# Genome-wide identification of the LexA-mediated DNA damage response in *Streptomyces venezuelae*

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**ABSTRACT**

DNA damage triggers a widely conserved stress response in bacteria called the SOS response that involves two key regulators, the activator RecA and the transcriptional repressor LexA. Despite the wide conservation of the SOS response, the number of genes controlled by LexA varies considerably between different organisms. The filamentous soil-dwelling bacteria of the genus *Streptomyces* contain LexA and RecA homologs but their roles in *Streptomyces* have not been systematically studied. Here, we demonstrate that RecA and LexA are required for the survival of *Streptomyces venezuelae* during DNA damaging conditions and for normal development during unperturbed growth. Monitoring the activity of a fluorescent *recA* promoter fusion and LexA protein levels revealed that the activation of the SOS response is delayed in *S. venezuelae*. By combining global transcriptional profiling and ChIP-seq analysis, we determined the LexA regulon and defined the core set of DNA damage repair genes that are expressed in response to treatment with the DNA alkylating agent mitomycin C. Our results show that DNA damage-induced degradation of LexA results in the differential regulation of LexA target genes. Using Surface Plasmon Resonance, we further confirm the LexA DNA binding motif (SOS box) and demonstrate that LexA displays tight but distinct binding affinities to its target promoters, indicating a graded response to DNA damage.

**IMPORTANCE**

The transcriptional regulator LexA functions as a repressor of the bacterial SOS response, which is induced during DNA damaging conditions. This results in the expression of genes important for survival and adaptation. Here, we report the regulatory network controlled by LexA in the filamentous antibiotic producing *Streptomyces* bacteria and establish the existence of the SOS response in *Streptomyces*. Collectively, our work reveals significant insights into the DNA damage response in *Streptomyces* that will promote further studies to understand how these important bacteria adapt to their environment.

**KEY WORDS**

*Streptomyces*, DNA damage, SOS response, LexA, RecA

46 **INTRODUCTION**

47 Exposure to genotoxic agents can cause DNA damage with potentially lethal consequences
48 and therefore the ability to restore genome integrity is an essential task for all living
49 organisms. In bacteria, DNA damage triggers a widely conserved cellular stress response
50 called the SOS response (1). The SOS response is regulated by two key proteins, the activator
51 RecA (a multifunctional recombinase) and the transcriptional repressor LexA. During
52 unperturbed growth, LexA binds as a dimer to a 16-bp palindromic sequence motif, the SOS
53 box, located in the promoter region of its target genes, thereby preventing their expression.
54 Elevated levels of DNA damage can lead to the accumulation of single-stranded (ss) DNA,
55 which is sensed and bound by RecA, resulting in the formation of a nucleoprotein filament.
56 This activated form of RecA functions as a co-protease and promotes the self-cleavage of
57 LexA. Consequently, LexA repressor levels decrease in the cell, leading to the induction of the
58 SOS response regulon. The gene products of the SOS regulon are involved in a range of cellular
59 functions critical for survival, adaptation, and DNA repair (2). Specifically, SOS induction
60 triggers the expression of genes involved in nucleotide excision and recombination repair
61 (*uvrABD, recN, ruvCAB*), the removal of alkylation damage (*ada*, *alkAB, aidB*) and translesion
62 synthesis (*polB, dinP, umuDC, dnaE2*) (3). In addition, the LexA regulon often activates
63 species-specific cell cycle checkpoints that stall cellular development to allow sufficient time
64 for DNA repair to occur (4). LexA also negatively autoregulates its own transcription and *recA*
65 expression (5), thereby ensuring that the SOS response is rapidly turned off once genome
66 integrity has been restored.

67 The two principal regulators of the SOS response, RecA and LexA, are evolutionary
68 well conserved and present in almost all free-living bacteria, including the ubiquitous Gram-
69 positive Actinomycetota (previously Actinobacteria) (3). Prominent members of this phylum
70 are the streptomycetes, which are renowned for their extraordinary potential to produce
71 specialised metabolites of economic and medical importance (6). *Streptomyces* are
72 characterized by a sophisticated developmental life cycle that starts with the germination of
73 spores. Emerging germ tubes grow by apical tip extension and hyphal branching to establish
74 a dense vegetative mycelial network that scavenges for nutrients. In response to nutrient
75 starvation, hyphae escape surface tension and grow upwards to form so-called aerial hyphae.
76 These hyphae subsequently undergo reproductive cell division, resulting in the production of

77 chains of equally-sized unigenomic spores that are eventually released into the environment
78 (7).

79 *Streptomyces* are commonly found in sediments, a heterogeneous and competitive
80 habitat where they are constantly exposed to a range of environmental stressors, including
81 genotoxic agents. Earlier studies showed that a *Streptomyces recA* null mutant is more
82 sensitive to DNA damaging agents and that DNA damaging antibiotics can inhibit sporulation,
83 suggesting the existence of an SOS-like stress response (8, 9). However, the underlying
84 regulatory network that underpins such a stress response is not understood. Furthermore,
85 work over the last two decades has revealed that the SOS motif recognized by LexA and the
86 number of genes directly controlled by LexA vary between bacterial genera and cannot simply
87 be predicted bioinformatically (2, 3).

88 In this work we confirm the existence of the SOS response in *Streptomyces venezuelae*,
89 one of the key model organisms for this genus (7, 10), and demonstrate that RecA and LexA
90 are important for survival during DNA damaging growth conditions. By combining global
91 transcriptomic profiling and ChIP-seq analysis, we identify the LexA regulon and the core set
92 of SOS genes involved in DNA repair. Using Surface Plasmon Resonance, we determine the
93 LexA binding kinetics to selected target promoters and confirm the specific interaction of LexA
94 with the identified SOS box. Our *in vivo* and *in vitro* work further reveals that in *S. venezuelae*
95 LexA displays a differential DNA binding affinity that implies a fine-tuned activation of the SOS
96 regulon in response to DNA damage.

97 **RESULTS**

98 **Deletion of *recA* and *lexA* affects sporulation and sensitizes *S. venezuelae* to DNA damage**

99 *The S. venezuelae* genome contains a single *recA* gene (*vnz_26845*) and a single *lexA* gene
100 (*vnz_27115*). To characterize the SOS response in *S. venezuelae*, we first constructed a Δ*recA*
101 mutant by replacing the *recA* coding region with an apramycin resistance cassette. Deletion
102 of *recA* resulted in a growth defect and the formation of two colony types: minute nonviable
103 colonies and slow-growing colonies with delayed and irregular sporulation septation. A similar
104 Δ*recA* phenotype was previously described for *S. coelicolor* (8). The developmental defects of
105 the Δ*recA* mutant could be fully complemented by expressing *recA in trans* from its native
106 promoter (Figure 1A and Supplementary Figure 1A). To exclude the possibility that a
107 compensating mutation allowed for more robust growth in the slow-growing Δ*recA* mutant,
108 we sequenced the genomic DNA isolated from two representative colonies and the parental
109 strain. We did not detect a suppressor mutation in the Δ*recA* mutant, suggesting that both
110 colony phenotypes are a direct consequence of the *recA* deletion.

111 Next, we generated a *S. venezuelae lexA* null mutant. Using a three-step procedure, a
112 second copy of *lexA* was first integrated at the phage BT1 attachment site of wild-type *S.*
113 *venezuelae*, followed by the deletion of the native *lexA* gene and the insertion of an apramycin
114 resistance cassette. The apramycin-marked Δ*lexA* allele was then moved back into wild-type
115 *S. venezuelae*, which lacked a second copy of *lexA*, using generalized SV1 phage transduction,
116 generating the Δ*lexA* mutant strain. Notably, deletion of *lexA* resulted in a severe growth
117 defect and a "white" phenotype (Supplementary Figure 1A), indicating that sporulation was
118 impaired in this genetic background. Scanning electron micrographs of colony surfaces
119 confirmed that the Δ*lexA* mutant failed to deposit regularly spaced sporulation septa,
120 resulting in undifferentiated aerial hyphae and virtually no spores (Figure 1A). Normal
121 sporulation and pigmentation were restored to the Δ*lexA* mutant when a single copy of wild-
122 type *lexA* under the control of its native promoter was introduced *in trans* (Figure 1A and
123 Supplementary Figure 1A).

124 We then determined the viability of the wild type, the Δ*recA* mutant, the Δ*lexA*
125 mutant and the corresponding complemented mutant strains in the presence of DNA
126 damaging agents, including mitomycin C (MMC), ciprofloxacin (Cipro) and methyl

127 methanesulfonate (MMS). MMC and MMS are DNA alkylating agents that cause DNA double-
128 strand breaks while ciprofloxacin targets DNA gyrase and inhibits DNA replication, leading to
129 the accumulation of single-stranded DNA. For this we either performed multiwell-based liquid
130 growth assays or spotted serial dilutions of the individual strains onto solid medium
131 supplemented with MMC, ciprofloxacin or MMS. Based on our initial liquid growth assays we
132 found that treatment of wild-type *S. venezuelae* with 0.25 µg $ml^{-1}$ MMC, 3 µg $ml^{-1}$
133 ciprofloxacin, and 3-5 mM MMS, strongly inhibited growth (Figure 1B and Supplementary
134 Figure 1B). As expected, the absence of either *recA* or *lexA* dramatically reduced the viability
135 of *S. venezuelae* when grown in the presence of MMC, ciprofloxacin or MMS and thus, as in
136 other bacteria, RecA and LexA are essential for the survival of genotoxic stress in *S.*
137 *venezuelae*.

138

139 **Induction of the SOS response requires prolonged exposure to DNA damaging agents**

140 The *Streptomyces* life cycle includes a multicellular growth phase during which growing
141 multinucleoid filaments are segmented by cross-walls. To gain an initial insight into the spatial
142 and temporal activation of the SOS response within the mycelium, we constructed a strain in
143 which expression of an ectopically inserted copy of *mcherry* was driven by the *recA* promoter
144 and monitored the increase of mCherry fluorescence in response to MMC-induced DNA
145 damage. We found that exposure of vegetative growing *S. venezuelae* cells to 0.25 µg $ml^{-1}$
146 MMC for 1h or 2h did not lead to marked activation of the $P_{recA}$-*mcherry* reporter fusion.
147 However, when *S. venezuelae* was grown in the presence of MMC for 14h, a clear increase in
148 cytoplasmic mCherry fluorescence within the entire mycelium was detectable (Figure 2A).
149 Notably, we obtained similar results when treating *S. venezuelae* with 3 µg $ml^{-1}$ ciprofloxacin
150 or 3 mM methyl methansulfonate for 1h or 14h, respectively, indicating that the delayed
151 induction of *recA* expression was not specific to mitomycin C (Supplementary Figure 2A). We
152 also repeated the live cell imaging experiments using a 10-fold higher MMC concentrations
153 (2.5 µg $ml^{-1}$) to test if this would trigger a more rapid induction of the SOS response but did
154 not detect increased expression of the *recA-mcherry* promoter fusion (Figure 2B).

155 To corroborate these findings, we engineered a functional LexA-FLAG fusion that
156 complemented the growth and sporulation defect in hyphae lacking *lexA* (Supplementary
157 figure 2B). Vegetatively growing cultures expressing a single copy of *lexA* fused to the *3xFLAG*

158 tag were challenged with 0.25 µg $ml^{-1}$ MMC for 1h, 2h or 14h and subsequently, LexA-FLAG
159 abundance in the corresponding cell lysates was analysed by automated Western blotting. In
160 line with fluorescent *recA* reporter fusion results (Figure 2A), the LexA-FLAG protein level
161 remained largely stable following treatment with MMC for 1 and 2 hours. However, LexA-
162 FLAG was almost completely absent in cell lysates of samples grown in the presence of MMC
163 for 14h (Figure 2C). To test the possibility that the FLAG tag was responsible for the slow
164 degradation of LexA-FLAG fusion, we used a polyclonal LexA antibody to examine the
165 decrease in native LexA levels in wild-type *S. venezuelae* following the same MMC treatment.
166 We observed the same slow decrease in native LexA levels as we saw in the strain producing
167 the LexA-FLAG fusion, showing that the FLAG tag did not affect the rate of degradation of
168 LexA (Supplementary Figure 2C). Together, these results indicate that the activation of the
169 two key components involved in launching the SOS response requires a chronic exposure to
170 genotoxic stress in *Streptomyces*.

171

172 **Defining the LexA DNA damage response regulon**

173 To identify genes directly under the control of LexA, we performed chromatin
174 immunoprecipitation sequencing (ChIP-seq), using anti-FLAG antibody on the Δ*lexA* strain
175 that expressed a functional LexA-FLAG fusion (Supplementary Figure 2C). Wild-type
176 *S. venezuelae* was used as a negative control to eliminate any false signals that might arise
177 from cross-reaction of the anti-FLAG antibody with other DNA binding proteins. ChIP-seq
178 revealed numerous LexA binding sites distributed across the *S. venezuelae* genome in the
179 absence of MMC (Figure 3A). No significant enrichment was observed in the wild-type control,
180 suggesting that there was no cross-reaction of the anti-FLAG antibody leading to non-specific
181 enrichment (Figure 3B). In total, 498 ChIP-seq peaks were identified, corresponding to LexA
182 binding sites that were enriched more than 2-fold relative to the wild-type control. Of these,
183 we selected all the binding sites that were located within a potential regulatory region of -
184 200 to 100 bp relative to the start codon of the first gene in a transcriptional unit
185 (Supplementary Table 1). Through this route, we determined 270 putative LexA binding sites.

186 Furthermore, we detected 176 intragenic LexA binding sites that were in proximity of
187 previously mapped transcription start sites (11), indicating that LexA might also control the
188 activity of intragenic promoters. We note that the transcription start site mapping was

189 performed in the absence of DNA damage, a condition during which LexA remains bound to
190 its target operators and largely prevents transcription of the associated genes. Closer
191 inspection of the putative intragenic promoters associated with LexA enrichment sites
192 revealed, that six out of the 176 putative intragenic promoters were located within genes
193 with a proposed function in DNA replication and repair (vnz_05400, vnz_06010, 08430,
194 vnz_25520, vnz_29285) and within one gene that shares similarity with the cell division
195 protein ZapE (*vnz_29020*) (12). In addition, two noticeable intragenic LexA binding sites for
196 which no transcription start site data is available were detected within the DNA helicase *uvrD*
197 (*vnz_23895*) and the error-prone DNA polymerase *dnaE2* (*vnz_06640*) (Supplementary Table
198 2). However, with the exception of dnaE2, none of the genes associated with an intragenic
199 LexA binding sites were differentially regulated in response to DNA damage (Supplementary
200 Table 3).

201 In order to identify LexA target genes that showed a clear transcriptional response to
202 DNA damage, we performed RNA sequencing on wild-type *S. venezuelae* treated with 0.25 µg
203 $ml^{-1}$ MMC, combined with an untreated wild-type control. Because we only observed
204 significant induction of *recA* expression and LexA degradation after prolonged exposure to
205 MMC, we conducted RNA-seq in three independent biological samples obtained from
206 cultures that were grown in the presence and absence of MMC for 14h. Due to the severely
207 reduced viability of the Δ*recA* mutant when grown in medium containing MMC (Figure 1B),
208 we were unable to include this strain in the RNA-seq analysis. In this way, we were able to
209 identify 175 LexA binding sites that were associated with differentially expressed
210 transcriptional units upon treatment with MMC (adjusted *p*-value $p<0.05$, |log2-fold
211 change|>1) (Figure 3C and Supplementary Table 3). Of these, 97 transcriptional units were
212 upregulated, including one putative LexA-target of unknown function located on the
213 *S. venezuelae* plasmid pSVIJ1 and 78 transcriptional units were downregulated following
214 MMC treatment (10). Although LexA generally functions as transcriptional repressor (13–15),
215 the MMC-induced downregulation of genes associated with a LexA binding site is indicative
216 of a direct or indirect positive regulation of these genes by LexA. We note that our global
217 transcriptional profiling analysis further showed that several genes required for sporulation
218 were significantly downregulated following the treatment with MMC, including the classical
219 *Streptomyces* developmental regulatory genes *whiI*, *whiH* and *whiB,* genes involved in cell

220 division *(ssgB)* and chromosome segregation *(smeA-sffA)* and several genes within the *whiE*
221 locus, which encodes the biosynthesis machinery for the production of the polyketide
222 pigment associated with mature spores (16–19). These findings are in agreement with other
223 recent studies, reporting that subinhibitory concentrations of MMC inhibit sporulation in *S.*
224 *venezuelae* (9, 20).

225 Functional categorization of the 97 LexA target genes derepressed in response to
226 MMC stress did not suggest an enrichment of a specific gene category and revealed that the
227 function of most genes associated with a LexA binding site is unknown and (Figure 3D and
228 Table 1). Among the LexA targets that showed the highest upregulation were several genes
229 belonging to a predicted prophage cluster (*vnz_21525, vnz_21545, vnz_21550*), genes
230 functioning in protein translation and degradation (*vnz_05845* and *vnz_00275*), metabolism
231 (*vnz_22770*), molecule transport (*vnz_15995 and vnz_26265*). Importantly, our integrated
232 RNA-seq and ChIP-seq approach led to the identification of a core set of LexA targets (3),
233 including the *recA-recX operon*, *lexA* and several conserved DNA repair genes encoding the
234 translesion DNA polymerases DnaE2 and DinP, RuvC and RuvA required for recombination
235 and repair, and the alkylation repair protein AlkB. In addition, our data suggest that *uvrA*
236 (nucleotide excision repair) may also be part of the LexA-controlled DNA damage response as
237 we observed a clear LexA ChIP-seq peak and a change in *uvrA* expression although this was
238 just beneath our applied cut-off in the RNA-seq analysis (|log2-fold change|>1).

239 Moreover, the LexA regulon includes additional genes which could further contribute
240 to the relief of DNA damage stress. These genes include *parE* (*vnz_27225*) which encodes an
241 additional topoisomerase IV subunit; *vnz_31145* which shares similarity to radical SAM
242 enzymes that repair DNA cross-links between thymine bases caused by UV radiation and an
243 operon located on S*. venezuelae plasmid* pSVIJ1 consisting of a hypothetical protein and a
244 error-prone DNA polymerase (*pvnz_37140-37135*).

245

246 **SOS boxes of DNA damage repair genes are tightly bound by LexA**

247 Since the SOS-response involves the derepression of LexA target genes, we sought to study
248 the effect of MMC-treatment on LexA binding *in vivo*. Therefore, and in parallel with our
249 earlier experiments conducted under non-DNA damaging growth conditions, we performed
250 ChIP-seq experiments following MMC-induced stress and subsequently compared LexA

251 enrichment across the genome in untreated samples to ChIP-seq samples that had been
252 obtained from cultures grown in the presence of 0.25 µg $ml^{-1}$ MMC for 14h. In contrast to our
253 untreated ChIP-seq experiments which demonstrated widespread binding of LexA across the
254 genome, there was an observable decrease in genome-wide LexA binding in the presence of
255 MMC (Figure 4A). This is in line with the observation that LexA protein levels are decreased
256 under DNA-damaging conditions (Figure 2C). As in our earlier experiments, no significant
257 enrichment was observed in the wild-type negative control under these conditions (Figure
258 4B). Of the 175 LexA binding sites determined here to be part of the SOS-response, MMC
259 treatment resulted in reduced local enrichment of LexA at 112 genomic positions and the
260 complete dissociation of LexA at 61 chromosomal loci. Both sets of loci also included the core
261 DNA damage repair genes described above (Table 1 and Supplementary Figure 3A). Two
262 chromosomal loci retained a similar level LexA enrichment under the conditions tested
263 (Supplementary Table 4).

264 These findings prompted us to further examine the sequence-specificity of LexA
265 binding. Using the motif-based sequence analysis tool MEME (21) and 100 nucleotide
266 sequences located under the summit of each LexA ChIP-seq peak (n=175), we first identified
267 the 16-bp consensus LexA DNA-binding motif tCGAAC-$N_4$-GNNCGa (Figure 4C), which is an
268 imperfect palindromic sequence, similar to previously reported LexA binding motifs from
269 related and distant bacterial systems (3). We then compared this consensus LexA binding
270 motif to the motifs generated from 100 nucleotide sequences associated with either ChIP-seq
271 peaks that decreased (n=112) or disappeared (n=61) after MMC stress. This analysis did not
272 reveal any significant differences in the sequence specificity of LexA binding *in vivo*
273 (Supplementary figure 3B), indicating that additional factors might contribute to LexA
274 enrichment and the differential response to MMC.

275 To further validate our *in vivo* ChIP-seq analysis, we examined the DNA binding
276 specificity of purified *S. venezuelae* LexA using Surface Plasmon Resonance (SPR). 23-bp
277 double-stranded oligonucleotides containing the consensus LexA DNA binding motif were
278 tethered to a chip surface within an SPR flow cell. Purified $(His)_6$-LexA was flowed over the
279 test DNA and LexA binding was recorded by measuring the change in response units during
280 LexA injection. Control experiments using double-stranded DNA with a scrambled LexA DNA
281 binding sequence showed only a very weak association of LexA, supporting the idea that the

282 observed binding was sequence-specific (Figure 4D). In addition, we performed SPR analysis
283 using 23-bp duplex DNA that contained the putative LexA binding motifs identified upstream
284 of *lexA*, *recA* and the two SOS genes *dnaE2* and *uvrA.* Based on our initial MEME analysis, we
285 identified two potential LexA binding sites in the promoter region of *lexA* (*lexA1 and lexA2*)
286 and three for *dnaE2 (dnaE2-1, dnaE2-2, dnaE2-3)*. In agreement with our ChIP-seq results, we
287 observed clear binding of LexA to the predicted binding sites except for the *dnaE2-3* motif,
288 suggesting that either this sequence may not represent a relevant LexA binding site or
289 additional factor(s) are required for binding *in vivo* (Figure 4D). We repeated the SPR
290 experiments, using sequentially increasing concentrations of LexA to enable the
291 determination of the binding affinity ($K_D$). These analyses showed a differential but overall
292 strong binding of LexA to the tested duplex DNA, indicating that the induction of LexA target
293 genes *in vivo* is under tight control in *Streptomyces* (Figure 4D and Supplementary Figure 3C).
294 This may provide an explanation for the requirement of a prolonged exposure to genotoxic
295 agents to induce the SOS response in *S. venezuelae* since LexA needs to first disassociate from
296 its target sequence to become susceptible to RecA-stimulated self-cleavage (22). However,
297 we cannot exclude the possibility that the overall stability of LexA also contributes to the
298 delayed induction of the SOS response

299

300 **DISCUSSION**

301 In this work, we confirm the presence of a functional DNA damage response and identify the
302 core set of LexA-controlled SOS genes in *S. venezuelae.* While deletion of *recA* is not
303 detrimental for most bacteria under non-DNA damaging growth conditions, deletion of *lexA*
304 and the resulting constitutive activation of the SOS response is lethal in *E. coli* or causes cell
305 filamentation in other bacterial species (23–26). *S. venezuelae* strains lacking either *recA* or
306 *lexA* are viable, however, growth and sporulation are severely compromised in these mutant
307 strains even under non-DNA damaging conditions. Available microarray data indicates that
308 *recA* is constitutively expressed throughout the *S. venezuelae* life cycle (27). Given the
309 importance of *recA* in genome maintenance (28), it is likely that the observed phenotypic
310 defects in the *recA* mutant are a direct consequence of genome instability, which could result
311 in large deletion in the chromosomal arms or defective chromosome segregation (8, 29).
312 Furthermore, in the *lexA* null mutant permanent induction of the SOS response may result in

313 the activation of a cell-cycle checkpoint that suppresses sporulation analogous to the SOS-
314 dependent cell division inhibitors identified in diverse bacteria (30–32, 32, 33). Notably,
315 *Streptomyces* lack clear homologs of these known cell division regulators, indicating the
316 presence of a yet unidentified mechanism involved in coordinating DNA damage repair and
317 cell division.

318 Our investigations revealed that the activation of the SOS response is delayed and
319 requires a chronic exposure to DNA damaging agents in *S. venezuelae*. A similar slow
320 activation of the SOS response were previously reported for other Streptomyces species and
321 related Actinomycetota, showing that short treatments with DNA damaging agents were not
322 sufficient to induce *recA* expression (34–36). The basis for this phenomenon is not fully
323 understood but several factors may contribute to the delayed induction of the SOS response.
324 For example, basal level of RecA is sufficiently high to repair DNA damage caused by short-
325 term genotoxic stress without the need of inducing the SOS. It is also conceivable that LexA
326 auto-cleavage is intrinsically slow compared to LexA from other organisms which is degraded
327 within minutes (37, 38) or the co-protease function of RecA is inhibited by RecX, a negative
328 regulator of RecA activity (36, 39). Although we cannot exclude the possibility that the
329 intracellular accumulation of genotoxic agents is slowed down by increased efflux activity or
330 properties of the *Streptomyces* cell envelope that limit the uptake of toxic compounds, these
331 findings suggest that the regulation of the SOS response might be different in *Streptomyces*.
332 Notably, the related Actinomycetota species *Mycobacterium smegmatis* employs two DNA
333 damage response pathways, a RecA/LexA-dependent SOS response and a RecA/LexA-
334 independent DNA damage response, with the latter one controlling the vast majority of DNA
335 repair genes (40, 41). This alternative stress response involves a protein complex comprised
336 of the two transcriptional regulators PafB and PafC, which bind to a DNA sequence motif that
337 is distinct from the SOS box to activate the mycobacterial DNA damage response presumably
338 upon binding single-stranded DNA (40–42). Although *Streptomyces* encode homologs of the
339 PafBC system, an initial motif search suggested that the PafBC binding motif is not conserved
340 in *S. venezuelae.* Thus, it remains to be shown whether a similar RecA/LexA-independent DNA
341 response exists in *Streptomyces*.

342 Our results, however, establish the presence of a LexA-dependent DNA damage
343 response in *Streptomyces.* In total, we identified 175 LexA binding sites that were associated

344 with DNA-damage responsive genes that were both up and down-regulated following MMC-
345 induced degradation of LexA. While the majority of these genes are of unknown function,
346 about 36% of all differentially regulated genes are predicted to encode proteins involved in
347 metabolism, signalling and other cellular processes. This clearly highlights the functional
348 diversity of the gene categories that comprise the LexA regulon in *Streptomyces*. Importantly,
349 our work identifies a core set of SOS-regulated DNA response genes involved in the regulation
350 of the SOS response (*lexA*, *recA),* DNA mutagenesis (*dnaE2-dinP*) and DNA repair (*ruvC-ruvA*
351 operon and *alkB*) (3). We also identified several additional genes and previously unrecognized
352 LexA target genes, involved in DNA decatenation (*parE*) (43, 44), UV-damage repair
353 (*vnz_31145*) (45) and mutagenesis (*pvnz_37140-37135*) which could further contribute to
354 restoring the integrity of the genome.

355 Furthermore, we detected a significant number of positively regulated LexA targets.
356 Although we cannot exclude the possibility that the MMC-induced downregulation of LexA
357 target genes might reflect the requirement for an additional transcriptional regulator(s) for
358 induction, several examples of positive regulation by LexA have now been identified, (23, 46–
359 49). Two noticeable LexA targets in *S. venezuelae* that showed a significant downregulation
360 upon MMC treatment include the structural genes for the developmental regulator WhiI and
361 the small membrane protein SmeA which, together with its partner protein SffA, is involved
362 in chromosome segregation during sporulation (18, 50). However, *whiI* and *smeA* expression
363 is also controlled by additional developmental regulators, indicating the presence of a multi-
364 layered regulatory network that controls the initiation of sporulation (16, 51). Together, our
365 findings support the idea of a much more diverse cellular role of LexA in DNA damage stress
366 resistance and *Streptomyces* development.

367 Our work further revealed that LexA displays a differential binding behaviour *in vivo*,
368 indicating a graded response to DNA damage. These observations are in line with a
369 hierarchical induction of LexA targets which is dependent upon both the level of DNA damage
370 and the LexA dissociation rate constant at a given promoter (52–54). Indeed, our *in vitro*
371 assays confirmed that in *S. venezuelae*, LexA binds tighter to the *recA, lexA2* and *dnaE2-2*
372 operator sequences compared to the *lexA1/dnaE2-1* motif and the SOS box located in the
373 promoter region of *uvrA*. This is likely to allow for a finely tuned response, balancing DNA
374 damage surveillance, rapid repair of DNA lesions and mounting a full SOS response.

375 Collectively, our work provides significant insight into the SOS response and how it might
376 enable *Streptomyces* to survive in diverse environments.

377

378 **METHODS**

379 **Bacterial strains and growth conditions**

380 Strains, plasmids and oligonucleotides used in this work are listed in Supplementary Tables 5
381 and 6, respectively. *E. coli* strains were grown in LB or LB agar at 37°C supplemented with the
382 following antibiotics when necessary: 100 µg $ml^{-1}$ carbenicillin 50 µg $ml^{-1}$ kanamycin, 25 µg
383 $ml^{-1}$ hygromycin, 50 µg $ml^{-1}$ apramycin, or 25 µg $ml^{-1}$ chloramphenicol. *S. venezuelae* NRRL B-
384 6544 was grown in maltose-yeast extract-malt extract medium (MYM), composed of 50% tap
385 water and 50% reverse osmosis water, and supplemented with R2 trace element (TE) solution
386 at a ratio of 1:500. Liquid cultures were grown under aeration at 30 °C and at 250 rpm. MYM
387 agar was supplemented with the following antibiotics when required: 5 µg $ml^{-1}$ kanamycin,
388 25 µg $ml^{-1}$ hygromycin, or 50 µg $ml^{-1}$ apramycin.

389

390 **Construction and complementation of the *recA* null mutant**

391 The Δ*recA* mutant strain (KS3) was generated using 'Redirect' PCR targeting (Gust et al., 2003
392 and 2004). The *recA* coding sequence (*vnz_26845*) on the cosmid vector Pl1-B2
393 (http://strepdb.streptomyces.org.uk/) was replaced by an *oriT*-containing apramycin
394 resistance cassette, which was amplified from pIJ773 using the primer pair ks6 and ks7. The
395 resulting disrupted cosmid (pKS100) was introduced into *S. venezuelae* by conjugation via *E.*
396 *coli* ET12567/pUZ8002. Resulting exconjugants were screened for double crossover events
397 ($Apr^R$, $Kan^S$) and deletion of *recA* was verified by PCR analysis using the primers ks8, ks9 and
398 ks10. A representative *recA* null mutant was designated KS3. For complementation, plasmid
399 pKS2 ($P_{recA}$-*recA*) was introduced into the Δ*recA* mutant by conjugation.

400 Genomic DNA for genome sequencing of the ΔrecA mutant (KS3) and the parental
401 wild-type strain was performed using the FastDNA kit for soil (MP Biomedicals Germany).
402 Genomic DNA of biological replicate samples per strain was sequenced by MiGS (Pittsburgh,
403 USA).

404

405 **Construction and complementation of the *lexA* null mutant**

406 To generate the *ΔlexA* mutant strain (KS44), a plasmid carrying a second copy of *lexA* (pKS1)
407 was first integrated at the phage BT1 attachment site of wild-type *S. venezuelae*. In the
408 resulting strain (KS9), the native *lexA* coding sequence *(vnz_27115*) was then replaced by the
409 *apr-oriT* cassette using the REDIRECT PCR targeting technology and cosmid Pl2-B2 as
410 described above, using the primer pair ks1 and ks2 to PCR amplify the apr-oriT cassette from
411 pIJ773. The mutagenized cosmid (pKS200) was introduced into KS9 by conjugation via
412 ET12567/pUZ8002 and exconjugants that had successfully undergone double crossover
413 events were identified by screening for apramycin-resistance and kanamycin sensitivity
414 yielding strain KS25. The Δ*lexA::apr* allele of KS25 was then moved back into wild-type *S.*
415 *venezuelae* via generalised SV1 phage transduction as described by Tschowri et al. (55).
416 Transduction of the Δ*lexA::apr* allele was confirmed by PCR analysis using the primers ks3,
417 ks5, and ks32. A representative Δ*lexA* null mutant strain was designated KS44. For
418 complementation, plasmid pKS1 ($P_{lexA}$-*lexA*) or pKS3 ($P_{lexA}$-*lexA-3xFLAG*) was introduced into
419 the Δ*lexA* mutant by conjugation, yielding strain KS57 and KS74, respectively.

420

421 **RNA preparation, sequencing (RNA-seq) and analysis**

422 RNA-seq was conducted in biological triplicate using the protocol described by Bush et al. (56).
423 Triplicate cultures of wild-type *S. venezuelae* were grown in 30 ml MYM cultures with shaking
424 (250 rpm) for 14h at 30°C, "treated" cultures were grown with the addition of 0.25 µg $ml^{-1}$
425 mitomycin C (MMC) (Merck Life Sciences UK Ltd). Mycelial pellets from untreated and treated
426 cultures were washed in 1x PBS before lysis and RNA purification using the RNEasy Kit
427 (Qiagen). Purified RNA samples were treated with on-column DNase I (Qiagen), followed by
428 an additional DNase I treatment (Turbo DNA-free, Invitrogen) and the absence of DNA
429 contamination was confirmed by PCR amplification of the house-keeping gene *hrdB*
430 (*vnz_27210*) using the primer pair mb1 and mb2. Library construction, rRNA depletion and
431 paired-end Illumina Hiseq sequencing (2x150 bp configuration) was performed by Genewiz
432 (NJ, USA).

433 The quality of the obtained sequencing reads was checked using FastQC
434 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and the reads were mapped
435 to the genome of *S. venezuelae* NRRL B-65442 (NCBI Reference Sequence: NZ_CP018074.1)

436 using the Bowtie2 alignment tool (57) and subsequently sorted and indexed using the
437 Samtools package (58). A custom Perl script was used to make a saf file for genes in the *S.*
438 *venezuelae* genome. The featureCounts tool of the BioConductor package "Rsubread" was
439 used to count the reads mapping to every gene in the *S. venezuelae* genome. The counts were
440 read into a DGEList object of the edgeR package of R and a quasi-likelihood negative binomial
441 generalized log-linear model was fitted to the data using the glmQLFit function of edgeR.
442 Genewise statistical tests were conducted using the glmQLFTest function of edgeR (59). The
443 "topTags" function was used to arrive at a table of differentially expressed genes (59). Genes
444 were classed as differentially expressed genes (DEGs) if the log2-fold-change
445 (treated/untreated) was greater than or equal to 1, or less than or equal to -1 and had a false
446 discovery rate (FDR, adjusted *p*-value) of less than or equal to 0.05.

447

448 **Chromatin immunoprecipitation-sequencing (ChIP-seq) and analysis**

449 ChIP-seq was performed in biological replicates as described previously (16) using a
450 *S. venezuelae* Δ*lexA* strain that was complemented with a *lexA-3xFLAG* fusion (KS74). Cells
451 were grown in MYM medium in the absence (untreated) and presence of 0.25 µg $ml^{-1}$ MMC
452 for 14h (14-MMC) at 30°C. Library construction and sequencing were performed by Genewiz
453 (NJ, USA) using Illumina Hiseq (2 x 150bp configuration, trimmed to 100bp). Obtained
454 sequencing reads were aligned to the *S. venezuelae* NRRL B-65442 genome (NCBI Reference
455 Sequence: NZ_CP018074.1) using the Bowtie 2 alignment tool (57). The resultant bam files
456 were sorted and indexed using the Samtools package (58). A custom Perl script was used to
457 make a saf file for every 30-nucleotide section in the *S. venezuelae* genome with adjacent
458 sections overlapping by 15 nucleotides. The featureCounts tool of the R package Rsubread
459 was used to count the number of reads mapping to every 30-nucleotide section in the saf file
460 made above. A custom R script was then employed to calculate a local enrichment for every
461 30-nucleotide section (overlapping by 15 nucleotides) by comparing the read density in the
462 section to the read density in the 3000 nucleotides around the section. The local enrichment
463 in the corresponding sections of the controls were subtracted from the local enrichment of
464 the ChIP samples. Custom Perl and R scripts were used to list out genes to the left, right, and
465 overlapping genome sections with a local enrichment of 2 or more. In addition, genes had to
466 be in the right orientation and within 300 nucleotides of the enriched region for association
467 with LexA-3xFLAG binding sites. Intragenic LexA bindings sites (local enrichment of >2) were

468 defined as genomic positions that were located more than 100 bp downstream of the
469 annotated start codon of a gene and which were not considered to be part of a promoter
470 region of the downstream gene. Of the 208 intragenic LexA-3xFLAG binding sites, 176 binding
471 sites were detected in proximity of a transcription start site (based on the "14h" TSS dataset
472 publicly available at EMB-EBI E-MTAB-10690) (11).

473 To assess the effect of MMC, the enrichment of the 175 LexA-3xFLAG ChIP-seq peaks
474 located upstream of transcriptional units was assessed based on the differential response to
475 MMC stress (adjusted *p*-value $p<0.05$ and fold change cut-off |log2-fold change|>1) and on
476 the following criteria: (i) Complete dissociation of LexA-3xFLAG from a binding site upon
477 MMC-treatment was judged to have occurred when the enrichment value (log2-fold-change)
478 in the MMC-treated ChIP-seq dataset (LexA-3xFLAG-14hMMC/WT-14hMMC) was less than
479 0.25. (ii) No dissociation of LexA-3xFLAG from a binding site upon MMC-treatment was called
480 when the enrichment value (log2-fold-change) calculated from the comparison of the treated
481 and untreated ChIP-seq datasets (LexA-3xFLAG-14hMMC/LexA-3xFLAG-untreated) was less
482 than 0.2. (iii) Partial dissociation of LexA-3xFLAG from a binding site upon MMC-treatment
483 was defined when peaks displayed a local enrichment value (log2-fold-change) in the MMC-
484 treated ChIP-seq dataset (LexA-3xFLAG-14hMMC/WT-14hMMC) of more than 0.25 and the
485 enrichment value (log2-fold change) calculated from the comparison of the treated and
486 untreated ChIP-seq datasets (LexA-3xFLAG-14hMMC/LexA-3xFLAG-untreated) was greater
487 than 0.2.

488

489 **Functional analysis of LexA regulon**

490 Analysis for the enrichment of functional classes was performed on proteins encoded by the
491 176 genes that were associated with intragenic LexA binding sites and the 175 differentially
492 expressed genes that are subject to LexA regulation (Supplementary Table 2 and 4). To predict
493 conserved protein features and to assign COG identifiers (clusters of orthologous groups), the
494 queries were searched against the Position Specific Scoring Matrix (PSSM) database (NCBI)
495 using the rpsblast program (60). Where more than one COG identifier was reported, genes
496 were inspected for their genetic context and predicted function and were manually assigned
497 a COG class.

498

499 **Motif prediction from LexA-enrichment sites**

500 100 nucleotide sequences surrounding the centre position of each of the 175 enrichment sites
501 determined by our ChIP-analysis were used as input for the MEME suite (21) (Supplementary
502 Table 4), with an *S. venezuelae* background model, to search for a conserved LexA binding
503 motif (SOS box). Site distribution was set to "Zero" or "One Occurrence Per Sequence", and
504 the MEME algorithm was set to search for three motifs on both strands with a width of 6-500
505 nucleotides.

506

507 **Automated Western blotting (WES)**

508 For determining LexA and LexA-FLAG levels, the Δ*lexA*/*lexA-3xFLAG* strain (KS74), and the wild
509 type were used. To confirm specific binding of the antibodies, the wild type or the Δ*lexA*
510 deletion strain (KS44) were used as the respective negative control. Duplicate cultures of each
511 strain were grown in liquid MYM medium for 14h without (untreated) and with 0.25 µg $ml^{-1}$
512 MMC, 3 µg $ml^{-1}$ ciprofloxacin, or 3 mM MMS. After 14h, untreated cultures were exposed for
513 1 and 2h to 0.25 µg $ml^{-1}$ or 2.5 µg $ml^{-1}$ MMC, 3 µg $ml^{-1}$ ciprofloxacin, and 5 mM MMS,
514 respectively. At the end of the incubation time, 2-5 ml of each MYM culture was removed and
515 washed once with 1x PBS. Samples of mycelium were resuspended in 0.4 ml ice-cold
516 sonication buffer [20 mM Tris pH 8.0, 5 mM EDTA, 1x EDTA-free protease inhibitors (Sigma
517 Aldrich)] and sonicated (5x 15 sec on/15 sec off) at 4.5-micron amplitude. Lysates were then
518 centrifuged at 16,000 x g for 15 min at 4$^{o}$C to remove cell debris. Total protein concentration
519 was determined using the Bradford assay (Biorad). To detect LexA-FLAG or LexA abundance
520 in cell lysates, 2 µg of total protein for each sample was loaded in duplicate into a microplate
521 (ProteinSimple #043-165) together with either anti-FLAG antibody (Sigma F4725) diluted
522 1:100 or polyclonal anti-LexA antibody diluted 1:200. LexA-FLAG and LexA levels, were then
523 assayed using the automated Western blotting machine (ProteinSimple, San Jose, CA),
524 according to the manufacturer's guidelines.

525 Virtual Western blots related to Figure 2C and Supplementary Figure 2C were
526 generated using the Compass software for simple western (Version 6.0.0) and uncropped
527 images of representative blots are shown in Supplementary Figure S4.

528
529

530 **Fluorescence microscopy**

531 To visualize the expression of the $P_{recA}$-*mcherry* fusion in *S. venezuelae* (KS80), cells were
532 grown in triplicate in liquid MYM medium with and without MMC, ciprofloxacin and MMS as
533 described. Cells obtained from each culture were immobilized on a 1% agarose pad and
534 visualized using a Zeiss Axio Observer Z.1 inverted epifluorescence microscope fitted with a
535 sCMOS camera (Hamamatsu Orca FLASH 4), a Zeiss Colibri 7 LED light source, a Hamamatsu
536 Orca Flash 4.0v3 sCMOS camera. Images were acquired using a Zeiss Alpha Plan-Apo
537 100x/1.46 Oil DIC M27 objective with an excitation/emission bandwidth of 577-603 nm/614-
538 659 nm to detect fluorescence emitted by the mCherry reporter fusion. Still images were
539 collected using the Zen Blue software and further analyzed using the Fiji imaging software
540 (61). To normalise the mCherry fluorescence intensity across different MMC treatments,
541 images were corrected for background fluorescence.

542

543 **Cryo-scanning electron microscopy**

544 *Streptomyces* samples were mounted on an aluminium stub using Tissue Tek$^{R}$ (Agar Scientific
545 Ltd, Stansted, England) and plunged into liquid nitrogen slush. The sample was transferred
546 onto the cryo-stage of an ALTO 2500 cryo-system (Gatan, Oxford, England) attached to an FEI
547 Nova NanoSEM 450 field emission SEM (Thermo Fisher Scientific, Eindhoven, The
548 Netherlands). Sublimation of surface frost was performed at -95$^{o}$C for four minutes before
549 sputter coating with platinum for 150 sec at 10 mA. The sample was moved onto the main
550 cryo-stage in the microscope, held at -125$^{o}$C, and imaged at 3 kV, spot 3, and digital TIFF files
551 were stored.

552

553 **Growth assays**

554 To determine viability of *S. venezuelae* strains on solid medium 10e$^{5}$ colony forming units per
555 ml$^{-1}$ (spores or mycelia fragments) were used to prepare a 10-fold serial dilution series and
556 3 µl of each dilution series was subsequently spotted onto MYM agar containing either no
557 antibiotic, 0.25 µg ml$^{-1}$ MMC, 3 µg ml$^{-1}$ ciprofloxacin, or 3 mM MMS. Plates, in biological
558 triplicate, were incubated at 30°C for 3-5 days and subsequently imaged.

559 To assay the growth of *S. venezuelae* in liquid MYM, $OD_{600}$ measurements were made using a
560 plate reader (SPECTROstar Nano by BMG Labtech) set at 30°C and shaking at 700 rpm. 800 µl

561 of MYM supplemented with increasing concentrations of MMC, ciprofloxacin, or MMS was
562 loaded into the wells of a 48-well-plate (677102 Greiner Bio-One) and inoculated with either
563 5 µl spores or 10 µl of mycelium. $OD_{600}$ measurements were recorded every 15 min for 24
564 hours and data was subsequently visualized using Graphpad Prism (Version 9.3.1). Each
565 experiment was performed in triplicate.

566

567 **Purification of $His_6$-LexA**

568 To purify $His_6$-LexA, *E. coli* NiCo21 (DE3) cells were transformed with the pLysS and the pKS16
569 plasmid. Cells were grown at 30°C in LB medium containing 25 µg $ml^{-1}$ chloramphenicol and
570 100 µg $ml^{-1}$ carbenicillin until $OD_{600}$ 0.5 before the addition of 0.5 mM IPTG (isopropyl β -D-1-
571 thiogalactopyranoside) to induce protein production. Cultures were incubated with shaking
572 at 30°C for 4h and then harvested by centrifugation. Cell pellets were resuspended in 50mM
573 Tris pH 10.5, 1 M NaCl, 10% glycerol with protease inhibitor (EDTA-free, Merck) and lysed by
574 sonication for 14 cycles at 18-micron amplitude, 15 sec on/ 10 sec off. Lysates were
575 centrifuged at 26,000 x g for 45 min at 4°C to remove cell debris and passed through a 0.45
576 µm filter before loading on a HisTrap column via the ÄKTA pure system. (GE Healthcare). $His_6$-
577 LexA was then eluted using an increasing concentration of imidazole. Fractions containing
578 $His_6$-LexA were pooled and subjected to size exclusion chromatography on a HiLoad 16/600
579 Superdex 200 pg column (GE Healthcare) in 50mM Tris pH 10.5, 200 mM NaCl, 10% glycerol,
580 0.5 mM DTT. The concentration of pooled protein fractions was determined by Bradford assay
581 and $His_6$-LexA was subsequently stored at -80°C until further use. To produce antibodies
582 against LexA from *Streptomyces*, $His_6$-LexA was overexpressed and purified as described
583 above, and a total amount of 3.54 mg of purified protein was sent to Cambridge Research
584 Biochemicals (UK) to be used to raise antibodies in rabbits.

585

586 **Surface Plasmon Resonance (SPR)**

587 All SPR experiments were performed using a Biacore 8K instrument (Cytiva). All
588 measurements were performed with a single sensor chip SA (Cytiva), using the ReDCaT
589 system as described previously (62). Complementary single-stranded oligonucleotides
590 containing the individual LexA binding sequences, or a scrambled binding motif were
591 annealed and subsequently diluted to a working concentration of 1 µM in HPS-EP+ buffer

592 (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% v/v Surfactant P20). Each DNA was
593 designed to contain the sequence of interest, and a single stranded extension on the reverse
594 primer which anneals to the ReDCaT sequence on the chip, enabling DNA of interest to be
595 bound and stripped after each experiment. For each experiment double-stranded DNA (with
596 20 base single-strand extension) was flowed over one flow cell on the chip at a flow rate of
597 10 µl $min^{-1}$ for 60 sec to allow annealing to the ReDCaT linker via the complementary DNA.
598 Purified *S. venezuelae* $His_6$-LexA (diluted in HBS-EP+ buffer) was flowed over both the blank
599 cell surface and the one containing the DNA to ensure the response seen was specific to
600 binding to the DNA. To assess whether LexA bound the DNA, 1 µM and 0.5 µM $His_6$-LexA was
601 used at a flow rate of 50 µl $min^{-1}$ for 120 sec before switching back to buffer flow to allow
602 dissociation for 120 sec. The DNA and any remaining protein were then removed with a wash
603 over both flow cells at 10 µL $min^{-1}$ with 1 M NaCl, 50 mM NaOH. DNA sequences were tested
604 in triplicate at two different protein concentrations and the amount of binding was recorded
605 as response units (RUs).

606 To determine LexA binding kinetics, a single cycle kinetics approach was used. DNA
607 was initially captured as previously described on the test flow cell and then increasing
608 concentrations of $His_6$-LexA (0.78125 nM, 1.5625 nM, 3.125 nM, 6.25 nM, 12.5 nM, and 25
609 nM) were injected over both the blank and DNA bound flow cells at a flow rate of 100 µl $min^{-1}$
610 for 120 sec. At the end of all the $His_6$-LexA injections, HPS-EP+ buffer was flowed over the
611 surface for 1200 sec to measure the dissociation. The DNA and any remaining bound protein
612 were then removed using the 1M NaCl and 50 mM NaOH wash. The recorded sensorgrams
613 were analyzed using Biacore Insight Evaluation software version 3.0.11.15423 (Cytiva). The
614 following formulae were used to calculate $R_{max}$ and $\%R_{max}$-values, respectively:
615 $Rmax=(Mwt\ LexA)/(Mwt\ DNA) \times RU \times n \times 0.78$ and $\%R_{max}= (Analyte\ binding\ RU)/R_{max} \times 100$.
616 Where Mwt is the molar mass, RU is the DNA capture response, 0.78 is a constant used for
617 estimating responses for DNA/protein interactions and n is the binding stoichiometry. Raw
618 data used to calculate $R_{max}$ and $\%R_{max}$ is shown in Supplementary Table 6. To calculate LexA
619 binding kinetics, a fit was applied using a predefined kinetics 1 to 1 binding model provided
620 with the Biacore 8K Evaluation Software 3.0.11.15423 (Cytiva). Data was plotted using
621 Graphpad Prism (Version 9.3.1).

622 **Conflicts of interest**

623 The authors declare no competing interests.

624

625 **Data summary**

626 The authors confirm that all supporting data have been provided within the article or through
627 supplementary data files. Raw RNA-seq and ChIP-seq data have been deposited in the
628 MIAME-compliant ArrayExpress database (https://www.ebi.ac.uk/arrayexpress/) under
629 accession number E-MTAB-11556 and E-MTAB-11603.

630

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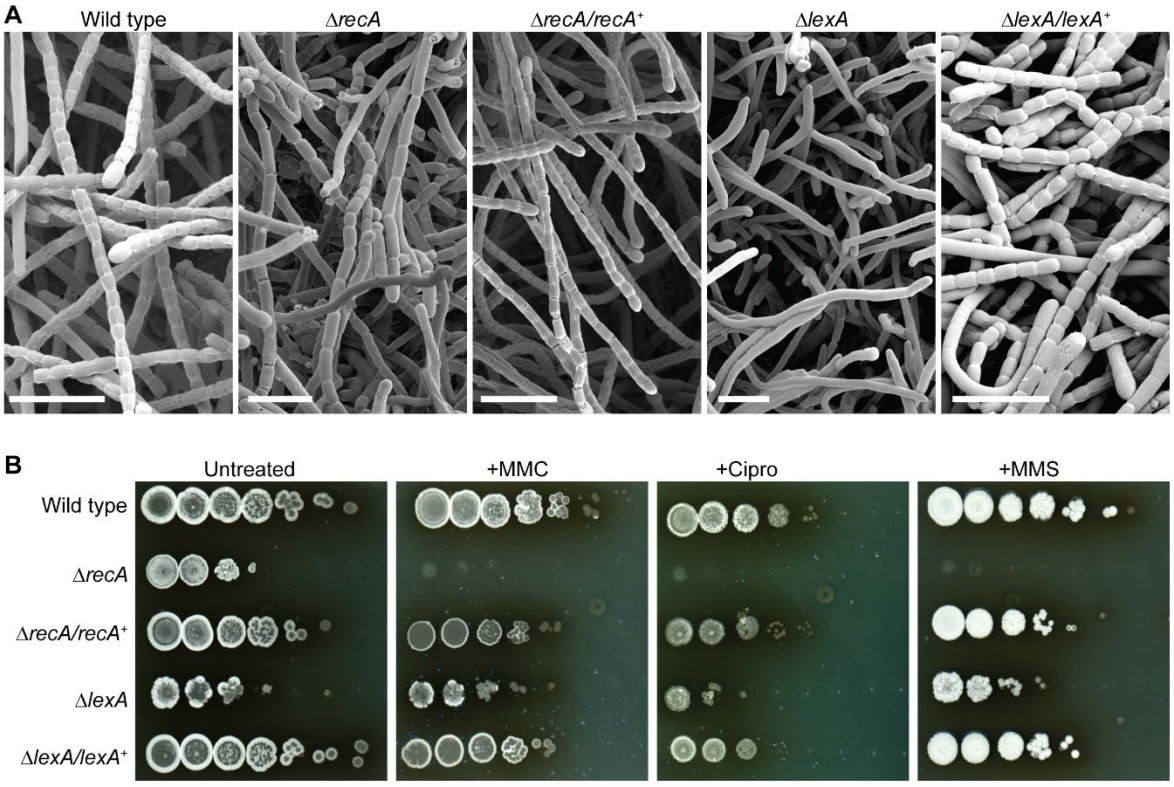
800 FIGURES & TABLE 1

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802 **Figure 1: Deletion of *recA* and *lexA* impair cellular development and increase sensitivity to DNA**
803 **damaging agents in *S. venezuelae*. A)** Representative cryo-scanning electron micrographs showing
804 aerial hyphae from the wild type, the Δ*recA* mutant (KS3), the Δ*lexA* mutant (KS44) and
805 complemented mutant strains Δ*recA/recA*$^+$ (KS14) and Δ*lexA/lexA*$^+$ (KS57). Scale bars: 5 μm. **B)**
806 Viability of the same strains as above when grown on solid MYM in the absence and presence of 0.25
807 μg ml$^{-1}$ mitomycin C (MMC), 3 μg ml$^{-1}$ ciprofloxacin (Cipro) and 3 mM methyl methanesulfonate
808 (MMS). Equal concentrations of spores were used, and a 10-fold serial dilution series of the spore
809 suspension was spotted onto MYM agar and incubated for 3-4 days. Shown are representative images
810 from biological triplicate experiments.

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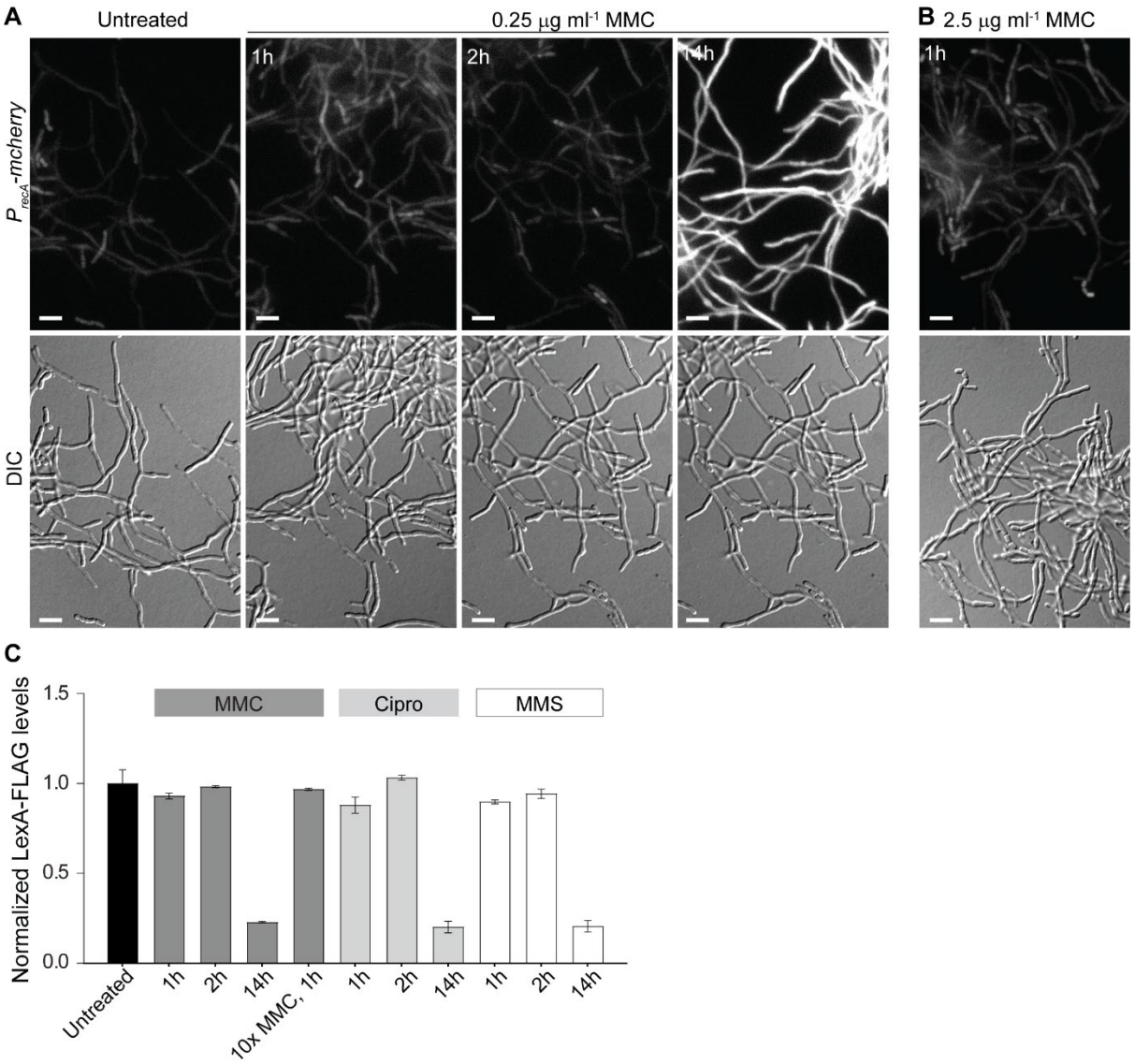


**Figure 2: Induction of the SOS response requires prolonged exposure to DNA damaging agents. A-B)** Fluorescence micrographs and corresponding differential interference contrast (DIC) images showing the induction of the *recA-mcherry* promoter fusion ($P_{recA}$-*mcherry*) in wild-type *S. venezuelae* hyphae (KS80) grown in the absence and presence of **(A)** 0.25 μg ml$^{-1}$ mitomycin C (MMC) for 1, 2 and 14h or **(B)** with 2.5 μg ml$^{-1}$ mitomycin C for 1h. Shown are representative images from biological triplicate experiments. Scale bars: 5 μm. **C)** Automated western blot analysis of LexA-FLAG protein levels in *S. venezuelae* (Δ*lexA/lexA-FLAG*$^{+}$, KS74) grown without and with 0.25 μg ml$^{-1}$ and 2.5 μg ml$^{-1}$ MMC, 3 μg ml$^{-1}$ ciprofloxacin (Cipro) and 5 mM methyl methanesulfonate (MMS) for 1h, 2h and 14h. LexA-FLAG abundance was determined using a monoclonal anti-FLAG antibody. Protein levels were normalized to the untreated control samples. Analysis was performed in biological duplicate. Representative virtual Western blots are shown in Supplementary Figure 4A.

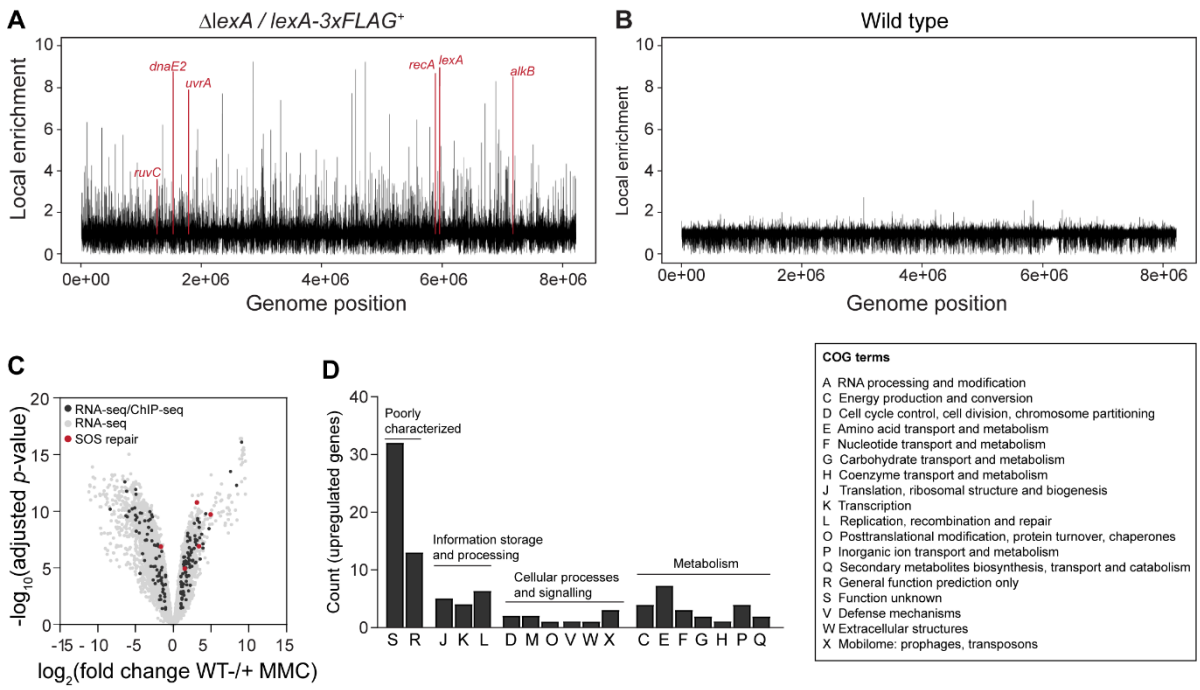


**Figure 3: Genome-wide identification of the LexA regulon. (A-B)** Genome-wide ChIP peak distribution of the LexA regulon in the **(A)** Δ*lexA/lexA-FLAG*$^{+}$ strain (KS74) and **(B)** the wild type. Strains were grown for 14h in MYM. Peaks of selected LexA regulon genes involved in DNA repair are labelled and coloured in red lines. ChIP-seq results were obtained from biological replicate experiments. **(C)** Volcano plot comparing RNA-seq profiles of the wild type grown with or without 0.25 µg ml$^{-1}$ MMC for 14h. Differentially expressed genes (grey dots) are defined by the adjusted *p*-value ($p<0.05$) and a fold change cut-off (|log2-fold change|>1). LexA target genes identified by ChIP-seq are labelled in black (n=175). Red-labelled dots indicate core DNA repair genes in the SOS regulon (Table 1). RNA-seq data was obtained from biological triplicate experiments. **(D)** Functional classification of genes that were upregulated following the degradation of LexA in response to MMC-induced stress (n=97, log2-fold change>1, adjusted *p*-value<0.05) were assigned Cluster of Orthologous Groups (COG) identifiers and their distribution was plotted. A legend for the corresponding COG annotations is presented to the right of the graph. Source data used to generate panel A-D can be found in Supplementary Table 1,3 and 4.

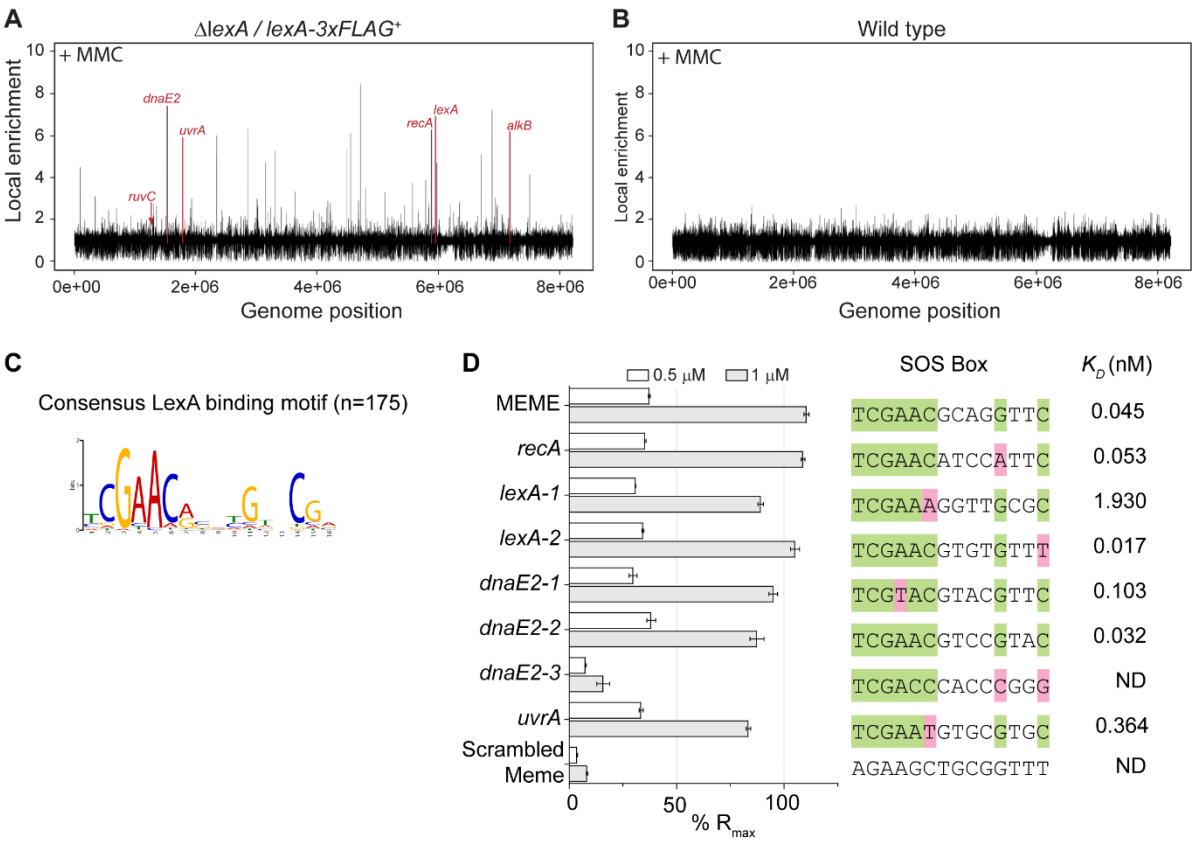


**Figure 4: LexA binds tightly to SOS boxes of core DNA damage genes *in vitro*.** Genome-wide ChIP peak distribution of the LexA regulon in the **(A)** Δ*lexA/lexA-FLAG*$^{+}$ strain (KS74) and **(B)** the wild type. Strains were grown for 14h in the presence of 0.25 μg ml$^{-1}$ MMC for 14h. Peaks of selected LexA regulon genes are labelled and coloured in red lines. **(C)** Identification of the LexA consensus motif (E-value 1.0e-252) by the MEME algorithm. The input of the analysis comprised ChIP-seq sequences of 100 nt length. The total number of sequences (n) is indicated. **(D)** Surface Plasmon Resonance (SPR) was used to determine the binding kinetics of LexA (0.5 μM and 1 μM) to 23-bp double-stranded DNA that contained individual target sequences located upstream of SOS genes involved in DNA repair. The target sequences (SOS box) used in these experiments are presented. Highly conserved nucleotides are shown in green, mismatches to the LexA consensus motif are shaded in magenta. A wider range of LexA concentrations was used to determine the binding affinity $K_D$ of LexA to the individual target sequences (Supplementary Figure S3). The affinity for the *dnaE2-3* binding site and the scrambled MEME motif were not determined (ND) due to very little specific binding of LexA.

859 **Table 1:** Selected members of the SOS regulon and co-regulated genes.

| Gene | Locus | Description | RNA-seq FC* | ChIP-seq FC** | SOS Box | Position (bp) *** |
|---|---|---|---|---|---|---|
| **Information storage and processing** | | | | | | |
| *recA* | *vnz_26845* | Recombinase A and LexA co-protease | 9.07 | 1.84 | TCGAACATCCATTC‡ | -162 |
| *recX* | *vnz_26850* | Recombination regulator | 6.28 | | | |
| *lexA* | *vnz_27115* | Transcriptional repressor | -3.10 | 1.34 | *lexA-1:* TCGAAAGGTTGCGC‡ *lexA-2:* TCGAACGTGTGTTT‡ | -53 |
| *dnaE2* | *vnz_06640* | DNA polymerase III, α subunit | 30.62 | 1.39 | *dnaE2-1:* TCGTACGTACGTTC‡ *dnaE2-2:* TCGAACGTCCGTAC‡ | -68 |
| *dinP* | *vnz_06635* | DNA polymerase IV | 13.74 | | | |
| *ruvC* | *vnz_05440* | Resolvase endonuclease subunit | 2.78 | 1.84 | TCCACCCCGAGAAC‡ | -23 |
| *ruvA* | *vnz_05435* | Holliday junction DNA helicase | 2.13 | | | |
| *alkB* | *vnz_32390* | Alkylated DNA repair protein | 11.02 | 1.39 | GGTTACGCTGGATC‡ | -20 |
| *infC* | *vnz_05845* | Translation initiation factor IF-3 | 3.05 | 2.39 | TCGTACTCCTGTGC | 91 |
| *parE* | *vnz_27225* | DNA topoisomerase IV subunit B | 3.78 | 1.18 | TCGAACTTACGTCC | -157 |
| **Cellular processes and signalling** | | | | | | |
| | | Prophage cluster | | | | |
| | *vnz_21525* | Putative phage tail fiber protein | 526.40 | 2.24 | TCGAACACCGGCGC | -174 |
| | *vnz_21545* | Hypothetical protein | 190.02 | 1.00 | TCGAACACAAGGCC | -123 |
| | *vnz_21550* | Hypothetical protein | 190.02 | 1.00 | TCGAACACAAGGCC | -182 |
| | *vnz_00275* | Serine protease | 10.93 | 1.92 | TCGAACGTTTGACG | -42 |
| **Metabolism** | | | | | | |
| | *vnz_22770* | Alcohol dehydrogenase | 26.72 | 1.62 | CCGGACACCGGGAC | -105 |
| | *vnz_15995* | ABC transporter permease | 15.14 | 2.01 | CCGAACGCATGTGT | -30 |
| | *vnz_26265* | Thiamine ABC transporter substrate-binding protein | 9.00 | 1.79 | TCGAACACATCTCC | -199 |

| General prediction | | | | | |
|---|---|---|---|---|---|
| *pvnz_37140* | Hypothetical protein | 15.35 | 1.22 | TCGAACACGTGCTC | -14 |
| *pvnz_37135* | DNA polymerase IV | 7.52 | | | |
| *vnz_31145* | Radical SAM protein | 19.97 | 1.31 | TCGACCGTTCGCTC | -61 |

860 *RNA-seq data: Relative fold-changes in gene expression of the wild-type cells grown for 14h in the
861 presence of MMC ($WT^{+MMC}$) compared to untreated cells ($WT^{-MMC}$) (adjusted *p*-value<0.05).
862 **Chip-seq data: Relative fold-change in local LexA-FLAG enrichment of cells expressing *lexA*-FLAG,
863 which were grown for 14h in the absence of MMC (LexA-$FLAG^{-MMC}$) compared to cells grown in the
864 presence of MMC (LexA-$FLAG^{+MMC}$) (|log2-FC|>1).
865 ***The position of the first base of the SOS box relative to the predicted start codon is indicated.
866 ‡ SOX box experimentally validated (Figure 4D)
867
868