Diversity in mutation rates between *Listeria monocytogenes* isolates exposed to stress

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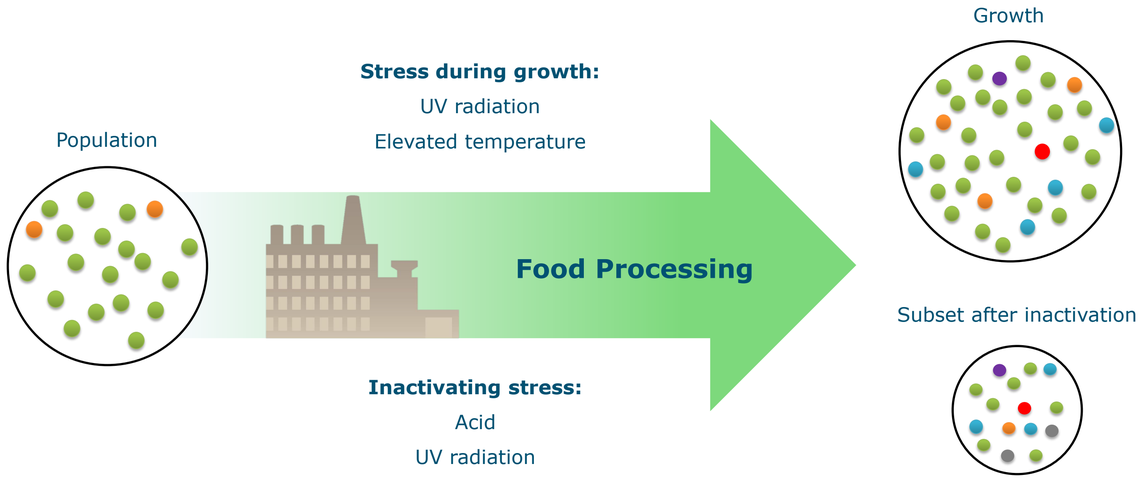
Background

During food processing, *Listeria monocytogenes* is exposed to a range of stressful conditions specifically designed to inactivate cells and limit potential outgrowth in the food product. However, this stress exposure may induce mutations that fuel adaptive evolution towards improved fitness. By selecting for specific mutations in the *rpoB* gene that provide rifampicin resistance, we were able to quantify the mutation rate under stress during growth, and the mutant fraction in the surviving cells after inactivating stress.



Methods

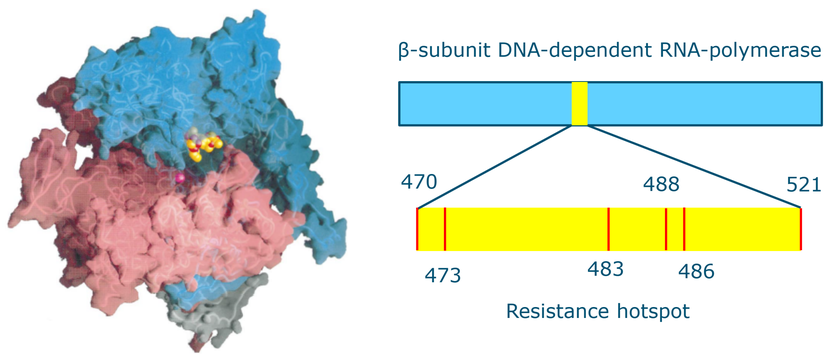


Figure 1. The RpoB protein and its β-subunit. The RpoB protein is shown in complex with rifampicin (Campbell et al. 2001). The β-subunit of *rpoB* is represented by the blue bar, its resistance hotspot by the yellow insert. Numbers represent base positions, starting from the first base of the *rpoB* β-subunit. Indicated mutations in this region provide rifampicin resistance and are a proxy for mutation rate.

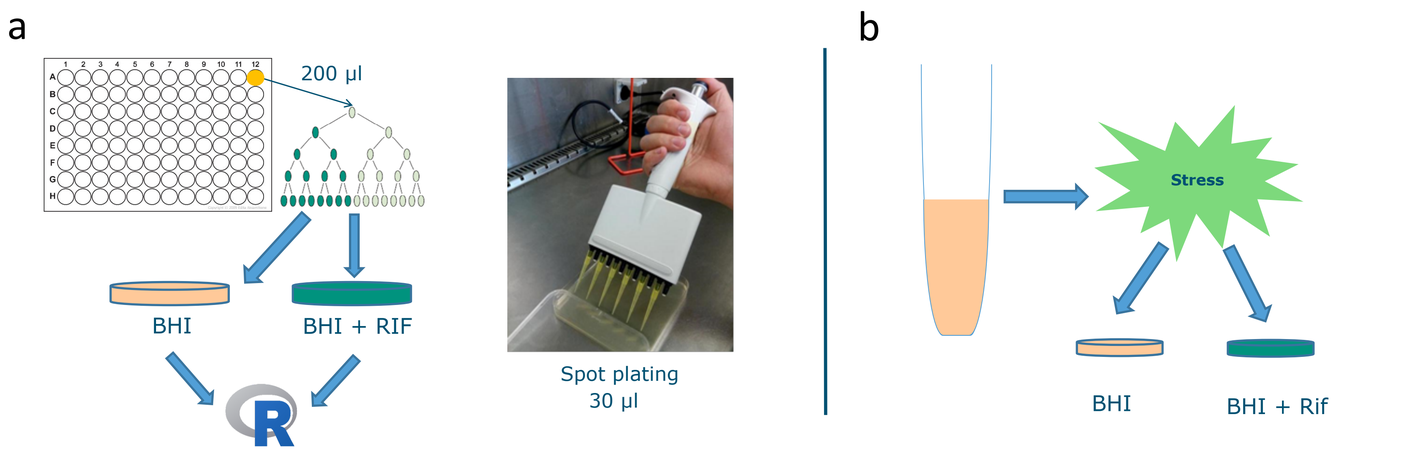


Figure 2. Quantification of both mutation rate during growth, and mutant fraction after inactivating stress. (a) A high throughput adaptation of the Luria-Delbrück protocol was used to quantify the mutation rate of 20 strains of *Listeria monocytogenes* during growth. (b) Stress exposed cells were plated on non-selective (BHI) and selective (BHI + Rif) medium to quantify differences in mutant fraction after inactivating stress treatment.

Results

Mutation rate during growth

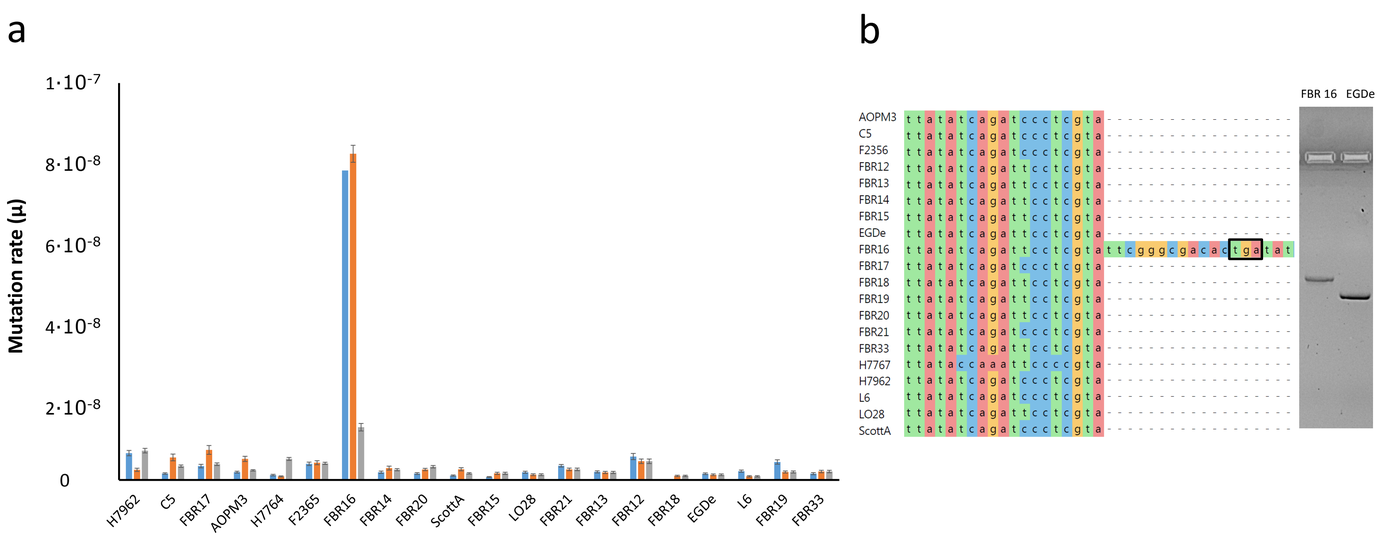


Figure 3. Mutation rate during growth. (a) Mutation rates during growth at 30°C (blue bar), 37°C (orange bar), and after UV exposure (grey bar) in 20 isolates of *Listeria monocytogenes.* Mutation rates varied from $7.6*10^{-10}$ to $6.7*10^{-9}$ mutations per cell per generation, except strain FBR 16, which generated $7.8*10^{-8}$ mutations per cell per generation. (b) Whole genome sequencing revealed an insertion and a premature stopcodon (black box) in the *mutS* DNA repair gene of strain FBR16. PCR and gel electrophoresis confirmed the 178 bp size difference between *mutS* of strain FBR16 and EGDe. Error bars represent 95% confidence intervals.

Mutant fractions after inactivating stress

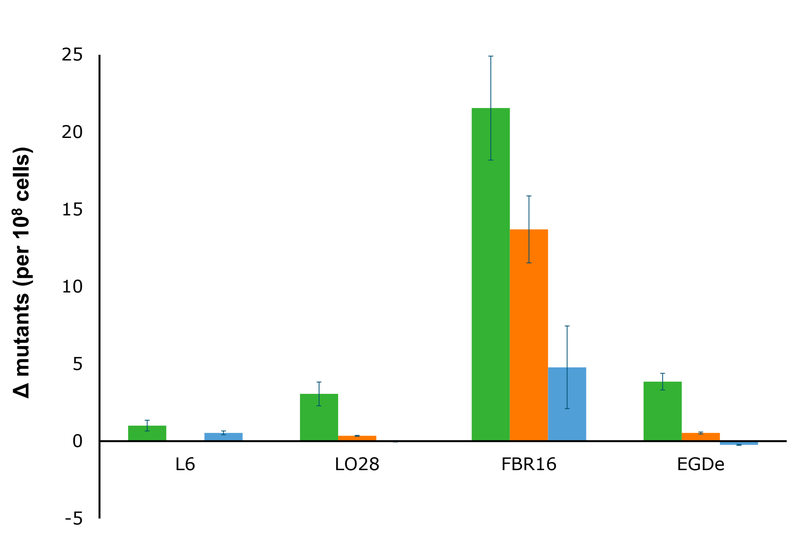


Figure 4. Impact of selected stresses on mutant fractions. The effect of inactivation at pH 2.5 (green bar), pH 3.0 (orange bar) and pH 3.5 (blue bar) on mutant fractions, measured after inactivating 50% of *Listeria monocytogenes* cells. Δ mutants was expressed as the fraction of mutants after stress exposure, compared to the fraction of mutants before stress exposure. Error bars represent standard deviations.

Conclusions

- Mutation rates were rather comparable for 19 of the 20 tested strains of *Listeria monocytogenes*, while strain FBR 16 had a significantly higher mutation rate
- The FBR 16 strain has an insertion in the *mutS* DNA mismatch repair gene
- None of the tested stresses significantly increases mutant fraction under inactivating stress, except for strain FBR 16
- Only strain FBR 16 had a higher fraction of mutants after inactivating stress

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References
Campbell, E. A., N. Korzheva, A. Mustaev, K. Murakami, S. Nair, A. Goldfarb & S. A. Darst (2001) Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. *Cell,* 104**,** 901-912.