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Nucleotide sequence of the lexA gene of Escherichia coli K-12

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ABSTRACT

A number of *E. coli* genes exhibit increased expression when the cellular DNA is damaged. In undamaged cells, *lexA* repressor limits the extent of their transcription, whereas, in damaged cells, the repressor is cleaved by a cellular protease, the product of the *recA* gene. We have sequenced 943 base pairs of cloned *E. coli* DNA containing the *lexA* gene. A regulatory region has been identified, followed by a translational open reading frame which encodes a polypeptide of 202 amino acids with a molecular weight of 22,300. The protein contains a single alanyl-glycyl peptide near its middle. This peptide is also found in certain phage repressors which are cleaved by the *recA* protease and has been shown to be the site of cleavage in these repressors. We have determined the nucleotide sequence of a portion of the *lexA*3 gene, whose product is 100-fold less susceptible to *recA* protease than the wild type repressor. We report a single base change (G to A) which alters the unique alanine-glycine sequence to alanine-aspartic acid.

INTRODUCTION

Following damage to DNA or impairments of replication, *E. coli* cells express a number of new functions, including such diverse phenomena as induced mutagenesis, enhanced DNA repair capacity, inhibition of cell division and prophage induction (1). At least part of this response is controlled by a complex system of transcriptional regulation involving the products of two genes, *recA* and *lexA*. Genetic and biochemical evidence supports the model that *lexA* protein is a repressor of many unlinked cellular genes and that, after inducing treatments, *recA* protein inactivates repressor function by means of a specific protease activity which cleaves the repressor into two fragments (2,3,4). Expression of new functions thus ensues.

We wished to determine the nucleotide sequence of the *lexA* gene for several reasons. First, it has proven difficult to do fine structure mapping of the gene because the available mutants have complex phenotypes. Moreover, *lexA* alleles can influence expression of the *recA* gene, whose

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product is required for genetic recombination in *E. coli*. As an alternative to classical genetic methods such as deletion mapping, it is possible to sequence mutant *lexA* genes and, by comparison with the wild type sequence, determine the alterations in the mutant. The availability of the sequence and of changes in various mutant proteins would allow a structure-function correlation to be made.

A second reason for determining the nucleotide sequence of *lexA*, and the corresponding amino acid sequence, is that it would facilitate a study of repressor cleavage by the *recA* protease. The N-terminal amino acid sequences of the cleavage products would localize the cleavage site in the sequence, as has been done with the lambda repressor (5).

A third reason originates from the close analogy between the proteolytic cleavage of the *lexA* and lambda repressors. The fact that these two substrates, along with a few other prophage repressors, are the only known substrates for this highly specific reaction (3, 6-11, B. Markham, unpublished data) suggests that *lexA* protein might be functionally and structurally similar to the well-studied lambda repressor. Lambda repressor is known to have two functional domains, and cleavage separates them (12). It would be of interest to know whether *lexA* protein is similarly organized. Moreover, the *lexA* protein and lambda repressor are similar in size: lambda repressor is 26.2 kd in size (13) and the *lexA* protein has been estimated at 24-25 kd (2,3,14). It is therefore possible that the genes coding for two proteins share homologies, a proposition testable by comparing their nucleotide sequences. Mutant lambda and *lexA* repressors which are resistant to cleavage (lambda *ind*$^-$ and *lexA*3 mutants) have been identified, and comparison of these mutant repressor sequences should provide information as to the mechanism of cleavage.

Finally, knowing the sequence would facilitate *in vitro* manipulations to create altered forms of the protein by site-directed mutagenesis, reassortment of restriction fragments from various mutant genes, and specific deletions.

In this report we give the complete sequence of the wild type *lexA* gene and a partial sequence of the *lexA*3 mutant gene which encodes a protease-resistant repressor.

MATERIALS AND METHODS

Bacteral strains and plasmids.

The bacterial strains used and their relevant genetic markers were

GM119 dam-3 dcm-1, from M. Marinus; the "maxicell" strain CSR603 recA1 uvrA6 phr-1 (15); DM511 lexA3 tsl-1 : tsl is a recessive allele of lexA which causes thermosensitive growth at 42.5° (16); plasmids with lexA function can be selected by their tsl+ phenotype (17); F' lacI^{Q1}, which overproduces the lac repressor 100-fold (18), was obtained from M. Calos; JL438 (DM511/F' lacI^{Q1}) and JL441 (CSR603/F'lacI^{Q1}) were prepared by conjugation. Multicopy plasmids used were pLJ3, carrying the lacUV5 promoter (19), from T. Roberts; pJL21 and pJL26, carrying lexA+ and lexA3, respectively (17); and pJL42, pJL44 and pJL45, described below. Multicopy plasmids were introduced into various hosts by transformation (17).

Restriction enzyme analysis.

Restriction endonucleases were obtained from the following sources: AluI, BclI, BstNI, DdeI, HaeII, HaeIII, HincII, HinfI, HpaI, HphI, MboI, MspI, PvuII, RsaI, and TaqI were obtained from New England Biolabs; ClaI, EcoRI and HindIII from Boehringer Mannheim. These enzymes were used as directed by the supplier. Restriction enzyme analysis of the lexA gene carried on pJL21 was done either by single and double digests of DNA, or by the method of Smith and Birnstiel (20) for frequent cutters, using DNA labeled at the EcoRI site. Single and double restriction digests of pJL21 and pJL26 were used to compare the lexA genes carried on these plasmids.

Enzymes and radioactive label.

T4 DNA ligase, polynucleotide kinase and DNA polymerase I (large fragment) were from New England Biolabs; bacterial alkaline phosphatase was from Millipore. Gamma [^{32}P]-ATP (1000-3000 Ci/mmole) was obtained from New England Nuclear.

Plasmid preparation and gel electrophoresis.

Plasmid preparations and gel electrophoresis were carried out as described (17).

Construction of pJL42.

The insert in the lexA+ plasmid pJL21 contains a single ClaI site about 1150 bp from the EcoRI site. pJL21 grown in a dam+ dcm+ host is not cleaved at this site by ClaI, and it is likely that an MboI site mapped at or near this ClaI site (not shown) lies adjacent to it in the sequence -ATCGATC-; methylation of the A residue in the GATC sequence might block ClaI action, as has been observed for other enzymes (21). To construct pJL42, pBR322 and unmethylated pJL21 DNA's were cut with EcoRI and ClaI, mixed and ligated; pJL42 was isolated following transformation of DM511 and selection for ampicillin resistance and tsl+ as described (17).

Construction of pJL44.

Plasmid pLJ3 was cut with *EcoRI* and *PvuII* to generate two fragments (95 and 190 bp) bearing the *lac*UV5 promoter (T. Roberts, personal communication). The 95-bp *EcoRI-PvuII* fragment, with the *lac*UV5 promoter directed toward the *PvuII* site, was isolated from an 8% acrylamide gel (22). pJL42 contains a single *HpaI* site at position 39 of the *lexA* sequence (Fig. 2), and a single *EcoRI* site; it was cut with these enzymes, mixed with the 95-bp *lac*UV5 promoter-fragment, treated with T4 DNA ligase, and treated with *HpaI*. After transformation of the *tsl* strain JL438, ampicillin-resistant clones were isolated at the permissive temperature (30°C); plasmid preparations were made and characterized by restriction mapping with *MspI*, which cuts within the *lac* promoter (not shown).

Construction of pJL45.

To fuse *lexA* to the *lac* promoter we wished to cut pJL21 at the *BstNI* site just before the start of the *lexA* coding sequence. Since this plasmid contains about 12 *BstNI* sites, pJL21 DNA (20 µg/ml) was cut with *BstNI* at 60°C in the presence of 1.6 mg/ml ethidium bromide; about half of the plasmid molecules were cut only once, and the rest were cut several times. DNA was treated successively with DNA polymerase I large fragment in the presence of dATP and dTTP, phenol and ether extraction, ethanol precipitation, alkaline phosphatase, phenol and ether extraction and ethanol precipitation, and then mixed with the 95 bp *lac*UV5 promoter fragment described above, and treated with T4 DNA ligase; after transformation of strain JL438, tsl^{+} tet^{r} clones were selected. Since this host expresses *lac* repressor at a high level, expression of *lexA* should be at very low levels. Consequently the selection for functional *lexA* protein ensured that the gene survived the manipulations intact. From 20 such clones, plasmids were isolated and screened by digestion with *MspI*; most were like pJL21, but one plasmid, pJL45, had the expected restriction pattern. It expresses *lexA* protein at very high levels upon treatment with the inducer IPTG (4).

When we sequenced the fusion, we found that the *lac* promotor had been fused not at the *BstNI* site but at position -9 of the *lexA* sequence (Fig. 3), 27 bp upstream from the *BstNI* site, generating a *HinfI* site. How this fusion arose is unclear.

Sequencing techniques.

Determination of DNA sequences was carried out according to Maxam and Gilbert (22) using the G, G+A, C+T and C reactions (procedures 10-13) except

as follows: the G+A reaction was incubated at 37°C for 7 min.; following the piperidine reaction, reaction mixtures were ethanol-precipitated (23); and the alkaline phosphatase reaction was as described (24). The sequencing strategy for the *lexA*$^+$ gene is shown in Fig. 1. The DNA used for sequencing the *lexA*$^+$ gene was an 1150-bp EcoR1-HindIII fragment from pJL42. A 624-bp *MspI* fragment (from position 209 to 833) from pJL26 containing the central portion of the *lexA*3 gene was used to sequence over an altered *MspI* site. The *lacP*-*lexA*$^+$ fusions were sequenced from a 278-bp *EcoRI*-*HinfI* fragment of pJL44 and a 445-bp *EcoRI*-*DdeI* fragment of pJL45.

RESULTS

Restriction endonuclease mapping.

The 5' end of the *lexA* gene has been located to within 140-bp of an *EcoRI* site (2), and *lexA* mRNA starts 74-bp from this site (25,26). A restriction map of the gene was prepared and those sites which proved useful for sequencing are shown in Fig. 1.

Nucleotide sequence of the lexA gene.

Fig. 2 shows the complete nucleotide sequence of the *lexA* gene, and DNA proximal and distal to it, determined on DNA obtained from pJL42. The

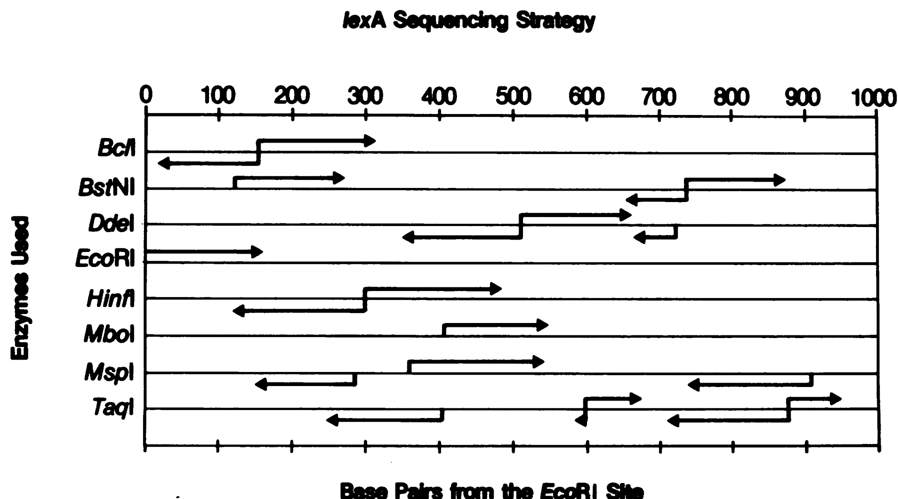

Figure 1. Sequencing strategy for the *lexA* gene. The arrows indicate the direction of sequencing and the length of sequence determined. Secondary restriction cuts are not shown. The map positions are shown relative to the *EcoRI* site.

numbering of the sequence is relative to the mRNA start site (25,26). The regulatory region contains the following control elements: 1) a possible -35 region (27) from positions -37 to -26 and a Pribnow box (28,29), -TATACTC- from positions -12 to -6; 2) two lexA protein binding sites (see Discussion); and 3) a putative Shine-Dalgarno sequence (30), -AGGGGG-, from +19 to +24.

An open reading frame was found beginning at position +29 and extending 606 bp to a TAA termination codon. Following this nonsense codon, we found a 14-bp region of hyphenated dyad symmetry from +650 to +676; contained within this (+651 to +675) is an 83% G+C rich sequence immediately followed by -TTTGTTTTT-. These features are common to transcription termination sites (31).

A partial verification of the sequence in Fig. 2 came from the restriction mapping data (not shown). Single BclI, HaeIII, HpaI, RsaI and multiple DdeI sites were located with single and double digests; all the AluI, BstNI, HaeII, HhaI, HinfI, MspI, MboI and TaqI sites, and all but one HphI (position 410) site were localized by the method of Smith and Birnstiel (20).

The start point of the lexA coding sequence.

Sequence analysis of two lacUV5 promoter-lexA fusion plasmids (Fig. 3) confirmed the location given for the start of the lexA coding sequence. In plasmid pJL44, the lac promoter is fused to position 40 of the lexA gene, and the sequence is that expected from the construction, in which two blunt ends from PvuII and HpaI cuts were joined (see Materials and Methods). Two lines of evidence indicated that this plasmid does not express functional lexA protein: first, it did not complement the recessive tsl mutation; second, lexA protein could not be observed in maxicell strains carrying this plasmid (data not shown). In plasmid pJL45, by contrast, the lac promoter was fused upstream from the presumed lexA coding sequence (see Materials and Methods and Fig. 3 for description of this fusion). This plasmid did express lexA function; even in a host overproducing the lac repressor 100-fold, pJL45 complemented the tsl defect. In maxicell strains carrying plasmid pJL45 and episome F'$lacI^{Q1}$, lexA protein was expressed at low levels in the absence of IPTG and at very high levels in the presence of IPTG (4). The phenotypes of these two plasmids indicate that the start point of the lexA coding sequence lies before position 40 in the mRNA, and the only AUG codon in this interval is the one indicated at position 29.

The predicted amino acid sequence of the lexA protein.

The amino acid sequence and the amino acid composition derived from it are shown

```
          -60                             -30                  1
GAATTCGATAAATCTCTGGTTTATTGTGCAGTTTATGGTTCCAAAATCGCCTTTTGCTGTATATACTCACAGCATAA

               20                            50                            80
CTGTATATACACCCAGGGGGCGGAATGAAAGCGTTAACGGCCAGGCAACAAGAGGTGTTTGATCTCATCCGTGATCAC
                             MetLysAlaLeuThrAlaArgGlnGlnGluValPheAspLeuIleArgAspHis

               100                           130                           160
ATCAGCCAGACAGGTATGCCGCCGACGCGTGCGGAAATCGCGCAGCGTTTGGGGTTCCGTTCCCCAAACGCGGCTGAA
IleSerGlnThrGlyMetProProThrArgAlaGluIleAlaGlnArgLeuGlyPheArgSerProAsnAlaAlaGlu

                 180                           210

GAACATCTGAAGGCGCTGGCACGCAAAGGCGTTATTGAAATTGTTTCCGGCGCATCACGCGGGATTCGTCTGTTGCAG
GluHisLeuLysAlaLeuAlaArgLysGlyValIleGluIleValSerGlyAlaSerArgGlyIleArgLeuLeuGln

                   260                     (Asp)  290
                                             A
GAAGAGGAAGAAGGGTTGCCGCTGGTAGGTCGTGTGGCTGCCGGTGAACCACTTCTGGCGCAACAGCATATTGAAGGT
GluGluGluGluGlyLeuProLeuValGlyArgValAlaAlaGlyGluProLeuLeuAlaGlnGlnHisIleGluGly

                     340                           370

CATTATCAGGTCGATCCTTCCTTATTCAAGCCGAATGCTGATTTCCTGCTGCGCGTCAGCGGGATGTCGATGAAAGAT
HisTyrGlnValAspProSerLeuPheLysProAsnAlaAspPheLeuLeuArgValSerGlyMetSerMetLysAsp

                     420                           450
ATCGGCATTATGGATGGTGACTTGCTGGCAGTGCATAAAACTCAGGATGTACGTAACGGTCAGGTCGTTGTCGCACGT
IleGlyIleMetAspGlyAspLeuLeuAlaValHisLysThrGlnAspValArgAsnGlyGlnValValValAlaArg

                       500                           530
ATTGATGACGAAGTTACCGTTAAGCGCCTGAAAAAACAGGGCAATAAAGTCGAACTGTTGCCAGAAAATAGCGAGTTT
IleAspAspGluValThrValLysArgLeuLysLysGlnGlyAsnLysValGluLeuLeuProGluAsnSerGluPhe

                         580                           610
AAACCAATTGTCGTTGACCTTCGTCAGCAGAGCTTCACCATTGAAGGGCTGGCGGTTGGGGTTATTCGCAACGGCGAC
LysProIleValValAspLeuArgGlnGlnSerPheThrIleGluGlyLeuAlaValGlyValIleArgAsnGlyAsp

                           660                           690
TGGCTGTAACATATCTCTGAGACCGCGTGCCGCCTGGCGTCGCGGTTTGTTTTTCATCTCTCTTCATCAGGCTTGTCT
TrpLeuEND

 710                           740                           770
GCATGGCATTCCTCACTTCATCTGATAAAGCACTCTGGCATCTCGCCTTACCCATGATTTTCTCCAATATCACCGTTC

   790                           820                           850
CGTTGCTGGGACTGGTCGATACGGCGGTAATTGGTCATCTTGATAGCCCGGTTTATTTGGGCGGCGTGGCGGTTGGCG

     870
CAACGGCG
```

Figure 2. Nucleotide sequence of the lexA DNA anticoding strand and the amino acid sequence derived from it. The underlined regions are those indicated in the text (see RESULTS). The boxed regions are binding sites for lexA protein (4). Nucleotides are numbered from the site corresponding to the 5' end of the lexA mRNA (25,26) and in each case the number refers to the base below the last digit. The unique alanine-glycine bond in the lexA protein is also underlined. The sequence derived from pJL26 (lexA3) is indicated by a line over the sequence. The only nucleotide change and the resulting amino acid change are indicated.

Plasmid	Sequence		lex function
	lacP	lexA	
		40	
pJL44	CAGGAAACAG	AACGGCCAGGC	-
		-9	
pJL45	CAGGAAACAG	ACTCACAGCAT	+

Figure 3. Sequence of the lacP-lexA fusions in pJL44 and pJL45. The nucleotide sequence in the region of the fusions are shown. The numbers indicate the position in the lexA sequence to which the lac promoter was fused.

in Fig. 2 and Table 1, respectively. The molecular weight calculated from the amino acid composition is 22,300. There is a unique alanine-glycine bond near the middle of the protein (Figs. 2 and 4) which may be a feature related to sensitivity to recA protease (see Discussion).

DNA sequence of the lexA3 mutant gene.

We determined the nucleotide sequence from the 5' end of a 624-bp MspI fragment of pJL26 (position +209 to +833) since a comparison of the MspI restriction digest pattern of pJL21 (lexA+) and pJL26 (lexA3) indicated the loss of an MspI site, within this region, at position 281 in the lexA3 mutant gene. This change was of special interest because it might result in a change of one of the amino acids in the unique alanine-glycine sequence. A single base change, G to A, was found at position 282. This was the only alteration from the wild type sequence that was observed in the 166 bases sequenced (from +209 to +375, Fig. 2). As a result of this difference the unique ala-gly sequence of the lexA+ protein is changed to alanine-aspartic acid in the mutant lexA3 protein.

DISCUSSION

We have determined the nucleotide sequence of the lexA gene and identified the regulatory region, the structural gene and a region containing features common to transcription termination sites (Fig. 2).

Table I Amino acid composition of the <u>lexA</u> protein derived from the nucleotide sequence

TYPE	AMINO ACID	#RESIDUES IN lexA PROTEIN
Non Polar	Alanine	16
	Isoleucine	13
	Leucine	22
	Methionine	5
	Phenylalanine	6
	Proline	9
	Valine	19
	Tryptophan	1
Polar (uncharged)	Asparagine	6
	Cysteine	0
	Glutamine	13
	Glycine	17
	Serine	9
	Tyrosine	1
	Threonine	6
Acidic	Aspartic acid	12
	Glutamic acid	16
Basic	Arginine	15
	Histidine	5
	Lysine	11
TOTAL		202

A previous comparison (4) of the *lexA* and *recA* operator sequences has revealed a single 20 bp region in the *recA* operator (not shown) which is closely homologous with two such regions in the *lexA* regulatory region. Each of these 20 bp regions share the sequences -CTGTAT- and -CAG- and have been shown to bind the *lexA* repressor; this binding prevents transcription

of distal sequences (4).

The translational start site was localized to the AUG codon at position 29 past the start of the lexA message. The lexA gene codes for a 22.3 kd protein as determined by the amino acid composition derived from the nucleotide sequence. The molecular weight of this protein was previously estimated, by its mobility in SDS polyacrylamide gels to be 25 kd (3,14). The reason for this discrepancy is unknown, but a similar disparity has been observed for the P22 repressor (32). No cysteine residues were found in the protein indicating that disulfide bridges have no role in stabilizing the tertiary structure or in the protein - protein interactions of the lexA product.

The fact that E. coli lexA and phage lambda and P22 repressors are susceptible to cleavage by the recA protease (3,7-11,32) prompted a comparison of their respective amino acid sequences. Interestingly, little obvious homology was found between lexA repressor and the phage repressors, although the phage repressors contain substantial homology to each other (32; unpublished observations). Each of these repressors, however, does contain an alanine-glycine bond located near the middle of the molecule (Fig. 4). recA protease cuts lambda and P22 repressors between these two residues (5, 32) and the size of lexA cleavage fragments are consistent with cleavage at or near this bond (3).

In the lexA3 gene, a single base change (G to A) was observed at a position corresponding to 281 of the lexA$^+$ gene (Fig. 3). This nucleotide

lexA repressor sequence

```
                                                Asp
                                                GAT(lexA3)
GAA GAA GGG TTG CCG CTG GTA GGT CGT GTG GCT GCC GGT GAA CCA CTT CTG GCG
Glu Glu Gly Leu Pro Leu Val Gly Arg Val Ala Ala Gly Glu Pro Leu Leu Ala
 *               *                   *       *   *
Glu Tyr Glu Tyr Pro Val Phe Ser His Val Gln Ala Gly Met Phe Ser Pro Glu
GAG TAT GAG TAC CCT GTT TTT TCT CAT GTT CAG GCA GGG ATG TTC TCA CCT GAG
                                                                    AAG(ind-)
                                                                    Lys
```

Lambda repressor sequence

Figure 4. Comparison of the presumed cleavage site of the lexA protein with the cleavage site of the lambda repressor. The nucleotide and amino acid sequence of the lambda repressor are from Sauer (33). The sequences are aligned relative to the unique alanine-glycine bonds. Asterisks are placed between common amino acids in the two proteins. The ind$^-$ mutation of the lambda cI gene and the lexA3 mutation are indicated.

change altered the amino acid at residue 84 from glycine to aspartic acid thereby eliminating the alanine-glycine dipeptide in the lexA3 gene. Interestingly, the lexA3 product has been found to be about 100-fold less susceptible to recA protease cleavage than the lexA+ repressor (3). Based on the observation that the protease-resistant lexA3 gene product lacks an alanine-glycine bond, it seems plausible that this bond is the specific site of cleavage of the lexA repressor by the recA protease.

In the lambda ind- mutant, which also produces a repressor resistant to cleavage, the amino acid residue at position 117, 5 codons away from the cleavage site at residues 111 and 112, is altered from glutamic acid to lysine (33) (see Fig. 4). We have sequenced an area of the lexA3 gene spanning the presumed cleavage site and have found no difference between it and the lexA+ gene aside from the one described (Fig. 2). Electrophoretic analysis on SDS polyacrylamide gels of the lexA3 cleavage products (3) suggests that the altered amino acid lies in the larger fragment, since the lexA3 repressor large fragment has a lower mobility than the equivalent lexA+ repressor fragment; this finding is consistent with the proposed location for the cleavage site.

It was previously determined (3,14) that the lexA3 protein migrated more slowly upon electrophoresis in SDS-polyacrylamide gels than did the wildtype protein. This finding would be compatible with a small insertion into the lexA gene; however, we have seen no difference in the restriction patterns of the lexA gene in pJL21 and pJL26, for all of the restriction digestions done except for two. The MspI and HphI patterns were altered as expected from the sequence shown. The apparent lack of a sizable insertion argues, as do other electrophoretic data (3) and similar changes in other systems (34,35) that the altered mobility reflects a simple amino acid change.

After we had completed sequencing the wild-type lexA gene, another independent sequence of the lexA+ gene was reported (26). Within the proposed lexA coding sequence, and upstream to the EcoRI site our sequence agrees with that given. It differs downstream at positions +677, +707, +708, +833 and +866, where Horii et al. (26) found C, C, C, G, and insertion of a C respectively. These changes at positions +707 and +708 would create a TaqI site at +706. We have sequenced unmethylated DNA over this area from a TaqI site at position +800. Our ability to use this strategy indicates that no TaqI site is present at +706.

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