JOURNAL OF BACTERIOLOGY, Nov. 1991, p. 7345–7350
0021-9193/91/227345-06$02.00/0

Vol. 173, No. 22

# One-Step Cloning System for Isolation of Bacterial *lexA*-Like Genes

SEBASTIÁN CALERO, XAVIER GARRIGA, AND JORDI BARBÉ*

*Department of Genetics and Microbiology, Autonomous University of Barcelona, 08193 Bellaterra, Barcelona, Spain*

Received 19 June 1991/Accepted 17 September 1991

**A system to isolate *lexA*-like genes of bacteria directly was developed. It is based upon the fact that the presence of a *lexA*(Def) mutation is lethal to SulA$^+$ cells of *Escherichia coli*. This system is composed of a SulA$^-$ LexA(Def) HsdR$^-$ strain and a *lexA*-conditional killer vector (plasmid pUA165) carrying the wild-type *sulA* gene of *E. coli* and a polylinker in which foreign DNA may be inserted. By using this method, the *lexA*-like genes of *Salmonella typhimurium*, *Erwinia carotovora*, *Pseudomonas aeruginosa*, and *P. putida* were cloned. We also found that the LexA repressor of *S. typhimurium* presented the highest affinity for the SOS boxes of *E. coli* in vivo, whereas the LexA protein of *P. aeruginosa* had the lowest. Likewise, all of these LexA repressors were cleaved by the activated RecA protein of *E. coli* after DNA damage. Furthermore, under high-stringency conditions, the *lexA* gene of *E. coli* hybridized with the *lexA* genes of *S. typhimurium* and *E. carotovora* but not with those of *P. aeruginosa* and *P. putida*.**

In *Escherichia coli*, treatments which damage DNA or block DNA replication produce coordinate induction of a set of at least 18 genes called the SOS network, or the SOS response, of which the products directly repair DNA or allow the cell to tolerate the DNA lesion until the repair may be accomplished (20, 35). Biochemical and genetic data have shown that the SOS response is controlled by the RecA and LexA proteins. LexA protein is the common repressor of the SOS genes, which include both *lexA* and *recA*. The basal RecA protein is reversibly activated by an inducing signal after DNA damage. The characteristics of this inducing signal are unknown, although it has been proposed that it consists of single-stranded DNA regions resulting from inhibition of DNA replication (7, 30). Activated RecA protein has apoprotease activity which, with the help of the Ssb protein (17, 36), facilitates autocatalytic cleavage of the LexA repressor (7, 18, 19), resulting in expression of the SOS genes. After DNA repair, the RecA protein is no longer activated and the level of the LexA protein increases, again repressing the SOS genes.

The existence of similar DNA damage-inducible responses in other bacterial species has been widely reported (25). Thus, and by interspecies functional complementation of *E. coli recA* mutations, the *recA* genes of many species of eubacteria, such as *Erwinia carotovora* (15), *Pseudomonas aeruginosa* (16), *Rhizobium meliloti* (4), and *Agrobacterium tumefaciens* (23), among others, have recently been cloned. Bacterial *recA*-like genes may easily be isolated directly by using the resistance to DNA damage that a plasmid harboring the heterologous *recA* gene confers on *recA* mutants of *E. coli*. Therefore, construction of a plasmid-genomic library of a given bacterium, introduction of recombinant plasmids in an *E. coli* RecA$^-$ strain, and plating in ethyl methanesulfonate- or methyl methanesulfonate-supplemented plates are all that is required to obtain *recA*-like genes. Comparison of all of the known sequences of different *recA* genes reveals that their divergences seem to be confined to position 3 of codons, giving rise to significant conservation of amino acid sequences (25). Furthermore, in every species of prokaryotes with which an exhaustive investigation has been carried out, *recA* gene analogs with important similar biological and physical properties have been identified.

The question of whether a LexA repressor also exists in species and genera containing *recA*-like genes is not clear in all cases. The DNA sequences of some *recA*-analogous genes show possible LexA-binding sites, but others do not (25). In this respect, and by employing a broad-range host plasmid containing a fusion of both the *recA* and *lacZ* genes of *E. coli*, we have recently shown that the LexA-binding site is present in many bacterial species (9). To determine whether or not the LexA repressor is present in several bacteria, it is necessary to isolate *lexA*-like genes. Nevertheless, and because the *lexA* gene has a negative regulatory role in the SOS pathway, no method for direct cloning of bacterial *lexA* genes has been developed. Furthermore, the lack of homology between the *recA* gene of *E. coli* and other *recA* genes (4, 23) suggests that *lexA* DNA probes cannot be used to obtain *lexA*-like genes from genomic libraries.

In this report, we present a one-step cloning system for direct isolation of *lexA*-like genes. It enabled us to obtain the *lexA* genes of *S. typhimurium*, *E. carotovora*, *P. aeruginosa*, and *P. putida*.

## MATERIALS AND METHODS

**Bacterial strains, plasmids, and growth conditions.** The bacterial strains and plasmids used in this work are listed in Table 1. To obtain strain UA4793 [LexA(Def) SulA$^-$ HsdR$^-$], the *lexA*(Def) allele was introduced into strain UA4567, which is an HsdR$^-$ mutant containing a *sulA*::Mud1Amp*lac* fusion, by using a *lexA71*::Tn*5* P1 donor strain. The UA4793 strain was then made RecA$^-$ by P1-mediated transduction of the Δ*recA306* mutation by using a Δ*recA306 srl*::Tn*10* strain as the donor. This gave rise to the UA4794 strain.

All of the strains, except temperature-sensitive mutants which were at 30°C, were normally grown at 37°C in LB rich medium (24) or AB minimal medium (6) supplemented with glucose (0.2%, wt/vol) or Casamino Acids (0.4%, wt/vol).

**Plasmid constructions.** The strategy developed to obtain plasmid pUA165 is shown in Fig. 1. Plasmid pUA94 was digested with *Bam*HI, and the 1.78-kb fragment carrying both the *sulA* and the *ompA* genes was cloned into the *Bgl*II

* Corresponding author.

TABLE 1. Bacterial strains and plasmids used in this work

| Strain or plasmid | Relevant characteristic(s) | Source or reference |
|---|---|---|
| *E. coli* strains | | |
| AB1157 | *recA*$^+$ *lexA*$^+$ | This laboratory |
| MC1061 | *recA*$^+$ *hsdR* | This labortory |
| UA4567 | Same as MC1061 but *sulA*::*lacZ* | 37 |
| SC30sp | *lexA71*::Tn*5* | 21 |
| EST1450 | Δ*recA306 srl*::Tn*10* | 34 |
| JL2301 | *lexA300*(Del) *recA*$^+$ *sulA*::*lacZ* | 12 |
| DM986 | *lexA41* | 27 |
| DH5α | *recA1 hsdR17 gyrA* | This laboratory |
| UA4793 | Same as UA4567 but *lexA71*::Tn*5* | P1, SC30sp × UA4567 |
| UA4794 | Same as UA4793 but Δ*recA306 srl*::Tn*10* | P1, EST1450 × UA4793 |
| *S. typhimurium* LT2 | Wild type | This laboratory |
| *E. carotovora* ATCC 15713 | Wild type | F. Uruburu |
| *P. aeruginosa* PAO | Wild type | M. Tsuda |
| *P. putida* mt-2 KT2440 | Wild type | K. Timmis |
| Plasmids | | |
| pCB267 | Amp$^r$ Lac$^-$ PhoA$^-$ | 32 |
| pPR292 | Cm$^r$ Amp$^r$ | 29 |
| pACYC184 | Tc$^r$ Cm$^r$ | This laboratory |
| pRL538 | Amp$^r$ | 8 |
| pHP45Ω | Amp$^r$ Sp$^r$; contains Ω interposon | 28 |
| pUA75 | Same as pPR292 but harbors *lexA* gene of *E. coli* | 11 |
| pUA94 | Same as pCB267 but harbors *sulA* and *ompA* genes of *E. coli* | 10 |

site of the pCB267 plasmid to obtain pUA161. Plasmid pUA162 was then built by insertion of a 1.8-kb *Bam*HI-*Hin*dIII pUA161-derivative fragment between the *Bam*HI and *Hin*dIII sites of pACYC184. A 2.1-kb *Bgl*II fragment containing the Ω interposon flanked by a polylinker on each side was inserted into the *Bam*HI site of pUA162 to give pUA163. This 2.1-kb *Bgl*II fragment had previously been constructed by subcloning of the Ω interposon from pHP45Ω in the pRL538 plasmid (Fig. 1). Plasmid pUA163 was then digested with *Bam*HI and self-ligated to eliminate the interposon, giving rise to pUA164. Finally, to delete the *ompA* gene, pUA164 was digested with both *Cla*I and *Nde*I, and after filling in of the ends, it was self-ligated and transformed into *E. coli* DH5α. pUA165 plasmid carries the *sulA* gene and the polylinker shown in Fig. 1 and encodes chloramphenicol resistance. The LexA(Def) SulA$^-$ HsdR$^-$ recipient strain which is required to obtain *lexA*-like genes is sensitive to this antibiotic (Table 1).

**Genetic and biochemical methods.** P1*vir* transductions were performed basically as described by Miller (24). When transducing cells to kanamycin resistance (Km$^r$), we plated the mixture of cells and phage on AB minimal glucose plates containing kanamycin directly after infection. For transduction to tetracycline resistance (Tc$^r$), the infected cells were plated directly onto LB plates containing tetracycline. UV irradiation and mitomycin C treatment were performed as previously described (2, 11). The β-galactosidase assay was performed as reported by Miller (24). Enzyme concentrations (units per milliliter) were calculated from the formula given by Casaregola et al. (5).

**DNA manipulations.** The plasmid gene bank of genomic DNAs of *E. coli* AB1157, *S. typhimurium* LT2, *E. carotovora*, *P. aeruginosa* PAO, and *P. putida* mt-2 was constructed by using the pUA165 vector (this work). Chromosomal DNA was obtained in all cases as previously reported (1). *Sau*3AI partially digested donor DNA about 5 to 10 kb long was purified by fractionation on a 10 to 40% sucrose gradient as described by Sambrook et al. (1). The conditions used for restriction endonuclease digestion, agarose gel electrophoresis, and isolation and ligation of DNA fragments have been described elsewhere (31). Transformation of genomic libraries into *E. coli* cells was performed by electroporation using a Bio-Rad Gene Pulser as previously described (26).

Southern hybridizations were performed as follows. Plasmid DNA was digested with a fivefold excess of the appropriate restriction enzyme. Digested DNA was electrophoresed in agarose gels (0.8%), denatured in situ, and transferred to nitrocellulose as described by Sambrook et al. (31). The DNA probe was labelled with digoxigenin-11-dUTP by using the Boehringer Mannheim random-primer system and following the supplier's instructions. Nitrocellulose filters were hybridized from 22 to 42 h at 42°C in a solution containing 50% formamide, 5× SSPE (0.9 M NaCl, 5 mM EDTA, 50 mM $NaPO_4$ [pH 7.7]), 500 μg of heat-denatured calf thymus DNA per ml, and 0.3% sodium dodecyl sulfate (SDS). The filters were washed at 68°C in 2× SSPE–0.1% SDS (60 min) and 0.1× SSPE–0.1% SDS (60 min). Detection of hybridized DNA was carried out as recommended by the supplier of the labelling kit (Boehringer Mannheim).

## RESULTS

**Rationale.** In a SulA$^+$ genetic background, *lexA*(Def) mutations are lethal to *E. coli*. This is because expression of the *sulA* gene is repressed by binding of the LexA protein to the SOS box located in the *sulA* promoter (13). In the absence of the LexA repressor, accumulation of the 18-kDa SulA protein causes rapid cessation of cell division by binding to the FtsZ protein (14). This inhibition of cell division gives rise to long, nonseptate filaments, leading to cell death (14). Therefore, we reasoned that when a plasmid harboring the wild-type *sulA* gene of *E. coli* was introduced

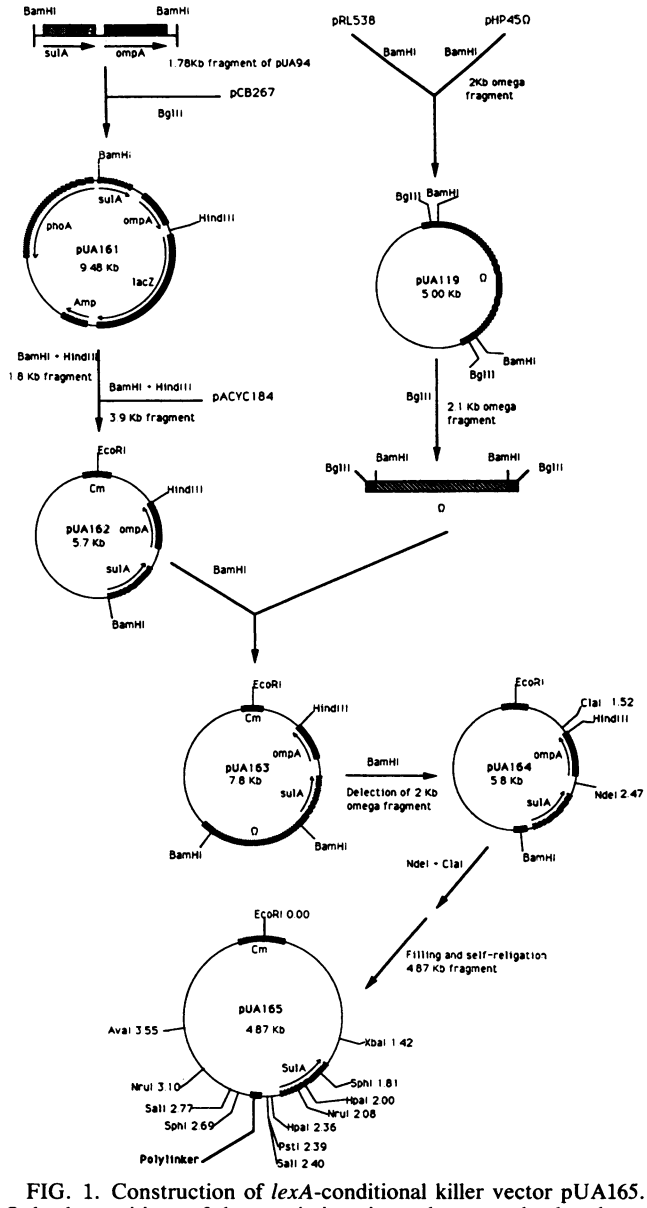


FIG. 1. Construction of *lexA*-conditional killer vector pUA165. Only the positions of the restriction sites relevant as landmarks to plasmid construction are given. The directions of transcription of several genes are indicated by the arrows. The restriction points present in the polylinker of this plasmid are *Xho*I, *Nru*I, *Hin*dIII, *Sph*I, *Pst*I, *Sal*I, *Xba*I, *Bam*HI, *Xba*I, *Sal*I, *Pst*I, *Sph*I, *Hin*dIII, *Nru*I, and *Xho*I. Plasmid pUA165 is not cut by *Bgl*II, *Pvu*I, *Stu*I, *Kpn*I, *Sfi*I, *Cla*I, *Nde*I, *Sma*I, or *Xma*I. The plasmids are not drawn to scale.

by transformation into a LexA(Def) SulA$^-$ mutant it should not be possible to detect transformants, since the cell division should be constitutively inhibited by the plasmid-codified wild-type SulA protein. On the other hand, by using a plasmid containing both *sulA* and *lexA* genes it should be possible to obtain transformants in a LexA(Def) SulA$^-$ mutant. To corroborate this hypothesis, we verified that whereas plasmid pUA165 (Fig. 1) yields transformants when introduced into the UA4567 strain (SulA$^-$), this is not the case in either the UA4793 [SulA$^-$ LexA(Def)] or the UA4794 [SulA$^-$ LexA(Def) RecA$^-$] strain (data not shown). On the other hand, transformants in all three strains were detected when a pUA165 derivative plasmid harboring the wild-type *lexA* gene of *E. coli* was employed (data not shown). We used killer vector pUA165 to isolate *lexA*-like genes of several bacteria by using a LexA(Def) SulA$^-$ HsdR$^-$ mutant as the recipient.

TABLE 2. Effects of *lexA*-like genes of several bacterial species on the basal levels of transcription of the *sulA* gene in two different LexA(Def) mutants of *E. coli*[a]

| Source of *lexA* gene (plasmid) | Specific units of β-galactosidase[b] | |
|---|---|---|
| | *lexA300*(Del) | *lexA71*::Tn*5*(Def) |
| None (pACYC184) | 5,750 | 5,900 |
| *P. putida* (pUA166) | 650 | 690 |
| *E. carotovora* (pUA167) | 440 | 500 |
| *S. typhimurium* (pUA168) | 350 | 360 |
| *E. coli* (pUA169) | 230 | 215 |
| *P. aeruginosa* (pUA170) | 750 | 800 |

[a] Cells were grown in minimal AB medium supplemented with glucose and Casamino Acids.
[b] All values were reproducible to within an error of ±10%.

**Isolation of *lexA*-like genes of *S. typhimurium*, *E. carotovora*, *P. aeruginosa*, and *P. putida*.** Two members of the family *Enterobacteriaceae* (*S. typhimurium* and *E. carotovora*) and two members of the family *Pseudomonadaceae* (*P. aeruginosa* and *P. putida*) were chosen to clone the *lexA*-like genes of bacteria different from *E. coli*. These species were selected because they have a *lexA*-like gene capable of repressing expression of the SOS genes of *E. coli* in vivo (9). In addition, two of them are closely related to *E. coli*, whereas the other two are not. Chromosomal DNAs from these bacteria, as well as from *E. coli* AB1157 as a positive control, were partially digested with *Sau*3AI, and fragments in the range of 5 to 10 kb were purified and ligated to plasmid pUA165, which had previously been digested with *Bam*HI. The ligation products were transformed by electroporation in strain UA4794. Two kinds of clones were detected. One of them presented a high basal level of expression of the *sulA*::*lacZ* fusion contained in strain UA4794, whereas the second showed lower expression of this fusion when plated on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside plates. Plasmid DNA isolation and restriction analysis of both kinds of colonies showed that the first type was a spontaneous deletion derivative of plasmid pUA165 lacking a fragment of the *sulA* gene. The second kind contained a pUA165 plasmid derivative carrying a heterologous fragment of DNA. From these last clones, plasmids pUA166, pUA167, pUA168, pUA169, and pUA170 harboring DNAs from *P. putida* (6.2 kb), *E. carotovora* (7.4 kb), *S. typhimurium* (8.3 kb), *E. coli* (8.4 kb), and *P. aeruginosa* (6.2 kb), respectively, were purified and used for further experiments.

**Behavior of the *lexA*-like genes isolated.** The above-mentioned plasmids were transformed in both the UA4793 and JL2301 strains of *E. coli* to analyze their effects on the basal level of *sulA* gene expression in two different LexA(Def) backgrounds. The data obtained showed that in both the *lexA71*::Tn*5* and *lexA300*(Del) mutants, the presence of recombinant plasmids gave rise to a dramatic decrease in basal expression of the *sulA* gene (Table 2). The plasmid carrying the *lexA* gene of *S. typhimurium* produced the largest decrease in *sulA* gene transcription, and the *lexA* gene of *P. aeruginosa* gave rise to the smallest decrease. These data agree with previous results indicating that the affinity for the SOS boxes of *E. coli* is higher for the LexA

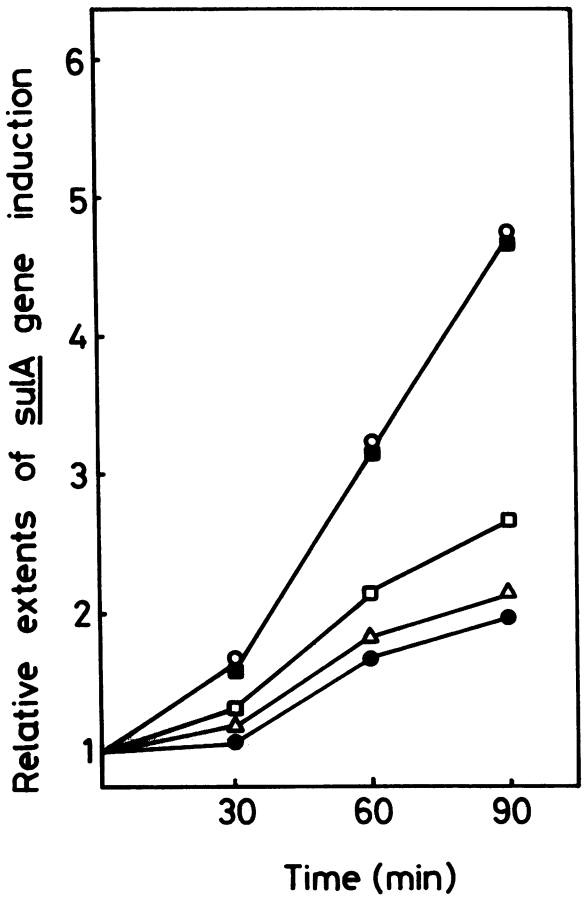


FIG. 2. Induction of a *sulA-lacZ* fusion in *E. coli* UA4793 (*lexA71*::Tn*5* *recA*$^+$) carrying the *lexA* gene of *E. coli* (○), *S. typhimurium* LT2 (■), *E. carotovora* (□), *P. aeruginosa* (●), or *P. putida* (△) after treatment with mitomycin C at 40 μg/ml. The relative extent of gene induction for each strain is the ratio between specific units of β-galactosidase of the mitomycin C-treated cells and the specific enzyme units of the untreated cells. All values were reproducible to within an error of ±10%.

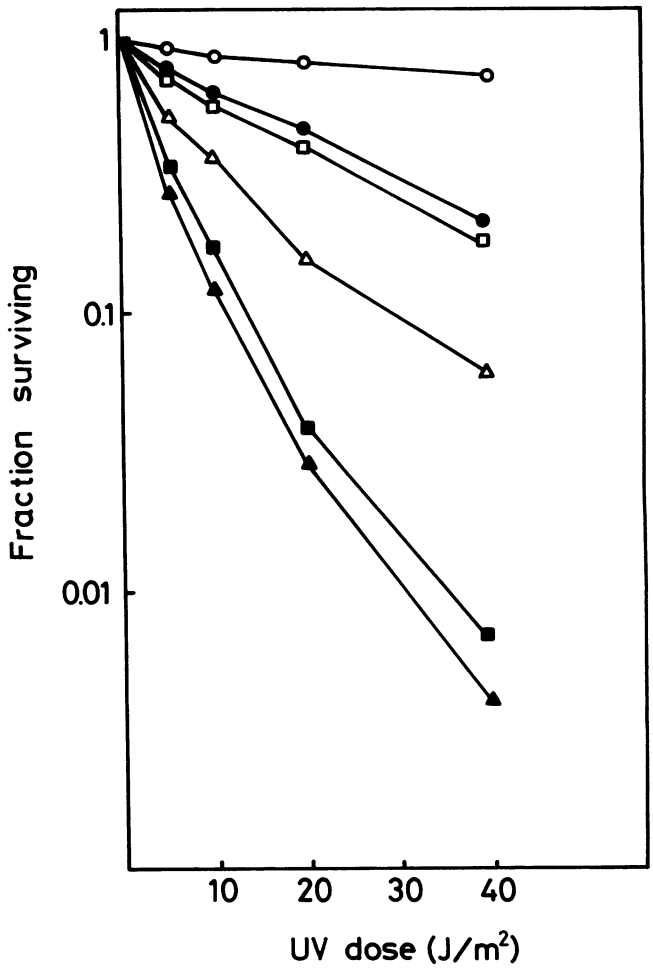


FIG. 3. Survival of *E. coli* UA4793 (*lexA71*::Tn*5* *recA*$^+$) carrying the *lexA* gene of *E. coli* (▲), *S. typhimurium* LT2 (■), *E. carotovora* (△), *P. aeruginosa* (●), or *P. putida* (□) after UV irradiation at different doses. The survival of strain UA4793 containing plasmid pACYC184 with no *lexA* gene is also shown as a control (○). All values were reproducible to within an error of ±10%.

repressor of *S. typhimurium* than for that of *P. aeruginosa* in vivo (9). Subsequently, a *lexA41* mutant of *E. coli* was transformed with all of these plasmids to confirm that the *lexA*-like genes obtained were capable of suppressing any *lexA*(Def) mutation. This mutant produces a partially defective LexA repressor which enables growth of the cells at 30°C but not at 42°C (27). The temperature sensitivity phenotype of this strain was eliminated by the presence of all of the *lexA*-like genes tested (data not shown).

All of these results indicated that the products of the various *lexA* genes isolated were able to bind to the SOS boxes of *E. coli*. Nevertheless, they provided no indication of whether cleavage of these LexA repressors could be promoted by the activated RecA protein of *E. coli*. For this reason, mitomycin C-mediated induction of *sulA* gene expression in the UA4793 strain containing these *lexA*-like genes was analyzed. The data obtained show that in all cases there was an increase in the expression of *sulA*::*lacZ* after DNA damage (Fig. 2), indicating that all LexA repressors may be hydrolyzed in RecA$^+$ cells of *E. coli*. It is worth noting that LexA proteins supporting the highest basal level of the *sulA*::*lacZ* fusion (those of *P. aeruginosa* and *P. putida*) showed the lowest relative extents of DNA damage-mediated induction. This is probably due to the fact that the more distantly related LexA repressors are less efficiently hydrolyzed by the coprotease RecA of *E. coli*.

It is known that in *E. coli*, the presence of a multicopy plasmid harboring the *lexA* gene enhances the sensitivity of the cells to DNA damage as a consequence of stronger repression of SOS genes, such as *uvrABD*, which directly participate in DNA repair (33). We therefore studied the behavior of all of the *lexA*-like genes isolated. Figure 3 shows that all of them caused UV sensitization of the UA4793 strain. LexA repressors showing the highest affinity for the SOS boxes of *E. coli* (*S. typhimurium* and *E. carotovora*) also produced the highest radiosensitization. These differences between the several *lexA* genes must be attributed to the fact that the multicopy-mediated overrepression of SOS genes is stronger in closely *E. coli*-related *lexA*-like genes (*S. typhimurium* and *E. carotovora*) than in distantly related ones (*P. aeruginosa* and *P. putida*), as shown in Table 2.

Finally, we used *E. coli lexA* gene DNA to determine

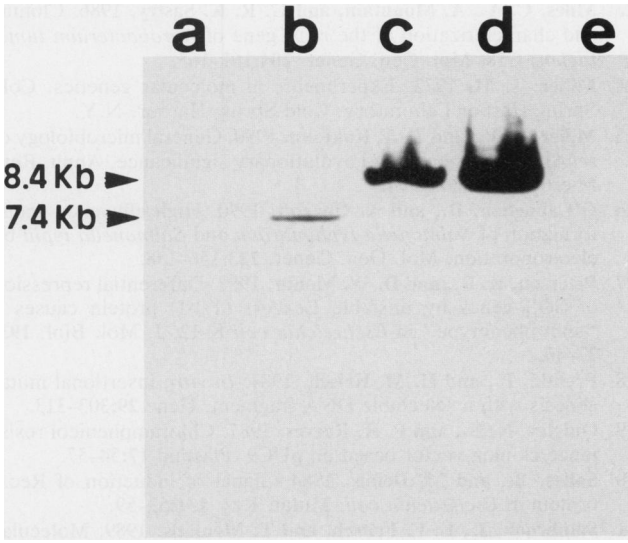


FIG. 4. Southern blot analysis of DNAs of plasmids pUA166, pUA167, pUA168, pUA169, and pUA170 containing, respectively, the *lexA* genes of *P. putida*, *E. carotovora*, *S. typhimurium*, *E. coli*, and *P. aeruginosa*. Lanes: a, pUA166 digested with *Hin*dIII; b, pUA167 digested with *Pst*I; c, pUA168 digested with *Xho*I; d, pUA169 digested with *Hin*dIII; e, pUA170 digested with *Hin*dIII. The internal 538-bp *Hin*cII fragment of the *lexA* gene of *E. coli* obtained from plasmid pUA75 was used as a probe. The quantity of plasmid DNA applied in each lane was 100 ng.

whether there was homology with the isolated *lexA* genes. The results obtained (Fig. 4) indicated that, as expected under high-stringency conditions, the *lexA* genes of *S. typhimurium* and *E. carotovora* present homology with the *E. coli lexA* gene. However, and according to the intensity of the hybridization, the homology of *S. typhimurium* must be higher than that of *E. carotovora*. On the other hand, the *lexA* genes of both *P. putida* and *P. aeruginosa* did not show hybridization with the *lexA* gene of *E. coli* (Fig. 4). These data agree with previous results concerning the *recA* genes of these bacteria. Thus, it has been shown that the nucleotide sequence of the *recA* gene of *E. carotovora* is 78% identical to that of *E. coli recA* (25), whereas the identity at the DNA level between the *P. aeruginosa* and *E. coli recA* genes is only 57% (25).

## DISCUSSION

The aim of this study was to develop a system which would allow direct isolation of bacterial *lexA* genes. For this purpose, we constructed *lexA*-conditional killer vector pUA165 carrying the wild-type *sulA* gene of *E. coli* and a broad polylinker to insert heterologous DNA. When pUA165 is introduced into a SulA$^-$ LexA(Def) mutant, cells die with the exception of those containing a *lexA*-like gene inserted into this plasmid. This method will be of great use in cloning *lexA* genes different from that of *E. coli*. In fact, we isolated this gene from two members of the family *Enterobacteriaceae* and two of the family *Pseudomonadaceae*; these are the first bacterial *lexA* genes different from that of *E. coli* which have been cloned. Our results also show that all of the resulting LexA proteins can block expression of the SOS genes of *E. coli* and may be cleaved by the activated RecA protein of *E. coli*. LexA repressors of *S. typhimurium* and *E. carotovora* seem to have a stronger affinity for the SOS box of *E. coli* than do the LexA repressor of *P. aeruginosa* and *P. putida*, as shown by both the basal level of *sulA* gene expression (Table 2) and multicopy *lexA*-mediated radiosensitization (Fig. 3). These data agree with previous findings on the regulation of expression of the *recA* gene of *E. coli* in those bacterial species in vivo (9).

Isolation of *lexA*-like genes is important in that it will allow us to analyze the conservation of the sequence of this gene in several bacteria, as well as to know in which species it is present. In this respect, studies of the sequences of several *lexA* genes can provide many data concerning the several domains of the LexA protein implied in both SOS box interaction and RecA-mediated cleavage. In a similar way, the comparison of sequences of several *recA*-like genes has been very helpful in determining the several functional domains of the RecA protein (25).

Other phenomena besides DNA repair have been associated with the SOS network. In the phytopathogenic bacterium *E. carotovora*, for instance, synthesis of pectin lyase and carotovoricin is induced by DNA damage in RecA$^+$ strains but not in RecA$^-$ mutants (22, 38). Similarly, syntheses of bacteriocins other than colicins are also induced by DNA damage (3). In this field, it is known that many colicin genes have a LexA-binding region in their respective promoters (35), although there is no information about whether this region is also present in bacteriocin genes belonging to another bacterial genus. Isolation of *lexA* genes of the bacterial species involved in these unrelated DNA repair processes may be useful for further study. In the same way, by introducing inactivated *lexA*-like genes into the original hosts, it will be possible to determine whether the relationship between the SOS response and the *sulA* system is limited to *E. coli* or is widespread. In summary, the possibility of directly obtaining bacterial *lexA* genes by the method reported in this work may facilitate the evolutive analysis of one of two regulatory genes of the SOS response, as well as other SOS-related bacterial behaviors.

## ACKNOWLEDGMENTS

This work was supported by grant PB88-0246 of the Comisión Interministerial de Ciencia y Tecnologia (CICYT), Spain. Sebastián Calero and Xavier Garriga were supported by predoctoral fellowships from the Spanish Ministry of Education and Science and the Autonomous University of Barcelona, respectively.

## REFERENCES

1. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1990. Current protocols in molecular biology. John Wiley & Sons, Inc., New York.
2. **Barbé, J., I. Gibert, M. Llagostera, and R. Guerrero.** 1987. DNA repair systems in the phototrophic bacterium *Rhodobacter capsulatus*. J. Gen. Microbiol. **133**:961–966.
3. **Benbrook, D. M., and R. V. Miller.** 1986. Effects of norfloxacin on DNA metabolism in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **29**:1–6.
4. **Better, M., and D. R. Helinski.** 1983. Isolation and characterization of the *recA* gene of *Rhizobium meliloti*. J. Bacteriol. **155**:311–316.
5. **Casaregola, S., R. D'Ari, and O. Huisman.** 1982. Quantitative evaluation of *recA* gene expression in *Escherichia coli*. Mol. Gen. Genet. **185**:430–439.
6. **Clark, D. J., and O. Maaløe.** 1967. DNA replication and the division cycle of *Escherichia coli*. J. Mol. Biol. **23**:99–112.
7. **Craig, N. L., and J. W. Roberts.** 1980. *E. coli recA* protein-directed cleavage of phage λ repressor requires polynucleotide. Nature (London) **283**:26–30.
8. **Elhai, J., and C. P. Wolk.** 1988. A versatile class of positive-selection vectors based on the nonviability of palindrome-

containing plasmids that allows cloning into long polylinkers. Gene **68**:119–138.

9. **Fernandez de Henestrosa, A. R., S. Calero, and J. Barbé.** 1991. Expression of the *recA* gene of *Escherichia coli* in several species of gram-negative bacteria. Mol. Gen. Genet. **226**:503–506.
10. **Gibert, I., and J. Barbé.** 1990. Cyclic AMP stimulates transcription of the structural gene of the outer-membrane protein OmpA of *Escherichia coli*. FEMS Microbiol. Lett. **68**:307–312.
11. **Gibert, I., S. Calero, and J. Barbé.** 1990. Measurement of *in vivo* expression of *nrdA* and *nrdB* genes of *Escherichia coli* by using *lacZ* gene fusions. Mol. Gen. Genet. **220**:400–408.
12. **Hill, S. A., and J. W. Little.** 1988. Allele replacement in *Escherichia coli* by use of a selectable marker for resistance to spectinomycin: replacement of the *lexA* gene. J. Bacteriol. **170**:5913–5915.
13. **Huisman, O., and R. D'Ari.** 1981. An inducible DNA replication-cell division coupling mechanism in *E. coli*. Nature (London) **290**:797–799.
14. **Huisman, O., R. D'Ari, and S. Gottesman.** 1984. Cell-division control in *Escherichia coli*: specific induction of the SOS *sfiA* protein is sufficient to block septation. Proc. Natl. Acad. Sci. USA **81**:4490–4494.
15. **Keener, S. L., K. P. McNamee, and K. McEntee.** 1984. Cloning and characterization of *recA* genes from *Proteus vulgaris*, *Erwinia carotovora*, *Shigella flexneri*, and *Escherichia coli* B/r. J. Bacteriol. **160**:153–160.
16. **Kokjohn, T. A., and R. V. Miller.** 1985. Molecular cloning and characterization of the *recA* gene of *Pseudomonas aeruginosa* PAO. J. Bacteriol. **163**:568–572.
17. **Lieberman, H. B., and E. M. Witkin.** 1981. DNA degradation, UV sensitivity and SOS-mediated mutagenesis in strains of *Escherichia coli* deficient in single-strand DNA binding protein: effects of mutations and treatments that alter levels of exonuclease V or *recA* protein. Mol. Gen. Genet. **190**:92–100.
18. **Little, J. W.** 1984. Autodigestion of *lexA* and phage λ repressors. Proc. Natl. Acad. Sci. USA **81**:1375–1379.
19. **Little, J. W., S. H. Edmiston, L. Z. Pacelli, and D. W. Mount.** 1980. Cleavage of the *Escherichia coli lexA* protein by the *recA* protease. Proc. Natl. Acad. Sci. USA **77**:3225–3229.
20. **Little, J. W., and D. W. Mount.** 1982. The SOS regulatory system of *Escherichia coli*. Cell **29**:11–22.
21. **McCall, J. O., E. M. Witkin, T. Kogoma, and V. Roegner-Maniscalco.** 1987. Constitutive expression of the SOS response in *recA718* mutants of *Escherichia coli* requires amplification of RecA718 protein. J. Bacteriol. **169**:728–734.
22. **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.
23. **Miles, C. A., A. Mountain, and G. R. K. Sastry.** 1986. Cloning and characterization of the *recA* gene of *Agrobacterium tumefaciens* C58. Mol. Gen. Genet. **204**:161–165.
24. **Miller, J. M.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Miller, R. V., and T. A. Kokjohn.** 1990. General microbiology of *recA*: environmental and evolutionary significance. Annu. Rev. Microbiol. **44**:365–394.
26. **O'Callaghan, D., and A. Charbit.** 1990. High efficiency transformation of *Salmonella typhimurium* and *Salmonella typhi* by electroporation. Mol. Gen. Genet. **223**:156–158.
27. **Peterson, K. R., and D. W. Mount.** 1987. Differential repression of SOS genes by unstable LexA41 (Tsl-1) protein causes a "split-phenotype" in *Escherichia coli* K-12. J. Mol. Biol. **193**:27–40.
28. **Prentki, P., and H. M. Krisch.** 1984. *In vitro* insertional mutagenesis with a selectable DNA fragment. Gene **29**:303–313.
29. **Quigley, N. B., and P. R. Reeves.** 1987. Chloramphenicol resistance cloning vector based on pUC9. Plasmid **17**:54–57.
30. **Salles, B., and M. Defais.** 1984. Signal of induction of RecA protein in *Escherichia coli*. Mutat. Res. **131**:53–59.
31. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. **Schneider, K., and C. F. Beck.** 1987. New expression vectors for identifying and testing signal structures for initiation and termination of transcription. Methods Enzymol. **153**:452–461.
33. **Sedgwick, S. G., and P. A. Goodwin.** 1985. Interspecies regulation of the SOS response by the *E. coli lexA*$^+$ gene. Mutat. Res. **145**:103–106.
34. **Tessman, E. S., and P. Peterson.** 1985. Plaque color method for rapid isolation of novel *recA* mutants of *Escherichia coli* K-12: new classes of protease-constitutive *recA* mutants. J. Bacteriol. **163**:677–687.
35. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48**:60–93.
36. **Weinstock, G. M., and K. McEntee.** 1981. RecA protein dependent proteolysis of bacteriophage lambda repressor. Characterization of the reaction and stimulation by DNA-binding proteins. J. Biol. Chem. **256**:10883–10888.
37. **Ysern, P., B. Clerch, M. Castaño, I. Gibert, J. Barbé, and M. Llagostera.** 1990. Induction of SOS genes in *Escherichia coli* and mutagenesis in *Salmonella typhimurium* by fluoroquinolones. Mutagenesis **5**:63–66.
38. **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.