Genetic Evidence for an Activator Required for Induction of Pectin Lyase in *Erwinia carotovora* subsp. *carotovora* by DNA-Damaging Agents†

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In *Erwinia carotovora* subsp. *carotovora* 71, the induction of pectin lyase (Pnl), the bacteriocin carotovoricin (Ctv), and cellular lysis (Lls) requires a RecA function. We obtained mutants wherein a pleiotropic defect, i.e., the lack of induction with mitomycin C, is not restored by the recA+ DNA. From a genomic library of strain 71, a cosmid (pAKC280) that restored induction of Pnl, Ctv, and Lls by mitomycin C was isolated. The activator function, designated Rdg for regulator of damage-inducible genes, was localized by subcloning and insertional mutagenesis to a 2.6-kb region within a 6.7-kb EcoRI fragment. An rdg-lacZ operon fusion was inducible by mitomycin C in RecA+ but not RecA− derivatives of *E. carotovora* subsp. *carotovora* 71 and *Escherichia coli*. A RecA+ *E. coli* strain carrying only a PnlA+ plasmid was not inducible for Pnl production; however, when both a PnlA+ plasmid and a Rdg+ plasmid were present, the transcription of *pnlA* and the production of the enzyme were activated by mitomycin C. The size of the *pnlA* transcript produced in *E. coli* was identical to that of the transcript produced by *E. carotovora* subsp. *carotovora* 71, suggesting that the same promoter and termination sequences were being utilized in these bacteria.

Pectin lyase (Pnl) production occurs in many soft-rot *Erwinia* strains in response to DNA-damaging agents (13, 23). In *Erwinia carotovora* subsp. *carotovora* 71, a function of RecA is required for transcriptional activation of the Pnl structural gene, *pnlA* (13, 26). RecA is also required for the production of the bacteriocin carotovoricin (Ctv) and cellular lysis (Lls), which occur concomitantly with Pnl production (26). These recA-mediated damage-inducible phenootypes of strain 71 were initially thought to be analogous to the SOS region of *Escherichia coli*. In the SOS system, a signal generated by DNA damage activates the production of the recA gene, which is then involved in cleaving LexA, the repressor of a number of unrelated genes (for a review, see reference 24).

Several lines of evidence, however, implicated a different regulatory mechanism in the expression of *pnlA*. Whereas the expression of several genes in *E. carotovora* subsp. *carotovora* 71 was repressed by the *E. coli* lexA+, Pnl production was not inhibited in the presence of the lexA+ DNA (14). Furthermore, *pnlA* contained no sequence resembling a LexA binding site (7) and was expressed only at a basal level in a recA− lexA+ *E. coli* strain in the presence of DNA-damaging agents (13). Collectively, these findings suggested the requirement of an activator in the regulation of *pnlA*. The isolation of (i) mutants wherein Pnl and Ctv production and cellular lysis are not induced by mitomycin C and (ii) a DNA segment that corrects this deficiency and activates *pnlA* transcription, as reported here, provides further support for this hypothesis.

For the isolation of mutants altered in *pnlA* regulation, ACS022, a *pnlA-lacZ* derivative of the LacZ− Ctv− strain ACS006 (16), was used. ACS022 was constructed by marker-exchange recombination, in which *pnlA* on the chromosome was replaced with *pnlA-lacZ* from the plasmid pAKC277 (13). This strain produced no detectable Pnl activity and was inducible by more than 50-fold for β-galactosidase production by 500 ng of mitomycin C per ml, i.e., a basal level of ca. 50 U was inducible to ca. 2,600 U. ACS022 lysed, as did strain 71, when mitomycin C was added to the culture medium at a final concentration of 500 ng/ml. These data and Southern blot analysis of EcoRI-digested ACS022 DNA with a *pnlA* probe confirmed that a homologous exchange of *pnlA-lacZ* for *pnlA* had occurred.

Two different mutagenesis schemes were utilized to isolate mutants defective in Pnl expression. In the first scheme, ethyl methanesulfonate mutagenesis (15) of ACS022 and screening on Luria-Bertani agar containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside and mitomycin C were used to obtain mutants that were defective in the production of β-galactosidase in response to mitomycin C at a frequency of 0.03%. These mutants, which were also defective in cellular lysis, could not be restored to their parental phenotype by the introduction of a recA+ plasmid or a *pnlA-lacZ* plasmid. The mutants were not sensitive to various DNA-damaging agents, including mitomycin C, ethyl methanesulfonate, and UV light. One such pleiotropic regulatory mutant, ACS132, produced about 70 U of β-galactosidase in the presence or absence of mitomycin C.

The second mutagenesis scheme utilized the transposon delivery vector pJB4J1 (3) and plate assays for Pnl (13) and Ctv (12) production to obtain Tn5 insertional mutants of *E. carotovora* subsp. *carotovora* 71 (27). One mutant, ACS130, produced a low level of Pnl and an undetectable level of Ctv in the presence or absence of mitomycin C (Table 1). In addition, there was no apparent loss of culture turbidity when the mutant was grown in the presence of mitomycin C (500 ng/ml). Thus, the characteristics of this mutant were very similar to those of ACS132. The pleiotropic nature of these mutants suggested that Ctv production and cellular lysis and Pnl production require the hypothesized activator.
TABLE 1. Complementation of pleiotropic regulatory mutant AC5130 by the Rdg+ cosmid pAKC280*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sp act (U/mg) of protein with mitomycin C at:</th>
<th>Pnl</th>
<th>Ctv</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0 µg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>71pSF6β</td>
<td></td>
<td>0.6</td>
<td>&lt;10</td>
</tr>
<tr>
<td>AC5130/pSF6</td>
<td></td>
<td>1.1</td>
<td>&lt;10</td>
</tr>
<tr>
<td>AC5130/pAKC280</td>
<td></td>
<td>1.8</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

* Cultures were grown, induced, and assayed for pectin lyase (Pnl) and carotovorin (Ctv) activities as previously described (13).

A library of the strain 71 genome (13) in the low-copy-number vector pSF6 (20) was mobilized into AC5130. After a screen for Ctv production (12), we detected a cosmid that contained approximately 40 kb of strain 71 DNA and restored Ctv production in response to mitomycin C (Table 1). This cosmid, pAKC280, also restored mitomycin C-inducible Pnl production (Table 1) and cellular lysis in AC5130 and β-galactosidase production in the pleiotropic mutant AC5132 (ca. 1,300 Miller units). pAKC280 did not restore Pnl production in the pnnA::Tn5 strain AC5121, Ctv production in the Ctv- mutant AC5127, or a RecA+ phenotype in the recA::Tn5 strain AC5117, strongly suggesting that pAKC280 carries a dominant gene for a regulatory function.

The regulatory locus on pAKC280 was designated rdg for regulator of damage-inducible genes. The Rdg function was specified by a 6.7-kb EcoRI fragment as determined by subcloning of this fragment into the low-copy-number vector pRK415 (8). The resulting plasmid, pAKC700, was used in saturation mutagenesis of the rdg region with MudI1734 (6, 13). This analysis allowed localization of Rdg function within a 2.6-kb segment of DNA (Fig. 1).

The properties of Rdg are consistent with its being an activator, since all of the Rdg- mutants are recessive. This conclusion was independently confirmed by examining the effect of pAKC280 on the activation of pnnA transcription in an E. coli recA+ lexA+ strain, MC4100. The presence of a Rdg+ plasmid (pAKC280) or a PnnA+ plasmid (pAKC278) alone in MC4100 was not sufficient to allow the production of Pnl in response to mitomycin C. However, when both plasmids were present, Pnl activity was inducible by about 10-fold (from 13 U to 122 U). Nearly all (>95%) of the Pnl produced by E. coli was cell associated (data not shown). When the pnnA-lacZ plasmid pAKC277 was substituted for the pnnA plasmid in MC4100 carrying pAKC280, β-galactosidase production increased from a basal level of ca. 20 Miller units to 16,000 Miller units in response to mitomycin C. We obtained similar results with pAKC700, a 6.7-kb Rdg+ subclone derived from pAKC280 (see above). The activation of pnnA transcription in E. coli in the presence of Rdg+ DNA but not in its absence provides strong evidence for a direct action of Rdg. It should be noted that activation of pnnA transcription did not occur in an E. coli recA lexA- strain harboring pAKC280 and pAKC277 (data not shown), indicating that the induction in E. coli, like that in E. carotovora subsp. carotovora, is RecA dependent.

As a further test of the fidelity of the Pnl system in E. coli, Northern RNA blots of total RNAs isolated from MC4100 (1) harboring the pnnA+ plasmid pAKC278 (13) and the pnnA- plasmid pAKC280 and E. carotovora subsp. carotovora 71 were hybridized with a 0.39-kb PvuII-KpnI fragment internal to pnnA (7). The amount of the pnnA transcript increased in both E. carotovora subsp. carotovora 71 and E. coli MC4100 after treatment with mitomycin C (Fig. 2). Additionally, the sizes of the transcripts (approximately 0.9 kb) from both strains appeared to be identical, suggesting that the transcriptional start and termination sequences utilized in E. coli are the same as those in E. carotovora subsp. carotovora 71.

An examination of the expression of rdg was possible, since two of seven MudI1734 insertions in rdg (Fig. 1) were oriented such that they created rdg-lacZ transcriptional fusions. Whereas both fusions were inducible in AC5130 by mitomycin C, subsequent studies utilized the plasmid pAKC704 carrying the MudI1734 insertion noted in Fig. 1. β-Galactosidase production by AC5130 harboring pAKC704 was 53 U in the absence of mitomycin C and 304 U when induced with 500 ng of the drug per ml. A similar induction of rdg-lacZ was seen in E. carotovora subsp. carotovora 71 and its LacZ+ derivative AC5006. The rdg-lacZ fusion also was inducible in the recA+ lexA+ strain E. coli M8820; in the absence of mitomycin C, M8820 harboring pAKC704 produced 64 U of β-galactosidase, whereas 363 U of activity was produced in response to mitomycin C. However, cultures of the E. coli recA strain DH5α or the E. carotovora subsp. carotovora 71 recA derivative AC5117 harboring pAKC704 were noninducible for β-galactosidase production; they produced less than 50 U in the presence or absence of mitomycin C. To test the possibility that rdg is negatively controlled by LexA, we examined the effects of E. coli lexA+ DNA and lexA mutations on the expression of the rdg-lacZ operon fusion in pAKC704. Our preliminary results suggest that the E. coli LexA can partially repress rdg expression (data not shown). This partial repression may be attributed to the use of heterologous lexA DNA or to an indirect effect of LexA via repression of recA. To avoid the use of heterologous DNA, we are utilizing a one-step cloning...
FIG. 2. Northern analysis of pnlA transcripts produced by E. coli MC4100 carrying PnlA'+ and Rdg' plasmids and E. carotovora subsp. carotovora 71. Cultures were grown and induced with mitomycin C as previously described (13), except that E. coli cultures were allowed to reach an A$_{600}$ of 1.0 before mitomycin C was added at a final concentration of 2.5 μg/ml. RNA was isolated, electrophoresed, and blotted as previously described (1, 7). A 32P-labeled 0.39-kb PvuII-KpnI fragment internal to pnlA was used as the probe (7). Lanes: 1, RNA isolated from a culture of MC4100/pAKC278+pAKC280 induced with mitomycin C; 2, RNA isolated from a culture of MC4100/pAKC278+pAKC280 grown in the absence of mitomycin C; 3, RNA isolated from a culture of E. carotovora subsp. carotovora 71 induced with mitomycin C; 4, RNA isolated from a culture of E. carotovora subsp. carotovora 71 grown in the absence of mitomycin C. Approximately 1 μg of RNA was loaded in each lane.

of the gene product(s), should provide clues as to whether Rdg is an RNA polymerase sigma factor or a DNA-binding protein.

Several other key issues also await clarification. For example, the role of RecA in Rdg-stimulated transcription of pnlA is not known. This work has shown that RecA is necessary for the DNA damage induction of rdg; thus, it is possible that the coproteolytic activity of RecA (9-11) determines the level of Rdg via modification of a regulator of rdg.

It is also possible that RecA is required for posttranslational activation of Rdg, as is the case for the UmuD protein (17, 21). In addition, the requirement of DNA-damaging agents in the activation of Pnl production will have to be reconciled in the context of a regulatory role of the RecA-LexA pathway. The resolution of those issues and further analyses of damage-inducible production of a plant tissue-macerating enzyme are now greatly facilitated with the reconstitution of a Pnl-producing system in E. coli.

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REFERENCES


