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The *adnAB* Locus, Encoding a Putative Helicase-Nuclease Activity, Is Essential in *Streptomyces*

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Homologous recombination is a crucial mechanism that repairs a wide range of DNA lesions, including the most deleterious ones, double-strand breaks (DSBs). This multistep process is initiated by the resection of the broken DNA ends by a multisubunit helicase-nuclease complex exemplified by *Escherichia coli* RecBCD, *Bacillus subtilis* AddAB, and newly discovered *Mycobacterium tuberculosis* AdnAB. Here we show that in *Streptomyces*, neither *recBCD* nor *addAB* homologues could be detected. The only putative helicase-nuclease-encoding genes identified were homologous to *M. tuberculosis adnAB* genes. These genes are conserved as a single copy in all sequenced genomes of *Streptomyces*. The disruption of *adnAB* in *Streptomyces ambofaciens* and *Streptomyces coelicolor* could not be achieved unless an ectopic copy was provided, indicating that *adnAB* is essential for growth. Both *adnA* and *adnB* genes were shown to be inducible in response to DNA damage (mitomycin C) and to be independently transcribed. Introduction of *S. ambofaciens adnAB* genes in an *E. coli recB* mutant restored viability and resistance to UV light, suggesting that *Streptomyces* AdnAB could be a functional homologue of RecBCD and be involved in DNA damage resistance.

Cells are under constant genotoxic pressure from both endogenous and exogenous sources. DNA damage needs to be repaired to avoid the formation of deleterious mutations, abortion of replication, and lethal chromosomal breakage. Homologous recombination (HR) is a crucial mechanism that repairs a variety of DNA lesions, including DNA double-strand breaks (DSBs), single-strand DNA gaps, and interstrand cross-links.

DSBs are probably the most deleterious DNA damage that a cell can encounter. They are induced in cells by physical agents such as ionizing radiation or UV light, chemical agents, and natural products such as mitomycin C (MMC) or bleomycin. DSBs also result from replication fork collapse during chromosomal replication (1). The failure to repair DSBs can lead to cell death and, in the case of disrepair, can trigger large-scale chromosome rearrangements, favoring the generation of genetic diversity.

In bacteria, DSBs are for the most part processed through HR, which requires a homologous DNA template to carry out faithful repair of the damaged DNA duplex. In intensively replicating cells (vegetative growth phase) or immediately after the passing of the replication fork, the sister chromatid can be used as an intact template. In nonreplicating phases, such as late stationary phase or in spores (which contain a single copy of the chromosome), DSBs are more likely repaired by an illegitimate repair pathway. Indeed, illegitimate recombination (IR) does not require an intact homologous sequence but as a consequence has reduced fidelity.

The involvement of DSB repair by HR in a range of fundamental cellular processes (e.g., chromosome integrity, replication, segregation, etc.) reveals that HR is conserved in all living organisms. The initiating step of the repair mechanism consists of the resection of the DSB end to generate a 3' single-stranded tail onto which a synaptic protein (e.g., RecA in bacteria, RAD51 in humans) is loaded to form a nucleoprotein filament. The nucleoprotein filament will be used to perform homology search and strand invasion within a homologous DNA sequence. Subsequently, resolvase activity releases two intact DNA duplexes.

The end resection step is performed by a multisubunit heli-

case-nuclease complex, such as RecBCD in *Escherichia coli* (2, 3) and AddAB in *Bacillus subtilis* (4–6). In *E. coli*, defects in *recB* or *recC* impair HR, reduce cell viability, and increase sensitivity to DNA-damaging agents (7–9). In *B. subtilis*, a defect in AddAB moderately decreases DSB repair efficiency (10). RecBCD and AddAB are functional homologues, given that heterologous expression of *B. subtilis* AddAB restores cell viability, the ability to repair UV-damaged DNA, and recombination in the *E. coli recBCD* null mutant (11). Examination of the phylogenetic distribution of RecBCD and AddAB revealed that one or both of the complexes are encoded in the majority of sequenced bacterial genomes (12). Using a proteomic approach, Sinha et al. (13) uncovered a new heterodimeric DNA motor-driven nuclease complex called AdnAB (ATP-dependent nuclease) in *Mycobacterium smegmatis*. This is the third helicase-nuclease complex identified that has been shown to be involved in DSB repair, as an *M. smegmatis adnAB*-null mutant is hypersensitive to ionizing radiation (14).

Although all three of these helicase-nuclease enzymes appear to be involved in DSB repair in their respective context, they differ in distribution of the functional domains (helicase motor and nuclease domains within the different subunits of the complex). RecBCD is composed of two helicase motor domains carried by RecB and RecD (15, 16) and one nuclease domain located at the C

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terminus of RecB. For its part, AddAB possesses a single helicase motor at the N terminus of the AddA subunit, plus two distinct RecB-like nuclease domains located at the C terminus of AddA and AddB (6). The AdnA and AdnB subunits are each composed of an N-terminal UvrD-like motor domain and a C-terminal RecB-like nuclease domain (13).

In *E. coli* and *B. subtilis*, an alternative pathway (the RecFOR pathway) can substitute for RecBCD and AddAB, respectively, in the end resection step. In RecBCD-deficient *E. coli* cells, the RecFOR pathway, known to be involved in single-strand-break repair, can compensate for the RecBCD defect and promote recombinational DSB repair (8, 17–19). This pathway combines the single-strand DNA exonuclease RecJ, the RecQ helicase, and the RecF, RecO, and RecR proteins that act together to promote loading of RecA onto single-stranded DNA. In *B. subtilis*, genetic studies revealed that *addAB* or Δ *recJ* mutations moderately impact DSB repair, whereas the *addAB* Δ *recJ* double mutation has a synergistic negative effect (10).

In mycobacteria, both AdnAB and RecBCD complexes cohabit: while AdnAB is required for HR (14), the RecBCD complex is involved in a RecA-independent illegitimate recombination pathway called single-strand annealing (SSA) (20). Moreover, the RecFOR pathway is absent, since neither RecJ nor RecQ has been identified in mycobacterial genomes thus far (21).

Streptomyces are actinobacteria that are well known for their high genetic plasticity (22, 23), yet their recombination pathways remain poorly understood. *Streptomyces* are Gram-positive soil bacteria possessing a large linear chromosome (24) showing a remarkable genetic organization. While the central region is highly syntenic at the intraspecific level and gathers the essential genes, the terminal parts are variable and prone to chromosomal rearrangements affecting several hundreds of kilobases under laboratory conditions. Interestingly, comparative genomics reveals that synteny in the terminal regions does not abruptly stop but rather fades progressively toward the terminal regions. This degenerated synteny consists of the accumulation over evolutionary times of insertions and deletions (indels) that follows an increasing gradient toward the chromosomal ends (25). This phenomenon can be put into perspective with the recent report of high intraspecies and interspecies HR levels within *Streptomyces* revealed by multilocus sequence analysis (MLSA) (26). Further, the intraspecies recombination rate exceeded the interspecies rate by 2 orders of magnitude, supporting the possibility that gene exchange and recombination may have shaped the genome of streptomycetes. These genomic characteristics prompted us to investigate HR in *Streptomyces* and to search for helicase-nuclease-encoding genes and their involvement in survival, recombination, and DNA repair pathways.

MATERIALS AND METHODS

Bacterial strains and media. The strains and plasmids used in this study are listed in Table 1. The DH5 α *E. coli* strain was used as a cloning host for plasmid construction. *E. coli* ET12567/pUZ8002 and S17-1 are the non-methylating plasmid donor strains used for intergeneric conjugation with *Streptomyces ambofaciens* and *Streptomyces coelicolor*. The *E. coli* strains JJC40 and JJC315 used for nuclease activity, cell viability, and UV sensitivity assays were kindly provided by Bénédicte Michel (CNRS, Gif-sur-Yvette, France). All *E. coli* strains were grown in Luria-Bertani (LB) broth at 37°C, with the exception of strain DY330, used for PCR targeting, which was grown at 30°C and transferred to 42°C for 17 min before transformation with linear DNA (27). Soya flour mannitol (SFM) agar medium was

TABLE 1 Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)	Source or reference
<i>Streptomyces</i>		
<i>S. ambofaciens</i> ATCC 23877	Used as wild-type strain	51
<i>S. coelicolor</i> A3(2)	Used as wild-type strain	52
<i>E. coli</i>		
DH5 α	F ⁻ ϕ dlacZ Δ M15 <i>endA1 supE44 thi-1 recA1 relA1 gyrA96 deoR nupG</i> Δ (<i>lacZYA-argF</i>)U169 λ ⁻ <i>hsdR17</i> ($r_K^- m_K^+$) <i>phoA relA1</i>	53
DY330	W3110 Δ <i>lacU169 gal490</i> λ c1857 Δ (<i>cro-bioA</i>)	27
ET12567/pUZ8002	<i>dam-13::Tn9 dcm cat tet hsdM hsdR zjj-201::Tn10 tra neo</i> RP4	54, 55
S17-1	<i>recA pro hsdR</i> RP4–2-Tc::Mu Km::Tn7	56
JJC40	<i>leu6 his4 argE3 lacY1 galk2 ara14 xyl5 mtl1 tsx33 rpsL31 supE44 hsdR</i>	Provided by B. Michel (CNRS)
JJC315	JJC40 derivative <i>recB268::Tn10</i> (Tet)	
BACs and plasmids		
BAB15E8	Resource of <i>adnAB</i> locus for construction of pSET152- <i>adnAB</i>	25
pSET152	Integrative vector for actinomycetes; <i>oriT</i> (RK2), <i>int</i> and <i>attP</i> (ϕ C31), <i>acc(3)</i> IV	57
pSET153	pSET152 derivative containing <i>neo</i> instead of <i>acc(3)</i> IV	This work
pSET152- <i>adnAB</i>	pSET152 derivative containing the <i>adnAB</i> locus	This work
pSET153- <i>adnAB</i>	pSET152- <i>adnAB</i> derivative containing <i>neo</i> instead of <i>acc(3)</i> IV	This work
pIJ6902-LCN	pIJ6902 derivative	Unpublished data
pIJ6902-LCN- <i>adnAB</i>	pIJ6902-LCN derivative containing <i>adnAB</i>	This work

used for *Streptomyces* sporulation, and liquid Hickey-Tresner (HT) medium was used for growth of mycelia for RNA extraction (28). When appropriate, antibiotics were added to the media at concentrations of 50 μ g \cdot ml⁻¹ kanamycin, 50 μ g \cdot ml⁻¹ apramycin, 100 μ g \cdot ml⁻¹ puromycin, 25 μ g \cdot ml⁻¹ nalidixic acid, and 12 μ g \cdot ml⁻¹ tetracycline.

Plasmid constructions. The pNSV005 plasmid was designed for the deletion of *S. ambofaciens adnAB*: the *adnAB* flanking regions were amplified by PCR. The 5' and 3' regions were amplified from *S. ambofaciens* ATCC 23877 genomic DNA by PCR. The two PCR fragments were cloned into the pGEM-T Easy vector and then released by HindIII/EcoRV or EcoRV/NheI digestions. The excisable cassette *att3-aac* conferring apramycin resistance was excised from pOSV234 (29) by EcoRV digestion. The above-mentioned three fragments were simultaneously ligated with the HindIII/NheI-digested vector pWED2 (30), which confers puromycin resistance to generate an *adnAB* deletion vector.

Derivatives of the 2St3B6 cosmid carrying a Tn5062 insertion (31) in either the *adnA* or *adnB* gene were purchased from Paul Dyson, University of Swansea (<http://strepdb.streptomyces.org.uk>). These cosmids were used for *adnA* and *adnB* inactivation attempts in *S. coelicolor*.

For *Streptomyces* complementation experiments, the *S. ambifaciens* *adnAB* locus, including its own promoter, was isolated as a 11.4-kb NcoI fragment from a recombinant bacterial artificial chromosome (BAC) and cloned in pSL1180 (32). The locus was then excised as an XbaI/EcoRI fragment to be inserted into pSET152 and to yield pSET152-*adnAB*. A kanamycin-resistant version of pSET152 called pSET153 was constructed by replacement of the *acc(3)IV* gene by the *neo* gene from supercos1 (Agilent Technologies).

For *E. coli* complementation experiments, pIJ6902-LCN (unpublished), a low-copy-number plasmid derived from pIJ6902 (33), was used as a cloning vector. In plasmid pIJ6902-LCN, the high-copy-number pMB1 origin was replaced by the low-copy-number replication origin of the F factor (derived from the pBeloBAC11 vector). In order to avoid amplification of the long GC-rich fragment, including the *adnAB* genes (7.6 kb), we opted for the replacement of the sequence of pSET153 by pIJ6902-LCN in the pSET153-*adnAB* construct by using a PCR targeting procedure. By this approach, the *adnAB* genes were placed immediately downstream of the pTipA promoter, which was shown to be expressed in *E. coli* in our previous work (unpublished data).

Genetic procedures. Plasmids, BACs, or cosmids were introduced into *S. ambifaciens* and *S. coelicolor* strains by intergeneric conjugation with *E. coli* ET12567/pUZ8002 or S17-1 (28). Single-crossover exconjugants leading to integration of the deletion construct into the chromosome were selected on plates containing apramycin. Double-crossover exconjugants leading to gene replacement and concomitant loss of the cloning vector were then screened for puromycin or kanamycin sensitivity according to the vector used (pNSV005 or 2St3B6 derivatives). Replacement events were confirmed by PCR. The frequencies of a second crossover were calculated from three independent experiments. *P* values were calculated by using the chi-square test.

Complemented *E. coli* strains were obtained by transformation of JJC315 with pIJ6902-LCN-*adnAB*.

RNA isolation and quantitative RT-PCR. Pregerminated spores (28) of *S. ambifaciens* ATCC 23877 were grown until an optical density at 600 nm (OD_{600}) of ~ 0.2 was reached in HT liquid medium. RNA was harvested from cell aliquots treated with $1 \mu\text{g} \cdot \text{ml}^{-1}$ mitomycin C (MMC) for 30 min. Total RNA was isolated from the harvested cells using the Aurum total RNA minikit (Bio-Rad). Reverse transcription (RT) reactions were performed using $1 \mu\text{g}$ of RNA and an iScript advanced cDNA synthesis kit from Bio-Rad according to the manufacturer's instructions. After quantitative RT-PCR was carried out on cDNA from each condition using iQ SYBR green supermix from Bio-Rad for each PCR, normalization of gene expression was achieved using *hrdB* as a reference. Primers and their positions in the target sequences are given in Table S1 in the supplemental material.

UV sensitivity and viability assays. Overnight cultures of *E. coli* were diluted at 1:100 into fresh LB medium with appropriate antibiotics and grown to an OD_{600} of ~ 0.3 . After dilution in 100 mM phosphate buffer (pH 7.0), $100 \mu\text{l}$ of the 10^{-3} dilution was spread on LB plates. Plates were irradiated with 0, 10, or $30 \text{ J} \cdot \text{m}^{-2}$ using a 254-nm UV light delivering $1 \text{ J} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and then incubated for 24 h at 37°C in the dark to avoid photoreactivation. Survival was determined as the number of CFU after a given UV dose divided by the CFU of nonirradiated cells. Cell viability was defined, in the absence of stress, as the ratio of CFU per ml to the expected cell number per ml as estimated by OD_{600} measurement.

Cell extract preparation and nuclease activity assay. *E. coli* bacteria were harvested from an overnight culture and resuspended in a lysis buffer containing 1 mM dithiothreitol (DTT), 1 mM EDTA, 0.05 M Tris (pH 8), and $1 \text{ mg} \cdot \text{ml}^{-1}$ lysozyme. Cell extracts were obtained after sonication. Nuclease activity assay mixtures contained 50 mM Tris-HCl (pH 8), 10 mM MgCl_2 , 50 μM ATP, 200 ng linear DNA (a 3,734-bp PCR product), and $1 \mu\text{g}$ cell extract, and the mixtures were incubated at 37°C for 0 h, 1.5 h, and 3 h. After electrophoretic separation and ethidium bromide staining, the intensity of each DNA band was estimated using ImageJ (<http://rsb.info.nih.gov/ij/>).

TABLE 2 AdnAB in *Streptomyces* and other actinomycete sequenced genomes^a

Species and strain	Locus tag for sequence	
	AdnA	AdnB
<i>Streptomyces avermitilis</i> MA-4680	SAV_3077	SAV_3076
<i>Streptomyces bingchenggensis</i> BCW-1	SBI_04047	SBI_04046
<i>Streptomyces cattleya</i> NRRL 8057 (=DSM 46488)	SCAT_4044	SCAT_4047
<i>Streptomyces coelicolor</i> A3(2)	SCO5183	SCO5184
<i>Streptomyces flavovirgatus</i> ATCC 33331	Sfla_2108	Sfla_2107
<i>Streptomyces griseus</i> subsp. <i>griseus</i> NBRC 13350	SGR_2342	SGR_2341
<i>Streptomyces scabiei</i> 87.22	SCAB_30771	SCAB_30761
<i>Streptomyces</i> sp. SirexAA-E	SACTE_4413	SACTE_4414
<i>Streptomyces violaceusniger</i> Tu 4113	Strvi_1501	Strvi_1502
<i>Streptomyces clavuligerus</i> ATCC 27064	SCLAV_4047	SCLAV_4048
<i>Streptomyces lividans</i> TK24	SLI_5469	SLI_5470
<i>Streptomyces davawensis</i> JMC 4913	BN159_3205	BN159_3204
<i>Streptomyces collinus</i> Tu 365	B446_24410	B446_24415
<i>Streptomyces hygroscopicus</i> subsp. <i>Jinggangensis</i> TL01	SHJGH_6050	SHJGH_6052
<i>Streptomyces hygroscopicus</i> subsp. <i>Jinggangensis</i> 5008	SHJG_6289	SHJG_6291
<i>Streptomyces fulvissimus</i> DSM 40593	SFUL_4994	SFUL_4995
<i>Streptomyces albus</i> J1074	XNR_1620	XNR_1619
<i>Streptomyces</i> sp. PAMC26508	F750_4709	F750_4710
<i>Streptomyces venezuelae</i> ATCC 10712	SVEN_4833	SVEN_4835 ^b
<i>Streptomyces rapamycinicus</i> NRRL 5491	M271_51085 ^c	M271_18000
<i>Saccharopolyspora erythraea</i> NRRL2338	SACE_1077	SACE_1078
<i>Rhodococcus erythropolis</i> PR4	RER_22180	RER_22190
<i>Frankia</i> sp. CcL3	Francic3_3800	Francic3_3799

^a Threshold e-value, $<10^{-20}$ for AdnA and $<10^{-40}$ for AdnB.

^b The aa identity with AdnB starts in SVEN_4835 and ends in SVEN_4836 (including the nuclease motif). The multiple homopolymers present in this region may contain a sequence error resulting in the predicted frameshift. Thus, these two coding sequences may form a single one.

^c M271_51085 of *S. venezuelae* includes a stretch of undetermined nucleotides interrupting the AdnA homologue.

RESULTS AND DISCUSSION

AdnAB is predicted to be the only helicase-nuclease activity in *Streptomyces*. Twenty complete *Streptomyces* genomes were searched for genes encoding helicase-nuclease activity. BLASTP searches confirmed a previous report (21) that no homologues for RecB, RecC, AddA, and AddB are present in *Streptomyces*. A weak homologue of RecD with partial amino acid identities was identified using Rv0629c (575 amino acids [aa]) encoding the RecD homologue of *M. tuberculosis* as the query sequence (36% aa identity over 73% of the protein length). This suggests the absence of a RecBCD-like and an AddAB-like helicase-nuclease complex in *Streptomyces*. Furthermore, searches for the RecQ-RecJ helicase-nuclease complex, known to carry out DNA end resection in the absence of RecBCD and AddAB in *E. coli* and *B. subtilis*, respectively (10, 34), resulted in the identification of a sole RecQ homologue in *Streptomyces*. In the absence of the RecJ component (exonuclease activity), this pathway is not expected to be functional.

In contrast, *Streptomyces* genomes showed the presence of genes encoding homologues of AdnA (Rv3202c) and AdnB (Rv3201c) from *M. tuberculosis* (complex called MtuAdnAB), with 35 to 40% amino acid identities (Table 2). Between *Streptomyces* species, AdnA-like and AdnB-like proteins are highly con-

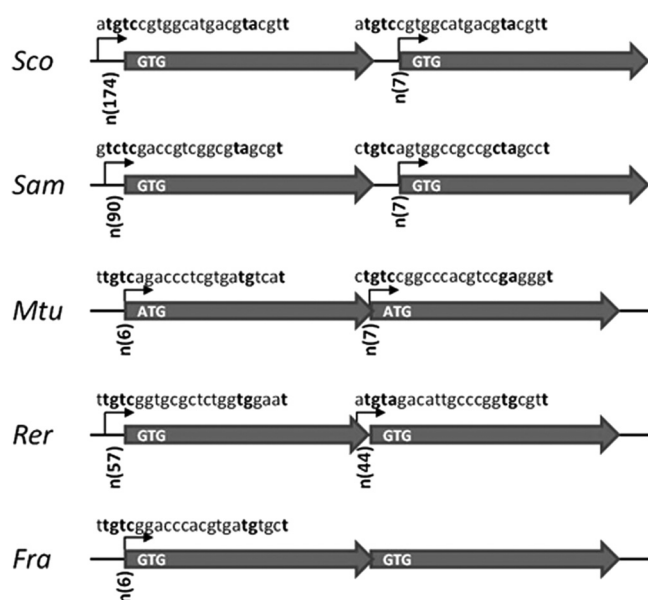


FIG 1 Organization of the *adnAB* locus in some actinomycetes. Putative promoter motifs are represented by arrows in front of both *adnA* and *adnB* genes of *S. coelicolor* (*Sco*), *S. ambifaciens* (*Sam*), *M. tuberculosis* (*Mtu*), *Rhodococcus erythropolis* (*Rer*), and *Frankia* sp. (*Fra*). The boldface nucleotides correspond to the strictly defined positions described by Gamulin et al. (5'-TGTC-12nt-TAnnT-3') (44). The distances between putative promoter motifs, indicated as n(number of nucleotides), and predicted start codons are indicated at the beginning of each arrow symbolizing the genes. The presence or absence of intergenic space is also indicated by a straight line between the arrows or by overlapping arrows, respectively.

served (from 72% to 95% when using the *S. ambifaciens* gene products as references), and the amino acid identities tend to reflect the phylogenetic relationships. These two proteins will be here called AdnA and AdnB. The level of amino acid identity of *Streptomyces* AdnA and AdnB proteins with those of other actinomycetes (*Saccharopolyspora erythraea*, *Rhodococcus erythropolis*, and *Frankia* sp.) falls below 50%. In all *Streptomyces* species, with the exception of *Streptomyces cattleya* and *Streptomyces venezuelae*, the genes encoding the AdnA and AdnB homologues are adjacent and show the same transcriptional orientation (Fig. 1), as in *Mycobacterium* species (13). In *S. cattleya* and *S. venezuelae*, a single putative gene of 702 and 834 nucleotides, respectively, is inserted between the *adnA* and *adnB* genes.

Streptomyces AdnAB proteins share structural similarities with MtuAdnAB (13) and with other actinomycete AdnAB homologues (see Fig. S1 in the supplemental material): all *Streptomyces* AdnA and AdnB proteins possess the 8 helicase motifs (I, Ia, II to VI, and Q) (35, 36) in the N-terminal domain as well as the 3 motifs of the RecB nuclease active site in the C-terminal domain (37, 38). These data strongly suggest that *Streptomyces* AdnA and AdnB both contain an N-terminal UvrD-like motor domain and a C-terminal RecB-like nuclease domain, as in MtuAdnAB (13). Hence, in the absence of all other reported complexes (i.e., RecBCD, AddAB, and RecQ-RecJ), AdnAB may be the only DNA end-processing helicase-nuclease in *Streptomyces*.

The *adnAB* locus is essential in *Streptomyces*. DNA end resection deficiency has a significant impact on bacterial cell viability and growth: *E. coli recBC recJ* (39, 40) and *B. subtilis addAB ΔrecJ*

double mutants (10) are deeply affected, and the *Acinetobacter baylyi* $\Delta recBCD \Delta recJ$ double mutant is not viable (41).

Hence, a strategy of deletion of the *adnAB* locus of *S. ambifaciens* was developed to assess the consequence of AdnAB deficiency on growth and survival against DNA-damaging agents. The *aac(3)IV* gene conferring apramycin resistance that is bordered by the 5'-end-flanking region (region U [upstream], 1,501 nt) and 3'-end-flanking region (region D [downstream], 1,790 nt) of the *adnAB* locus was inserted in the suicide plasmid pWED2 (carrying the *pac* gene, conferring resistance to puromycin). Apramycin- and puromycin-resistant (*Apra^r Pur^r*) clones resulting from integration via a single crossover could be easily selected, and PCR analysis showed that this crossover occurred evenly in U and D regions. Therefore, there was no obstacle to a crossover event in either of the two regions, and the length of the regions was fully sufficient to support HR. In contrast, deletion of the *adnAB* locus by the formation of a second crossover (in the U region when the deletion cassette was initially inserted via the D region or vice versa) giving rise to *Apra^r Pur^s* clones could not be obtained. Formally, no *Apra^r Pur^s* clones could be found among 386 and 366 derivatives of 3 independent clones harboring the deletion construct in the U and D region, respectively.

In order to determine whether the failure to obtain *adnAB* gene replacement in *S. ambifaciens* results from the lethality of the *adnAB* deletion, an integrative plasmid (pSET153, conferring resistance to kanamycin) carrying an intact copy of the *adnAB* locus (as an 11.4-kb DNA fragment; see Materials and Methods) was inserted into the ϕ C31 phage integration site of the chromosome. This insertion was carried out in strains harboring the *adnAB* deletion construct inserted by single crossover in the U or D region (3 strains for each). In the presence of this ectopic copy of *adnAB*, double crossovers resulting in *Apra^r Pur^s Kan^r* clones were selected at high frequencies. The loss of the puromycin resistance gene corresponding to the formation of the second crossover was observed at similar frequencies from strains with U (420 of 1,314 colonies tested, 32%) and D (464 of 1,472 colonies tested, 31.5%) region insertions and was confirmed by PCR analyses. The observation that the double crossover could be selected only in the presence of an ectopic extra copy of the *adnAB* genes indicates that the *adnAB* locus is essential in *S. ambifaciens*.

Similar experiments were carried out in the closely related strain *S. coelicolor* A3 (2). For this purpose, recombinant cosmids, including the *adnAB* locus interrupted by the Tn5062 transposon (in either *adnA* or *adnB*), were used in attempts to replace the *adnA* and *adnB* genes independently. Again, while single-crossover events resulting in *Apra^r Kan^r* clones were scored at high frequencies, no gene replacement by double crossover could be selected (*Apra^r Kan^s*). In these experiments, the homologous upstream and downstream regions were at least 15 kb long and were thus obviously sufficient to support HR. From these data, we can conclude that both *adnA* and *adnB* are essential for viability, since this strategy targeted either open reading frame independently. The possibility that *adnA* mutation could have polar effects on *adnB* gene expression was excluded, since these two genes are independently transcribed (see below).

This essential nature of *adnAB* in *Streptomyces* is in contrast to all the other bacterial species investigated so far. Hence, the *E. coli recBCD* mutant, *B. subtilis addAB* mutant, and *A. baylyi ΔrecBCD* mutant are viable, probably as a result of the presence of alternative helicase-nuclease activities (e.g., RecJ/Q). *E. coli recBC recJ*

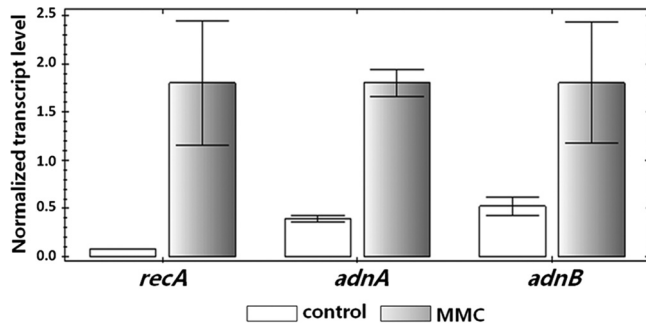


FIG 2 Induction of *adnAB* by exposure to mitomycin C. Quantitative RT-PCR experiments were carried out to test the induction of *adnA* and *adnB* in response to MMC. *S. ambifaciens* cultures were exposed to MMC ($1 \mu\text{g} \cdot \text{ml}^{-1}$) or not exposed for 30 min. *recA* served as a positive control. *hrdB* was used for normalization as a reference gene. This experiment was performed in triplicate.

and *B. subtilis addAB ΔrecJ* mutants are still viable although drastically impaired in growth, which suggests that they contain additional back-up systems. This is consistent with the lethality of *E. coli recD recJ* mutants in which additional mutations in the exonuclease I-encoding gene (*xonA*) and the exonuclease VII-encoding gene (*xseA*) are introduced (42). While an *xseA* homologue is found in *S. ambifaciens* and *S. coelicolor* genomes, it clearly does not provide the same back-up activity.

***adnAB* expression is induced in response to genotoxic agents.** The *adnAB* genes of *M. tuberculosis* were shown to be induced (9-fold) in response to MMC, a DNA-damaging agent causing genotoxic stress (43). In order to test whether *adnAB* genes are induced by DNA damage in *Streptomyces*, the level of expression of *adnAB* was measured by quantitative RT-PCR in response to MMC. The *recA* gene involved in the positive regulation of the SOS system in bacteria was chosen as a positive control in these experiments. The *hrdB* gene was used for normalization of gene expression levels. As expected, induction of *recA* was observed after exposure to $1 \mu\text{g} \cdot \text{ml}^{-1}$ MMC (about 20-fold). Under the same conditions, a significant induction of about 4-fold was observed for both *adnA* and *adnB* genes (Fig. 2). In order to establish whether *adnA* and *adnB* constitute an operon, the transcription of the intergenic sequence (250 bp) was assessed in the presence or absence of MMC. No transcript was observed, indicating that both genes were independently expressed.

Consistent with these data, we could identify putative motifs upstream of both genes that strongly resemble the consensus promoter sequence 5'-tTGTCRgtg-8nt-TAnnnT-3' (where upper- and lowercase letters correspond to conserved and variable nucleotide positions, respectively) described by Gamulin et al. (44) in *M. tuberculosis*. This promoter motif was involved in the RecA-independent inducible response to DNA-damaging agents (MMC) (43, 44). In our analysis, a sequence approaching this consensus can be found just upstream of the translation start codon of both *adnA* and *adnB* (6 and 7 nucleotides, respectively) (Fig. 1). This promoter motif was also detected upstream of *adnA* in *Frankia* sp. and of *adnA* and *adnB* in *Rhodococcus erythropolis*, both actinomycetes. Interestingly, the promoter motifs are positioned only 7 nucleotides upstream from the predicted translational start codon of *adnB* in both *S. coelicolor* and *S. ambifaciens*, suggesting that these genes may be leaderless genes that are widespread in actinobacteria (45).

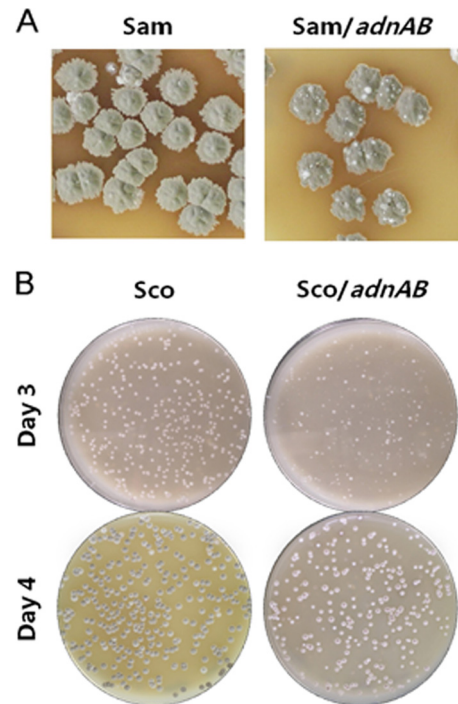


FIG 3 Phenotypes of *Streptomyces* harboring two copies of the *adnAB* locus. The *S. ambifaciens* (A) and *S. coelicolor* (B) strains harboring an empty plasmid pSET153 (Sam and Sco, respectively) as a control or harboring plasmid pSET153-*adnAB* (Sam/*adnAB* and Sco/*adnAB*, respectively) were grown on SFM agar. Genetic instability (represented by papillae) in *S. ambifaciens* was observed after 12 days of growth. The growth defect of *S. coelicolor* harboring two *adnAB* loci was visible from the third day of growth.

Phenotypic characterization of a *Streptomyces* mutant carrying two *adnAB* copies. *S. ambifaciens* and *S. coelicolor* strains containing two copies of the *adnAB* locus were obtained during this work and were exploited to assess the impact of *adnAB* overexpression. *S. ambifaciens* harboring two copies of *adnAB* exhibited a colonial phenotype typical of genetic instability, i.e., with a high frequency of unpigmented papillae on bacterial colonies (Fig. 3A) (46). Colonies of *S. coelicolor* strains harboring two copies of the *adnAB* locus showed a marked impairment of their development (Fig. 3B). Furthermore, a small but reproducible increase in sensitivity to MMC was noticed in *S. ambifaciens* clones harboring two *adnAB* loci (not shown). Quantitative RT-PCR analysis showed that the presence of two copies of *adnAB* was associated with 6- and 3-fold increases in transcript quantity for *adnA* and *adnB*, respectively, compared to the wild-type (WT) context. Therefore, the observed phenotypes may result from the modulation of the homeostasis of helicase-nuclease cellular activity. The excess of nuclease activity may be harmful and lead to an unexpected degradation of DNA during chromosomal replication, affecting cell growth. In the same way, genome instability and sensitivity to genotoxic agents may result from impairment of the HR process. A parallel could be drawn with *E. coli*, where overexpression of *recBCD* reduces resistance to UV exposure (47).

***Streptomyces adnAB* restores survival and UV light resistance to *E. coli recB*.** In *E. coli*, RecBCD (exonuclease V) is crucial for the initiation of HR at DNA double-strand ends, and its absence leads to a drastic decrease in survival compared to that of the

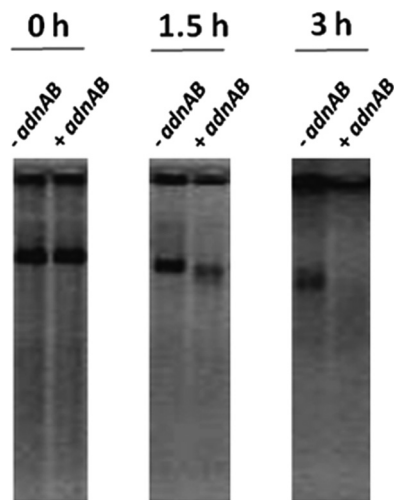


FIG 4 *Streptomyces adnAB* can restore the nuclease activity of an *E. coli* $\Delta recB$ mutant. Cell extracts were prepared from an *E. coli* $\Delta recB$ mutant strain carrying an empty plasmid ($-adnAB$) or a plasmid that included the locus *adnAB* from *S. ambofaciens* ($+adnAB$). Reaction mixtures were incubated for 0 h, 1.5 h, or 3 h at 37°C.

WT strain in response to genotoxic agents (for a review, see reference 48). In order to test whether *Streptomyces* AdnAB works as a functional homologue of *E. coli* RecBCD, complementation of an *E. coli* $\Delta recB$ mutant (JJC315) was examined. The $\Delta recB$ mutant (allele *recB268*) is suggested to be deficient for the B and D subunits of the RecBCD complex due to a polar effect on the adjacent *recD* gene (49).

Plasmid pIJ6902-LCN either empty or containing the *S. ambofaciens adnAB* locus was used to transform *E. coli* JJC40 (WT) and JJC315 ($\Delta recB$) strains. First, crude extracts of *E. coli* clones harboring these plasmids were assayed for exonuclease activity. For that purpose, 300 ng of linear DNA was exposed to crude extracts. After 3 h of exposure, the degradation was more visible with extracts from cells containing the *adnAB* locus (Fig. 4). The degradation provided by the *adnAB*-complemented extract was roughly estimated to be 40 to 60% higher than that of the noncomplemented strain. This result strongly suggested that *Streptomyces adnAB* can be expressed in *E. coli* and exhibits an exonuclease activity.

To examine whether the viability of an *E. coli* $\Delta recB$ mutant could be restored by the *Streptomyces adnAB* genes, the plating efficiency of *E. coli* cultures grown to an OD_{600} of ~ 0.3 was measured. Cell viability was expressed as the number of CFU $\cdot ml^{-1}$ per unit OD_{600} . $\Delta recB$ mutation in *E. coli* resulted in a 20-fold reduction of cell viability. In contrast, in the *E. coli* $\Delta recB$ mutant carrying *S. ambofaciens adnAB*, the viability was partially restored, reaching 60% of the WT level of survival (Fig. 5A).

The role of *Streptomyces* AdnAB in DNA repair was studied by examining whether *S. ambofaciens adnAB* genes could restore the impaired ability of the *E. coli* $\Delta recB$ mutant to repair UV-induced DNA damage. Samples of cultures in exponential phase were irradiated with UV light, and cell survival was determined (Fig. 5B). In our experimental conditions, the *E. coli* $\Delta recB$ mutant was highly sensitive to UV, as cell survival was reduced about 200-fold compared to that of the WT at $10 J \cdot m^{-2}$. However, the strain complemented with *S. ambofaciens adnAB* was about 60-fold

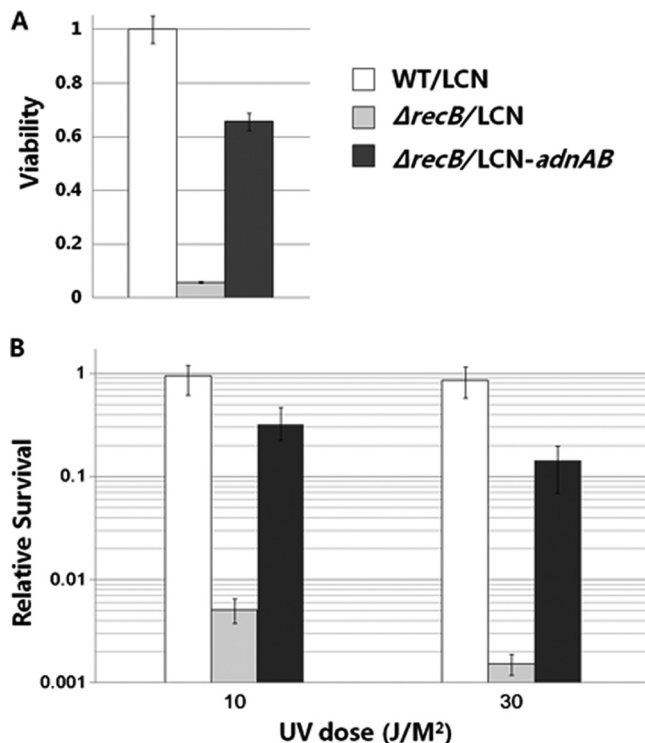


FIG 5 *S. ambofaciens adnAB* can restore cell viability and UV resistance of *E. coli* $\Delta recB$. The wild-type *E. coli* strain carrying pIJ6902-LCN (WT/LCN) and the *E. coli* $\Delta recB$ strain carrying pIJ6902-LCN ($\Delta recB$ /LCN) or pIJ6902-LCN-*adnAB* ($\Delta recB$ /LCN-*adnAB*) were plated to measure cell viability (A) and exposed to UV at $10 J \cdot m^{-2}$ and $30 J \cdot m^{-2}$ to measure relative survival (B). This experiment was performed in triplicate.

more resistant to UV than the *E. coli* $\Delta recB$ mutant, which demonstrates that *Streptomyces adnAB* could restore the impaired ability of $\Delta recB$ mutant to repair UV lesions. Altogether, these data suggest that *S. ambofaciens* AdnAB is a functional homologue of RecBCD and is likely to be involved in DNA repair.

In conclusion, the helicase-nuclease encoded by *adnAB* of *Streptomyces* is assumed to be involved in HR. Therefore, *adnAB* essentiality may be linked to some HR-dependent critical cell processes such as DNA replication, DNA repair, or chromosome segregation. It is also tempting to speculate that integrity and maintenance of the chromosomal linearity and bacterial telomeres could require a functional HR pathway. The terminal redundancies, characteristic of the *Streptomyces* chromosome, may result from HR or require HR for its maintenance. However, HR does not seem to be essential, since *recA*-null mutants can be generated in *Streptomyces* (50). Hence, the essentiality of AdnAB would result from the involvement of AdnAB in an unknown crucial cell process.

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REFERENCES

- Michel B, Ehrlich SD, Uzzet M. 1997. DNA double-strand breaks caused by replication arrest. *EMBO J.* 16:430–438. <http://dx.doi.org/10.1093/emboj/16.2.430>.
- Singleton MR, Dillingham MS, Gaudier M, Kowalczykowski SC, Wigley DB. 2004. Crystal structure of RecBCD enzyme reveals a machine for processing DNA breaks. *Nature* 432:187–193. <http://dx.doi.org/10.1038/nature02988>.
- Dillingham MS, Kowalczykowski SC. 2008. RecBCD enzyme and the repair of double-stranded DNA breaks. *Microbiol. Mol. Biol. Rev.* 72:642–671. <http://dx.doi.org/10.1128/MMBR.00020-08>.
- Haijema BJ, Meima R, Kooistra J, Venema G. 1996. Effects of lysine-to-glycine mutations in the ATP-binding consensus sequences in the AddA and AddB subunits on the *Bacillus subtilis* AddAB enzyme activities. *J. Bacteriol.* 178:5130–5137.
- Haijema BJ, Venema G, Kooistra J. 1996. The C terminus of the AddA subunit of the *Bacillus subtilis* ATP-dependent DNase is required for the ATP-dependent exonuclease activity but not for the helicase activity. *J. Bacteriol.* 178:5086–5091.
- Yeles JTP, Dillingham MS. 2007. A dual-nuclease mechanism for DNA break processing by AddAB-type helicase-nucleases. *J. Mol. Biol.* 371:66–78. <http://dx.doi.org/10.1016/j.jmb.2007.05.053>.
- Capaldo-Kimball F, Barbour SD. 1971. Involvement of recombination genes in growth and viability of *Escherichia coli* K-12. *J. Bacteriol.* 106:204–212.
- Horii Z-I, Clark AJ. 1973. Genetic analysis of the RecF pathway to genetic recombination in *Escherichia coli* K12: isolation and characterization of mutants. *J. Mol. Biol.* 80:327–344. [http://dx.doi.org/10.1016/0022-2836\(73\)90176-9](http://dx.doi.org/10.1016/0022-2836(73)90176-9).
- Kushner SR. 1974. *In vivo* studies of temperature-sensitive *recB* and *recC* mutants. *J. Bacteriol.* 120:1213–1218.
- Sanchez H, Kidane D, Castillo Cozar M, Graumann PL, Alonso JC. 2006. Recruitment of *Bacillus subtilis* RecN to DNA double-strand breaks in the absence of DNA end processing. *J. Bacteriol.* 188:353–360. <http://dx.doi.org/10.1128/JB.188.2.353-360.2006>.
- Kooistra J, Haijema BJ, Venema G. 1993. The *Bacillus subtilis* *addAB* genes are fully functional in *Escherichia coli*. *Mol. Microbiol.* 7:915–923. <http://dx.doi.org/10.1111/j.1365-2958.1993.tb01182.x>.
- Cromie GA. 2009. Phylogenetic ubiquity and shuffling of the bacterial RecBCD and AddAB recombination complexes. *J. Bacteriol.* 191:5076–5084. <http://dx.doi.org/10.1128/JB.00254-09>.
- Sinha KM, Unciuleac M-C, Glickman MS, Shuman S. 2009. AdnAB: a new DSB-resecting motor-nuclease from mycobacteria. *Genes Dev.* 23:1423–1437. <http://dx.doi.org/10.1101/gad.1805709>.
- Unciuleac M-C, Shuman S. 2010. Double strand break unwinding and resection by the mycobacterial helicase-nuclease AdnAB in the presence of single strand DNA-binding protein (SSB). *J. Biol. Chem.* 285:34319–34329. <http://dx.doi.org/10.1074/jbc.M110.162925>.
- Dillingham MS, Spies M, Kowalczykowski SC. 2003. RecBCD enzyme is a bipolar DNA helicase. *Nature* 423:893–897. <http://dx.doi.org/10.1038/nature01673>.
- Taylor AF, Smith GR. 2003. RecBCD enzyme is a DNA helicase with fast and slow motors of opposite polarity. *Nature* 423:889–893. <http://dx.doi.org/10.1038/nature01674>.
- Clark AJ, Sandler SJ, Willis DK, Chu CC, Blamar MA, Lovett ST. 1984. Genes of the RecE and RecF pathways of conjugational recombination in *Escherichia coli*. *Cold Spring Harbor Symp. Quant. Biol.* 49:453–462. <http://dx.doi.org/10.1101/SQB.1984.049.01.051>.
- Nakayama H, Nakayama K, Nakayama R, Irino N, Nakayama Y, Hanawalt PC. 1984. Isolation and genetic characterization of a thymineless death-resistant mutant of *Escherichia coli* K12: identification of a new mutation (*recQ1*) that blocks the RecF recombination pathway. *Mol. Gen. Genet.* 195:474–480. <http://dx.doi.org/10.1007/BF00341449>.
- Kolodner R, Fishel RA, Howard M. 1985. Genetic recombination of bacterial plasmid DNA: effect of RecF pathway mutations on plasmid recombination in *Escherichia coli*. *J. Bacteriol.* 163:1060–1066.
- Gupta R, Barkan D, Redelman-Sidi G, Shuman S, Glickman MS. 2011. Mycobacteria exploit three genetically distinct DNA double-strand break repair pathways. *Mol. Microbiol.* 79:316–330. <http://dx.doi.org/10.1111/j.1365-2958.2010.07463.x>.
- Rocha EPC, Cornet E, Michel B. 2005. Comparative and evolutionary analysis of the bacterial homologous recombination systems. *PLoS Genet.* 1:e15. <http://dx.doi.org/10.1371/journal.pgen.0010015>.
- Kirby R. 2011. Chromosome diversity and similarity within the *Actinomycetales*. *FEMS Microbiol. Lett.* 319:1–10. <http://dx.doi.org/10.1111/j.1574-6968.2011.02242.x>.
- Thibessard A, Leblond P. 2014. Subtelomere plasticity in the bacterium *Streptomyces*, p 243–258. In Louis EJ, Becker MM (ed), *Subtelomeres*. Springer, Berlin, Germany.
- Lin YS, Kieser HM, Hopwood DA, Chen CW. 1993. The chromosomal DNA of *Streptomyces lividans* 66 is linear. *Mol. Microbiol.* 10:923–933. <http://dx.doi.org/10.1111/j.1365-2958.1993.tb00964.x>.
- Choulet F, Aigle B, Gallois A, Mangenot S, Gerbaud C, Truong C, Francou F-X, Fourrier C, Guérineau M, Decaris B, Barbe V, Pernodet J-L, Leblond P. 2006. Evolution of the terminal regions of the *Streptomyces* linear chromosome. *Mol. Biol. Evol.* 23:2361–2369. <http://dx.doi.org/10.1093/molbev/msl108>.
- Doroghazi JR, Buckley DH. 2010. Widespread homologous recombination within and between *Streptomyces* species. *ISME J.* 4:1136–1143. <http://dx.doi.org/10.1038/ismej.2010.45>.
- Yu D, Ellis HM, Lee E-C, Jenkins NA, Copeland NG, Court DL. 2000. An efficient recombination system for chromosome engineering in *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* 97:5978–5983. <http://dx.doi.org/10.1073/pnas.100127597>.
- Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA. 2000. *Practical Streptomyces genetics*. John Innes Foundation, Norwich, United Kingdom.
- Nguyen HC, Karray F, Lautru S, Gagnat J, Lebrhi A, Huynh TDH, Pernodet J-L. 2010. Glycosylation steps during spiramycin biosynthesis in *Streptomyces ambofaciens*: involvement of three glycosyltransferases and their interplay with two auxiliary proteins. *Antimicrob. Agents Chemother.* 54:2830–2839. <http://dx.doi.org/10.1128/AAC.01602-09>.
- Karray F, Darbon E, Oestreicher N, Dominguez H, Tuphile K, Gagnat J, Blondelet-Rouault M-H, Gerbaud C, Pernodet J-L. 2007. Organization of the biosynthetic gene cluster for the macrolide antibiotic spiramycin in *Streptomyces ambofaciens*. *Microbiology* 153:4111–4122. <http://dx.doi.org/10.1099/mic.0.2007/009746-0>.
- Fernández-Martínez LT, Del Sol R, Evans MC, Fielding S, Herron PR, Chandra G, Dyson PJ. 2011. A transposon insertion single-gene knockout library and new ordered cosmid library for the model organism *Streptomyces coelicolor* A3(2). *Antonie Van Leeuwenhoek* 99:515–522. <http://dx.doi.org/10.1007/s10482-010-9518-1>.
- Brosius J. 1989. Superpolylinkers in cloning and expression vectors. *DNA* 8:759–777. <http://dx.doi.org/10.1089/dna.1989.8.759>.
- Huang J, Shi J, Molle V, Sohlberg B, Weaver D, Bibb MJ, Karoonuthaisiri N, Lih C-J, Kao CM, Buttner MJ, Cohen SN. 2005. Cross-regulation among disparate antibiotic biosynthetic pathways of *Streptomyces coelicolor*. *Mol. Microbiol.* 58:1276–1287. <http://dx.doi.org/10.1111/j.1365-2958.2005.04879.x>.
- Ivancić-Baće I, Peharec P, Moslavac S, Skrobot N, Salaj-Smic E, Brci-Kosti K. 2003. RecFOR function is required for DNA repair and recombination in a RecA loading-deficient *recB* mutant of *Escherichia coli*. *Genetics* 163:485–494.
- Gorbalenya AE, Koonin EV. 1993. Helicases: amino acid sequence comparisons and structure-function relationships. *Curr. Opin. Struct. Biol.* 3:419–429. [http://dx.doi.org/10.1016/S0959-440X\(05\)80116-2](http://dx.doi.org/10.1016/S0959-440X(05)80116-2).
- Tanner NK, Cordin O, Banroques J, Doere M, Linder P. 2003. The Q motif: a newly identified motif in DEAD box helicases may regulate ATP binding and hydrolysis. *Mol. Cell* 11:127–138.
- Yu M, Souaya J, Julin DA. 1998. Identification of the nuclease active site in the multifunctional RecBCD enzyme by creation of a chimeric enzyme. *J. Mol. Biol.* 283:797–808. <http://dx.doi.org/10.1006/jmbi.1998.2127>.
- Wang J, Chen R, Julin DA. 2000. A single nuclease active site of the *Escherichia coli* RecBCD enzyme catalyzes single-stranded DNA degradation in both directions. *J. Biol. Chem.* 275:507–513. <http://dx.doi.org/10.1074/jbc.275.1.507>.
- Lloyd RG, Evans NP, Buckman C. 1987. Formation of recombinant *lacZ*⁺ DNA in conjugational crosses with a *recB* mutant of *Escherichia coli* K12 depends on *recF*, *recJ*, and *recO*. *Mol. Gen. Genet.* 209:135–141. <http://dx.doi.org/10.1007/BF00329848>.

40. Lovett ST, Clark AJ. 1984. Genetic analysis of the *recJ* gene of *Escherichia coli* K-12. *J. Bacteriol.* 157:190–196.
41. Kickstein E, Harms K, Wackernagel W. 2007. Deletions of *recBCD* or *recD* influence genetic transformation differently and are lethal together with a *recJ* deletion in *Acinetobacter baylyi*. *Microbiology* 153:2259–2270. <http://dx.doi.org/10.1099/mic.0.2007/005256-0>.
42. Dermić D. 2006. Functions of multiple exonucleases are essential for cell viability, DNA repair and homologous recombination in *recD* mutants of *Escherichia coli*. *Genetics* 172:2057–2069.
43. Rand L, Hinds J, Springer B, Sander P, Buxton RS, Davis EO. 2003. The majority of inducible DNA repair genes in *Mycobacterium tuberculosis* are induced independently of RecA. *Mol. Microbiol.* 50:1031–1042. <http://dx.doi.org/10.1046/j.1365-2958.2003.03765.x>.
44. Gamulin V, Cetkovic H, Ahel I. 2004. Identification of a promoter motif regulating the major DNA damage response mechanism of *Mycobacterium tuberculosis*. *FEMS Microbiol. Lett.* 238:57–63. <http://dx.doi.org/10.1111/j.1574-6968.2004.tb09737.x>.
45. Zheng X, Hu G-Q, She Z-S, Zhu H. 2011. Leaderless genes in bacteria: clue to the evolution of translation initiation mechanisms in prokaryotes. *BMC Genomics* 12:361. <http://dx.doi.org/10.1186/1471-2164-12-361>.
46. Martin P, Dary A, Decaris B. 1998. Generation of a genetic polymorphism in clonal populations of the bacterium *Streptomyces ambofaciens*: characterization of different mutator states. *Mutat. Res.* 421:73–82. [http://dx.doi.org/10.1016/S0027-5107\(98\)00156-0](http://dx.doi.org/10.1016/S0027-5107(98)00156-0).
47. Dermić D, Halupecki E, Zahradka D, Petranović M. 2005. RecBCD enzyme overproduction impairs DNA repair and homologous recombination in *Escherichia coli*. *Res. Microbiol.* 156:304–311. <http://dx.doi.org/10.1016/j.resmic.2004.10.005>.
48. Kuzminov A. 1999. Recombinational repair of DNA damage in *Escherichia coli* and bacteriophage λ . *Microbiol. Mol. Biol. Rev.* 63:751–813.
49. Lloyd RG, Buckman C, Benson FE. 1987. Genetic analysis of conjugational recombination in *Escherichia coli* K12 strains deficient in RecBCD enzyme. *J. Gen. Microbiol.* 133:2531–2538.
50. Huang T-W, Chen CW. 2006. A *recA* null mutation may be generated in *Streptomyces coelicolor*. *J. Bacteriol.* 188:6771–6779. <http://dx.doi.org/10.1128/JB.00951-06>.
51. Pinnert-Sindico, S. 1954. Une nouvelle espèce de *Streptomyces* productrice d'antibiotiques: *Streptomyces ambofaciens* n. sp. caractères cultureux. *Ann. Inst. Pasteur* 87:702–707.
52. Hopwood DA. 1999. Forty years of genetics with *Streptomyces*: from *in vivo* through *in vitro* to *in silico*. *Microbiology* 145:2183–2202.
53. Hanahan D. 1983. Studies on transformation of *Escherichia coli* with plasmids. *J. Mol. Biol.* 166:557–580. [http://dx.doi.org/10.1016/S0022-2836\(83\)80284-8](http://dx.doi.org/10.1016/S0022-2836(83)80284-8).
54. MacNeil DJ, Gewain KM, Ruby CL, Dezeny G, Gibbons PH, MacNeil T. 1992. Analysis of *Streptomyces avermitilis* genes required for avermectin biosynthesis utilizing a novel integration vector. *Gene* 111:61–68. [http://dx.doi.org/10.1016/0378-1119\(92\)90603-M](http://dx.doi.org/10.1016/0378-1119(92)90603-M).
55. Paget MSB, Chamberlin L, Atrih A, Foster SJ, Buttner MJ. 1999. Evidence that the extracytoplasmic function sigma factor σ^E is required for normal cell wall structure in *Streptomyces coelicolor* A3(2). *J. Bacteriol.* 181:204–211.
56. Simon R, Priefer U, Pühler A. 1983. A broad host range mobilization system for *in vivo* genetic engineering: transposon mutagenesis in gram negative bacteria. *Nat. Biotechnol.* 1:784–791. <http://dx.doi.org/10.1038/nbt1183-784>.
57. Bierman M, Logan R, O'Brien K, Seno ET, Rao RN, Schonher BE. 1992. Plasmid cloning vectors for the conjugal transfer of DNA from *Escherichia coli* to *Streptomyces* spp. *Gene* 116:43–49. [http://dx.doi.org/10.1016/0378-1119\(92\)90627-2](http://dx.doi.org/10.1016/0378-1119(92)90627-2).