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► To cite this version:

Ira Tanneur, Etienne Dervyn, Matthieu Jules, Pierre Nicolas. Mismatch repair in *Bacillus subtilis* compensates for the correction bias of PolC exonuclease. *Society for Molecular Biology and Evolution* (*SMBE*) 2023, Jul 2023, Ferrara, Italy. 2023. hal-04163622

HAL Id: hal-04163622

<https://hal.science/hal-04163622>

Submitted on 17 Jul 2023

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Mismatch repair in *Bacillus subtilis* compensates for the correction bias of PolC exonuclease

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SMBE — Ferrara — July 23 - 27, 2023

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Context and purpose

Mutations are one of the main sources of genetic variation, and a major component of evolutionary adaptation. Most of them occur during DNA replication due to the errors of DNA polymerases. These mutations are counterbalanced by two dedicated repair systems:

- the capacity for proofreading by exonuclease, an enzyme associated with the polymerase;
- the mismatch repair system (MMR) that detects and corrects errors on the nascent strand during and after DNA replication.

⇒ To better understand mutational processes: *B. subtilis* strains impaired for one or both of these systems and showing inducible mutation rates.

Strains

- $\Delta mutS$ and $\Delta mutL$ - expected : **constitutive increase in spontaneous mutation rate**
- integration at an ectopic locus under the control of IPTG of:
 - a known dominant negative *mutL* allele: L^* strain;
 - a *polC* mutant with a deficient exonuclease: C^* strain;
 - a *mutL^*/polC^** operon using the above alleles : LC^* strain.

Fluctuation assays confirmed the increase in spontaneous mutation rate, and its **inductibility** by IPTG up to 100 μM in L^* , C^* and LC^* strains.

Evolution of high mutation rates in MA

Premise of Mutation Accumulation (MA): enforce repeated random bottlenecks on a population to accumulate mutations in a neutral way, before sequencing the genome of the evolved population and compute the mutation rate.

Experimental design

- Strains evolved on LB (+100 μM IPTG when necessary), in 4 independent lines, for 280 - 540 generations;
- random picking of a colony each day;
- populations sequenced at intermediate and final points.

Evolution of mutation rates

- Decrease in the mutation rate of 1 C^* line and the 4 LC^* lines during the experiment.

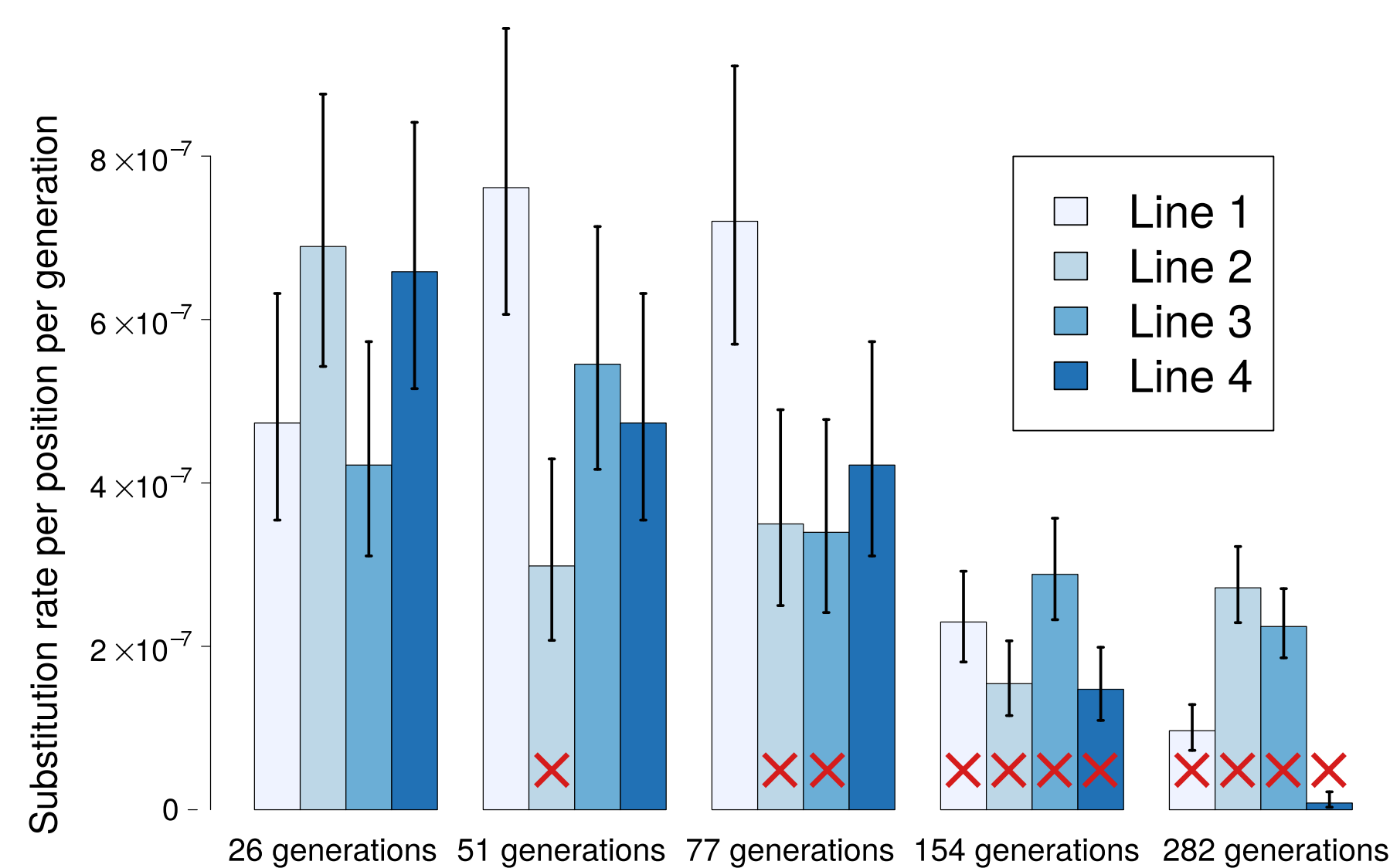


Figure 1: Spontaneous substitution rate per line depending on the number of elapsed generations for the LC^* strain. Sequencing intervals for which the mutations were discarded are indicated with a red cross. 95% CIs are indicated.

⇒ Mutations found after the decrease in mutation rate discarded from the analysis.

⇒ **High mutation rates are deleterious**, and lead to counter-selection despite the experimental design.

⇒ Intermediate sequencings → accurate measurement of mutation rates.

Mutation and reparation biases

Substitution rates and transition / transversion biases

Strain	Subs. rate σ (95% CI)	Tv. proportion (95% CI)
CS	$7.0 (0.18 - 39) \cdot 10^{-11}$	0 (0 - 0.98)
WT 3610 ^[1]	$3.4 (3.0 - 3.8) \cdot 10^{-10}$	0.25 (0.21 - 0.31)
$\Delta mutS$ 3610 ^[1]	$3.4 (3.3 - 3.5) \cdot 10^{-8}$	0.03 (0.02 - 0.03)
$\Delta mutL$	$1.8 (1.5 - 2.1) \cdot 10^{-8}$	0.01 (0.00 - 0.05)
$\Delta mutS$	$1.9 (1.6 - 2.2) \cdot 10^{-8}$	0.01 (0.00 - 0.05)
L^*	$1.4 (1.1 - 1.7) \cdot 10^{-8}$	0.02 (0.00 - 0.06)
C^*	$5.5 (5.0 - 6.1) \cdot 10^{-8}$	0.12 (0.09 - 0.16)
LC^*	$5.5 (5.2 - 6.3) \cdot 10^{-7}$	0.04 (0.02 - 0.06)

⇒ substitution rate of $LC^* \simeq \times 10^3$ higher than WT;

⇒ contrasts in transversions ratios, *e. g.* between C^* and MMR-deficient strains → **MMR has a strong tendency to repair transitions** rather than transversions

Impact of the replication strand and neighbouring nucleotides

Arbitrarily: mutated base considered to be the pyrimidine of the pair.

⇒ Strand orientation : substitutions are favored when **T is templating the leading strand** in all strains, and when **C is templating the lagging strand** in MMR-deficient strains.

⇒ Orientation of **replication, and not transcription**, impacts the local substitution rate, including in head-on genes;

⇒ **Adjacent nucleotides impact the substitution rate**, *e.g.* the presence of a G and/or a C increases the mutation rate.

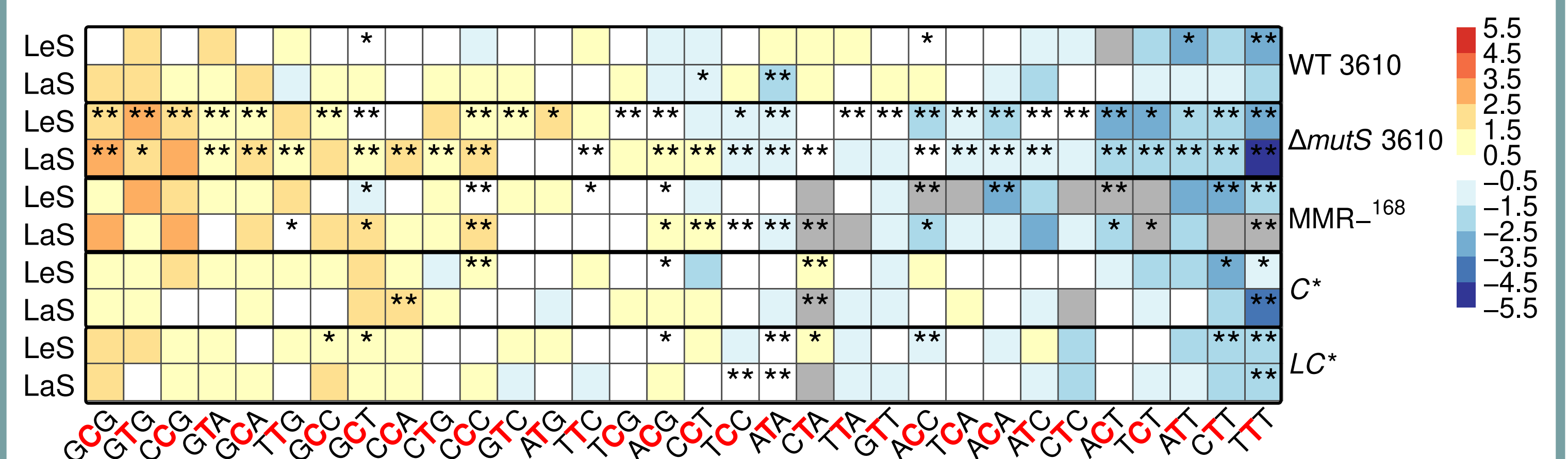


Figure 2: \log_2 of substitution rates by triplet and replication strand, centered by genotype. Triplets are indicated in the 5'→3' orientation with regards to the pyrimidine of the mutated pair (in the middle). LeS and LaS: resp., pyrimidine templating the leading or lagging strand. Test $\mu(\text{triplet, rep.strand}) = \mu(\text{global})$ according to the Poisson distribution: * : $0.001 \leq p\text{-value} < 0.01$; ** : $p\text{-value} < 0.001$.

⇒ Impact of **both triplet and replication strand** have to be taken into account

⇒ **Similarity with multiple organisms** including *Escherichia coli*^[2], *Saccharomyces cerevisiae*^[3] and *Deinococcus radiodurans*^[4] when it comes to the influence of neighbouring nucleotides.

Conclusions and perspectives

✓ A cautionary tale on mutation accumulation experiments with hypermutating strains

✓ A preliminary deciphering of parameters influencing the local mutation rate: **adjacent nucleotides** and **replication strand**;

→ Investigate the contribution of the **essential polymerase DnaE** to mutations and the possible role of the **MMR as a equalizer of mutation spectra**;

→ Characterize the contribution of **MMR saturation** to C^* and WT mutation rates;

→ Use the strains in experimental evolution with a well-suited IPTG concentration (*cf.* fluctuation assays), and the **knowledge of mutational spectra** to determine the appropriate strain.

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