Short term experimental evolution reveals the underlying mechanisms of multiple-stress resistance in a foodborne pathogen

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Background

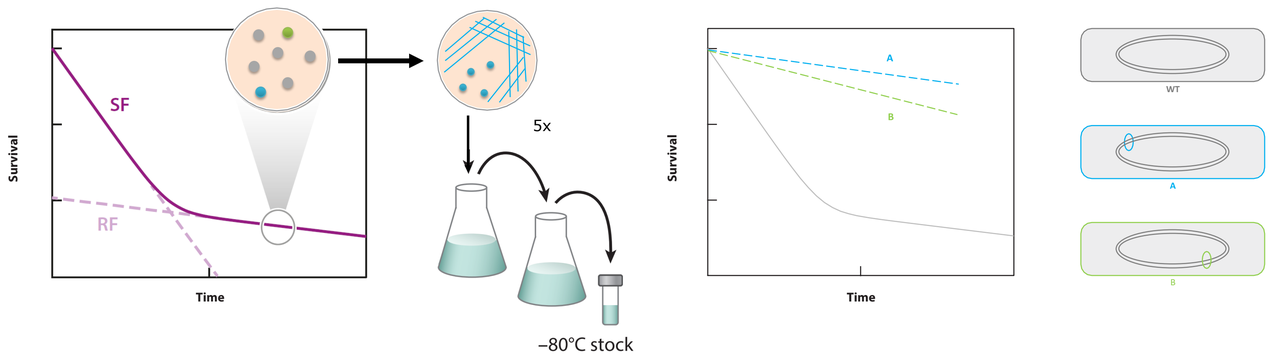

Figure 1. Schematic presentation of the isolation of stress resistant variants. Heterogeneity of *Listeria monocytogenes* populations leads to tailing of the inactivation curve and allows for the selection of stress resistant variants after a single stress exposure.

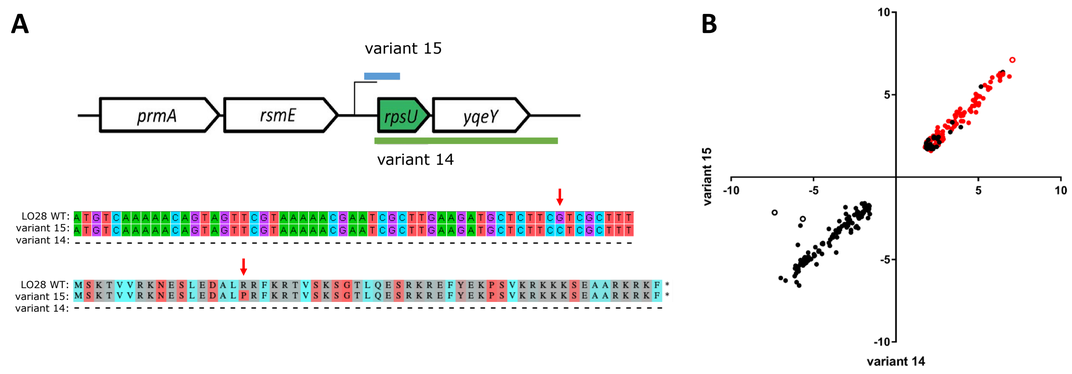

Figure 2. Genotyping of isolated stress resistant variants *L. monocytogenes* variant 14 and 15 share a mutation in ribosomal protein S21 (*rpsU)* (A), leading to increased expression of the Sigma B stress-resistance regulon (shown in red) in variants 14 and 15 compared to WT (B).

Experimental evolution

The stress resistant variants have a lower growth speed than the wild type (Figure 3), therefore we used experimental evolution in an attempt to select for WT-like growth behavior in both variant 14 and 15.

After around 90 and 200 generations, for V15 and V14 respectively, we compared the evolved variants to their respective ancestors by measuring growth speed and acid stress resistance (Figure 4 and 5).

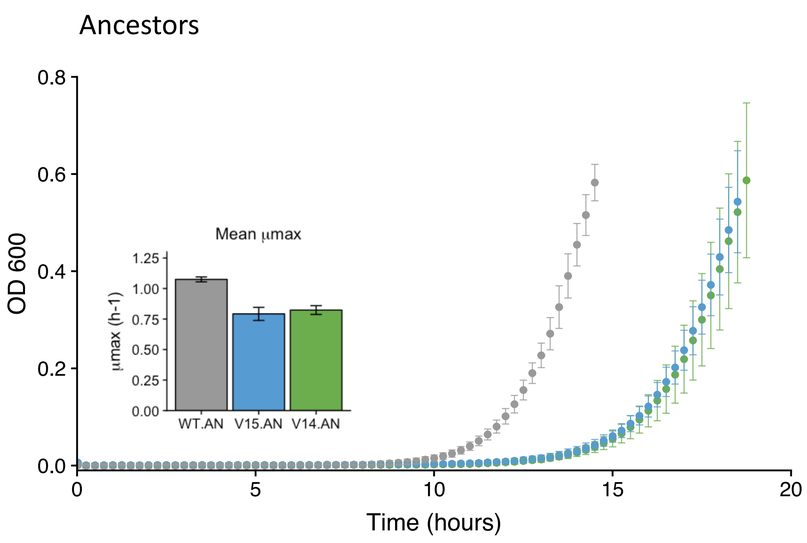

Figure 3. Growth behavior of *L. monocytogenes* variants. WT grows significantly faster than both variants, based on optical density and mean µmax.

Figure 4. Growth behavior of evolved *L. monocytogenes* variants. Variant 15.1 has regained WT growth kinetics, while variant 15.2, 14.1 and 14.2 have growth kinetics intermediate between WT and their respective ancestors.

Characterization of evolved strains

When evolved variant 14 and 15 were tested for acid stress resistance we found the evolved variants, with increased growth speed, to be as stress sensitive as the wild type (Figure 5).

Whole genome sequencing and SNP analysis revealed that in variant 14, carrying the *rpsU* deletion, compensatory mutations occurred in 30S ribosomal protein S2 (RpsB). Variant 14.1 and 14.2 both mutated in the same position: RpsB$^{22Arg\text{-}His}$ and RpsB$^{22Arg\text{-}Ser}$

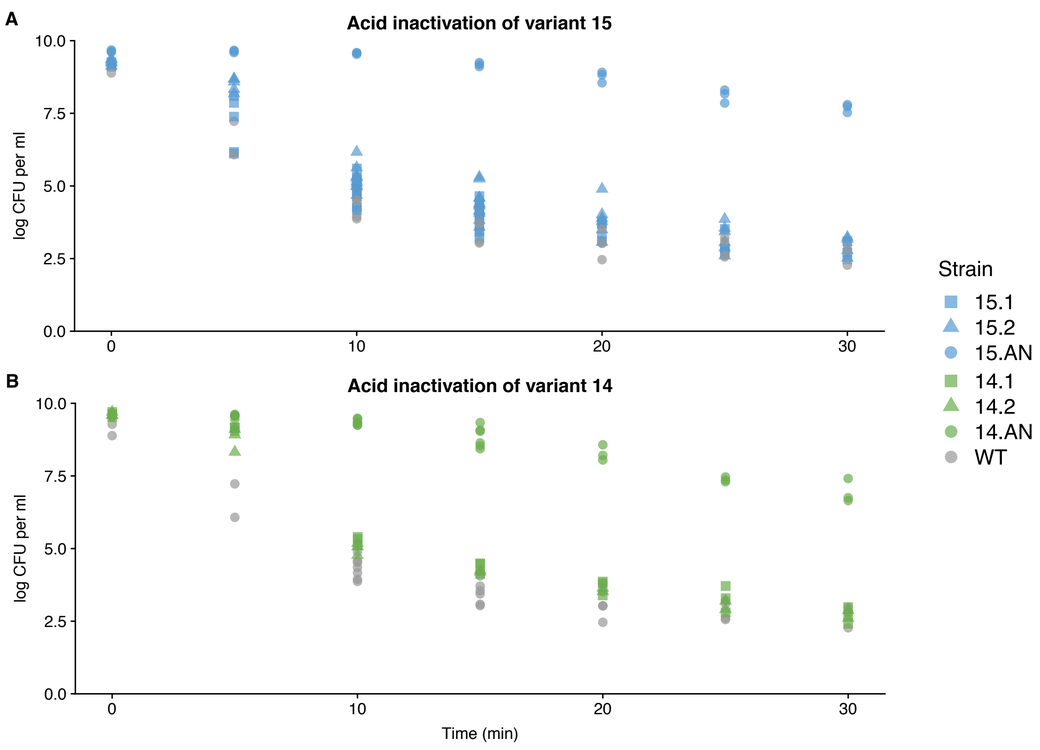

Figure 5. Acid stress resistance of *L. monocytogenes* variant 14 and 15. Tested at pH 3.0 and 37°C.

Variant 15, carrying the SNP in *rpsU* (RpsU^{17Arg} to RpsU^{17Pro}) mutated from RpsU^{17Pro} to RpsU^{17His} and RpsU^{17Thr} for variant 15.1 and 15.2, respectively (Figure 6 B).

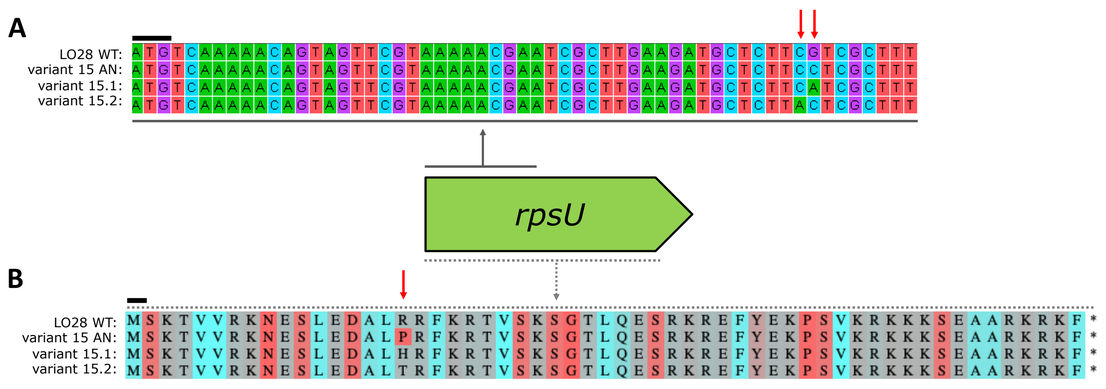

Figure 6. Mutations in *rpsU* of *L. monocytogenes* variant 15. Partial DNA sequence of variant 15 *rpsU* (A) and full RpsU protein sequence (B). The arrows indicate mutated codons/amino acids. The red color in the amino acid sequence indicates a predicted bend in the protein.

Conclusions

- Short term evolution experiments with variants 14 and 15 selecting for enhanced fitness enabled isolation of evolved strains showing WT-like fitness and loss of multiple stress resistance.
- RpsU^{17Thr} and RpsU^{17His} substitutions in variant 15 RpsU^{17Pro} are linked to restoration of WT (RpsU^{17Arg}) behavior.
- Mutations in ribosomal protein S2 (RpsB) are linked to WT-like growth behavior and loss of stress resistance in evolved variant 14 carrying the *rpsU* deletion.

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