC272 did not restore carotovoricin production in *ctv*::Tn*5* mutants (data not shown). PNL production in $RecA^+$ or $RecA^-$ *E. coli* strains harboring pAKC270 and

TABLE 2. PNL production by strain 193 and a *pnl*::Tn*5* mutant of strain 71 (AC5122) harboring Pnl^+ plasmids pAKC270 and pAKC272[a]

Plasmid	PNL activity[b] for strain:	
	193	AC5122
pSF6	20	<1
pAKC270	120	160
pAKC272	240	230

[a] Strains were grown in L broth containing 50 μg of spectinomycin and 100 μg of streptomycin per ml at 28°C with shaking. When cultures reached an A_{600} of 0.4, they were divided and MC (1 μg/ml, final concentration) was added to one half of each original culture. After 12 h of incubation, the cultures were assayed for PNL activity. PNL activity produced by strain 71 reached a maximum at 8 h, and loss of activity was not significant through 15 h after the addition of MC. Cultures which did not receive MC showed less than 1 U of activity, which is the limit of detection for these assay conditions.

[b] PNL activity is expressed as units of activity per milligram of culture protein. Results shown are for cultures which received MC.

TABLE 3. β-Galactosidase production by strain 71 and a *recA*::Tn*5* derivative of strain 71 carrying a *pnlA-lacZ* plasmid[a]

Strain (plasmid)	Sp act of β-galactosidase[b] in cultures containing MC at:		Induction ratio
	0 μg/ml	1 μg/ml	
71(pAKC277)	349	24,340	70
AC5117(pAKC277)	540	604	1

[a] Strains were grown as described in Table 2, footnote *a*.
[b] β-Galactosidase activity is expressed as units of activity [i.e., (1,000 × optical density at 420 nm)/(minutes × milliliters)] per milligram of culture protein. Values are corrected for background β-galactosidase activity produced by the strains harboring the vector pSF6.

pAKC272 was not detected. However, a subclone carrying a 3.4-kb *Eco*RI fragment of pAKC272 in the high-copy-number plasmid pBluescript SK$^-$ (pAKC278) did allow *E. coli* (RecA$^+$ or RecA$^-$) to constitutively produce 4.5 U of activity per mg of protein. This indicated that we had cloned the PNL structural gene, designated *pnlA*. PNL activity did not increase in the RecA$^+$ *E. coli* strain in the presence of MC. One explanation for this result may be that in the absence of an activator, *pnlA* transcription occurs at a very low efficiency.

Further localization of *pnlA* was undertaken by using *Exo*III-*Exo*VII deletion analysis of pAKC278 and pAKC279. Plasmids harboring unidirectional deletions extending from both ends of the 3.4-kb *Eco*RI Pnl$^+$ fragment were tested for their abilities to restore PNL production in the *pnlA*::Tn*5* mutant AC5122. Strain 71 DNA of relevant deletion derivatives used in localizing *pnlA* to an approximately 1.4-kb segment of DNA is depicted in Fig. 1.

Analysis of *pnlA-lacZ* fusions and their expression in *E. coli* and *E. carotovora* subsp. *carotovora* strains. Eleven Pnl$^-$ clones which contained Mu dI1734 insertions within a 1.1-kb segment of the insert DNA of pAKC272 (Fig. 1) were isolated. Of the 11 insertions, 6 resulted in *pnlA-lacZ* transcriptional fusions that were inducible in a RecA$^+$ *E. carotovora* subsp. *carotovora* strain following exposure to MC, demonstrating that regulation of PNL production occurs in part, if not solely, at the level of *pnlA* transcription. *Hin*dIII restriction analysis confirmed that these six mini-Mu–*lacZ* insertions were in one orientation while the remaining five insertions were in the opposite orientation (Fig. 1). pAKC277, harboring the *pnlA-lacZ* fusion (Fig. 1), was used in subsequent studies. The fusion in this plasmid, while being inducible in strain 71, was not inducible in a RecA$^-$ derivative of strain 71 (Table 3). This demonstrated that the effect of a *recA* mutation on PNL production, as noted by Zink et al. (24), is manifested at the level of transcription. However, the fact that a β-galactosidase specific activity of 540 to 604 (corrected for background activity) was noted in a RecA$^-$ derivative of strain 71 suggests that a basal level of *pnlA* transcription may occur in the absence of *recA* function.

In RecA$^+$ *E. coli* MC4100 carrying the fusion, β-galactosidase activity was lower (4.3 U/mg of protein) when MC was added to the culture than when the culture was grown in the absence of MC (14.3 U/mg of protein). The findings that *pnlA-lacZ* was not inducible in MC4100 and that the basal level of β-galactosidase activity was approximately 1/40 that found in *E. carotovora* subsp. *carotovora* are consistent with the hypothesis that PNL regulation involves a factor in addition to *pnlA* and *recA*. The fact that β-galactosidase production was lower in the presence of MC probably reflects the detrimental effects of the drug on the strain.

TABLE 4. PNL production and occurrence of *pnlA* homologous sequences in wild-type *Erwinia* strains[a]

Strain	Sp act of PNL[b] in cultures containing MC at:		Size (kb) of fragment hybridizing to *pnlA* probe
	0 μg/ml	0.5 μg/ml	
E. carotovora subsp. *carotovora*			
71	0.3	87.4	3.4
193	0.5	23.7	2.5
SCRI193	0.4	19.9	9.4
E. carotovora subsp. *atroseptica*			
5	<0.1[c]	0.5	5.9
12	0.3	0.5	9.6
31	0.4	0.2	9.6
198	0.4	12.8	2.6
E. chrysanthemi			
EC16	0.5	0.8	ND[d]
EC183	1.3	17.4	ND
D1	0.3	0.3	ND
D2	<0.1	0.7	ND
E. rhapontici			
1	0.6	123.3	5.5
102	1.1	175.0	ND
109	0.5	96.0	ND
112	1.0	277.8	ND

[a] Strains were grown in L broth at 28°C with shaking at 150 rpm. When cultures reached an A_{600} of 0.6, they were divided and MC was added to one half of each original culture. After 12 h of incubation, the culture supernatants and cell extracts were assayed for PNL activity. Values represent the sums of activities in culture supernatants and cell extracts.
[b] PNL activity is expressed as stated in Table 2, footnote *b*.
[c] The limit of detection for these assay conditions was 0.1 U of activity.
[d] ND, No hybridizing signal detected.

Homology between *pnlA* of strain 71 and DNA of various *Erwinia* strains. PNL is produced by many but not all soft-rot *Erwinia* species (19). Furthermore, among the PNL-producing strains of a given species there is great variability in the amount of PNL activity detected in cultures treated with DNA-damaging agents (Table 4) (19). Therefore, it was of interest to determine if various PNL-producing *Erwinia* strains contain sequences which are homologous to *pnlA* of the efficient PNL producer strain 71. Following hybridization of a ^{32}P-labeled 1.4-kb *Eco*RV-*Pvu*II fragment containing most of *pnlA* (Fig. 1) and a high-stringency wash (2× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate]–0.1% sodium dodecyl sulfate (SDS) for 30 min at 65°C and 0.1× SSC–0.1% SDS for 15 min at 65°C), *pnlA* homologs on various-size *Eco*RI fragments were detected in three *E. carotovora* subsp. *carotovora* and four *E. carotovora* subsp. *atroseptica* strains (Fig. 2 and Table 4). With a low-stringency wash (2× SSC–0.1% SDS for 30 min at 65°C and 0.5× SSC–0.1% SDS for 15 min at 65°C), *pnlA* hybridizing signals were detected in one of four PNL-producing *E. rhapontici* strains and no *E. chrysanthemi* strains (Table 4). Although *E. carotovora* subsp. *atroseptica* 5, 12, and 31 contained *pnlA* homologs, very little PNL was produced by these strains (Table 4). On the other hand, the *E. rhapontici* strains 102, 109, and 112, capable of producing PNL specific activity of between 95 and 275, contained no detectable *pnlA* homologous sequences. These findings suggest that *pnlA* has

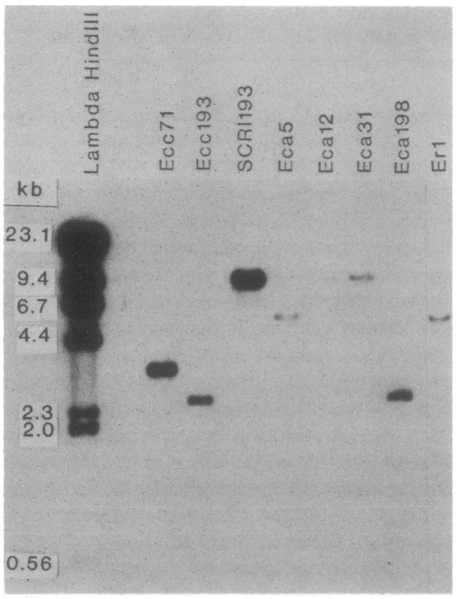

FIG. 2. Southern hybridization of *Eco*RI-digested DNAs from wild-type *Erwinia* strains. DNA fragments were transferred to nylon membranes (Biotrans; ICN Pharmaceuticals, Inc., East Hills, N.Y.) and probed with a [^{32}P]dCTP-labeled 1.4-kb *Pvu*II-*Eco*RV fragment containing most of the strain 71 *pnlA* gene (Fig. 1). Stringent wash conditions were used in all cases except *E. rhapontici* strain 1 DNA, which underwent a low-stringency wash. See Table 4 for the results of other hybridizations, the amounts of PNL produced by these strains, and the sizes of the hybridizing fragments. Abbreviations: Ecc, *E. carotovora* subsp. *carotovora*; Eca, *E. carotovora* subsp. *atroseptica*; Er, *E. rhapontici*.

diverged and that this divergence is much more pronounced between species boundaries. The low levels of PNL activities in strains containing *pnlA* homologs may be due to differences in the quality or the quantity of a PNL regulatory protein(s) influencing the synthesis of the PNL polypeptides. In addition, differences in *pnlA* may lead to the synthesis of PNL polypeptides with different substrate-binding or substrate-cleaving abilities, thereby affecting overall catalytic activity.

The findings presented here extend our understanding of the PNL system in strain 71 and provide us with information concerning the divergence of *pnlA* among various soft-rot *Erwinia* spp. We have demonstrated that PNL production is regulated at the level of transcription, shown that the positive effect of RecA on PNL production is exerted at the transcriptional level, provided evidence (albeit indirect) for the requirement of an additional factor (other than RecA) in the induction of *pnlA*, and documented the occurrence of *pnlA*-like sequences in *E. carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica*, and a strain of *E. rhapontici*. Further analysis of the regulation of PNL production will necessitate studies with PNL regulatory gene(s), which our laboratory is currently seeking.

ACKNOWLEDGMENTS

This investigation was supported by grant DMB-8796262 from the National Science Foundation and by the Food for the 21st Century program of the University of Missouri.

We thank Asita Chatterjee for her help with the hybridization studies and various aspects of manuscript preparation and our colleagues for generously providing us with bacterial strains and plasmids.

LITERATURE CITED

1. **Ames, B. N.** 1983. Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. Science **221**:1256–1264.
2. **Castilho, B. A., P. Olfson, and M. J. Casadaban.** 1984. Plasmid insertion mutagenesis and *lac* gene fusion with mini-Mu bacteriophage transposons. J. Bacteriol. **158**:488–495.
3. **Chatterjee, A. K.** 1980. Acceptance by *Erwinia* spp. of R plasmid R68.45 and its ability to mobilize the chromosome of *Erwinia chrysanthemi*. J Bacteriol. **142**:111–119.
4. **Chatterjee, A. K., and A. K. Vidaver.** 1986. Genetics of pathogenicity factors: application to phytopathogenic bacteria, p. 122–153. *In* D. S. Ingram and P. H. Williams (ed.), Advances in plant pathology, vol. 4. Academic Press, Inc. (London), Ltd., London.
5. **Collmer, A., and N. T. Keen.** 1986. The role of pectic enzymes in plant pathogenesis. Annu. Rev. Phytopathol. **24**:383–409.
6. **Figurski, D. H., and D. R. Helinski.** 1979. Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided in *trans*. Proc. Natl. Acad. Sci. USA **76**:1648–1652.
7. **Henikoff, S.** 1987. Unidirectional digestion with exonuclease III in DNA sequence analysis. Methods Enzymol. **155**:156–165.
8. **Hohn, B., and J. Collins.** 1980. A small cosmid for efficient cloning of large DNA fragments. Gene **11**:291–298.
9. **Itoh, Y., K. Izaki, and H. Takahashi.** 1980. Simultaneous synthesis of pectin lyase and carotovoricin induced by mitomycin C, nalidixic acid or ultraviolet light irradiation in *Erwinia carotovora*. Agric. Biol. Chem. **44**:1135–1140.
10. **Itoh, Y., J. Sugiura, K. Izaki, and H. Takahashi.** 1982. Enzymological and immunological properties of pectin lyases from bacteriocinogenic strains of *Erwinia carotovora*. Agric. Biol. Chem. **46**:199–205.
11. **Kamimiya, S., K. Izaki, and H. Takahashi.** 1972. A new pectolytic enzyme in *Erwinia aroideae* formed in the presence of nalidixic acid. Agric. Biol. Chem. **36**:2367–2372.
12. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **McEvoy, J. L., H. Murata, J. K. Engwall, and A. K. Chatterjee.** 1988. Genetics of damage-inducible pectin lyase (PNL) of *Erwinia carotovora* subsp. *carotovora*: analysis of Tn*5* insertion mutants and molecular cloning of *Pnl* DNA, p. 257–258. *In* R. Palacios and D. P. S. Verma (ed.), Molecular genetics of plant-microbe interactions. APS Press, St. Paul, Minn.
14. **McEvoy, J. L., K. K. Thurn, and A. K. Chatterjee.** 1987. Expression of the *E. coli lexA*$^+$ gene in *Erwinia carotovora* subsp. *carotovora* and its effect on production of pectin lyase and carotovoricin. FEMS Microbiol. Lett. **42**:205–208.
15. **Miller, J. F.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Selvaraj, G., Y. C. Fong, and V. N. Iyer.** 1984. A portable DNA sequence carrying the cohesive site (*cos*) of bacteriophage lambda and the *mob* (mobilization) region of the broad-host-range plasmid RK2: a module for the construction of new cosmids. Gene **32**:235–241.
17. **Staskawicz, B. J., D. Dahlbeck, and N. T. Keen.** 1984. Cloned avirulence genes of *Pseudomonas glycinea* pv. *glycinea* determines race-specific incompatability on *Glycine max* (L.) Merr. Proc. Natl. Acad. Sci. USA **81**:6024–6028.
18. **Tomizawa, H., and H. Takahashi.** 1971. Stimulation of pectolytic enzyme formation of *Erwinia aroideae* by nalidixic acid, mitomycin C and bleomycin. Agric. Biol. Chem. **35**:191–200.
19. **Tsuyumu, S., and A. K. Chatterjee.** 1984. Pectin lyase production in *Erwinia chrysanthemi* and other soft-rot *Erwinia* species. Physiol. Plant Pathol. **24**:291–302.
20. **Tsuyumu, S., T. Funakubo, K. Hori, Y. Takikawa, and M. Goto.** 1985. Presence of DNA damaging agents in plants as the possible inducers of pectin lyase of soft-rot *Erwinia*. Ann. Phytopathol. Soc. Jpn. **51**:294–302.
21. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol.

Rev. **48**:60–93.

22. **Walker, G. C.** 1987. The SOS response of *Escherichia coli*, p. 1346–1357. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.
23. **Whitford, W. G., and R. A. DiCioccio.** 1988. Alkaline blotting of DNA from SeaKem GTG agarose to a nylon membrane. Resolutions **4**:1–2.
24. **Zink, R. T., J. K. Engwall, J. L. McEvoy, and A. K. Chatterjee.** 1985. *recA* is required in the induction of pectin lyase and carotovoricin in *Erwinia carotovora* subsp. *carotovora*. J. Bacteriol. **164**:390–396.