Variability in lag duration of *Listeria monocytogenes* strains in half Fraser enrichment broth after stress affects the detection efficacy using the ISO 11290 method

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Background & objective

Testing food products for the presence of *Listeria monocytogenes* is crucial for controlling this important food pathogen. While advances in rapid techniques have tried to shorten the lengthy detection procedure, an enrichment step is still necessary to recover damaged cells and reach detectable levels.

However, recovery kinetics during enrichment are not well understood and insight in the recovery of *L. monocytogenes* is important to reduce the time-consuming enrichment step without forfeiting reliable detection. Therefore we wanted to quantify the growth kinetics of stressed *L. monocytogenes* cells during enrichment in half Fraser broth (HFB) according to ISO 11290-1:2017 to model the effect of strain diversity on recovery.

Methods

23 *L. monocytogenes* strains were enriched according to the first step of the ISO 11290-1:2017 protocol in HFB. Growth was followed over time for reference (without additional stress) and 60°C heat stressed cells after one log reduction.

Lag duration for each reproduction was estimated by the 3-phase model.

Results

Strains of *L. monocytogenes* differ significantly in their ability to recover in HFB after 60°C heat treatment (*figure 1*). The lag of reference cells ranges between 1.4 to 2.7 h, while after 60°C heat stress the lag ranges between 4.7 to 15.8 h.

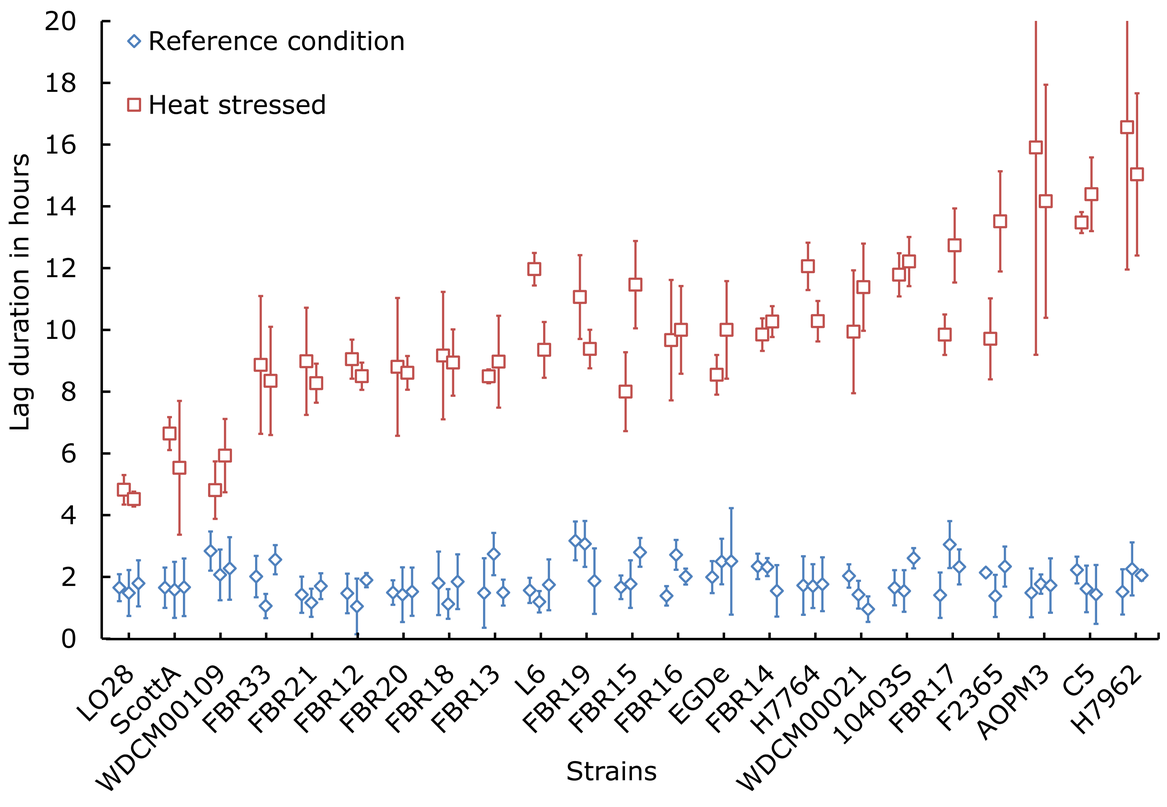

Figure 1. Lag duration in HFB in reference condition (with no additional stress) and after 60°C heat stress (one *D*-value reduction). The growth curve was fitted, and the lag duration was estimated for each biological reproduction. The 95% confidence interval was determined for each fitting and displayed as error bars**.**

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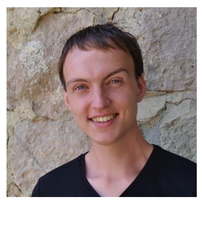

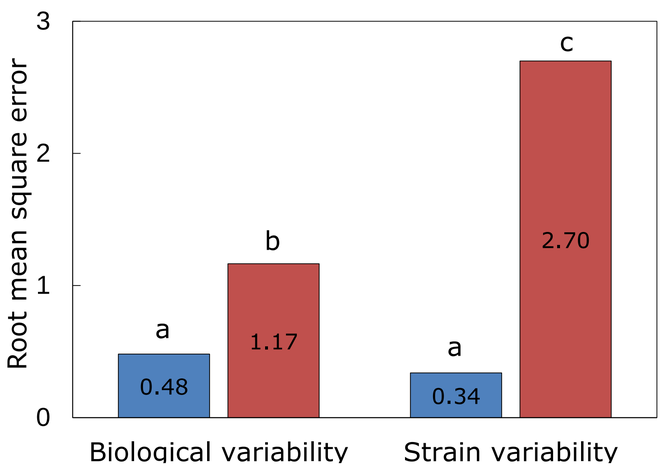

Although there is an increase in biological variability after 60°C heat stress compared to reference cells, the variability between strains increases significantly ($p<0.01$) (*figure 2*).

Strain diversity is thus an important factor in heat stress recovery.

Figure 2. The biological- and strain variability calculated as the root mean square error for both reference and 60°C heat stressed cells. Different letters indicate significant differences calculated by *F*-test with a *p*-value lower than 0.01.

The enrichment data was used for a scenario analysis of the primary enrichment step.

In *figure 3* the lag in HFB is shown for the fastest-, average- and slowest recovering strains starting with 1 cell in 250 ml enrichment broth.

Even strains with an average lag duration barely reach the 2 log CFU/ml detection limit within 24 hours that is necessary for efficient transfer to the secondary enrichment step.

In certain ready-to-eat food products there is the strict requirement of absence of *L. monocytogenes*. Variation in the ability of strains to recover from stress in HFB can mean that these cells are not detected in the 24 hour primary enrichment step and can thus cause a false-negative result.

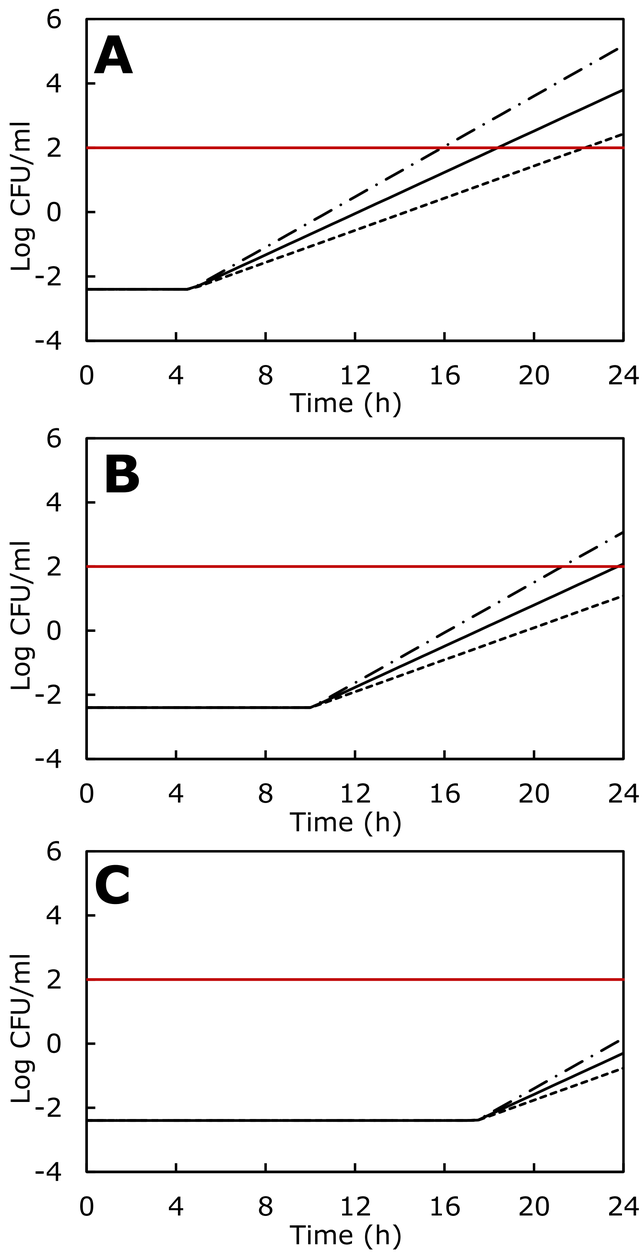

Figure 3. Scenario analysis of the primary enrichment in HFB starting with 1 cell in 250 mL broth with (**A**) the minimum recovery time with average growth rate ± 2 standard deviations (**B**) average recovery time with average growth rate ± 2 standard deviations (**C**) the maximum recovery time with average growth rate ± 2 standard deviations. In red the minimum threshold to transfer at least one cell to the secondary enrichment step.

Conclusions

- There is significant strain variation in recovery from 60°C heat stress in half Fraser enrichment broth.
- Strains with a long lag duration can fail to reach the detection threshold during the ISO 11290-1 primary enrichment and cause false-negatives.
- Strain variability is an important factor in the recovery and detection of stressed *L. monocytogenes* cells from food products.