Gene profiling-based phenotyping for identification of cellular parameters that contribute to fitness, robustness and virulence of acid resistant *Listeria monocytogenes* variants

Jeroen Koomen, Karin I. Metselaar, Marcel H. Tempelaars, Luc M. Wijnands, Marcel H. Zwietering, Heidy M.W. den Besten, and Tjakko Abee

WAGENINGEN
UNIVERSITY & RESEARCH

Rijksinstituut voor Volksgezondheid en Milieu
Ministerie van Volksgezondheid, Welzijn en Sport

Background

When bacterial populations are subjected to food relevant lethal stresses, such as heat and low pH, a small fraction of the population may survive. Previous studies reported isolation of multiple stress resistant *Listeria monocytogenes* variants after a single exposure to acid stress. Phenotypic clustering and subsequent whole genome sequencing of the multiple stress resistant variants revealed various mutations in *rpsU*, encoding ribosomal protein S21, in the largest phenotypic cluster[1].

Objective

To elucidate features that can contribute to fitness, robustness and host interaction of stress resistant *L. monocytogenes* LO28 *rpsU* variants via a comparative gene expression profiling and phenotyping approach.

Results

General transcriptome response of variants 14 and 15

In total, 169 and 150 genes were significantly upregulated (Fig. 1a) and downregulated (Fig. 1b) in the variants compared to the wild type, overall showing highly similar expression profiles.

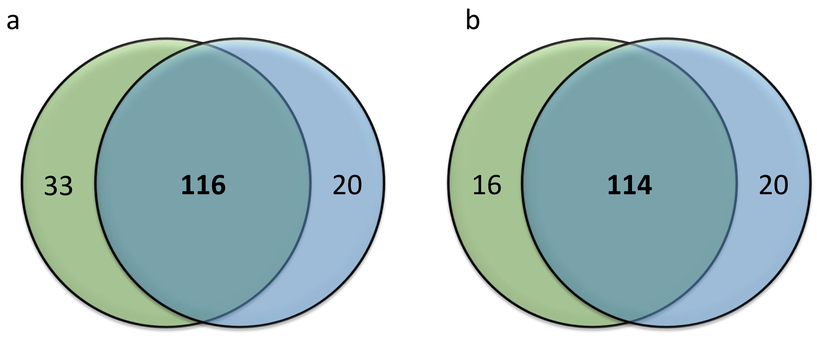

Figure 1. Differentially expressed genes in *L. monocytogenes* LO28 wild type and variants. The green circle represents variant 14, the blue circle represents variant 15. Panels represent genes that are upregulated (a) or downregulated (b) in the variants, showing an overlap of 116 and 114 genes respectively.

General stress response

Circa 70% of the genes of the general stress sigma factor SigB were upregulated (see Fig. 2). Pointing to an important role of the SigB-activated stress response in the multiple stress resistant phenotype of the variants. Among the upregulated genes are those involved in glycerol metabolism (*glpK*$_{1,}$ Fig. *3*), compatible solute uptake (*opuC,* Fig. 4) and attachment to human cells (*inlA/B* Fig. 5), while genes involved in motility (e.g. *flaA*) were downregulated (data not shown).

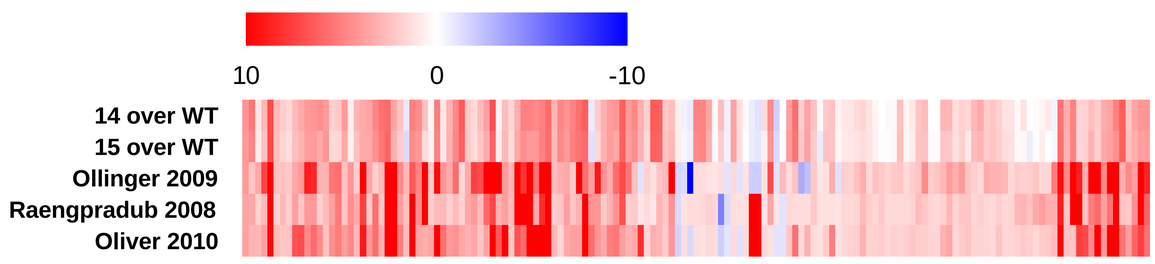

Figure 2. Heatmap of expression of SigB regulated genes in *L. monocytogenes* LO 28 variants 14 and 15, based on indicated references given in [2]. The colour scale indicates $\log_2$ expression in variants over wild type.

Glycerol consumption

Transcriptome analysis indicated higher expression of SigB-regulated putative glycerol uptake facilitator protein GlpF1 (lmo1539), along with other genes involved in glycerol catabolism (see fig. 3a). Comparative HPLC analysis revealed higher glycerol consumption by *L. monocytogenes* LO28 variants 14 and 15 than WT (Fig. 3b)

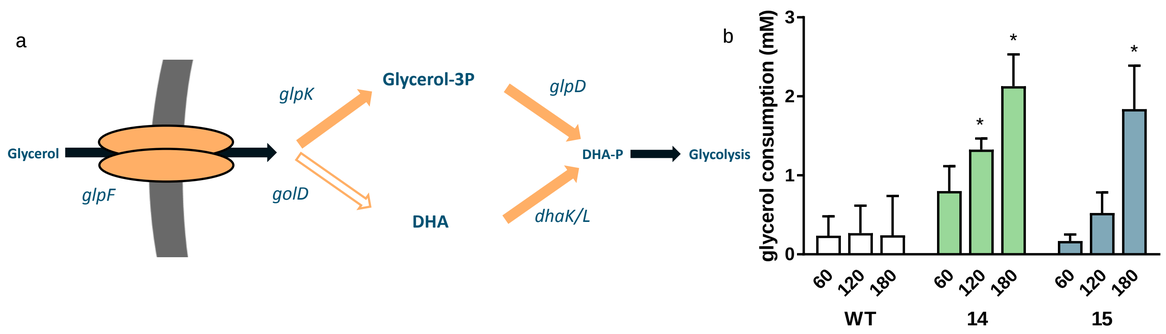

Figure 3: Glycerol consumption in *L. monocytogenes* LO28 wild type and variants. (a) Genes involved in glycerol uptake and catabolism. Solid arrows show upregulated genes in variants 14 and 15, open arrows indicate no significant change. (b) Glycerol consumption of late exponential cells incubated in nutrient broth with 25 mM glycerol for 60, 120 and 180 minutes in the presence of protein-synthesis inhibitor chloramphenicol. Error bars indicate standard errors. * Indicates significant difference over the same time point in the WT.

Freeze-thaw resistance

Upregulation of the *opuC* operon in variants 14 and 15 encoding the OpuC transporter could result in higher intracellular concentrations of compatible solutes and improved freezing-thawing resistance. Exposure of *L. monocytogenes* LO28 WT and variants 14 and 15 to consecutive cycles of freezing and thawing indeed revealed increased survival of the variants, signifying enhanced robustness (Fig. 4).

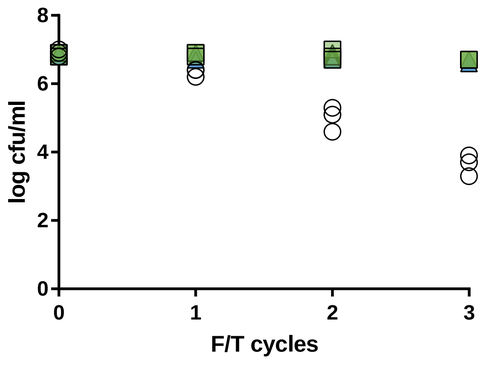

Figure 4: Survival of *L. monocytogenes* LO28 WT and variants 14 (green) and 15 (blue) after cycles of freezing and thawing.

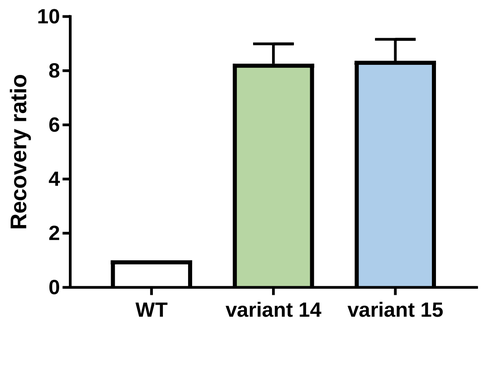

Figure 5: Recovery ratio of *L. monocytogenes* LO28 WT and variants after incubation with Caco-2 cells. Error bars denote standard errors.

Caco-2 cells attachment and invasion

In variants 14 and 15, expression of *inlA* and *inlB* was upregulated, consequently we tested attachment and invasion of Caco-2 cells by *L. monocytogenes* LO28 WT and variant 14 and 15. The recovery ratio of variants 14 and 15 was eight-fold higher in comparison to the WT (Fig. 5).

Conclusions

This study shows that the multiple-stress resistant phenotype of *L. monocytogenes rpsU* variants is caused by the massive upregulation of SigB regulon members. Future work aims to elucidate mechanisms underlying the activation of the SigB regulon in *rpsU* variants.

Acknowledgements

The authors would like to thank Jos Boekhorst for assistance in normalization of the microarray data, Natalia Crespo for figure 3a, and Maren Lanzl for assistance in Caco-2 experiments.

Wageningen University & Research
P.O. Box 17, 6700 AA Wageningen, The Netherlands
Contact: jeroen.koomen@wur.nl
T + 31 (0)317 485358, M +31 (0)641774260

References:

Metselaar KI, den Besten HMW, Boekhorst J, van Hijum SAFT, Zwietering MH and Abee T (2015) Diversity of acid stress resistant variants of *Listeria monocytogenes* and the potential role of ribosomal protein S21 encoded by *rpsU*. Front. Microbiol. 6:422.

Mujahid S, Orsi RH, Vangay P, Boor KJ, and Wiedmann M (2013). Refinement of the *Listeria monocytogenes* SigB regulon through quantitative proteomic analysis. Microbiology, 159:1109-1119.