January 23, 2003

# Geobacter Sulfurreducens Has Two Autoregulated LexA Genes Whose Products do not Bind the RecA Promoter: Differing Response of LexA and RecA to DNA Damage

Mónica Jara
Cinthia Núñez
Susan Campoy
Antonio R Fernández de Henestrosa
Derek Lovley, *University of Massachusetts - Amherst*, et al.

Journal of Bacteriology

# *Geobacter sulfurreducens* Has Two Autoregulated *lexA* Genes Whose Products Do Not Bind the *recA* Promoter: Differing Responses of *lexA* and *recA* to DNA Damage

**Mónica Jara, Cinthia Núñuz, Susana Campoy, Antonio R. Fernández de Henestrosa, Derek R. Lovley and Jordi Barbé**
***J. Bacteriol.*** **2003, 185(8):2493. DOI: 10.1128/JB.185.8.2493-2502.2003.**

JOURNAL OF BACTERIOLOGY, Apr. 2003, p. 2493–2502 Vol. 185, No. 8
0021-9193/03/$08.00+0 DOI: 10.1128/JB.185.8.2493–2502.2003


# *Geobacter sulfurreducens* Has Two Autoregulated *lexA* Genes Whose Products Do Not Bind the *recA* Promoter: Differing Responses of *lexA* and *recA* to DNA Damage

Mónica Jara,[1] Cinthia Núñez,[2,3] Susana Campoy,[1] Antonio R. Fernández de Henestrosa,[1] Derek R. Lovley,[2] and Jordi Barbé[1,4]*

*Department of Genetics and Microbiology, Universitat Autònoma de Barcelona,[1] and Centre de Recerca en Sanitat Animal, Universitat Autònoma de Barcelona-Institut de Recerca i Tecnologia Agroalimentària, Bellaterra,[4] 08193 Barcelona, Spain; Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01003[2]; and Departamento de Microbiologia Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos 62251, Mexico[3]*

Received 15 November 2002/Accepted 23 January 2003

**The *Escherichia coli* LexA protein was used as a query sequence in TBLASTN searches to identify the *lexA* gene of the δ-proteobacterium *Geobacter sulfurreducens* from its genome sequence. The results of the search indicated that *G. sulfurreducens* has two independent *lexA* genes designated *lexA1* and *lexA2*. A copy of a *dinB* gene homologue, which in *E. coli* encodes DNA polymerase IV, is present downstream of each *lexA* gene. Reverse transcription-PCR analyses demonstrated that, in both cases, *lexA* and *dinB* constitute a single transcriptional unit. Electrophoretic mobility shift assays with purified LexA1 and LexA2 proteins have shown that both proteins bind the imperfect palindrome $GGTTN_2CN_4GN_3ACC$ found in the promoter region of both *lexA1* and *lexA2*. This sequence is also present upstream of the *Geobacter metallireducens lexA* gene, indicating that it is the LexA box of this bacterial genus. This palindrome is not found upstream of either the *G. sulfurreducens* or the *G. metallireducens recA* genes. Furthermore, DNA damage induces expression of the *lexA-dinB* transcriptional unit but not that of the *recA* gene. However, the basal level of *recA* gene expression is dramatically higher than that of the *lexA* gene. Likewise, the promoters of the *G. sulfurreducens recN*, *ruvAB*, *ssb*, *umuDC*, *uvrA*, and *uvrB* genes do not contain the LexA box and are not likely to bind to the LexA1 or LexA2 proteins. *G. sulfurreducens* is the first bacterial species harboring a *lexA* gene for which a constitutive expression of its *recA* gene has been described.**

*Geobacter sulfurreducens* is an Fe(III)-reducing microorganism belonging to the δ subdivision of *Proteobacteria* that is able to completely oxidize organic compounds to carbon dioxide with fumarate, Fe(III), elemental sulfur, or malate as terminal electron acceptors (16). Although this organism was previously classified as strict anaerobe, recent evidence indicates that it can tolerate oxygen exposure. It has recently been demonstrated that a brief exposure to oxygen can generate reactive oxygen species ($O_2^-$ and $H_2O_2$) in anaerobic bacteria (28). Furthermore, it is known that Fe(II) in combination with these oxygen species can result in DNA damage via the Fenton reaction (1, 9, 12, 18). These iron-mediated DNA damage-induced lesions seem to be repaired mainly by recombination (27, 32). Even in the absence of oxygen, other intracellular reductants, such as NADH and glutathione, can damage DNA in vitro and in vivo in the presence of iron (8, 23, 35).

Bacterial cells contain several DNA repair pathways, with those of *Escherichia coli* being the most studied. Among them, the SOS network is probably the most versatile DNA repair system. The *E. coli* SOS system contains about 40 genes that display several cellular activities, including recombination increase, error-prone DNA replication, inhibition of cell division, and prophage induction (5, 7, 34). These genes are regulated by the *recA* and *lexA* products, which also belong to this regulon (14). The *E. coli* LexA protein specifically binds the consensus sequence $CTGTN_8ACAG$, called the *E. coli* SOS box (34). When the DNA is not injured, LexA blocks SOS gene transcription. However, when DNA damage is present, the RecA protein acquires an active conformation and binds single-stranded DNA regions produced by DNA damage-mediated inhibition of replication (26). After activation, RecA promotes the autocatalytic cleavage of LexA at the $Ala^{84}$-$Gly^{85}$ bond with the participation of its own $Ser^{119}$ and $Lys^{156}$ residues (13, 17). The LexA cleavage, whose mechanism is very similar to that of serine proteases, triggers the induction of the SOS genes. After repair of DNA, the single-stranded regions disappear and the RecA protein is no longer activated resulting in an increase in the LexA repressor level which in turn inhibits SOS gene expression.

The *E. coli* SOS box is also present in the promoter of DNA damage-inducible genes in many members of γ-*Proteobacteria*, including those belonging to the *Enterobacteriaceae*, *Pseudomonadaceae*, *Vibrionaceae*, and *Pasteurellaceae* families, among others. Nevertheless, in other γ-*Proteobacteria*, such as *Xylella fastidiosa* and *Xanthomonas campestris*, the LexA-binding sequence ($TTAGN_6TACTA$) is different from that of *E. coli* (3). This fact indicates that a significant heterogeneity in the LexA-binding sequence exists in the γ class. In other bacterial phy-

* Corresponding author. Mailing address: Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Bellaterra, 08193 Barcelona, Spain. Phone: 34-93-581-1837. Fax: 34-93-581-2387. E-mail: jordi.barbe@uab.es.

TABLE 1. Bacterial strains and plasmids used in this study

| Strain or plasmid | Relevant features[a] | Source or reference |
|---|---|---|
| Strains | | |
| *G. sulfurreducens* DL1 | Wild-type strain | 2 |
| *E. coli* | | |
| DH5α | *supE4* Δ*lacU169* (ϕ80 *lacZ*ΔM15) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1* | Clontech |
| BL21-CodonPlus (DE3) | *E. coli* B $F^-$ *ompT hsdS*($r_B$ $m_B$) *dcm*$^+$ $Tet^r$ *gal* λ(DE3)*endA hte* (*argU ileY leuW* $Cam^r$) | Clontech |
| Plasmids | | |
| pGEM-T | PCR cloning vector; $Ap^r$ | Promega |
| pGEX-4T-1 | Overexpression vector with GST tag; $Ap^r$ | Promega |
| pUA1021 | pGEM-T derivative carrying a 1,075-bp PCR fragment containing the *G. sulfurreducens* *lexA1* gene, including its own promoter region | This work |
| pUA1022 | pGEM-T derivative carrying a 710-bp PCR fragment containing the *G. sulfurreducens* *lexA2* gene, including its own promoter region | This work |
| pUA1023 | pGEM-T derivative carrying a 639-bp PCR fragment containing the *G. sulfurreducens* *lexA1* coding region | This work |
| pUA1024 | pGEM-T derivative carrying a 606-bp PCR fragment containing the *G. sulfurreducens* *lexA2* coding region | This work |
| pUA1025 | Derived from pGEX-4T-1 carrying an *Eco*RI-*Sal*I fragment containing *G. sulfurreducens* *lexA1* gene from pUA1023 | This work |
| pUA1026 | Derived from pGEX-4T-1 carrying an *Eco*RI-*Sal*I fragment containing *G. sulfurreducens* *lexA2* gene from pUA1024 | This work |

[a] $Cam^r$, chloramphenicol resistance; $Ap^r$, ampicillin resistance.

logenetic groups, such as the gram-positive and α-class *Proteobacteria*, the sequence of the LexA-binding region also differs (CGAACRNRYGTTYC and $GTTCN_7GTTC$, respectively) (29, 35).

Despite the fact that *G. sulfurreducens* lives under conditions likely to promote DNA damage, nothing is known about the DNA repair mechanisms of this microorganism. In fact, no information is available concerning the DNA repair system in any of the δ-*Proteobacteria*. For this reason and in order to take advantage of the fact that the *G. sulfurreducens* genome is being sequenced, the *lexA* gene of this organism was cloned, its DNA-binding sequence was identified, and the behaviors of several DNA repair-related genes were analyzed.

## MATERIALS AND METHODS

**Bacterial strains, plasmids, oligonucleotides, and DNA techniques.** Bacterial strains and plasmids used in this work are listed in Table 1. *E. coli* was grown at 37°C in Luria-Bertani medium (24). Antibiotics were added to the cultures at previously reported concentrations (24). *G. sulfurreducens* was cultured at 30°C in NBAF medium under strict anaerobic conditions as described previously (4), and *E. coli* cells were transformed with plasmid DNA as described previously (24). All restriction enzymes, PCR-oligonucleotide primers, T4 DNA ligase and polymerase, and the digoxigenin (DIG)-DNA labeling and detection kit were from Roche (Mannheim, Germany). Total *G. sulfurreducens* DNA was prepared by using the MasterPure complete DNA purification kit (Epicentre, Madison, Wis.).

The synthetic oligonucleotide primers used for PCR amplification are listed in Table 2. To facilitate subcloning of some PCR-DNA fragments, specific restriction sites (identified in Table 2) were incorporated into the oligonucleotide primers. Mutations were introduced into the *G. sulfurreducens lexA1* promoter by PCR mutagenesis with oligonucleotides carrying designed substitutions (Table 2). The DNA sequence of all PCR-mutagenized fragments was determined by the dideoxy method (25) on an ALF Sequencer (Pharmacia Biotech, Uppsala, Sweden). In all cases, both DNA strands were sequenced. Southern analysis was performed as previously described (30) with an internal 513-bp fragment of the *G. sulfurreducens recA* gene obtained with the primers indicated in Table 2.

**Cloning of *G. sulfurreducens lexA* genes.** The *G. sulfurreducens lexA1* and *lexA2* genes were identified by a TBLASTN search of its preliminary genome sequence (http://www.tigr.org) by using the *E. coli* LexA protein as a probe. Two independent regions of significant homology were detected, indicating the presence of two different *lexA* genes. In order to amplify these two genes, primers based upon their flanking regions were designed (Table 2). The resulting DNA fragments (1,075 and 710 bp for *lexA1* and *lexA2*, respectively) were then cloned into pGEM-T to yield plasmids pUA1021 and pUA1022. Both inserts contained in these plasmids were sequenced to confirm that no mutation had been introduced during the PCR.

**Purification of *G. sulfurreducens* LexA1 and LexA2 proteins.** The plasmids pUA1025 and pUA1026 were constructed in order to create and express Glutathione *S*-transferase (GST)-LexA1 and -LexA2 fusion proteins. The first step in the construction of these plasmids was to amplify the two *lexA* genes from plasmids pUA1021 and pUA1022 by using the primers LexA1EcoRI, LexA1SalI, LexA2EcoRI, and LexA2SalI. The resulting DNA fragments were cloned into pGEM-T to produce pUA1023 and pUA1024. After excision with *Eco*RI and *Sal*I, both *lexA* genes were inserted into the pGEX4T1 expression vector (Amersham-Pharmacia, Uppsala, Sweden) immediately downstream of the T7 promoter. The initiation codon of the LexA1 and LexA2 proteins was placed immediately downstream of the *Eco*RI sites in the LexA1EcoRI and LexA2EcoRI primers, such that the *lexA1* and *lexA2* genes could be fused to GST in frame. The inserts of pUA1025 and pUA1026 were sequenced in order to ensure that no mutations were introduced during amplification.

To overproduce the LexA-GST fusion protein, pUA1025 and pUA1026 plasmids were transformed into *E. coli* BL21-CodonPlus (Stratagene, La Jolla, Calif.). The two resulting BL21-CodonPlus strains were diluted in 0.5 liter of Luria-Bertani medium and incubated at 37°C until they reached an optical density at 600 nm ($OD_{600}$) of 0.8. Fusion protein expression was induced at this time by the addition of IPTG (isopropyl-β-D-thiogalactopyranoside) to a final concentration of 1 mM. After incubation for an additional 3 h at 37°C, cells were collected by centrifugation for 15 min at 3,000 × *g*. The bacterial pellet was resuspended in phosphate-buffered saline (PBS; 10 mM $Na_2HPO_4$, 1.7 mM $KH_2PO_4$, 140 mM NaCl, 2.7 mM KCl [pH 7.4]) containing Complete Mini protease inhibitor cocktail (Roche). The resulting cell suspensions were lysed by sonication. Unbroken cells and debris were removed by centrifugation for 20 min at 19,000 × *g*. The supernatants containing the GST-LexA1 and GST-LexA2 fusion proteins were incubated with PBS-glutathione-Sepharose 4B beads (Amersham-Pharmacia) for 2 h at 4°C in order to affinity purify the fusion protein. The beads were then washed twice with PBS containing 0.1% Triton and three times with PBS without detergent.

The sequence Leu-Val-Pro-Arg-Gly-Ser is located immediately downstream of the GST coding sequence in the pGEX4T vector series and serves as a linker between the LexA and GST moieties of the fusion proteins. This hexapeptide is recognized by the thrombin protease, which cleaves at the Arg-Gly bond. It was therefore possible to release the *G. sulfurreducens* LexA proteins from the Sepharose beads by incubating a 700-ml bed volume of beads with 25 U of

TABLE 2. Oligonucleotide primers used in this work

| Primer | Sequence (5′-3′)[a] | Position(s)[b] | Application |
|---|---|---|---|
| LexA1up | TCATCAACGTGGTATGTGG | −398 | Sense primer for cloning *G. sulfurreducens lexA1* gene |
| LexA1dw | TTGCTCATGCTTGAGTGCC | +639 | Antisense primer for cloning *G. sulfurreducens lexA1* gene |
| LexA1EcoRI | *GAATTC*ATGCAGGAACTTGCCCCCCGCCAGC | +1 | Sense primer for cloning *G. sulfurreducens lexA1* gene in the pGEX-4t-1 vector |
| LexA1SalI | *GTCGAC*CTACTCCAGGGGTCGGTAGATGCCG | +659 | Antisense primer for cloning *G. sulfurreducens lexA1* gene in the pGEX-4t-1 vector |
| LexA1+48 | GGTTATGAACTCAAGCAC | +48 | Antisense primer for cloning *G. sulfurreducens lexA1* promoter used as a specific competitor in an EMSA |
| LexA1+48dig | DIG-GGTTATGAACTCAAGCAC | +48 | Antisense primer used to obtain wild-type and mutagenized *lexA* probes (DIG 5′ end labeled) |
| LexA1-95 | TGACAGCAATTCCCGC | −95 | Sense primer to obtain LexA1.1 fragment |
| LexA1-74 | GTTTCACACCCTTGAC | −74 | Sense primer to obtain LexA1.2 fragment |
| LexA1-41a | CATGATAGGTTGACATATG | −41 | Sense primer to obtain LexA1.3 fragment |
| LexA1-34 | TGACATATGTCAACCA | −34 | Sense primer to obtain LexA1.4 fragment |
| LexA1-41+CCCC | CATGATAGGTTGACAT<u>CCCC</u>ATGTCAACCAAT | −41 | Sense primer to obtain the mutagenized probe containing an insertion in the middle of the proposed palindrome (LexA1.3mut fragment) |
| LexA1-41b | CATGATAGGTTGACATATGTCAACC | −41 | Sense primer to obtain LexA-40 fragment |
| LexA1.1 | CATGAT<u>G</u>GGTTGACATATGTCAACC | −41 | Sense primer to obtain single mutants in *lexA* promoter |
| LexA1.2 | CATGATA<u>G</u>GTTGACATATGTCAACC | −41 | Sense primer to obtain single mutants in *lexA* promoter |
| LexA1.3 | CATGATAG<u>G</u>TTGACATATGTCAACC | −41 | Sense primer to obtain single mutants in *lexA* promoter |
| LexA1.4 | CATGATAGG<u>G</u>TGACATATGTCAACC | −41 | Sense primer to obtain single mutants in *lexA* promoter |
| LexA1.5 | CATGATAGGT<u>G</u>GACATATGTCAACC | −41 | Sense primer to obtain single mutants in *lexA* promoter |
| LexA1.6 | CATGATAGGTT<u>C</u>ACATATGTCAACC | −41 | Sense primer to obtain single mutants in *lexA* promoter |
| LexA1.7 | CATGATAGGTTGA<u>G</u>ATATGTCAACC | −41 | Sense primer to obtain single mutants in *lexA* promoter |
| LexA1.8 | CATGATAGGTTGAC<u>G</u>TATGTCAACC | −41 | Sense primer to obtain single mutants in *lexA* promoter |
| LexA1.9 | CATGATAGGTTGACAT<u>C</u>TGTCAACCATGACTG | −41 | Sense primer to obtain single mutants in *lexA* promoter |
| LexA1.10 | CATGATAGGTTGACATA<u>C</u>GTCAACCATGACTG | −41 | Sense primer to obtain single mutants in *lexA* promoter |
| LexA1.11 | CATGATAGGTTGACATAT<u>C</u>TCAACCATGACTG | −41 | Sense primer to obtain single mutants in *lexA* promoter |
| LexA1.12 | CATGATAGGTTGACATATG<u>C</u>CAACCATGACTG | −41 | Sense primer to obtain single mutants in *lexA* promoter |
| LexA1.13 | CATGATAGGTTGACATATGTCA<u>C</u>CCATGACTGAT | −41 | Sense primer to obtain single mutants in *lexA* promoter |
| LexA1.14 | CATGATAGGTTGACATATGTCAA<u>G</u>CATGACTGAT | −41 | Sense primer to obtain single mutants in *lexA* promoter |
| LexA1.15 | CATGATAGGTTGACATATGTCAAC<u>G</u>ATGACTGAT | −41 | Sense primer to obtain single mutants in *lexA* promoter |
| LexA1.16 | CATGATAGGTTGACATATGTCAACC<u>G</u>TGACTGAT | −41 | Sense primer to obtain single mutants in *lexA* promoter |
| RecAup | AGAGTCTGCGGACGCCG | −213 | Sense primer for cloning the upstream region of *G. sulfurreducens recA* gene |
| RecAdw | CTATCTGGCTGAGCGCAAGC | +55 | Antisense primer for cloning the upstream region of *G. sulfurreducens recA* gene |
| RecAdw-dig | DIG-CTATCTGGCTGAGCGCAAGC | +55 | Antisense primer to obtain *recA* probes for EMSA (DIG 5′ end labeled) |
| RecAF | AGCCTTGGAAATTGCCGAG | +372 | Sense primer to obtain the internal region of *recA* gene used as a probe in Southern analysis |
| RecAR | TCCGCCGTAGGAGAACCAG | +885 | Antisense primer to obtain an internal region of *recA* gene used as a probe in Southern analysis |
| LexA2up | GTAACCCCCGCCCTGGCGG | −104 | Sense primer for cloning *G. sulfurreducens lexA2* gene |
| LexA2dw | GGGGCAAAGACGGCCGC | +606 | Antisense primer for cloning *G. sulfurreducens lexA2* gene |
| LexA2EcoRI | *GAATTC*ATGCAGGAACTTGCCCCCCGCCAGC | +1 | Sense primer for cloning *G. sulfurreducens lexA2* gene in the pGEX-4t-1 vector |
| LexA2SalI | *GTCGAC*CTACTCCAGGGGTCGGTAGATGCCG | +606 | Antisense primer for cloning *G. sulfurreducens lexA2* gene in the pGEX-4t-1 vector |
| LexA2+65 | TTTTCGGCGATGAAGCCGG | +65 | Antisense primer for cloning *G. sulfurreducens lexA2* promoter used as a specific competitor in EMSAs |
| LexA2+65-dig | DIG-TTTTCGGCGATGAAGCCGG | +65 | Antisense primer used to obtain wild-type *lexA2* probes (DIG 5′ end labeled) |
| LexAint | GTGACTCCATGATCAACGCCGC | +386 (*lexA1*), +353 (*lexA2*) | Sense primer to obtain the internal region of both *lexA-dinB* copies in an RT-PCR |
| DinBint | GCGATGCCGATGGAGCAGG | +1122 (*lexA1*), +1300 (*lexA2*) | Antisense primer to obtain the internal region of both *lexA-dinB* copies in an RT-PCR |
| LexA1 A | ATCGGCAAAGTTGTGGGTATT | +600 | Sense primer to obtain the internal region of *G. sulfurreducens lexA1* gene for mRNA quantification assays |
| LexA1 B | CTCGTAGGATGCGGTAGTGATGA | +735 | Antisense primer to obtain the internal region of *G. sulfurreducens lexA1* gene for mRNA quantification assays |
| LexA2 A | GGAGAACCGGGACATCGTG | +419 | Sense primer to obtain the internal region of *G. sulfurreducens lexA2* gene for mRNA quantification assays |
| LexA2 B | GGCTCCCCGGGGCAAAGAC | +667 | Antisense primer to obtain the internal region of *G. sulfurreducens lexA2* gene for mRNA quantification assays |
| RecA A | TGCGTCATCTTCATCAACCAG | +581 | Sense primer to obtain the internal region of *G. sulfurreducens recA* gene for mRNA quantification assays |
| RecA B | GTAAATGTCGAACTCCACCTCTT | +789 | Antisense primer to obtain the internal region of *G. sulfurreducens recA* gene for mRNA quantification assays |
| Trp A | GGACGACCCTGCCGAAGAT | +224 | Sense primer to obtain the internal region of *G. sulfurreducens* tryptophan synthase gene for mRNA quantification assays |
| Trp B | TGGGGGTGAGAAGGAAGATAACAT | +466 | Antisense primer to obtain the internal region of *G. sulfurreducens* tryptophan synthase gene for mRNA quantification assays |

[a] When present, added restriction sites are shown in italics and introduced nucleotide changes are underlined.
[b] Position(s) of the 5′ end(s) of the oligonucleotide with respect to the translational starting point of each *G. sulfurreducens* gene.

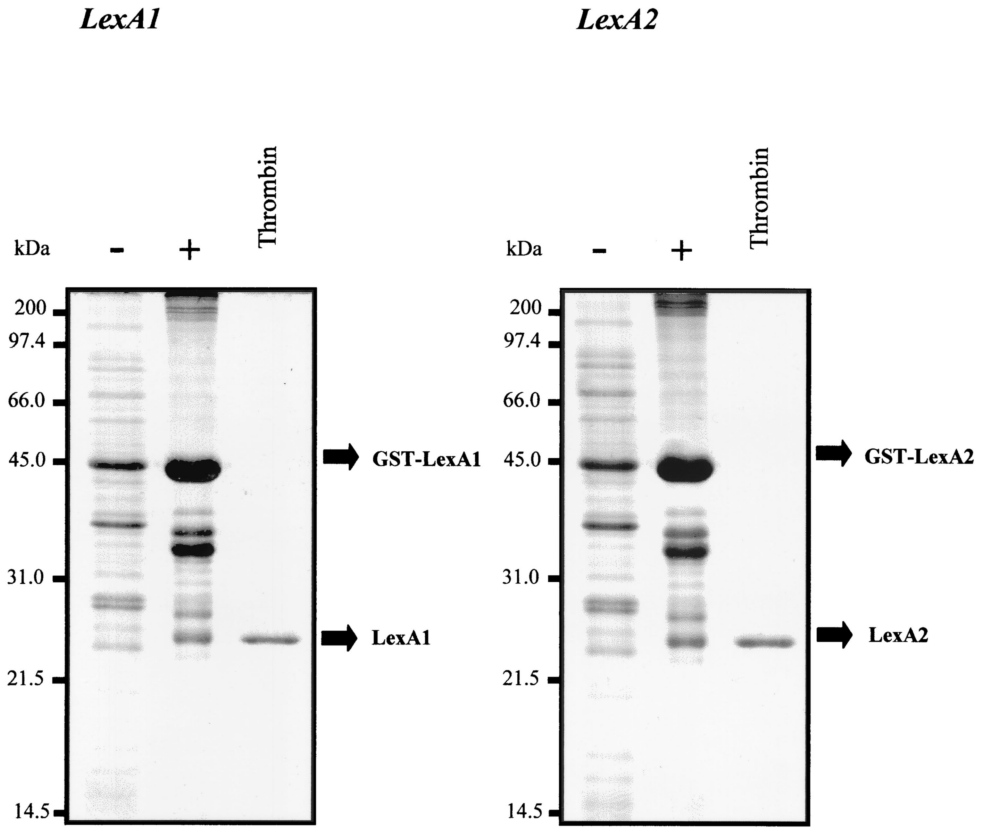


FIG. 1. Overproduction and purification of *G. sulfurreducens* LexA1 and LexA2 in *E. coli*. Samples were analyzed by sodium dodecyl sulfate–13% polyacrylamide gel electrophoresis. In both panels, crude extracts from *E. coli* BL21-CodonPlus (DE3) cells carrying either the pUA1025 or the pUA1026 plasmid in the absence (−) or presence (+) of IPTG (isopropyl-β-D-thiogalactopyranoside) are presented. Purified LexA1 and LexA2 proteins after treatment with glutathione-Sepharose 4B and thrombin protease are also shown. Molecular mass markers are indicated on the left sides of each panel in kilodaltons.

thrombin (Amersham-Pharmacia) in 1 ml of PBS. The supernatants containing the *G. sulfurreducens* LexA1 and LexA2 proteins with an additional five-amino-acid tail at their N termini (Gly-Ser-Pro-Glu-Phe) were visualized in a Coomassie blue-stained sodium dodecyl sulfate–13% polyacrylamide gel electrophoresis gel (11). Their purity was >95% (Fig. 1).

**EMSAs.** *G. sulfurreducens* LexA1 and LexA2-DNA complexes were detected by electrophoresis mobility shift assays (EMSAs) with the purified *G. sulfurreducens* LexA1 and LexA2 proteins (Fig. 1). DNA probes were prepared by PCR amplification with one primer labeled at its 5′ end with DIG (Table 2). Probes were purified in 2 to 3% low-melting-point agarose gels depending upon the size. DNA-protein-binding reactions (20 μl), typically containing 10 ng of DIG-DNA-labeled probe and 40 ng of purified *G. sulfurreducens* LexA1 or LexA2 protein, were incubated in binding buffer (10 mM HEPES-NaOH [pH 8], 10 mM Tris-HCl [pH 8], 5% glycerol, 50 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 20 μg of salmon DNA/ml, 50 μg of bovine serum albumin/ml) for 30 min at 30°C and loaded onto a 5% nondenaturing Tris-glycine polyacrylamide gel (prerun for 30 min at 10 V/cm in 25 mM Tris-HCl [pH 8.5]–250 mM glycine–1 mM EDTA). DNA-protein complexes were separated at 150 V for 1 h, followed by transfer to a Biodine B nylon membrane (Pall Gelman Laboratory). DIG-labeled DNA-protein complexes were detected by following the manufacturer's protocol (Roche). For the binding-competition experiments, a 300-fold molar excess of either specific or nonspecific unlabeled competitor DNA was also included in the mixture. All EMSAs were repeated a minimum of three times to ensure the reproducibility of the results.

**Mitomycin C-mediated DNA damage induction in *G. sulfurreducens*.** The DNA damage response was induced by mitomycin C (Sigma Chemical Co., St. Louis, Mo.) as previously described (3). In brief, mitomycin C was added to mid-log-phase NBAF cultures ($OD_{600}$ of 0.3) of *G. sulfurreducens* at a final concentration of 0.8 μg/ml. After 1.5 h of incubation at 30°C the cells were harvested, and the RNA was extracted as described below.

**RT-PCR analysis of *G. sulfurreducens* gene expression.** To determine the transcriptional organization of the *G. sulfurreducens lexA* and *dinB* genes, reverse transcriptase (Roche) was used to generate cDNA by reverse transcription (RT)-PCR with total RNA from *G. sulfurreducens* as a template and the pairs of primers indicated in Table 2. These primers were designed to amplify a PCR product of either 736- or 947-bp if *lexA1* and *dinB1* or *lexA2* and *dinB2* genes constituted a single transcription unit, respectively.

Total RNA from *G. sulfurreducens* was prepared from mid-log-phase ($OD_{600}$ of 0.3) cells grown in NBAF medium (4). After collection of the cells by centrifugation at 4°C for 10 min at 4,300 × *g*, the RNA was purified by using the RNeasy Midi Kit (Qiagen, Valencia, Calif.). The RNA was treated with RNase-free DNase I (Roche) to ensure the absence of contaminating DNA. RNA concentration and its integrity were determined by $A_{260}$ measurements and 1% formaldehyde-agarose gel electrophoresis, respectively (24). In all RT-PCR experiments, the absence of contaminating DNA in RNA samples after RNase-free DNase I (Roche) treatment was confirmed by carrying out PCR amplification without the addition of reverse transcriptase.

Mitomycin C-mediated induction of several genes studied here was carried out by real-time quantitative RT-PCR analysis of total *G. sulfurreducens* RNA with the LightCycler apparatus (Roche) by using the LC-RNA Master SYBR Green I kit (Roche) and the primers indicated in Table 2 according to the manufacturer's instructions. The concentration of total RNA from both treated and untreated cultures was adjusted to the same value. The amount of mRNA of each gene was determined by using a standard curve generated by the amplification of an internal fragment of the *G. sulfurreducens* 16S rRNA with the appropriate primers indicated in Table 2. In all determinations, the amount of mRNA of *G. sulfurreducens trpA* gene, encoding the tryptophan synthase, was also determined. This gene was used as the negative control because it is not directly related to any DNA repair system and its expression is not DNA damage inducible (2, 10).

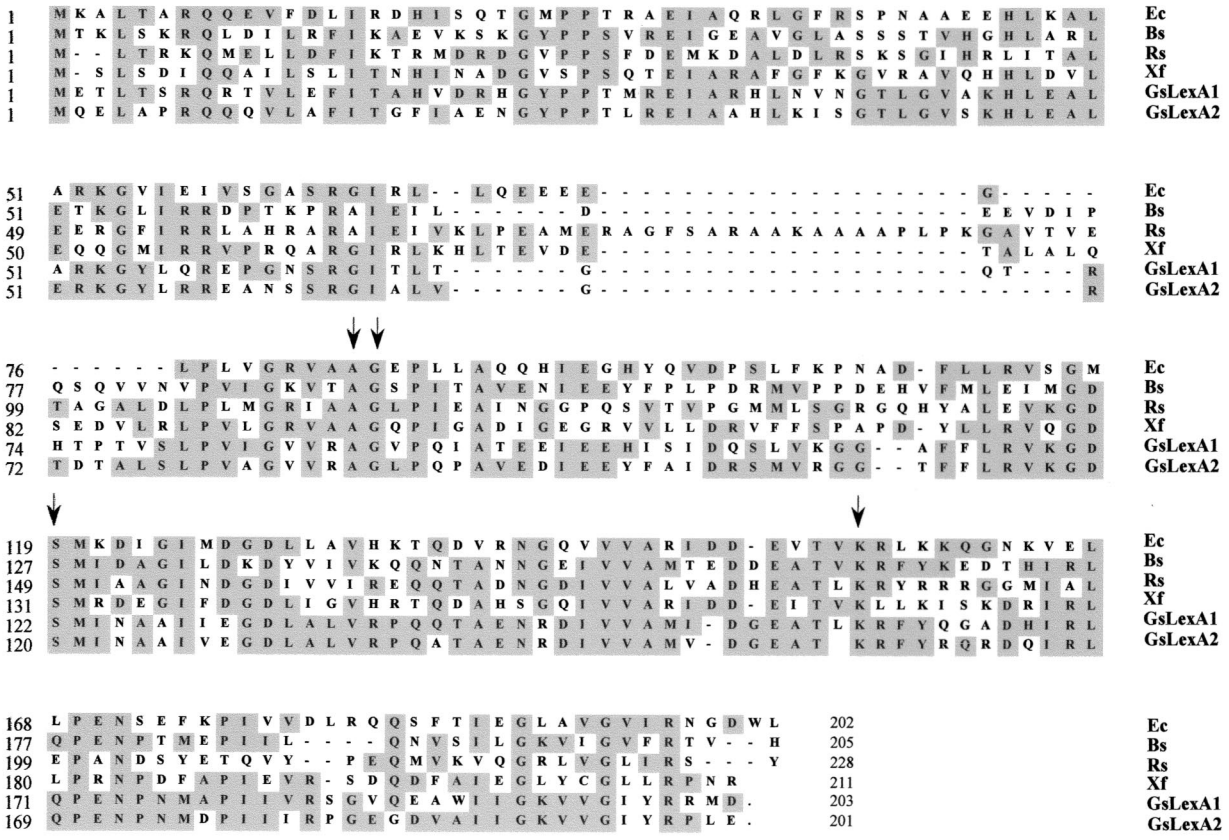


FIG. 2. Alignment of bacterial LexA proteins whose DNA-binding domains have been experimentally defined with *G. sulfurreducens* LexA1 and LexA2 proteins. Bs, *B. subtilis*; Ec, *E. coli*; Rs, *R. sphaeroides*; Xf, *X. fastidiosa*. The arrows indicate the residues involved in the autocatalytic cleavage of LexA.

## RESULTS AND DISCUSSION

***G. sulfurreducens* contains two independent *lexA* genes.** Genes for two proteins homologues to the LexA of *E. coli* were identified in the *G. sulfurreducens* genome when the *E. coli* LexA protein sequence was used as a query for TBLASTN analysis at the NCBI database (http://www.ncbi.nlm.nih.gov/Microb_blast/unfinishedgenome.html). The two homologs, designated LexA1 and LexA2, are predicted to be 201 and 203 amino acids long, respectively, and both contain the two characteristic domains of LexA repressors: the N-terminal DNA-binding domain harboring a helix-turn-helix motif and the C-terminal protease (13, 17). In addition to the Ala-Gly bond, at which the autocleavage takes place, the conserved Lys and Ser residues involved in hydrolysis are also present (Fig. 2). The two *G. sulfurreducens* LexA proteins are 68% identical and, curiously, are most closely related to *Thermotoga maritima* and *Bacillus subtilis* LexA proteins (43% identity). Not surprisingly, when *G. sulfurreducens* LexA1 and LexA2 are compared through CLUSTALW analysis (31) to other LexA proteins whose DNA-binding sequences have been determined (*B. subtilis*, *E. coli*, *Rhodobacter sphaeroides*, and *X. fastidiosa*), the highest degree of divergence is at the N-terminal DNA-binding domain (Fig. 2).

Downstream from both *G. sulfurreducens lexA* genes is an open reading frame encoding a protein with significant identity (41%) to *E. coli* DNA polymerase IV, the product of *dinB* (33) (Fig. 3A). The identity between both *G. sulfurreducens dinB* gene homologues was 64%. The fact that the distances between *lexA1* and *dinB1* and between *lexA2* and *dinB2* were very short (50 and 296 bp, respectively) suggested that, in both cases, the *lexA* and *dinB* genes were cotranscribed. To evaluate this hypothesis, an RT-PCR analysis of total *G. sulfurreducens* RNA was carried out with a single set of primers designed to amplify fragments of either 736 or 947 bp (Fig. 3A) if *lexA1-dinB1* or *lexA2-dinB2* were a transcriptional unit. The products recovered demonstrated that transcription of each one of the two *lexA* genes is linked to its respective *dinB* gene (Fig. 3B). *G. sulfurreducens* is the first bacterial species found to have this genetic organization. In *E. coli*, *lexD* is downstream of the *lexA* gene, whereas *recA* is downstream in *X. fastidiosa* (3, 19), but *dinB* does not appear to be immediately downstream of *lexA* in either organism. A TBLASTN search of the *Geobacter metallireducens* genome (http://www.jgi.doe.gov) with the *G. sulfurreducens* LexA1 protein as a query revealed that the *dinB* gene is also immediately downstream of the *lexA* gene in this *Geobacter* species (data not shown). This fact may indicate that the presence of a single transcriptional unit comprising both *lexA* and *dinB* genes is a common characteristic of the *Geobacter* genus. Nevertheless, we were unable to detect a second copy of the *lexA-dinB* operon in *G. metallireducens*. The ge-

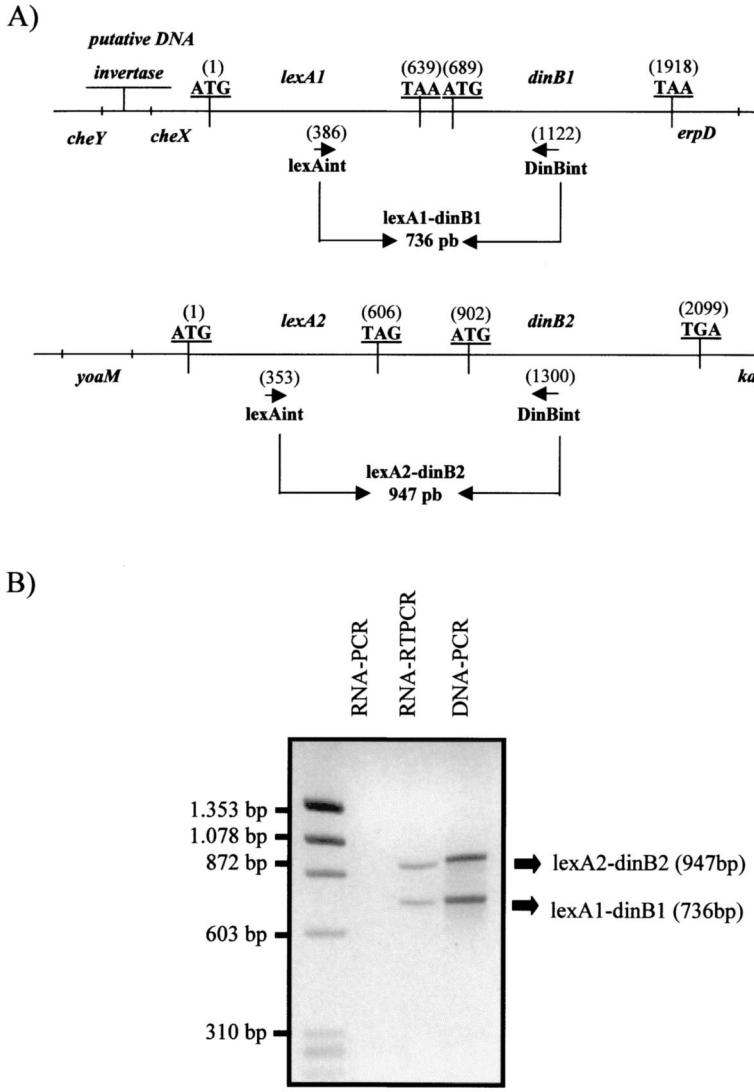


FIG. 3. (A) Structural arrangement of *G. sulfurreducens lexA1*, *dinB1*, *lexA2*, and *dinB2* genes. The translational starting point and stop codons of each one of these genes are in boldface and underlined. The positions of the primers (lexAint and dinBint) employed to determine the characteristics of transcripts are indicated by arrows. Numerical positions refer to either the *lexA1* or the *lexA2* translational starting codon. Genes found immediately upstream and downstream from *lexA1-dinB1* and *lexA2-dinB2* are also indicated. (B) RT-PCR analysis of *lexA1-dinB1* and *lexA2-dinB2* transcripts present in total RNA from *G. sulfurreducens* cells (RNA-RT-PCR). As a control PCR, experiments were carried out with the same primers, but without reverse transcriptase, with either RNA (RNA-PC) or DNA (DNA-PCR) as a template. The molecular mass marker used (*Hae*III-digested DNA of Φx174) is shown at the left of the gel in base pairs.

nome of this organism is not complete; thus, it is not yet certain whether a second *lexA-dinB* operon does not exist or its sequence is not yet available.

The fact that the regions surrounding the *G. sulfurreducens lexA1* and *lexA2* genes are different (Fig. 3A) indicates that the origin of these two genes is unlikely to be attributable to a recent single chromosomal duplication. However, the presence of an open reading frame encoding a putative DNA invertase immediately upstream of the *lexA1* gene suggests that a genetic rearrangement may have taken place. The G+C content of *G. sulfurreducens lexA2* and *G. metallireducens lexA*, as well as that of their surrounding region, is approximately 65%, whereas the G+C content of the *lexA1* gene is only 56%. Given this difference, the possibility that the *lexA1* gene was incorporated into the *G. sulfurreducens* genome by horizontal transfer cannot be excluded.

***G. sulfurreducens* LexA1 and LexA2 proteins bind the same DNA sequence.** All *lexA* genes that have been functionally studied so far are autoregulated. To analyze the possibility of LexA autoregulation in *G. sulfurreducens*, EMSAs with the purified LexA1 protein and the *lexA1* promoter as a probe were performed. The addition of LexA1 to a fragment extending from −95 to +48 with respect to its putative translational start point of the *lexA1* gene decreased its mobility (Fig. 4A). This DNA-protein interaction was specific because it was abolished by an excess of unlabeled *lexA1* fragment but not when an excess of nonspecific DNA was added (Fig. 4A). The addition of a 169-bp fragment spanning base pairs from −104 to

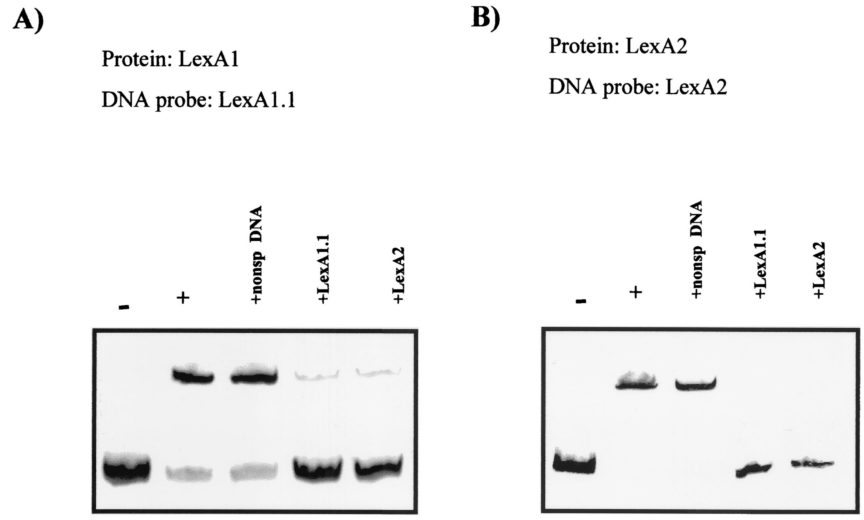


FIG. 4. EMSA of the *G. sulfurreducens lexA1* and *lexA2* promoters in the presence of 40 ng of either purified LexA1 (A) or purified LexA2 (B) proteins. In each case, the effect of a 300-fold molar excess of either unlabeled pGEM-T plasmid DNA (nonsp DNA) or LexA1.1 and LexA2 fragments on the migration of the *lexA1* and *lexA2* promoters is also shown.

+65 of the *lexA2* gene also abolished LexA1 binding to the LexA1 fragment. Likewise, LexA2 binds specifically to its own *lexA2* promoter, as well as to the *lexA1* promoter (Fig. 4B). These data clearly suggest that the sequence recognized by both LexA1 and LexA2 proteins is the same and that it is present in both the *lexA1* and the *lexA2* promoters.

To precisely define the *G. sulfurreducens* LexA1- and LexA2-binding sites, serial deletions of the upstream promoter region of the *lexA1* gene were generated and analyzed in EMSA experiments with the purified LexA1 protein (Fig. 5A). The electrophoretic mobilities of LexA1.1, LexA1.2, and LexA1.3 were retarded in the presence of the LexA1 protein, whereas the mobility of LexA1.4 was not affected (Fig. 5B). These data indicate that the LexA1-binding motif is located between the −34 and −41 positions. A search for potential binding motifs (i.e., either direct or inverted repeats) in this region revealed the presence of the perfect palindrome GGTTGACATATGT CAACC. In fact, the insertion of four cytosines into the middle of this palindrome (between the internal submotifs GACAT and ATGTC) abolished LexA1.3 fragment retardation (Fig. 5B). Furthermore, a sequence very close to this palindrome is also present upstream of the *lexA2* gene (Fig. 6). Then, using the LexA1.3 fragment, point mutations were introduced into each of the nucleotides of this palindrome, which are conserved in both *lexA* promoters and its surrounding bases, to determine whether they are effectively involved in the LexA1-binding reaction. The data obtained indicate that *G. sulfurreducens* LexA1, and consequently LexA2, binds the GGTTNNC NNNNGNNNACC motif (Fig. 6). An examination of the upstream region of the *G. metallireducens lexA* promoter revealed the presence of this sequence. All of these data led us to designate this imperfect palindrome as the LexA box of the *Geobacter* genus. It must be noted that other DNA-binding sequences of bacterial LexA repressors, such as those of *B. subtilis* and *X. fastidiosa*, are also imperfect palindromes in

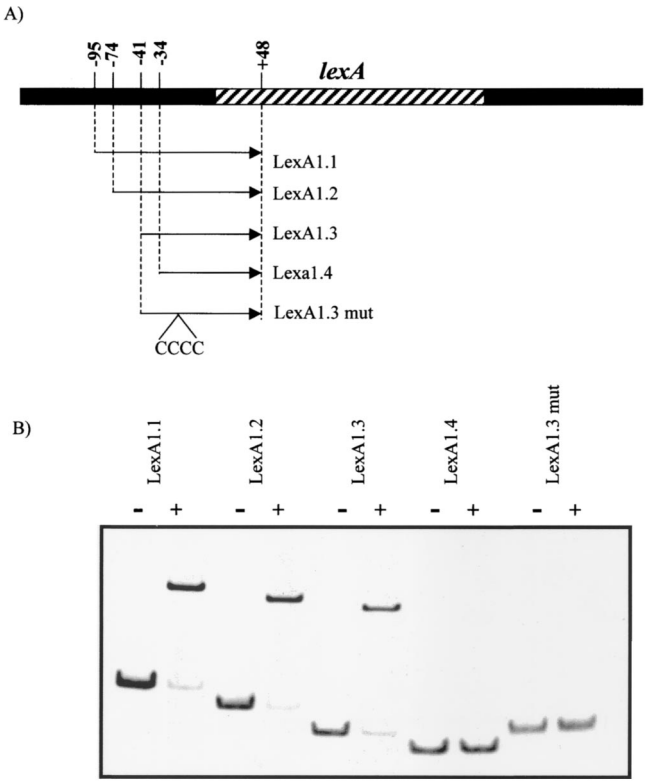


FIG. 5. (A) Diagram representing fragments amplified from the *G. sulfurreducens lexA1* promoter used in EMSA experiments. The LexA1.1, LexA1.2, LexA1.3, LexA1.4, and LexA1.3mut fragments were generated by PCR amplification with primers indicated in Table 2. In all cases, the positions indicated refer to the predicted translational starting point of the *G. sulfurreducens lexA* gene. (B) Electrophoretic mobilities of LexA1.1, LexA1.2, LexA1.3, LexA1.4, and LexA1.3mut fragments in the presence (+) or absence (−) of 40 ng of purifed *G. sulfurreducens* LexA1 protein.

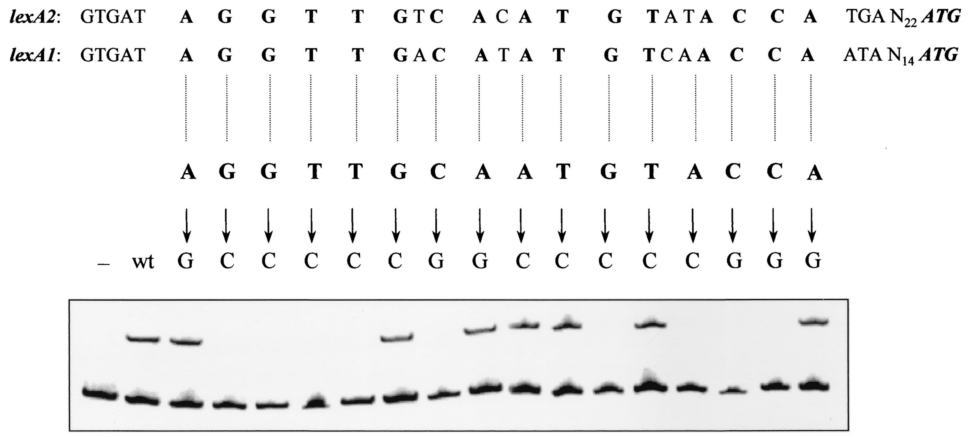


FIG. 6. Effect of mutagenesis of the putative *G. sulfurreducens* LexA box and its flanking nucleotides on the electrophoretic mobility of the LexA1.3 fragment in the presence of 40 ng of purified *G. sulfurreducens* LexA1 protein. The mobility of the wild-type LexA1.3 fragment (wt) in the absence (−) or in the presence (+) of the same amount of purified *G. sulfurreducens* LexA protein is also presented as a control. The translational starting codons of the *G. sulfurreducens lexA1* or *lexA2* genes are in italic and boldface type.

which internal bases are specifically required for LexA binding (3, 36).

**Characterization of *G. sulfurreducens recA* gene expression.** The product of the *E. coli recA* gene is involved in both recombination-mediated and error-prone DNA repair. Furthermore, *E. coli* RecA is also required for LexA autocleavage after DNA damage. These data indicate that the *G. sulfurreducens* LexA regulon is different from that of *E. coli*, at least regarding the genetic organization of the *lexA* gene. Therefore, regulation of *G. sulfurreducens recA* gene expression was analyzed. This gene was identified by a TBLASTN search by using the *E. coli* RecA protein as a query. The *G. sulfurreducens* RecA protein showed maximal identity to the *Myxococcus xanthus* RecA2 protein (73%), another member of the δ-*Proteobacteria*. The G+C content of the *G. sulfurreducens recA* gene (63%) was similar to that of the *lexA2* gene. An analysis of the sequence of the *recA* upstream region revealed that the imperfect palindrome required for LexA1 and LexA2 binding was not present. In agreement with this, the electrophoretic mobility of the *G. sulfurreducens recA* promoter was not affected by the presence of either the LexA1 or the LexA2 proteins (Fig. 7A). Likewise, the addition of the unlabeled *recA* promoter did not abolish retardation of the *lexA1* promoter in the presence of LexA1 protein (Fig. 7B). Furthermore, the upstream region of the *G. metallireducens recA* gene also lacks the LexA recognition sequence.

As a consequence of these results, we investigated whether the *G. sulfurreducens recA* gene is DNA damage inducible. Figure 8 shows that *recA* gene transcription is not stimulated by the DNA-damaging agent mitomycin C, whereas this compound triggers expression of the *lexA2-dinB2* transcriptional unit. These data indicate that the *lexA2* gene is induced by DNA damage and that both *G. sulfurreducens* LexA proteins contain all amino acid residues that are involved in the RecA-catalyzed autocleavage process (14). Thus, the absence of DNA damage-mediated expression of the *G. sulfurreducens*

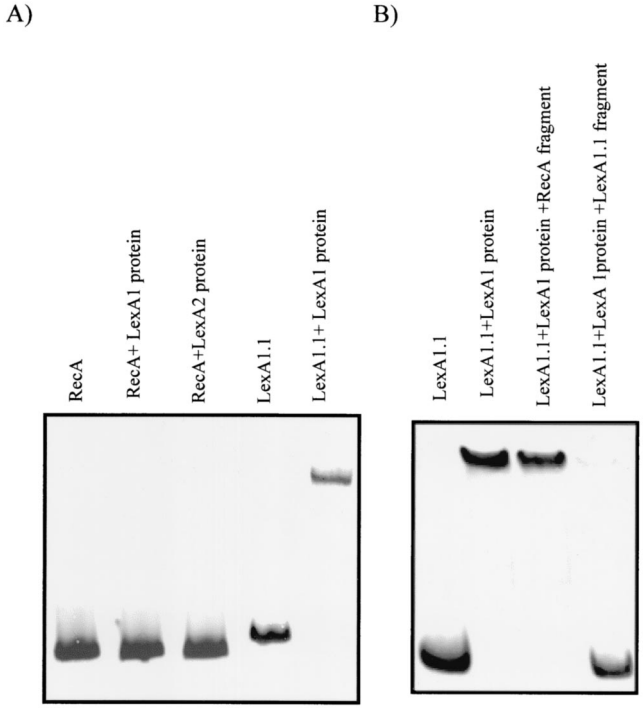


FIG. 7. (A) Electrophoretic mobilities of the *recA* promoter in the presence or absence of 40 ng of either purified *G. sulfurreducens* LexA1 or LexA2 protein. The mobility of the LexA1.1 fragment in the absence or in the presence of LexA1 protein is also shown as a control. (B) Effect of a 300-fold molar excess of unlabeled RecA DNA fragment on the electrophoretic mobility of the LexA1.1 fragment in the presence of 40 ng of LexA1 protein. As controls, the mobility of the LexA1.1 fragment in the absence of LexA1 protein, as well as in the presence of LexA1 protein but with or without the unlabeled LexA1.1 fragment as a competitor, are also shown.

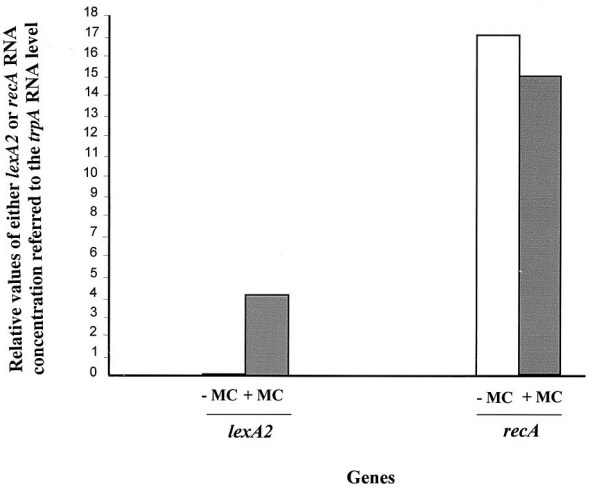


FIG. 8. Mitomycin C-mediated induction of *G. sulfurreducens lexA2* and *recA* genes measured by RT-PCR on line. The data presented are the ratios of the mRNA concentration of either the *lexA2* or the *recA* gene to the amount of the *trpA* gene, either in the presence (+) or in the absence (−) of mitomycin C (MC). Values were calculated 1.5 h after the addition of mitomycin C. In all cases, the data presented are the means of three independent PCRs (each performed in triplicate), and the standard deviation of any value was never greater than 10%.

*recA* gene cannot be attributed to a defect in the process of *lexA* regulated-gene induction.

It has been reported that some bacteria such as *M. xanthus* possess two distinct *recA* genes (20). To determine that this is not the case in *G. sulfurreducens*, Southern analysis was performed with a highly conserved internal region of the *G. sulfurreducens recA* gene as a probe. As shown in Fig. 9, a single band of the expected size was detected when *G. sulfurreducens* chromosomal DNA was digested with three restriction enzymes. These data unequivocally confirm that the *G. sulfurreducens* genome contains only one copy of the *recA* gene. In agreement with this result, TBLASTN analysis of the *G. sulfurreducens* sequence data bank revealed the presence of only one RecA-like protein when *E. coli* RecA was used as a query.

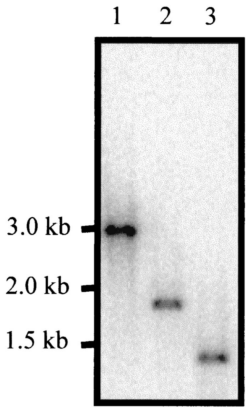


FIG. 9. Southern blot hybridization of *G. sulfurreducens* genomic DNA digested with *Sma*I (lane 1), *Bam*HI (lane 2), or *Sal*I (lane 3) restriction enzymes, with an internal fragment of the *G. sulfurreducens recA* gene as a probe.

To our knowledge, *G. sulfurreducens recA* is the first gene from a bacterial species possessing a LexA protein that is not DNA damage inducible. The *Porphyromonas gingivalis recA* gene is not stimulated by DNA damage (15), but the complete sequence of the genome of this microorganism has revealed that it lacks a *lexA*-like gene (http://www.tigr.org). It seems likely that the strong DNA-damaging potential of the *G. sulfurreducens* environment has directed an evolutionary process to attain a constitutive synthesis of RecA, which is probably the most important protein involved in the recombinational DNA repair pathway in bacteria. In fact, the transcriptional level of the *G. sulfurreducens recA* gene is dramatically higher than that of both the *lexA2* and the *trpA* genes (Fig. 8).

TBLASTN searches also identified several *G. sulfurreducens* genes, which in *E. coli* belong to the LexA regulon (*recN*, *ruvAB*, *ssb*, *umuDC*, *uvrA*, and *uvrB*). However, none of their promoters contained the *G. sulfurreducens* LexA-binding sequence. Likewise, a search in the *G. sulfurreducens* sequence database by using the Findpatterns program of the Genetics Computer Group package (6) has not revealed the presence of a LexA-binding sequence upstream of any of the either identified or known open reading frames. Since the sequence of the *G. sulfurreducens* genome is practically finished, this finding suggests that the gene content of the LexA regulon in this organism is very narrow. In agreement with this, only the single transcriptional unit *lexA-recA* and a gene encoding a putative DNA modification methylase are directly under the control of the LexA protein in *X. fastidiosa* (2). The fact that both the LexA box and the composition of the LexA regulon differ greatly in *X. fastidiosa*, *G. sulfurreducens*, and *E. coli* indicates that this network has been subjected to a great deal of variability throughout bacterial evolution. Interestingly, the level of divergence is not so high in other global bacterial regulons such as *fur*, which controls iron uptake (21, 22). The identification of new LexA-binding sites, as well as the elucidation of LexA regulon gene contents for other phylogenetic bacterial classes, will provide further understanding of its evolution.

## ACKNOWLEDGMENTS

This work was funded by grants BMC2001-2065 from the Ministerio de Ciencia y Tecnología (MCyT) de España and 2001SGR-206 from the Departament d'Universitats, Recerca i Societat de la Informació (DURSI) de la Generalitat de Catalunya. A.R.F.D.H. and M.J. were recipients of a postdoctoral contract and a predoctoral fellowship from the MCyT and DURSI, respectively. C.N. and D.R.L. were supported by the Genomes to Life Program, U.S. Department of Energy (grant DE-FC02-02ER63446). In addition, C.N. was also the recipient of a DGAPA/UNAM postdoctoral fellowship.

We are deeply indebted to M. Coppi for help in the revision of the manuscript and to Joan Ruiz and Pilar Cortés for excellent technical assistance.

## REFERENCES

1. **Brawn, K., and I. Fridovich.** 1981. DNA strand scission by enzymatically generated oxygen radicals. Arch. Biochem. Biophys. **206:**414–419.
2. **Caccavo, F. J., D. J. Lonergan, D. R. Lovley, M. Davis, J. F. Stolz, and M. J. McInerney.** 1994. *Geobacter sulfurreducens* sp. nov., a hydrogen and acetate oxidizing dissimilatory metal-reducing microorganism. Appl. Environ. Microbiol. **60:**3752–3759.
3. **Campoy, S., G. Mazón, A. R. Fernández de Henestrosa, M. Llagostera, P. Brant-Monteiro, and J. Barbé.** 2002. A new regulatory DNA motif of the gamma subclass *Proteobacteria*: identification of the LexA protein binding site of the plant pathogen *Xylella fastidiosa*. Microbiology **148:**3583–3597.
4. **Coppi, M. V., C. Leang, S. J. Sandler, and D. Lovley.** 2001. Development of a genetic system for *Geobacter sulfurreducens*. J. Bacteriol. **67:**3180–3187.

5. **Courcelle, J., A. Khodursky, B. Peter, P. O. Brown, and P. C. Hanawalt.** 2001. Comparative gene expression profiles following UV exposure in wild-type and SOS-deficient *Escherichia coli.* Genetics **158:**41–64.
6. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
7. **Fernández de Henestrosa, A. R., T. Ogi, S. Aoyogi, D. Chafin, J. J. Hayes, H. Ohmori, and R. Woodgate.** 2000. Identification of additional genes belonging to the LexA regulon in *Escherichia coli*. Mol. Microbiol. **35:**1560–1572.
8. **Imlay, J. A., and S. Linn.** 1988. DNA damage and oxygen radical toxicity. Science **240:**1302–1309.
9. **Keyer, K., and J. A. Imlay.** 1996. Superoxide accelerates DNA damage by elevating free-iron levels. Proc. Natl. Acad. Sci. USA **93:**13635–13640.
10. **Khil, P. P., and R. D. Camerini-Otero.** 2002. Over 1000 genes are involved in the DNA damage response of *Escherichia coli*. Mol. Microbiol. **44:**89–105.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
12. **Lesko, S. A., R. J. Lorentzen, and P. O. Ts'o.** 1980. Role of superoxide in deoxyribonucleic acid strand scission. Biochemistry **19:**3023–3028.
13. **Little, J. W.** 1991. Mechanism of specific LexA cleavage: autodigestion and the role of RecA coprotease. Biochimie **73:**411–422.
14. **Little, J. W., D. Mount, and C. R. Yanish-Perron.** 1981. Purified LexA protein is a repressor of the *recA* and *lexA* genes. Proc. Natl. Acad. Sci. USA **78:**4199–4203.
15. **Liu, Y., and H. M. Fletcher.** 2001. The *recA* gene in *Porphyromonas gingivalis* is expressed during infection of the murine host. Oral Microbiol. Immunol. **16:**218–233.
16. **Lovley, D. R.** 2000. Fe(III) and Mn(IV) reduction, p. 3–30. *In* D. R. Lovley (ed.), Environmental microbe-metal interactions. ASM Press, Washington, D.C.
17. **Luo, Y., R. A. Pfuetzner, S. Mosimann, M. Paetzel, E. A. Frey, M. Cherney, B. Kim, J. W. Little, and C. J. Strynadka.** 2001. Crystal structure of LexA: a conformational switch for regulation of self-cleavage. Cell **106:**585–594.
18. **McCord, J. M., and E. D. Day.** 1978. Superoxide-dependent production of hydroxyl radical catalyzed by iron-EDTA complex. FEBS Lett. **86:**139–142.
19. **Miki, T., Y. Shirabe, Y. Ebina, and A. Nakazawa.** 1984. Transcription analysis of the *lexA* gene of *Escherichia coli*: attenuation and cotranscription with the neighboring region. Nucleic Acids Res. **12:**1203–1217.
20. **Norioka, N., M. Y. Hsu, S. Inouye, and M. Inouye.** 1995. Two *recA* genes in *Myxococcus xanthus.* J. Bacteriol. **177:**4179–4182.
21. **Panina, E. M., A. A. Mironov, and M. S. Gelfand.** 2001. Comparative analysis of Fur regulons in γ-proteobacteria. Nucleic Acids Res. **29:**5195–5206.
22. **Ratledge, C., and L. G. Dover.** 2000. Iron metabolism in pathogenic bacteria. Annu. Rev. Microbiol. **54:**881–941.
23. **Rowley, D. A., and B. Halliwell.** 1982. Superoxide-dependent formation of hydroxyl radicals from NADH and NADPH in the presence of iron salts. FEBS Lett. **142:**39–41.
24. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1992. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Sanger, F., S. Nicklen, and S. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
26. **Sassanfar, M., and J. W. Roberts.** 1990. Nature of SOS-inducing signal in *Escherichia coli*: the involvement of DNA replication. J. Mol. Biol. **212:**79–96.
27. **Serkin, C. D., and H. S. Seifert.** 2000. Iron availability regulates DNA recombination in *Neisseria gonorrhoeae*. Mol. Microbiol. **37:**1075–1086.
28. **Takeuchi, T., N. Kato, K. Watanabe, and K. Morimoto.** 2000. Mechanism of oxidative DNA damage induction in a strict anaerobe, *Prevotella melaninogenica*. FEMS Microbiol. Lett. **192:**133–138.
29. **Tapias, A., and J. Barbé.** 1999. Regulation of divergent transcription from the *uvrA-ssb* promoters in *Sinorhizobium meliloti.* Mol. Gen. Genet. **262:**121–130.
30. **Tapias, A., S. Campoy, and J. Barbé.** 2000. Analysis of the expression of the *Rhodobacter spheroides lexA* gene. Mol. Gen. Genet. **263:**957–965.
31. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.
32. **Touati, D., M. Jacques, B. Tardat, L. Bouchard, and S. Despied.** 1995. Lethal oxidative damage and mutagenesis are generated by iron in Δ*fur* mutants of *Escherichia coli:* protective role of superoxide dismutase. J. Bacteriol. **177:**2305–2314.
33. **Wagner, J., P. Gruz, S. R. Kim, M. Yamada, K. Matsui, R. P. P. Fuchs, and T. Nohmi.** 1999. The *dinB* gene encodes a novel *E. coli* DNA polymerase, DNA polIV, involved in mutagenesis. Mol. Cell **4:**281–286.
34. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli.* Microbiol. Rev. **48:**60–93.
35. **Winterbourn, C. C.** 1979. Comparison of superoxide with other reducing agents in the biological production of hydroxyl radicals. Biochem. J. **182:**625–628.
36. **Winterling, K. W., D. Chafin, J. J. Hayes, J. Sun, A. S. Levine, R. E. Yasbin, and R. Woodgate.** 1998. The *Bacillus subtilis* DinR binding site: redefinition of the consensus sequence. J. Bacteriol. **180:**2201–2211.