A new regulatory DNA motif of the gamma subclass *Proteobacteria*: identification of the LexA protein binding site of the plant pathogen *Xylella fastidiosa*

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***Escherichia coli* LexA protein is the repressor of a gene network whose members are directly involved in the repair of damaged DNA and in the survival of bacterial cells until DNA lesions have been eliminated. The *lexA* gene is widely present in bacteria, although the sequences of only three LexA-binding sites are known: Gram-positive, alpha *Proteobacteria* and some members of gamma *Proteobacteria* represented by *E. coli*. Taking advantage of the fact that the genome sequence of the plant-pathogenic bacterium *Xylella fastidiosa* has been determined, its *lexA* gene has been cloned and overexpressed in *E. coli* to purify its product. After demonstration that *X. fastidiosa* *lexA* and *recA* genes are co-transcribed, gel mobility shift assays and directed mutagenesis experiments using the promoter of the *lexA-recA* transcriptional unit demonstrated that the *X. fastidiosa* LexA protein specifically binds the imperfect palindrome TTAGN₆TACTA. This is the first LexA binding sequence identified in the gamma *Proteobacteria* differing from the *E. coli*-like LexA box. Although a computational search has revealed the presence of TTAGN₆TACTA-like motifs upstream of *X. fastidiosa* genes other than *lexA*, *X. fastidiosa* LexA only binds the promoter of one of them, XF2313, encoding a putative DNA-modification methylase. Moreover, *X. fastidiosa* LexA protein does not bind any of the other genes whose homologues are regulated by the LexA repressor in *E. coli* (*uvrA*, *uvrB*, *ssb*, *ruvAB*, *ftsK*, *dinG*, *recN* and *ybfE*). RT-PCR quantitative analysis has also demonstrated that *lexA-recA* and XF2313 genes, as well as the *X. fastidiosa* genes which are homologues to those of *E. coli* belonging to the LexA regulon, with the exception of *ssb*, are DNA damage-inducible in *X. fastidiosa*.**

Keywords: DNA damage, gene expression, SOS system

INTRODUCTION

Bacteria have different pathways to repair DNA damage, the SOS system being one of the most important (Walker, 1984). In *Escherichia coli*, the SOS response has been widely studied, with at least 40 genes constituting this regulon (Fernandez de Henestrosa *et al.*, 2000; Courcelle *et al.*, 2001). The *recA* and *lexA* gene products control the expression of the *E. coli* SOS network to which both belong, LexA protein being the

negative regulator (Walker, 1984). It has been demonstrated that the RecA protein is converted into an active conformation after binding to single-stranded DNA regions generated by DNA damage-mediated inhibition of replication or by enzymic processing of broken DNA ends (Sassanfar & Roberts, 1990). After activation, RecA promotes autocatalytic cleavage of LexA, inducing the SOS genes (Walker, 1984). *E. coli* LexA cleavage occurs in the Ala₈₄-Gly₈₅ bond and is mediated by its Ser₁₁₉ and Lys₁₅₆ residues by a similar mechanism to that of serine proteases (Little, 1991; Luo *et al.*, 2001). Once DNA damage has been repaired, RecA is no longer activated and the LexA protein level increases again,

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Abbreviation: DIG, digoxigenin.

inhibiting expression of SOS genes. Recently, it has been suggested that the product of *dinI* is involved in the arrest of RecA protein activation (Voloshin *et al.*, 2001). Likewise, it has also been shown that some *E. coli* genes are DNA damage-inducible in a *lexA*-independent pathway, suggesting that other regulatory pathways could be involved in the *E. coli* DNA damage mediated response (Courcelle *et al.*, 2001; Khil & Camerini-Otero, 2002).

The *E. coli* LexA repressor specifically binds regions placed upstream of SOS genes. These LexA binding sites are imperfect 16-bp palindromes whose consensus sequence is CTGTN₈ACAG (Walker, 1984), which has been designated as the *E. coli* SOS box. The presence of a similar LexA regulon has been described in other bacterial groups, although the LexA-binding sequence is not conserved. Thus, the imperfect palindrome CGAA-CRNRYGTTYC and the GTTCN₇GTTC direct repeat are the LexA boxes of Gram-positive bacteria and the alpha subclass *Proteobacteria*, respectively (Winterling *et al.*, 1998; Fernandez de Henestrosa *et al.*, 1998; Tapias & Barbé, 1999). While the *recA* gene has been found in all sequenced eubacterial genomes, *lexA* appears to be absent in some of them. In this way, a *lexA*-like gene does not seem to be present in *Aquifex aeolicus*, *Borrelia burgdorferi*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Campylobacter jejuni*, *Helicobacter pylori* and *Porphyromonas gingivalis*. Moreover, and with the exception of the plant-pathogenic bacterium *Xylella fastidiosa*, all members of the gamma subclass *Proteobacteria* whose genome sequences have been published (*E. coli*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Pasteurella multocida* and *Vibrio cholerae*) present an *E. coli*-like LexA box upstream of the promoter genes belonging to the SOS system. Nevertheless, and despite the absence of this *E. coli*-like LexA box, a *lexA* homologue gene is present in the genome of *X. fastidiosa* (Simpson *et al.*, 2000). This fact indicates that, unlike what happens in both Gram-positive and alpha subclass *Proteobacteria* (Winterling *et al.*, 1998; Tapias & Barbé, 1999), a significant heterogeneity exists in the very compact phylogenetic gamma subclass, with respect to the sequence of the control region of the common LexA regulon.

Differences among the LexA networks of several bacteria seems to be not only limited to the LexA box, but also to the regulation mechanism. So, whereas the *E. coli* LexA represses gene transcription by precluding RNA polymerase binding to the promoter region (Brent & Ptashne, 1981; Little *et al.*, 1981; Bertrand-Burggraf *et al.*, 1987), *Rhodobacter sphaeroides* LexA interferes with the clearance process besides also being able to act as a transcriptional activator (Tapias *et al.*, 2002). All together indicate that it is not suitable to postulate a general model to explain how the LexA regulon functions in the Domain Bacteria, but this aspect must be studied in each one of their several phylogenetic groups. Likewise, the number of evolutionary analyses of regulatory sequences as well as of the constitution of different gene networks in the several bacterial phyla is

increasing (Eisen & Hanawalt, 1999; Tan *et al.*, 2001; Makarova *et al.*, 2001; Panina *et al.*, 2001; Rodionov *et al.*, 2001; Roy *et al.*, 2002). Nevertheless, and for this kind of studies to be carried out, a previous identification of new regulatory sequences, as well as of the genes which are under their control, is required.

In this context, and also to further characterize the differences existing among the LexA regulon of several groups of the gamma subclass *Proteobacteria*, the *X. fastidiosa* *lexA* gene has been cloned, overexpressed in *E. coli* and its product purified to determine the sequence to which it binds. Furthermore, the effect of DNA damage on the expression of *X. fastidiosa* genes whose homologues are under LexA regulation in *E. coli* has also been analysed.

METHODS

Bacterial strains, plasmids, oligonucleotides and DNA techniques. Bacterial strains and plasmids used in this work are listed in Table 1. *E. coli* and *X. fastidiosa* strains were grown at either 37 °C or 29 °C in LB or PW medium (Davis *et al.*, 1981; Sambrook *et al.*, 1992), respectively. Antibiotics were added to the cultures at reported concentrations (Sambrook *et al.*, 1992). *E. coli* cells were transformed with plasmid DNA as described by Sambrook *et al.* (1992). All restriction enzymes, PCR oligonucleotide primers, T4 DNA ligase and polymerase, and the DIG DNA labelling and detection kit were from Roche. DNA from *X. fastidiosa* cells was extracted as described by Monteiro *et al.* (2001).

The synthetic oligonucleotide primers used for PCR amplification are listed in Table 2. To facilitate subcloning of some PCR-amplified DNA fragments, specific restriction sites were incorporated into the oligonucleotide primers. These restriction sites are shown in Table 2. Mutants in the *X. fastidiosa* *lexA* promoter were obtained by PCR mutagenesis using oligonucleotides carrying designed substitutions (Table 2). The DNA sequence of all PCR-mutagenized fragments was determined by the dideoxy method (Sanger *et al.*, 1977) on an ALF Sequencer (Pharmacia Biotech). In all cases the entire nucleotide sequence was determined for both DNA strands.

Molecular cloning of the *X. fastidiosa* *lexA* gene. The *X. fastidiosa* *lexA* gene was amplified from the total DNA of the *X. fastidiosa* CVC 9a5c strain using the LexAup and LexAdw oligonucleotide primers (Table 2) corresponding to nucleotides -273 to -252 and +706 to +726, with respect to its proposed translational starting point (Simpson *et al.*, 2000). The 999 bp PCR fragment obtained was cloned into the pGEM-T vector giving plasmid pUA945. To confirm that no mutation was introduced during the amplification reaction, the sequence of the fragment was determined. pUA945 was used as a template in PCR amplifications with LexANdeI and LexABamHI primers (Table 2), and the fragment obtained was again cloned in pGEM-T. The putative ATG initial triplet of LexA is part of the NdeI restriction site of the LexANdeI primer which enables IPTG-mediated overexpression of the *X. fastidiosa* LexA protein. After digestion with NdeI and BamHI, the *lexA* gene was cloned downstream of the T7 promoter of the pET15b expression vector (Novagen) carrying a N-terminal tag containing six histidine residues according to standard procedures (Sambrook *et al.*, 1992), and the ligation mix was transformed into DH5 α competent cells. Once confirmed that no mutation had been introduced in the *lexA* gene contained in the pUA973 plasmid by DNA sequencing,

Table 1. Bacterial strains and plasmids used in this work

Strain/plasmid	Relevant features	Source/reference
<i>Xylella fastidiosa</i> CVC 9a5c	Wild-type strain	Fundecitrus
<i>E. coli</i> DH5 α	<i>supE4 ΔlacU169 (ϕ80 lacZΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1</i>	Clontech
BL21(DE3)	F ⁻ <i>ompT hsdS_B(r_B-r_B) dcm gal λ(DE3)</i>	Clontech
Plasmids		
pGEM-T	PCR cloning vector, Ap ^R	Promega
pET15b	Overexpression vector carrying a N-terminal tag containing six histidine residues; Km ^R	Novagen
pUA945	pGEMT derivative carrying a 999 bp PCR fragment containing the <i>X. fastidiosa</i> <i>lexA</i> gene including its own promoter region	This work
pUA973	pET15b derivative carrying a 764 bp <i>NdeI</i> – <i>Bam</i> HI fragment containing the <i>X. fastidiosa</i> <i>lexA</i> gene	This work

this was transformed into the *E. coli* BL21(DE3) strain for overexpression of the LexA protein.

Purification of the *X. fastidiosa* LexA protein. His-tag fusion protein was purified by using the Talon Metal Affinity Resin Kit (Clontech) as described by the manufacturer. To carry out this, *E. coli* BL21(DE3) cells containing pUA973 were grown in 250 ml LB broth with shaking until OD₅₅₀ 0.8. Protein expression was induced by adding 10 mM IPTG to the culture, which was incubated at 37 °C for an additional 3 h. Cells were harvested by centrifugation at 5000 *g* for 10 min at 4 °C, resuspended in 10 ml washing solution (50 mM NaH₂PO₄, 300 mM NaCl, pH 7) to which protease inhibitor cocktail (Complete Mini, EDTA free; Roche) was added to the concentration indicated by the supplier. After sonication of the cells, the lysate was centrifuged at 14000 *g* for 20 min, and the supernatant was collected. Metal Affinity Resin (Clontech; 2 ml), previously equilibrated with washing buffer, was added to the supernatant and the mix was agitated for 20 min at room temperature to allow binding of the tagged LexA protein to the resin. Afterwards, it was centrifuged and washed several times with washing buffer. The resin was then transferred to a gravity-flow column and washed again with 10 ml washing buffer. Tagged LexA protein was eluted by adding 10 ml elution buffer (50 mM NaH₂PO₄, 300 mM NaCl, 150 mM imidazole) and the eluate was collected in 500 μ l fractions. To determine the fraction containing the majority of the tagged LexA protein, SDS-PAGE in 15% polyacrylamide gels was performed according to standard procedures (Laemmli, 1970). The selected fraction was more than 98% pure as determined by Coomassie blue staining as shown in Fig. 1.

Mobility shift assays. *X. fastidiosa* LexA–DNA complexes were detected by electrophoretic mobility shift assays using purified *X. fastidiosa* LexA protein (Fig. 1). DNA probes were prepared by PCR amplification using one of the primers labelled at its 5' end with digoxigenin (DIG) (Table 2) and purifying each product in a 2–3% low-melting-point agarose gel depending on DNA size. DNA–protein reactions (20 μ l) typically containing 10 ng DIG-labelled DNA probe and 40 ng purified *X. fastidiosa* LexA 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 DTT, 2 μ g poly(dG–dC) and 50 μ g BSA ml. After 30 min at 30 °C, the mixture was loaded onto a 5% non-denaturing Tris/glycine polyacrylamide gel (pre-run for 30 min at 10 V cm⁻¹ 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-labelled 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 non-specific unlabelled competitor DNA was also included in the mixture. When used, the protein amount of crude extract of IPTG-induced *E. coli* BL21(DE3) cells carrying either the pUA973 plasmid or the pET15b vector alone added to the binding mixture was about 500 ng. Protein concentrations were determined as described by Bradford (1976). All mobility shift assays were repeated a minimum of three times to assure reproducibility of the results.

RT-PCR analysis of *X. fastidiosa* gene expression. To determine the transcriptional organization of *X. fastidiosa* *lexA* and *recA* genes, reverse transcriptase (Roche) was used to generate cDNA by RT-PCR using total RNA from *X. fastidiosa* as a template and the pair of primers indicated in Table 2 and designed to amplify a PCR product of 355 bp if the *lexA* and *recA* genes constituted a single transcription unit. Total RNA from *X. fastidiosa* was prepared with Trizol as described by the supplier (Gibco) and treated with RNase-free DNase I (Roche) to assure the absence of contaminating DNA. RNA concentration and its integrity were determined by A₂₆₀ measurements and 1% formaldehyde-agarose gel electrophoresis, respectively (Sambrook *et al.*, 1992). In all RT-PCR experiments, the absence of contaminating DNA in RNA samples after RNase-free DNase I (Roche) treatment was confirmed by processing a duplicate of them in the same way but without reverse transcriptase addition.

Mitomycin C-mediated induction of several genes studied in this work was carried out by real-time quantitative RT-PCR analysis of total *X. fastidiosa* RNA with the LightCycler apparatus (Roche), using the LC-RNA master SYBR green I kit (Roche) and primers indicated in Table 2, following

Table 2. Oligonucleotide primers used in this work

EMSA, electrophoretic mobility shift assay.

Primer	Sequence (5'–3')*	Posn†	Application
LexAup	GCTCAGTGTGATGCATCCATT	–273	Upper primer for cloning <i>X. fastidiosa</i> <i>lexA</i> gene
LexAdw	CAGCTTAAACTGCCAAGGCA	+726	Lower primer for cloning <i>X. fastidiosa</i> <i>lexA</i> gene
LexANdel	CATATGAGTTTGAGCGATATTCAG	+1	Upper primer for cloning <i>X. fastidiosa</i> <i>lexA</i> gene in the pET15b vector
LexABamHI	GGATCCCAGCTTAAACTGCCAAGGCA	+726	Lower primer for cloning <i>X. fastidiosa</i> <i>lexA</i> gene in the pET15b vector
LexA + 92	GTCTGCGAAGGAGAAACGCC	+92	Lower primer for cloning <i>X. fastidiosa</i> <i>lexA</i> promoter used as specific competitor in EMSA assays
LexA + 92dig	DIG-GTCTGCGAAGGAGAAACGCC	+92	Lower primer used to obtain wild-type and mutagenized <i>lexA</i> probes (digoxigenin 5'-end labelled)
LexA-87	GCTGGATCTGGCAGTATGG	–87	Upper primer to obtain the LexA2 fragment
LexA-25	GTGCTTATTAGTAATAATAC	–25	Upper primer to obtain the LexA3 fragment
LexA-10	AATACTAATTATGAGTTTG	–10	Upper primer to obtain the LexA4 fragment
Mut1	GTGCTTA ^{acc} TAATAATACTAATTATG	–25	Upper primer to obtain the mutagenized probe containing a change on the left motif of the proposed palindrome
Mut2	GTGCTTATTAGTAATAATA ^{aagg} TTATG	–25	Upper primer to obtain the mutagenized probe containing a change on the right motif of the proposed palindrome
Mut + ggg	GTGCTTATTAGT ^{ggg} AATAATACTAATTATG	–25	Upper primer to obtain the mutagenized probe containing an insertion in the middle of the proposed palindrome
LexA1	GTGCTT ^c TTAGTAATAATACTAATTATGAG	–25	Upper primer to obtain single mutants in <i>lexA</i> promoter
LexA2	GTGCTTA ^a TAGTAATAATACTAATTATGAG	–25	Upper primer to obtain single mutants in <i>lexA</i> promoter
LexA3	GTGCTTAT ^c AGTAATAATACTAATTATGAG	–25	Upper primer to obtain single mutants in <i>lexA</i> promoter
LexA4	GTGCTTATT ^c GTAATAATACTAATTATGAG	–25	Upper primer to obtain single mutants in <i>lexA</i> promoter
LexA5	GTGCTTATTA ^a TAATAATACTAATTATGAG	–25	Upper primer to obtain single mutants in <i>lexA</i> promoter
LexA6	GTGCTTATTAG ^c AATAATACTAATTATGAG	–25	Upper primer to obtain single mutants in <i>lexA</i> promoter
LexA7	GTGCTTATTAGT ^c ATAATACTAATTATGAG	–25	Upper primer to obtain single mutants in <i>lexA</i> promoter
LexA8	GTGCTTATTAGTA ^c TAATACTAATTATGAG	–25	Upper primer to obtain single mutants in <i>lexA</i> promoter
LexA9	GTGCTTATTAGTAA ^c AATACTAATTATGAG	–25	Upper primer to obtain single mutants in <i>lexA</i> promoter
LexA10	GTGCTTATTAGTAAT ^c ATACTAATTATGAG	–25	Upper primer to obtain single mutants in <i>lexA</i> promoter
LexA11	GTGCTTATTAGTAATA ^c TACTAATTATGAG	–25	Upper primer to obtain single mutants in <i>lexA</i> promoter
LexA12	GTGCTTATTAGTAATAA ^c ACTAATTATGAG	–25	Upper primer to obtain single mutants in <i>lexA</i> promoter
LexA13	5'-GTGCTTATTAGTAATAAT ^c CTAATTATGAG	–25	Upper primer to obtain single mutants in <i>lexA</i> promoter
LexA14	5'-GTGCTTATTAGTAATAATA ^a TAATTATGAG	–25	Upper primer to obtain single mutants in <i>lexA</i> promoter

Table 2 (cont.)

Primer	Sequence (5'–3')*	Posn†	Application
LexA15	GTGCTTATTAGTAATAATAC _a AATTATGAGATATTCAGC	–25	Upper primer to obtain single mutants in <i>lexA</i> promoter
LexA16	GTGCTTATTAGTAATAATACT _g ATTATGAGATATTCAGC	–25	Upper primer to obtain single mutants in <i>lexA</i> promoter
LexA17	GTGCTTATTAGTAATAATACT _g TTATGAGATATTCAGC	–25	Upper primer to obtain single mutants in <i>lexA</i> promoter
LexA18	GTGCTTATTAGTAATAATACTA _g TATGAGATATTCAGC	–25	Upper primer to obtain single mutants in <i>lexA</i> promoter
RecAup	GACCGGATTCGTTTGCTACC	+522‡	Upper primer for cloning the upstream region of <i>X. fastidiosa recA</i> gene, to analyse transcriptional organization of <i>recA</i> and <i>lexA</i> genes, and for mRNA quantification assays
RecAdw	CCCTTGCCGAACTAATCTGG	+877‡	Lower primer for cloning the upstream region of <i>X. fastidiosa recA</i> gene, to analyse transcriptional organization of <i>recA</i> and <i>lexA</i> genes, and for mRNA quantification assays
RecAdw-dig	dig-CCCTTGCCGAACTAATCTGG	+877‡	Lower primer used to obtain <i>recA</i> probes for EMSA assays (digoxigenin 5'-end labelled)
UvrAup	GAATTCTGAACAACGCGATATTGC	–387	Upper primer for cloning <i>X. fastidiosa uvrA</i> promoter for EMSA assays
UvrAdw	GGATCCGATTCTTAAGATTGTGTG	+53	Lower primer for cloning <i>X. fastidiosa uvrA</i> promoter for EMSA assays
UvrBup	GAATTCTCGCTAAGCAGGTGTTGC	–407	Upper primer for cloning <i>X. fastidiosa uvrB</i> promoter for EMSA assays
UvrBdw	GGATCCCTGCTTCTCAGTGAGATG	+39	Lower primer for cloning <i>X. fastidiosa uvrB</i> promoter for EMSA assays
Ssbup	GAATTCAGCTAACATCGTTAGC	–301	Upper primer for cloning <i>X. fastidiosa ssb</i> promoter for EMSA assays
Ssbdw	GGATCCGATCGTTCAAGCGGTTAC	+76	Lower primer for cloning <i>X. fastidiosa ssb</i> promoter for EMSA assays
DinGup	GAACCTCGTCACCAATGATCC	–394	Upper primer for cloning <i>X. fastidiosa dinG</i> promoter for EMSA assays
DinGdw	GTTGTTCAAAAATCTCGCC	+75	Lower primer for cloning <i>X. fastidiosa dinG</i> promoter for EMSA assays
RecNup	ACACGTAGCCGACGAATATC	–420	Upper primer for cloning <i>X. fastidiosa recN</i> promoter for EMSA assays
RecNdw	GATGTTACGGACAACAGCG	+49	Lower primer for cloning <i>X. fastidiosa recN</i> promoter for EMSA assays
RuvAup	CCGCCATGTCTACCATGC	–526	Upper primer for cloning <i>X. fastidiosa ruvA</i> promoter for EMSA assays
RuvAdw	CTCCACACACATCAACCACG	+69	Lower primer for cloning <i>X. fastidiosa ruvA</i> promoter for EMSA assays
YigNup	CGTTATGCCGAATACTTTGC	–466	Upper primer for cloning <i>X. fastidiosa yigN</i> promoter for EMSA assays
YigNdw	GTCATGAAGCACCATTGAGC	+66	Lower primer for cloning <i>X. fastidiosa yigN</i> promoter for EMSA assays
Ftskup	TACTGGTTGAAGGTTTGC	–451	Upper primer for cloning <i>X. fastidiosa ftsK</i> promoter for EMSA assays
FstKdw	GCGATCATGATCAACACC	+58	Lower primer for cloning <i>X. fastidiosa ftsK</i> promoter for EMSA assays
XF2313up	TAACCAGATCATTGCCGTGG	–303	Upper primer for cloning <i>X. fastidiosa</i> XF2313 promoter for EMSA assays
XF2313dw	AAGCAGCCGCAGTGCGTCGC	+59	Lower primer for cloning <i>X. fastidiosa</i> XF2313 promoter for EMSA assays
XF1822up	CCAACAGTGCATATGTTGGC	+51	Upper primer for cloning <i>X. fastidiosa</i> XF1822 promoter for EMSA assays
XF1822dw	ATTCTGCTATTACGGTAGCC	–549	Lower primer for cloning <i>X. fastidiosa</i> XF1822 promoter for EMSA assays
XF1614up	TCTTCTAGTGGAATTCTGTCC	–556	Upper primer for cloning <i>X. fastidiosa</i> XF1614 promoter for EMSA assays
XF1614dw	GCTCCAGTGCTTATAATCGG	–253	Lower primer for cloning <i>X. fastidiosa</i> XF1614 promoter for EMSA assays

Table 2 (cont.)

Primer	Sequence (5'–3')*	Posn†	Application
XF1417up	TGCTTGGCGTTATCGTCACC	– 126	Upper primer for cloning <i>X. fastidiosa</i> XF1417 promoter for EMSA assays
XF1417dw	AAAGGCCCTGTTCCAGCCTC	+ 180	Lower primer for cloning <i>X. fastidiosa</i> XF1417 promoter for EMSA assays
XF1271up	GTTCGATGTCGGAGGACGTC	– 317	Upper primer for cloning <i>X. fastidiosa</i> XF1271 promoter for EMSA assays
XF1271dw	ACCATGCTCAGAATGATCGG	+ 32	Lower primer for cloning <i>X. fastidiosa</i> XF1271 promoter for EMSA assays
XF2297up	CTCTACGTAGTTGTACGC	– 321	Upper primer for cloning <i>X. fastidiosa</i> XF2297 promoter for EMSA assays
XF2297dw	CGCCAATCAGTTAAACAC	+ 84	Lower primer for cloning <i>X. fastidiosa</i> XF2297 promoter for EMSA assays
XF2313/1	TTGTTGCGTCATCTGCGTCG	+ 5	Upper primer for cloning an internal region of <i>X. fastidiosa</i> XF2313 gene for mRNA quantification assays
XF2313/2	CTCTGTACGGCATCGGTGAG	+ 361	Lower primer for cloning an internal region of <i>X. fastidiosa</i> XF2313 gene for mRNA quantification assays
UvrA1	CGCGGACACACAATCTTAAG	+ 29	Upper primer for cloning an internal region of <i>X. fastidiosa</i> <i>uvrA</i> gene for mRNA quantification assays
UvrA3	TGCGCTCACGTACCACAG	+ 425	Lower primer for cloning an internal region of <i>X. fastidiosa</i> <i>uvrA</i> gene for mRNA quantification assays
UvrB1	TGAGCAGCGGCAATTGATCAG	+ 1	Upper primer for cloning an internal region of <i>X. fastidiosa</i> <i>uvrB</i> gene for mRNA quantification assays
UvrB2	GTCTGACCGGTAAGCGGATC	+ 202	Lower primer for cloning an internal region of <i>X. fastidiosa</i> <i>uvrB</i> gene for mRNA quantification assays
DinG1	TACCGGCACGTTCAACATGC	+ 63	Upper primer for cloning an internal region of <i>X. fastidiosa</i> <i>dinG</i> gene for mRNA quantification assays
DinG2	TAGATCGCGGTGGTAGAGCT	+ 431	Lower primer for cloning an internal region of <i>X. fastidiosa</i> <i>dinG</i> gene for mRNA quantification assays
RecN1	CAACATCACGATACACCACCC	+ 204	Upper primer for cloning an internal region of <i>X. fastidiosa</i> <i>recN</i> gene for mRNA quantification assays
RecN2	GATTCGCTCGGACATATCGC	+ 558	Lower primer for cloning an internal region of <i>X. fastidiosa</i> <i>recN</i> gene for mRNA quantification assays
Ssb1	TGGCCCGTGGTATCAATAAG	+ 7	Upper primer for cloning an internal region of <i>X. fastidiosa</i> <i>ssb</i> gene for mRNA quantification assays

Table 2 (cont.)

Primer	Sequence (5'–3')*	Posn†	Application
Ssb2	TTGTCATAGCGGATGCTCCC	+263	Lower primer for cloning an internal region of <i>X. fastidiosa</i> <i>ssb</i> gene for mRNA quantification assays
YigN1	GTTTGGATGAGCTGACTGCC	+204	Upper primer for cloning an internal region of <i>X. fastidiosa</i> <i>yigN</i> gene for mRNA quantification assays
YigN2	AGACACCGTTGAGATCACCG	+518	Lower primer for cloning an internal region of <i>X. fastidiosa</i> <i>yigN</i> gene for mRNA quantification assays
FtsK1	AGGCTGGTGCTTGGATTGCG	+81	Upper primer for cloning an internal region of <i>X. fastidiosa</i> <i>ftsK</i> gene for mRNA quantification assays
FtsK2	AAGCACAGCACGACCAATGC	+415	Lower primer for cloning an internal region of <i>X. fastidiosa</i> <i>ftsK</i> gene for mRNA quantification assays
RuvA1	CTCGTGGTTGATGTGTGTGG	+50	Upper primer for cloning an internal region of <i>X. fastidiosa</i> <i>ruvA</i> gene for mRNA quantification assays
RuvA2	CTCAACTCCACCAGCATACG	+386	Lower primer for cloning an internal region of <i>X. fastidiosa</i> <i>ruvA</i> gene for mRNA quantification assays
Thr1	TGCTCAAGCACAAACACC	+1059	Upper primer for cloning an internal region of <i>X. fastidiosa</i> threonine synthase gene for mRNA quantification assays
Thr2	CTGAACCTCATTGTGCCC	+685	Lower primer for cloning an internal region of <i>X. fastidiosa</i> threonine synthase gene for mRNA quantification assays
Trp1	TGCACAGGCCTATGATGC	+246	Upper primer for cloning an internal region of <i>X. fastidiosa</i> tryptophan synthase gene for mRNA quantification assays
Trp2	ACCCAACAACCTTCATCCG	+624	Lower primer for cloning an internal region of <i>X. fastidiosa</i> tryptophan synthase gene for mRNA quantification assays
rRNA1	ACGCTAATACCGCATACGACC	172439§	Upper primer for cloning an internal region of <i>X. fastidiosa</i> 16S rRNA gene for mRNA quantification assays (used as control)
rRNA _{dw2}	GAAGTTAGCCGGTGCTTATTC	173003§	Lower primer for cloning an internal region of <i>X. fastidiosa</i> 16S rRNA gene for mRNA quantification assays

* When present, added restriction sites are shown in italics and introduced nucleotide changes are shown in lower case and underlined.

† Position of 5' end of the oligonucleotide with respect to the proposed translational starting point of each *X. fastidiosa* gene.

‡ Position of 5' end of the oligonucleotide with respect to the proposed translational starting point of *X. fastidiosa* *lexA* gene.

§ Position of 5' end of the oligonucleotide in *X. fastidiosa* genome.

Fig. 1. Overproduction and purification of *X. fastidiosa* LexA in *E. coli*. Samples were analysed by SDS-PAGE (15% acrylamide). Lanes 1 and 2 contain crude extracts from *E. coli* BL21(DE3)/pUA973 cells in the absence or in the presence of IPTG, respectively. The purified His-tagged *X. fastidiosa* LexA protein after Co^{2+} -affinity chromatography is shown in lane 3. The molecular masses of protein markers are indicated on the left side.

Fig. 2. Electrophoretic mobility of the DNA fragment containing the *X. fastidiosa* *lexA* promoter in presence of increasing concentrations of purified *X. fastidiosa* His₆-LexA protein. In each binding reaction (end volume of 20 μl), amounts of purified His₆-LexA protein used were 5 ng (lane 1), 10 ng (lane 2), 20 ng (lane 3), 40 ng (lane 4) and 80 ng (lane 5). The mobility of the *X. fastidiosa* *lexA* promoter in the absence of purified His₆-LexA protein is also shown as a control (—).

supplier's instructions. The concentration of total RNA of both treated and untreated cultures was adjusted to the same value. The amount of mRNA of each gene was determined by plotting it against a standard curve generated by the amplification of an internal fragment of the *X. fastidiosa* 16S rRNA with the appropriate primers indicated in Table 2. In all determinations, the amount of mRNA of *X. fastidiosa* XF1375 and XF2223 genes, encoding tryptophan synthase and threonine synthase, respectively, was also determined. These two genes were used as negative controls because their expression is not DNA-damage-inducible in *E. coli* (Courcelle *et al.*, 2001).

RESULTS AND DISCUSSION

X. fastidiosa LexA protein specifically binds to its own promoter

Gel mobility shift assays with the purified *X. fastidiosa* LexA were used to determine the binding ability of this protein to its own promoter. Addition of increasing concentrations of LexA to a fragment extending from -273 to $+92$ of the *X. fastidiosa* *lexA* gene promoter (with respect to its proposed translational start point) produces one retardation band whose intensity is directly related to the amount of protein used (Fig. 2). To more exactly locate the binding region, the *X. fastidiosa* *lexA* promoter was divided into four fragments (designated as LexA1, LexA2, LexA3 and LexA4) obtained by PCR amplification with the oligonucleotides listed in Table 2, DIG end-labelled and then used as probes in gel retardation assays (Fig. 3a). A stable

DNA-protein complex was observed when fragments LexA1, LexA2 and LexA3 were incubated in the presence of purified *X. fastidiosa* LexA, but there was no change in the mobility of the LexA4 fragment under the same conditions (Fig. 3b). These data indicate that the region to which the LexA protein binds, or at least a portion of it, must lie between positions -25 and -10 of the *lexA* promoter. No DNA-protein complex was detected when a 355 bp fragment comprising 295 bp of the upstream region of *recA* gene was used as a probe (Fig. 3b). This is in agreement with the close proximity existing between the *X. fastidiosa* *lexA* and *recA* genes, which is only 184 bp (Fig. 3a) and with the fact that both constitute a single transcriptional unit in this organism (Fig. 4) as shown by RT-PCR analysis of the *X. fastidiosa* RNA with RecAup and RecAdw primers beginning 111 bp upstream and 60 bp downstream of the translational stop and starting codons of the *lexA* and *recA* genes, respectively (Fig. 3a). It is worth noting that this is the first bacterial species described so far in which *lexA* and *recA* genes constitute a single transcriptional unit.

The DNA-LexA complex was sensitive to competition by an excess of unlabelled LexA3 fragment, but not when non-specific DNA was used as competitor (Fig. 5a, lanes 3 and 4, respectively). The same DNA-LexA complex was obtained when, instead of purified LexA repressor, crude extracts from IPTG-induced *E. coli* BL21(DE3) cells overexpressing the *X. fastidiosa* LexA

Fig. 3. (a) Diagram representing fragments amplified from the *X. fastidiosa* *lexA-recA* promoter used in gel retardation experiments. LexA1, LexA2, LexA3 and LexA4 fragments were generated by PCR amplification. The primers RecAup and RecAdw were used for amplification of the RecA DNA fragment and to analyse the transcriptional organization of the *lexA* and *recA* genes. In all cases, positions indicated refer to the predicted translational starting point of the *X. fastidiosa* *lexA* gene (Simpson *et al.*, 2000). (b) Electrophoretic mobility of LexA1, LexA2, LexA3, LexA4 and RecA fragments in the presence (+) and in the absence (–) of 40 ng purified *X. fastidiosa* LexA protein.

(Fig. 1, lane 2) were used (Fig. 5a, lane 5). Moreover, no DNA–LexA complex was detected when crude extracts of *E. coli* BL21(DE3) cells carrying the pET15b plasmid alone were employed in gel retardation assays with the LexA3 fragment (Fig. 5a, lane 6). These results unequivocally demonstrate that the complex was indeed formed by the specific binding of the *X. fastidiosa* LexA protein to the *lexA* promoter.

Mutational analysis of the *lexA* promoter

A search for potential binding motifs (i.e. either direct or inverted repeats) in the sequence between positions –25 and the proposed ATG translational starting codon was carried out. The palindrome TTAGTAATAATACTAA, centred at the –11 position, was detected in the LexA3 fragment. Then, mutations were introduced into

the left (TTAG→ACCA) and the right (CTAA→AAGG) halves of this palindrome, and their effects on the mobility of the LexA3 fragment were analysed.

Results obtained indicated that no DNA–LexA complex was formed when probes containing substitutions in either the left or right halves of the palindrome were employed (Fig. 5b, lanes 3 and 5). Furthermore, a three-base insertion between the TTAG and CTAA motifs also abolished the gel retardation of the band (Fig. 5b, lane 4).

To determine which bases of this TTAGN₈CTAA palindrome were directly involved in the LexA interaction with the DNA, the effect of a single substitution in each one of the nucleotides of the palindrome and its immediately surrounding bases was tested. Results obtained indicated that a single nucleotide

Fig. 4. RT-PCR analysis of transcripts of the *lexA-recA* operon in *X. fastidiosa* cells using RecAup and RecAdw primers in the presence of total RNA (RNA-RT-PCR). Results obtained when the PCR experiment is carried out with the same primers, but without reverse transcriptase, using either RNA (RNA-PCR) or DNA (DNA-PCR) are shown as controls. As indicated in Fig. 3a, the beginning of RecAup and RecAdw primers are 111 bp upstream and 60 bp downstream of the translational stop and starting codons of the *lexA* and *recA* genes, respectively. M, marker.

substitution in every position of the TTAG motif, as well as in any of the first three nucleotides (CTA) of the second motif of the palindrome, eliminates the DNA–LexA complex formation (Fig. 6). Additionally, mutagenesis of either T or A placed immediately upstream of the CTA trinucleotide also prevents DNA–LexA complex formation (Fig. 6). In accordance with these data, neither of the mutant fragments that do not form the DNA–LexA complex was able to abolish the mobility shift of the wild-type fragment originated in the presence of purified *X. fastidiosa* LexA protein when used in competition experiments (data not shown). These results demonstrate that binding of the *X. fastidiosa* LexA protein to the *lexA* promoter requires the TTA-GN₆TACTA imperfect palindrome. In agreement with the fact that *lexA* and *recA* genes are co-transcribed (Fig. 3b and Fig. 4), no motif presenting a sequence related with this TTAGN₆TACTA palindrome is found immediately upstream of the *X. fastidiosa* *recA* gene.

It must be noted that the LexA binding site present upstream of the *lexA-recA* operon is very close to the translational start codon of the *lexA* gene. This is not a usual situation since LexA binding motifs of *E. coli*, Gram-positive bacteria and alpha *Proteobacteria* are typically located about 25 nt upstream of their transcriptional starting points. However, some exceptions exist since *Mycobacterium tuberculosis* Rv3074 and Rv3776 genes also present a LexA binding sequence near their translational starting points (Davis *et al.*, 2002).

LexA protein binds different gene promoters in *X. fastidiosa* and *E. coli*

As mentioned above, at least 40 genes are directly under the control of the LexA repressor in *E. coli* (Fernandez de Henestrosa *et al.*, 2000; Courcelle *et al.*, 2001). A

Fig. 5. (a) Effect of 300-fold molar unlabelled LexA1 fragment (lane 3) and pGEM-5Zf(+) plasmid DNA (lane 4) on the migration of the LexA3 fragment in the presence of 40 ng purified *X. fastidiosa* LexA protein. The electrophoretic mobility of the LexA3 fragment in the presence of crude extracts from IPTG-induced *E. coli* BL21(DE3) cells carrying either the pUA973 plasmid or the pET15b vector alone is also shown as a control

Fig. 6. Effect of single-nucleotide substitutions in the TTAG and CTAA motifs of the TTAGTAATAATACTAA palindrome as well as in its immediately surrounding bases in the electrophoretic mobility of the LexA3 fragment in the presence of 40 ng purified *X. fastidiosa* LexA protein. The mobility of the wild-type LexA3 fragment in absence (—) or in presence (wt) of the same amount of purified *X. fastidiosa* LexA protein is also presented as a control. The proposed translational starting codon (position +1) of the *X. fastidiosa* *lexA* gene (Simpson *et al.*, 2000) is in bold and italics and has been taken as the +1 position. Bases whose substitution abolish the LexA–DNA complex are boxed.

TBLASTX search using each one of the encoded products of these *E. coli* LexA-regulated genes as a query has enabled us to demonstrate that, besides *lexA* and *recA*, only eight of them (*uvrA*, *uvrB*, *ssb*, *recN*, *ruvAB*, *dinG*, *ftsK* and *yigN*) are present in the genome of *X. fastidiosa*. Sequence analysis of the upstream regions of these eight *X. fastidiosa* genes revealed that none has a sequence related to the TTAGN₆TACTA palindrome. In agreement with this fact, fragments containing 400 bp of the upstream region of these eight genes were not able to inhibit the DNA–*X. fastidiosa* LexA complex formation when used as unlabelled competitors in gel retardation experiments with the DIG-labelled LexA3 fragment as a probe (data not shown). Moreover, a search in the *X. fastidiosa* genome database using the Findpatterns Program of the Genetics Computer Group

package (Devereux *et al.*, 1984) has revealed the presence of the TTAGN₆TACTA palindrome upstream of six ORFs: *lexA–recA*, XF1271, XF1417, XF1614, XF1823 and XF2313. It was further verified by competitive gel retardation assays (Fig. 7) that the LexA repressor only binds to the promoter of XF2313 encoding a putative DNA-modification methylase (Simpson *et al.*, 2000). A similar case in which a putative LexA-binding sequence has been identified by *in silico* analysis, but which afterwards does not bind the LexA protein *in vitro*, has also been for the *E. coli* *dinJ* gene (Fernández de Henestrosa *et al.*, 2000). The reasons for this lack of binding have not been established although they could be attributed to additional factors such as the structure of the region or the secondary role of the neighbouring bases of the regulatory sequence, as has been demonstrated for several transcriptional regulators (Winterling *et al.*, 1998; Griffith & Wolf, 2001; Davis *et al.*, 2002). In agreement with this, the sequence between both motifs of the imperfect palindrome TTAGN₆TACTA is very different in XF1271, XF1417, XF1614 and XF1823 than in XF2313 and *lexA* (Fig. 7). It must be noted that in the *X. fastidiosa* genome there is another ORF (called XF2297) which is almost identical to XF2313 although it is slightly shorter probably due to a frameshift near its N terminus, suggesting that XF2297 derives from XF2313.

(lanes 5 and 6, respectively). (b) Electrophoretic mobility of mutant derivatives of the LexA3 fragment presenting substitutions in either the TTAG (Mut Left), CTAA (Mut Right) motif of the TTAGTAATAATACTAA palindrome or an insertion of 3 nt (+GGG) in its internal region in the presence of 40 ng purified *X. fastidiosa* LexA protein. In both panels, the mobility of the LexA3 fragment in the absence or in the presence of the same amount of purified *X. fastidiosa* LexA protein (lanes 1 and 2, respectively) is also shown as control.

Fig. 7. Electrophoretic mobility of the LexA3 fragment in the presence of purified *X. fastidiosa* LexA protein and 300-fold molar excess of unlabelled DNA fragments containing about 400 bp of the upstream region of XF2313, XF1822, XF1614, XF1417 and XF1271 genes of *X. fastidiosa*. The mobility of the LexA fragment in the absence of any additional DNA (+) or purified *X. fastidiosa* LexA protein (–) is shown as a control. The sequence of the region between both palindromic halves (boxed) of the TTAG₆TACTA motif, as well as the distance to their respective translational starting codons, are also shown for each fragment to illustrate differences existing among them.

Organism	Gene	Sequence																
<i>X. fastidiosa</i> CVC strain	<i>lexA</i>	TTA	T	T	A	G	T	A	A	T	A	A	T	A	C	T	A	ATTA
	XF2313	TAT	T	T	A	G	T	A	A	T	T	T	T	A	C	T	A	AATC
<i>X. fastidiosa</i> oleander strain	<i>lexA</i>	TTA	T	T	A	G	T	A	A	T	A	A	T	A	C	T	A	ATTA
	XF2313-like	TAT	T	T	A	G	T	A	A	A	A	A	T	T	A	C	T	A
<i>X. fastidiosa</i> almonder strain	<i>lexA</i>	TTA	T	T	A	G	T	A	A	T	A	A	T	A	C	T	A	ATTA
	XF2313-like	TAT	T	T	A	G	T	A	A	A	A	A	T	T	A	C	T	A
<i>Xanthomonas citri</i>	<i>lexA</i>	TCG	T	T	A	G	T	A	G	T	A	A	T	A	C	T	A	CTAA
<i>Xanthomonas campestris</i>	<i>lexA</i>	TCG	T	T	A	G	T	A	G	T	A	A	T	A	C	T	A	CTAA
<i>Xanthomonas oryzae</i>	<i>lexA</i>	TTA	T	T	A	G	T	A	A	T	A	A	T	A	C	T	A	AACA
Consensus sequence			T	T	A	G	T	A	R	W	A	W	T	A	C	T	A	

Fig. 8. Alignment of the sequence containing the TTAG₆TACTA motif present upstream of several genes of members of the *Xanthomonas* and *Xylella* genera. The consensus sequence includes nucleotides conserved in more than the 20% of 9 sequences considered. The GenBank accession numbers of *Xanthomonas citri*, *Xan. campestris* and *Xan. oryzae lexA* genes are AF081945, AF399933 and AF399932, respectively. The upstream sequences of XF2313-like genes of *Xyl. fastidiosa* pv. *oleander* and *Xyl. fastidiosa* pv. *almonder* strains were obtained by TBLASTN analysis against their respective genomes (<http://igweb.integratedgenomics.com/GOLD/prokaryagenomes.html>) using the *Xyl. fastidiosa* XF2313 gene as a query. In agreement with the standard nomenclature R is A or G and W is A or T.

Nevertheless, a 600 bp fragment containing the upstream region of XF2297 neither presents a copy of the TTAG₆TACTA motif nor binds the *X. fastidiosa* LexA protein (data not shown). The LexA-binding sequence was perhaps lost in the genetic rearrangement that produced XF2297 from XF2313.

Three proteins with a significant degree of identity (about 43%) to the putative *X. fastidiosa* DNA-modification methylase encoded by XF2313 have been detected in the *Streptomyces coelicolor* genome after a TBLASTX search in the GenBank database. However, upstream of these *S. coelicolor* genes a Gram-positive

LexA-binding sequence is not found, suggesting that the presence of a LexA-binding sequence upstream of orthologous genes encoding an XF2313-like protein is not a general phenomenon. The absence of a LexA box in the promoters of *E. coli* LexA-regulated orthologous genes has also been described for *Ps. aeruginosa* (*uvrA* and *uvrB*) and *M. tuberculosis* (*uvrA*, *recN*, *dinP* and *dinG*) (Rivera *et al.*, 1996, 1997; Brooks *et al.*, 2001).

Moreover, the TTAGN₆TACTA palindrome is also present in an upstream region 169 nt long in the *Xanthomonas campestris* *lexA* gene, in which it has been shown that its own LexA protein binds, although the precise binding sequence has not been established (Yang *et al.*, 2001). It must be noted that *Xyl. fastidiosa* and *Xan. campestris* LexA proteins present a high degree of identity in their N-terminal regions. According to this fact, the DNA binding sequence of both bacteria are expected to be very similar. Furthermore, the fact that the TTAGN₆TACTA palindrome is perfectly conserved in the *lexA* promoters of *Xanthomonas oryzae*, *Xyl. fastidiosa* pv. *oleander* and *Xyl. fastidiosa* pv. *almonder*, as well as in the promoter of the XF2313-like genes of these two former species (Fig. 8), leads us to propose this sequence as the LexA binding site of the Order Xanthomonadales. The TTAGN₆TACTA palindrome is the first LexA-binding sequence different from the *E. coli*-like one identified in the gamma subclass *Proteobacteria*.

X. fastidiosa *lexA-recA* and XF2313 transcriptional units are inducible by DNA damage

To further characterize the behaviour of *X. fastidiosa* genes binding to LexA protein, the effect of mitomycin C (20 µg ml⁻¹) on the expression of *lexA-recA* and XF2313 was analysed. Table 3 indicates that both transcriptional units are induced by DNA damage, whereas expression of two non-DNA-damage-related genes (those encoding tryptophan and threonine synthase) is not significantly stimulated by mitomycin C. It is worth noting that all LexA-regulated *E. coli* genes which are also present in *X. fastidiosa*, with the exception of *ssb*, are also DNA damage-inducible in this organism despite the fact they cannot bind to their own LexA protein (Table 3). A similar result has also been described for *M. tuberculosis* in which *uvrA* and *uvrB* are DNA damage-inducible but do not bind the LexA protein, whereas *recN*, *dinP* and *dinG* have their transcriptions neither stimulated by DNA injuries nor bind LexA (Brooks *et al.*, 2001). The DNA damage-mediated transcription of genes that do not bind LexA protein in either *M. tuberculosis* or *X. fastidiosa* could be explained by two different mechanisms: i) the existence of a *lexA*-independent pathway of induction, or ii) the presence of an additional transcriptional regulatory factor which is under LexA control. Nevertheless, this last possibility seems to be unlikely since a gene encoding a putative transcriptional regulator, aside from *lexA*, presenting its own LexA-binding sequence has not been detected in either the *M. tuberculosis* or *X. fastidiosa* genomes, (Simpson *et al.*,

Table 3. Mitomycin C-mediated induction of genes whose promoters bind either the *X. fastidiosa* or *E. coli* LexA proteins

Gene	<i>X. fastidiosa</i> LexA binding	Induction factor*
<i>lexA</i>	+	3·91
XF2313	+	15·98
<i>ssb</i>	—	1·36
<i>uvrA</i>	—	2·27
<i>uvrB</i>	—	6·11
<i>ftsK</i>	—	4·3
<i>recN</i>	—	6·4
<i>yigN</i>	—	6·52
<i>ruvAB</i>	—	6·67
<i>dinG</i>	—	7·27
XF1375†	—	1·27
XF2223†	—	1·66

* The induction factor is the ratio of mRNA concentration of each gene of cells treated with mitomycin C to that of untreated cells. Values were calculated 16 h after addition of mitomycin C. For all cases, data presented are the means of three independent experiments (each in triplicate), and the single standard deviation of any value was never greater than 10%.

† XF1375 and XF2223 genes of *X. fastidiosa* encoding tryptophan synthase and threonine synthase, respectively, were used as negative controls.

2000; Cole *et al.*, 1998). Additional investigations are necessary to better understand the molecular basis of this behaviour. Moreover, the fact that bacterial species belonging to phylogenetic groups as different as Gram-positive and the gamma subclass of *Proteobacteria* have DNA damage-inducible genes which are unable to bind the LexA repressor suggests that this phenomenon may be widespread in the bacterial world.

The presented results clearly indicate that gene composition of the LexA network can have significant variations depending on the bacterial species. The reasons for these differences are not yet known, but, besides evolutionary divergences, they could probably be related to the specific ecological and physiological niches of each bacterial group. Nevertheless, at the moment there is not enough information to precisely establish this relationship. Further work is needed to identify new LexA-binding sequences in other bacterial phylogenetic groups to be able to determine the gene composition of their corresponding LexA regulons for the understanding of the variability of this important gene network.

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