Supplementary Information

β-lactam antibiotics promote bacterial mutagenesis via an RpoS-mediated reduction in replication fidelity

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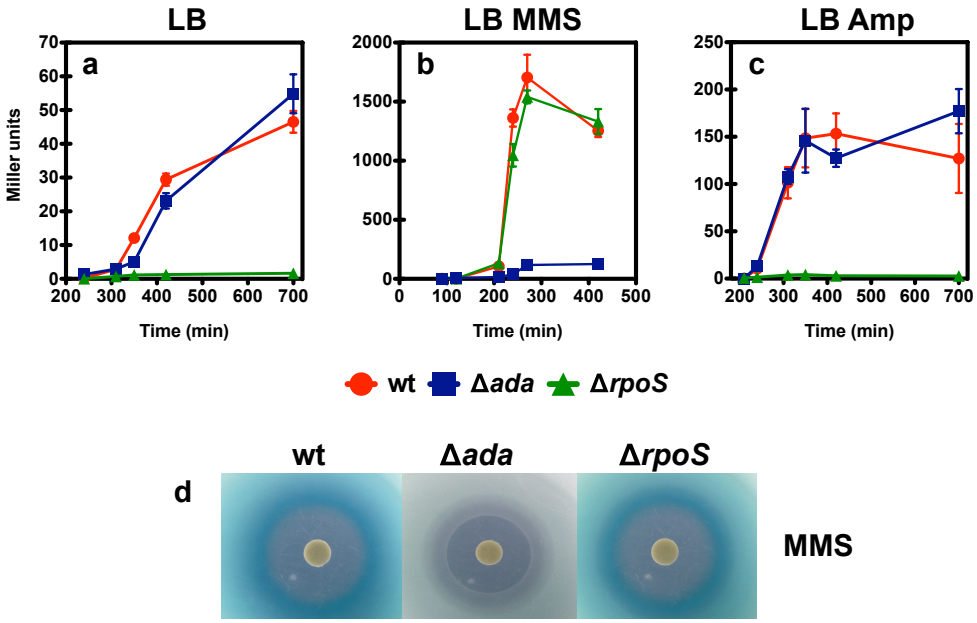
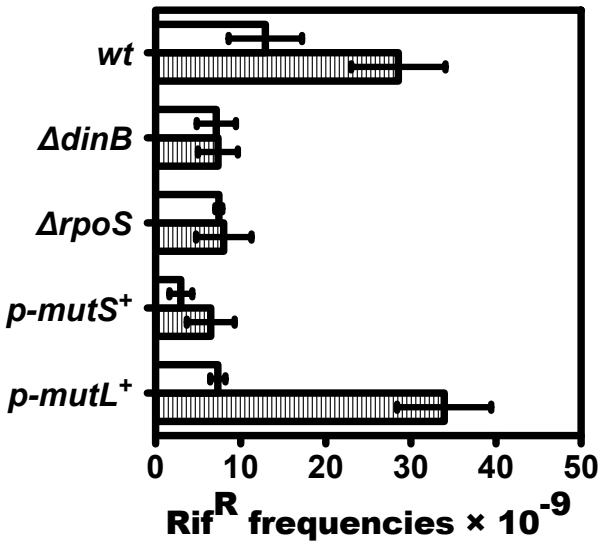
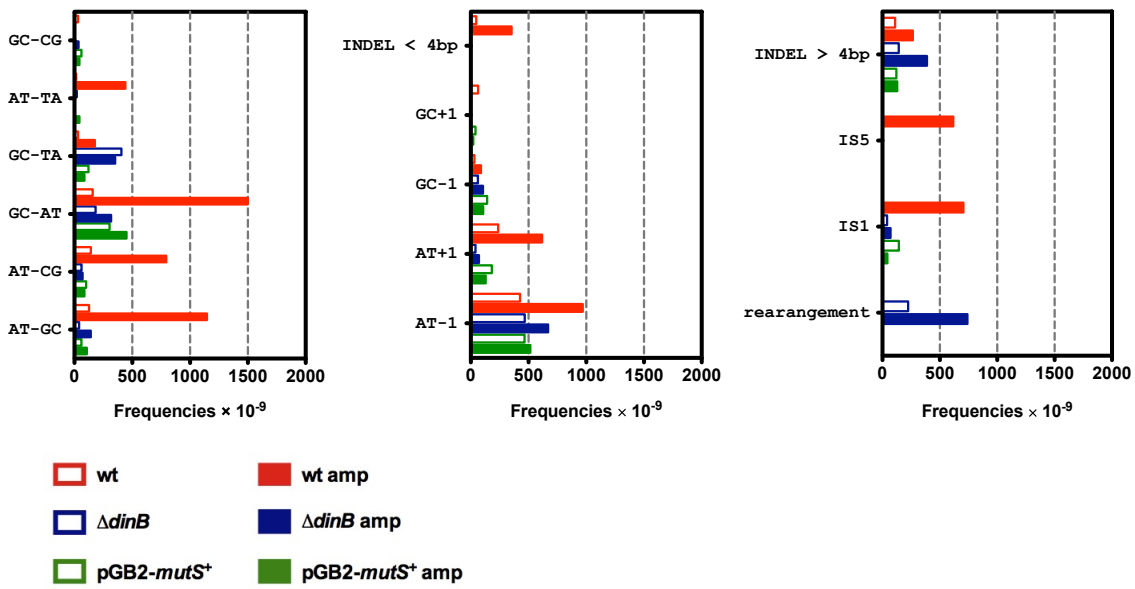
Supplementary Figures and Tables



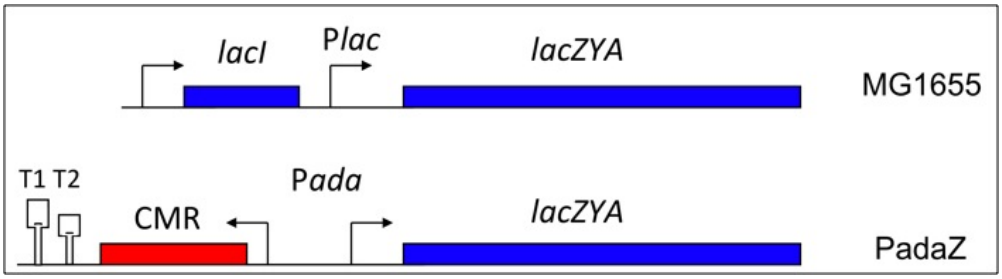
Supplementary Figure S1: Induction of the E. coli p*ada::lacZ* reporter fusion. (a-c) Kinetics of induction of the p*ada::lacZ* reporter fusion in different growth conditions. **(d)** Ada-dependent induction of the p*ada::lacZ* reporter fusion on LB X-Gal MMS plates.



Supplementary Figure S2: β-lactam antibiotic-induced mutagenesis in *E. coli*. Bacteria were grown in LB (open bar) or LB supplemented with 1 μg/ml of ampicillin (filled bar). The frequency of the rifampicin-resistant mutants were measured. Relevant genotypes are indicated. Each result represents the mean ± sem of at least three independent experiments.



Supplementary Figure S3: (a) Spectrum of base substitutions observed in the *cI* (ind$^-$) gene. **(b)** Small INDELS observed in the *cI* (ind$^-$) gene. **(c)** IS mobility and small genomic rearrangements observed in the *cI* (ind$^-$) gene.



Supplementary Figure S4: The *E. coli* p*ada::lacZ* reporter fusion.

Supplementary Table S1: *E. coli* p*ada::lacZ* reporter fusion induction by different antibiotics on LB X-Gal plates.

Antibiotic family	Antibiotics	Relevant genotype			
		wt	Δ*rpoS*	Δ*ada*	p-*clpPclpX*$^{+}$
Aminoglycoside	gentamycin	+	-	+	-
β-lactam	ampicillin	+	-	+	-
	carbenicillin	+	-	+	-
	ceftazidim	+	-	+	-
Quinolone	norfloxacin	+	-	+	-
	levofloxacin	+	-	+	-
	nalidixic acid	+	-	+	-

+ : high induction

- : reduced induction

Supplementary Table S2: Induction of the *E. coli* p*ada::lacZ* reporter fusion in different genetic backgrounds on LB X-Gal plates

Genotype	Spontaneous induction	MMS	Ampicillin
arcA-arcB	+	+	+
barA-uvrY	+	+	+
rcs phosphorelay	+	+	+
hfq	-	+	-
csdA	+	+	+
cspC	+	+	+
cspE	+	+	+
iraP iraM iraD	+	+	+
rssB	++	+	+

+ : high induction

- : reduced induction

Supplementary Table S3: Strains used in this study

Strains	Genotype	Sources
***E coli* MG1655**	*rph-1*	[35]
FF1	Δ*lacI,Z,Y,A*	INSERM lab collection
PadaZ	*pada* :: *lacZ,Y,A* Δ*lacI* :: cm^R	This study
PadaZ *ada*	PadaZ Δ*ada* :: kan^R	[44]
PadaZ *rpoS*	PadaZ Δ*rpoS* :: kan^R	[44]
PadaZ *ada rpoS*	PadaZ Δ*ada* :: FRT *rpoS* :: kan^R	[44]
PadaZ p-clpPX	PadaZ pGB2 p*clpP* :: *clpPclpX* $spec/str^R$	This study
PadaZ *arcA*	PadaZ Δ*arcA*::kan^R	[44]
PadaZ *arcB*	PadaZ Δ*arcB*::kan^R	[44]
PadaZ *barA*	PadaZ Δ*barA*::kan^R	[44]
PadaZ *uvrY*	PadaZ Δ*uvrY*::kan^R	[44]
PadaZ *rcsA*	PadaZ Δ*rcsA*::kan^R	[44]
PadaZ *rcsB*	PadaZ Δ*rcsB*::kan^R	[44]
PadaZ *rcsC*	PadaZ Δ*rcsC*::kan^R	[44]
PadaZ *rcsD*	PadaZ Δ*rcsD*::kan^R	[44]
PadaZ *rcsF*	PadaZ Δ*rcsF*::kan^R	[44]
PadaZ *hfq*	PadaZ Δ*hfq*::kan^R	[44]
PadaZ *csdA*	PadaZ Δ*csdA*::kan^R	[44]
PadaZ *cspC*	PadaZ Δ*cspC*::kan^R	[44]
PadaZ *cspE*	PadaZ Δ*cspE*::kan^R	[44]
PadaZ *iraP*	PadaZ Δ*iraP*::kan^R	[44]
PadaZ iraM	PadaZ Δ*iraM*::kan^R	[44]
PadaZ *iraD*	PadaZ Δ*iraD*::kan^R	[44]
PadaZ *rssB*	PadaZ Δ*rssB*::kan^R	[44]
MGCI	Attλ CI(Ind^-) λpR :: *tetA ara* :: FRT *metRE* :: FRT	[39]
MGCI *sulAgfp*	ΔattB :: sulAp :: *gfp*	[49]
MGCI *dinB*	MGCI Δ*dinB* :: FRT	[39]
MGCI $dinB^{Y79A}$	MGCI *dinB* :: $dinB^{Y79A}$ cm^R	[63]
MGCI $dinB^{F13V}$	MGCI *dinB* :: $dinB^{F13V}$ cm^R	[64]
MGCI *rpoS*	MGCI Δ*rpoS* :: kan^R	INSERM lab collection
MGCI $lexA_3$	MGCI $lexA_3$ *malB* :: Tn*9* Cm^R	INSERM lab collection
MGCI $lexA_3$ *rpoS*	MGCI $lexA_3$ *malB* :: Tn*9* Cm^R Δ*rpoS*::kan^R	[44]
MGCI $lexA_3$ *dinB*	MGCI $lexA_3$ *malB* :: Tn*9* Cm^R Δ*dinB*::kan^R	[44]
MGCI p$mutS^+$	MGCI pACYC184 pmutS :: mutS cm^R	INSERM lab collection
MGCI p$mutL^+$	MGCI pACYC184 pmutL :: mutL cm^R	INSERM lab collection
***P. aeruginosa* PA14**		
PA14 *dinB*	Δ*dinB* :: Gm^R	[64]
PA14 *rpoS*	Δ*rpoS* :: Gm^R	[64]
PA14 p$mutS^+$	pJM6αlac *mutS* Kan^R	[64]
PA14 p$mutL^+$	pJM6αlac *mutL* Kan^R	[64]
V. cholerae		
VC *dinB*	Δ*dinB* :: $Spec^R$	This study
VC *rpoS*	Δ*rpoS* :: $Spec^R$	This study
VC p$mutS^+$	pTOPO *mutS* kan^R	This study
VC p$mutL^+$	pTOPO *mutL* kan^R	This study

Supplementary Table S4: Primers used for qPCR

Gene	Sequence
dinB	5′GGCTGTATCCGGAACTTGAA 5′GGTGGTTTGCTGAAAATCGT
mutS	5′CCGGATGGGTGATTTTTATG 5′AGTTTTCCACCGCATGGTAG
rpoS	5′TATTCGTTTGCCGATTCACA 5′GGCTTATCCAGTTGCTCTGC
ada	5′CGATGATGACGCGACACTAA 5′TGAGGCTGGCGATCACTT
rrsB	5′TGCATCTGATACTGGCAAGC 5′ACCTGAGCGTCAGTCTTCGT

Supplementary Table S5: primer used for *in vitro* transcription

Oligonucleotides	Sequence 5' → 3'	Description
T7-SdrS	**GAAATTAATACGACTCACTATAGGCAAGGCAACTAAGCCTGC**	**Used for SdrS T7 template, Fwd**
3'-SdrS	**AAAAAGAGACCGAACACGATTCC**	**Used for T7 template, Rev**
T7-MutS	**GAAATTAATACGACTCACTATAGGCCCGTATGCGCCACGCTTT**	**Used for T7 template, Fwd**
3'-MutS	**GCATGTAGTTGATGGGTGCCAG**	**Used for T7 template, Rev**

Supplementary Methods

Construction of the *V. cholerae* Δ*dinB* strain

The deletion of the *V. cholerae* gene VC2287 (*dinB*) was achieved using gene replacement by homologous recombination. A linear DNA fragment containing the *aadA1* cassette flanked by two homologous regions upstream and downstream of the VC2287 gene was transformed, and spectinomycin-resistant recombinant mutants were selected on LB agar plates with 100 µg/ml of spectinomycin. The 500-bp regions upstream and downstream of VC2287 were amplified using the following primers: $P2287_{up1}$: ctggtcaactgctcacgatactgacgtacc; $P2287_{up2}$: gcgagcatcgtttgttcgcccagcttctgtatggaacggggccgatgcctctcttaaaacagacatcatggagtgggg; $P2287_{dw1}$: cgtgaaaggcgagatcaccaaggtagtcggcaaataatgtcagcccaccaatactgtatacataaacagtataataataagc and $P2287_{dw2}$: gacaggcttgatggtcatgggcgaagagc. The *aadA1* gene was amplified from the pAM34 plasmid using following primers: $Paada1_1$: tatcaccccactccatgatgtctgttttaagagaggcatcggccccgttc catacagaagctgggcgaacaaacgatgctcgc and

$Paada1_2$: cttattattatactgtttatgtatacagtattggtgggctgacattatttgccgactaccttggtgatctcgcctttcacg. The linear DNA fragment used for the recombination was generated using the tree PCR products described above and the following primers: $P2287_{rec1}$: ctggtcaactgctcacgatactgacgtacc and $P2287_{rec2}$: gacaggcttgatggtcatgggcgaagagc.

Construction of the *V. cholerae* Δ*rpoS* strain

The deletion of the *V. cholerae* gene VC0534 (*rpoS*) was accomplished as described above for the VC2287 gene using the following primers: $P0534_{up1}$: gtcaaaatttgactaaaaaagatccagttaagacgg; $P0534_{up2}$: gcgagcatcgtttgttcgcccagcttctgtatggaacgggagcggcctccccctggcaactttgcgagtcattg cgatttacaacc; $P0534_{dw1}$:cgtgaaaggcgagatcaccaaggtagtcggcaaataatgtctttttccagactcatccaaaactaaggcacccgg and $P0534_{dw2}$: ccgagtggcttgccaaagagattggtgcc for the upstream and downstream regions; $Paada1_3$

taaatcgcaatgactcgcaaagttgccaggggaggccgccccgttccatacagaagctgggcgaacaaacgatgctcgc and Paada1$_4$:gcaccccaccgggtgccttagttttggatgagtctggaaaaagacattatttgccgactaccttggtgatctcgcctttcacg for the *aadA1* gene; and P0534$_{rec1}$ gtcaaaatttgactaaaaaagatccagttaagacgg and P0534$_{rec2}$ ccgagtggcttgccaaagagattggtgcc for the recombining DNA fragment.

Construction of the vector to overexpress the *clpP clpX* genes

The plasmid overexpressing the *clpP clpX* operon was constructed by cloning a PCR fragment (Pclp$_{up}$: gcgaagcttcgtaatttacgcagcataac; Pclp$_{dw}$: gcgaagctttcaattacgatgggtcagaa) containing the 200 nt upstream the *clpP* gene followed by the *clpP clpX* operon into pGB2.

Construction of the vector to overexpress *V. cholerae mutL* and *mutS*

The two genes VC0345 (*mutL*) and VC0535 (*mutS*) were amplified by PCR from the *V. cholerae* N16961 genome using following primers: P0345$_1$: tgtaagtttatacataggcgagtactctgttatggatgacgattcgaatcctacccgcccgtttagc; P0345$_2$: tgagtcatga gtgtaatgctgtaattgaggcgg; P0535$_1$: tgtaagtttatacataggcgagtactctgttatggatgatgaaatcgaacgc ctcaccgagc and P0535$_2$: ctagagcagctttttcaattgatagagc. The resulting products were cloned into the pTOPO vector (Invitrogen) under the control of the *bla* promoter.

Construction of the RpoS-regulon induction reporter strain

The p*ada*::*lacZ* reporter fusion was constructed by allelic exchange of the *cis*-regulatory region of lactose operon with the 200 nucleotides upstream of the *ada* gene start codon (supplementary Fig. S4). The upstream region of the *ada* gene and the *cat* gene from the pKD3 vector[61] were sub-cloned into the vector pBAD24A[62] using following primers: Pada$_1$: gcgctcgaggctaaagaggttgttcgccg; Pada$_2$: gcggctagctacaggcgtttcctgttcca; Pcat$_1$: gcgctgcaggtgtaggctggagctgcttc and Pcat$_2$: gcgctcgagatgggaattagccatggtcc. A linear PCR

fragment containing both sequences was inserted in the *E. coli* MG1655 strain genome using *lacI* and *lacZ* gene sequences ($Pins_1$: attgactctcttccgggcgctatcatgccataccgcgaaaggttttgcgc Gtctcatgagcggatacata and $Pins_2$: cagtcacgacgttgtaaaacgacggccagtgaatccgtaatcatggtcat aatcagctccctggtta) as targets for the homologous recombination[61]. p*ada*::*lacZ* allele was then transferred using P1 transduction in the strain FF1 that is deleted for all lactose operon genes. The resulting strain was designated PadaZ (Supplementary Fig. S4).

Supplementary References

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