A point mutation in ribosomal gene rpsU enables SigB activation independently of stressosome and SigB regulator RsbV in Listeria monocytogenes

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# Abstract

Microbial population heterogeneity leads to different stress responses and growth behaviour of individual cells in a population. Previously, a point mutation in the *rpsU* gene (*rpsU*G50C) encoding ribosomal protein S21 was identified in *Listeria monocytogenes* LO28 variant 15, which leads to increased multi-stress resistance and a reduced maximum specific growth rate. However, mechanisms underlying the phenotypic changes in the *rpsU*G50C variant are still unknown. In *L. monocytogenes*, the general stress response is regulated by the alternative sigma factor SigB, and the activation of SigB is controlled by a series of Rsb proteins, including the stressosome protein RsbR, and the anti-anti-sigma factor RsbV. To confirm the SigB-mediated robustness increase, the *rpsU*G50C mutation was introduced to *L. monocytogenes* EGDe wild type the mutant stains ∆*sigB*, ∆*rsbV* and ∆*rsbR*. We combined a phenotype and proteomics approach to investigate the acid and heat stress resistance as well as the maximum specific growth rate of these mutants. As expected, the introduction of the *rpsU*G50C in the ∆*sigB* mutant did not trigger a SigB-mediated robustness increase. However, the introduction of *rpsU*G50C in the ∆*rsbV* and ∆*rsbR* mutants resulted in the activation of SigB and its regulon members and concomitant increased robustness, indicating that SigB is activated through another signaling pathway. Comparative analysis of maximum specific growth rates indicated that all *rpsU*G50C mutants had reduced maximum specific growth rates even with the deletion of *sigB*, *rsbV* or *rsbR*. In conclusion, the increased stress resistance of the *rpsU*G50C mutants is due to the activation of SigB by an unknown mechanism, which is different from the classical stressosome and RsbV/RsbW partner switching SigB activation model, and the reduced maximum specific growth rate of *rpsU*G50C mutants is independent of the SigB activation and conceivably due to reduced ribosomal functioning.

Keywords: *L. monocytogenes*, SigB, *rpsU*, heterogeneity

# 1. Introduction

*Listeria monocytogenes* is a ubiquitous foodborne pathogen, which can cause the disease listeriosis typically caused by ingestion of contaminated food (Radoshevich and Cossart 2018). In order to adapt and survive harsh environmental conditions during the transmission from the soil to the human gastrointestinal tract, *L. monocytogenes* has applied many protective strategies including population heterogeneity (Abee et al. 2016). Population heterogeneity includes genetic and non-genetic population variability, and both can generate phenotypic variation in a population and contribute to the overall fitness, adaptation and survival capacity of the population (Davidson and Surette 2008; Ryall, Eydallin, and Ferenci 2012; Smits, Kuipers, and Veening 2006 ). Pathogens may be inactivated during food processing, and differences in stress resistance between individual cells can result in a higher than expected number of surviving cells and selection of stress-resistant variants (K. I. Metselaar 2016).

Previously, 23 stable stress resistance *L. monocytogenes* variants have been isolated upon acid treatment of *L. monocytogenes* strain LO28 (Karin I. Metselaar et al. 2013). These variants showed a trade-off between reduced maximum specific growth rate and increased resistance against acid, heat, high hydrostatic pressure and benzalkonium chloride (Karin I. Metselaar et al. 2013, 2015). Whole genome sequencing analysis showed that 11 of the 23 variants had mutations in the *rpsU* gene locus, which codes the ribosome 30S small sub-unit protein S21 (RpsU) (Karin I. Metselaar et al. 2015). Two variants have been selected for further research, namely, variant V14 and variant V15 (Koomen et al. 2018). Variant V14 has a deletion of the whole *rpsU* and *yqeY* genes and half of *phoH* gene, while variant V15 has a nucleotide substitution from G to C in *rpsU* at position 50 (NC\_003210.1:g.1501930G*>*C p.(Arg17Pro), marked as *rpsU*G50C in this study), which may lead to an amino acid substitution from arginine to proline in the RpsU protein (marked as RpsU17Arg-Pro in this study) (Karin I. Metselaar et al. 2015). Comparative transcriptomic and phenotypic studies showed that variants V14 and V15 have a large overlap in the gene expression profiles and similar phenotypic results including increased stress resistance, higher glycerol utilization rates, flagella absence and higher Caco-2 cells attachment and invasion levels compared to the wild type (Koomen et al. 2018). These results suggest that the deletion of the whole *rpsU* and point mutation *rpsU*G50C may affect the phenotype by the same mechanism (Koomen et al. 2018). Additional studies following introduction of the *rpsU*G50C mutation into *L. monocytogenes* LO28 and EGDe wild type strains, confirmed that this mutation results in multiple stress resistance and reduced maximum specific growth rate in both mutant strains (Koomen et al. 2021).

SigB is considered as the regulator of general stress response and controls the transcription of approximately 300 genes that contribute to the stress response and virulence of *L. monocytogenes* (Liu et al. 2019; O’Byrne and Karatzas 2008; Toledo-Arana et al. 2009; Guerreiro, Arcari, and O’Byrne 2020). Indeed, previous transcriptomic and proteomic analyses showed that many SigB regulon genes and proteins coded by SigB regulon genes were strongly up-regulated in the *rpsU* variants, which suggests that the activation of SigB-mediated stress may explain the multiple stress resistance phenotype of *rpsU* variants (Koomen et al. 2018; Koomen et al. 2021). Generally, the activation of SigB is controlled at the post-translation level through the stressosome and a series of other Rsb proteins ([Figure 1](#fig-sigb-act)) (Becker et al. 1998; Guerreiro, Arcari, and O’Byrne 2020 ). Briefly, RsbT is captured by the stressosome which is composed of RsbS and RsbR in unstressed cells. Upon environmental stress, RsbR and RsbS are phosphorylated, and RsbT is released from the stressosome. The free RsbT can bind to RsbU and stimulate its phosphatase function. Then RsbV is dephosphorylated by RsbU and binds to RsbW, which releases the previously bound SigB. Thereby, the SigB is free to bind to RNA polymerase and initiate the transcription of SigB regulon genes. Once stress is removed, RsbX, which is co-expressed with SigB, can dephosphorylate RsbR and RsbS, and RsbT binds back to the stressosome and inactivates the signal transduction (Guerreiro, Arcari, and O’Byrne 2020). To date, it is unknown whether this stressosome-mediated signaling pathway is involved in SigB activation in the *L. monocytogenes* *rpsU*G50C mutant, and whether SigB activation leads to reduced fitness of this mutant.

Therefore, in the current study we aim to answer four questions ([Figure 1](#fig-sigb-act)): Q1, is the stressosome involved in activation of SigB in the *rpsU*G50C mutant? Or, Q2, is anti-sigma factor RsbV involved? Q3, do other factors contribute to (indirect) activation of SigB in the *rpsU*G50C mutant independently from RsbV? And, Q4, does activation of SigB and its regulon lead to reduced fitness of the *rpsU*G50C mutant? To address these questions, the *rpsU*G50C mutation was introduced in *L. monocytogenes* EGDe wild type (WT), and in the previously constructed RsbR, RsbV and SigB deletion mutants ([Figure 1](#fig-sigb-act)), which were used to study stressosome structure and functionality (Guerreiro et al. 2020; Utratna et al. 2012; Dessaux et al. 2020). Comparative phenotypic and proteomic study of *L. monocytogenes* EGDe WT, *rpsU*G50C single mutant, and ∆*rsbR*-*rpsU*G50C, ∆*rsbV*-*rpsU*G50Cand ∆*sigB*-*rpsU*G50C double mutants will shed light on the interaction between the ribosome and stressosome-dependent SigB activation and the fitness effect in cells with and without functional RpsU, and whether additional factors are involved.

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| Figure 1: Scheme to asses impact of *rpsU*G50C mutation on stressosome-dependent SigB activation in *L. monocytogenes* EGDe wild type and EGDe *rpsU*G50C mutants. Following perception of a stress signal, RsbT dissociates from the stressosome, which is formed by RsbR and RsbT. Free RsbT binds to RsbU. The phosphatase activity of RsbU is activated and removes a phosphate (P) group from RsbV. The anti-sigma factor RsbW has a higher affinity for the now dephosphorylated RsbV than for SigB, resulting in release of SigB allowing it to bind to RNA polymerase and initiate transcription of SigB regulon members. RpsU function is disrupted by a point mutation resulting in non-functional RpsU17Arg-Pro, relaying a signal to either the stressosome (Q1), directly to RsbV (Q2) or indirectly to RsbW (Q3). In addition, whether SigB-dependent stress defense activation underlies the fitness trade off marked by Q4. Δ1, Δ2 and Δ3 respectively indicate the positions of RsbR, RsbV and SigB in the signaling cascade, which are absent in the respective single and double mutants. See text for details. |

# 2. Materials and Methods

## 2.1 Bacterial strains, plasmids and mutant construction

Table 1: The plasmids and strains used in this study

| Plasmid or Strain | Description | Source or reference |  |
| --- | --- | --- | --- |
| Plasmids |  |  |  |
| pAULA-*rpsU*G50C |  |  |  |
|  |  |  |  |

# 3. Results

# 4. Discussion

# 5. Conclusions

Proteomic data of EGDe-rpsUG50C, ∆sigB-rpsUG50C and ∆rsbV-rpsUG50C compared to EGDe WT, ∆sigB and ∆rsbV, respectively. The p-value and log2 ratio in bold are considered significant. The gene locus, gene name and protein name in bold and underline are for proteins differentially expressed in all three of EGDe-rpsUG50C, ∆sigB-rpsUG50C and

∆rsbV-rpsUG50C.

# 6. Declaration of Competing Interest

# 7. Acknowledgments

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# 8. Supplementary Material