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Brief Report

The growth-survival trade-off is hard-wired in the *Lactococcus lactis* gene regulation network

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Summary

Most microbes reside in oligotrophic environments for extended periods of time, requiring survival strategies that maintain proliferative capacity. We demonstrate that the non-spore-forming *Lactococcus lactis* KF147 progressively activates the expression of stress-associated functions in response to the declining growth rate elicited by prolonged retentostat cultivation, which coincides with up to 10^4-fold increased stress tolerance. Our findings provide a quantified view of the transcription and stress-tolerance adaptations underlying the growth-survival trade-off in *L. lactis*, and exemplify the hard-wiring of this trade-off in the lactococcal gene regulation network.

Most aquatic and terrestrial ecosystems on earth are oligotrophic. Resident microbes have evolved a variety of strategies to maintain proliferative capacity under nutrient-poor conditions for long periods (Egli, 2010; Hoehler and Jorgensen, 2013; Hallsworth, 2021). They adapt to such conditions by minimizing their energy expenditure and metabolic activity, ultimately resulting in a slow- or non-growing state (Lennon and Jones, 2011; Boutte and Crosson, 2013; Ercan *et al*., 2013; Hoehler and Jorgensen, 2013; Kleerebezem *et al*., 2020). Distinct non-growing phenotypes have been described, including spores, akinetes, biofilms and viable but non-culturable states (Harrison *et al*., 2007; Navarro Llorens *et al*., 2010; Lennon and Jones, 2011; Boutte and Crosson, 2013; van Mastrigt *et al*., 2018; van Tatenhove-Pel *et al*., 2019). Despite this phenotypic variation slow- or non-growing phenotypes consistently show increased resistance to stress conditions (Dressaire *et al*., 2008; Lopez-Maury *et al*., 2008; Lu *et al*., 2009; Zakrzewska *et al*., 2011; Boutte and Crosson, 2013; Maharjan *et al*., 2013; Ercan *et al*., 2015b; Ferenci, 2016; Biselli *et al*., 2020; Abram *et al*., 2021), reflecting the growth-survival trade-off. Although the existence of such trade-off has been described, its quantitative assessment and molecular underpinning remain incomplete. Retentostat cultivation enables the study of microbes at progressively reduced growth rates that ultimately approximate zero-growth under controlled conditions, which coincides with the reallocation of cellular energy from growth to maintenance-related processes (Ercan *et al*., 2015b).

A slow-growing, glucose-limited anaerobic chemostat culture of the plant-isolate *Lactococcus lactis* KF147 (specific growth rate 0.025 h^{-1}) was switched to retentostat cultivation mode by effluent removal through a cross-flow filter while maintaining medium dilution at 0.025 h^{-1}, whereby microbial biomass is retained in the fermentor (Ercan *et al*., 2013; Ercan *et al*., 2015b). Previous retentostat studies showed that *L. lactis* KF147 growth gradually approximates a near-zero specific growth rate after 2–3 weeks, while maintaining high levels of cell viability and cultivability (Ercan *et al*., 2013) (Supplementary Fig. S1). In parallel, cellular energy expenditure gradually switches from growth towards maintenance-related processes (Ercan *et al*., 2013), coinciding with fluctuations in pyruvate dissipation between homolactic and mixed-acid fermentation and the activation of import and utilization pathways for alternative carbon sources (Ercan *et al*., 2015a). Strikingly, similar studies that used a dairy isolate of *L. lactis* (strain FM03-V1) demonstrated that this strain had an approximately sixfold higher energy requirement during near-zero growth rates

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(van Mastrigt *et al*., 2018; van Mastrigt *et al*., 2019; Kleerebezem *et al*., 2020). These observations could reflect that the plant isolate KF147 is more adapted to the energy-poor environment associated with plants, contrasting the adaptation of the dairy isolate FM03-V1 to long-term cultivation in the energy-rich dairy environment (Kleerebezem *et al*., 2020).

The physiological changes observed in *L. lactis* KF147 were in agreement with the progressive transcriptome changes observed at consecutive timepoints during retentostat cultivation (Ercan *et al*., 2015a). Further mining of the transcriptome data revealed that adaptation of *L. lactis* during retentostat cultivation includes a gradual alleviation of repression of stress-response associated functions (Fig. 1). These functions were previously assigned to the so-called 'stressome' of *L. lactis* and other lactic acid bacteria (Papadimitriou *et al*., 2016) and include canonical class I and class III heat shock proteins as well as several functions involved in cell membrane-, acid- and low-temperature stress responses, which were described to contribute to tolerance to various stress conditions such as heat-, acid- and oxidative-stress (Papadimitriou *et al*., 2016). Quantitative analysis showed that the specific growth rate and stressome gene expression levels displayed a strong negative correlation. The increase of expression of stressome genes was most prominent during the first 4 weeks of retentostat cultivation, closely approximating (~90%) final expression of these genes after 6 weeks of retentostat cultivation (>15-fold induction relative to chemostat conditions), and coincided with the strongest reduction (>95%) of the relative growth rate (Fig. 1). This finding establishes that a

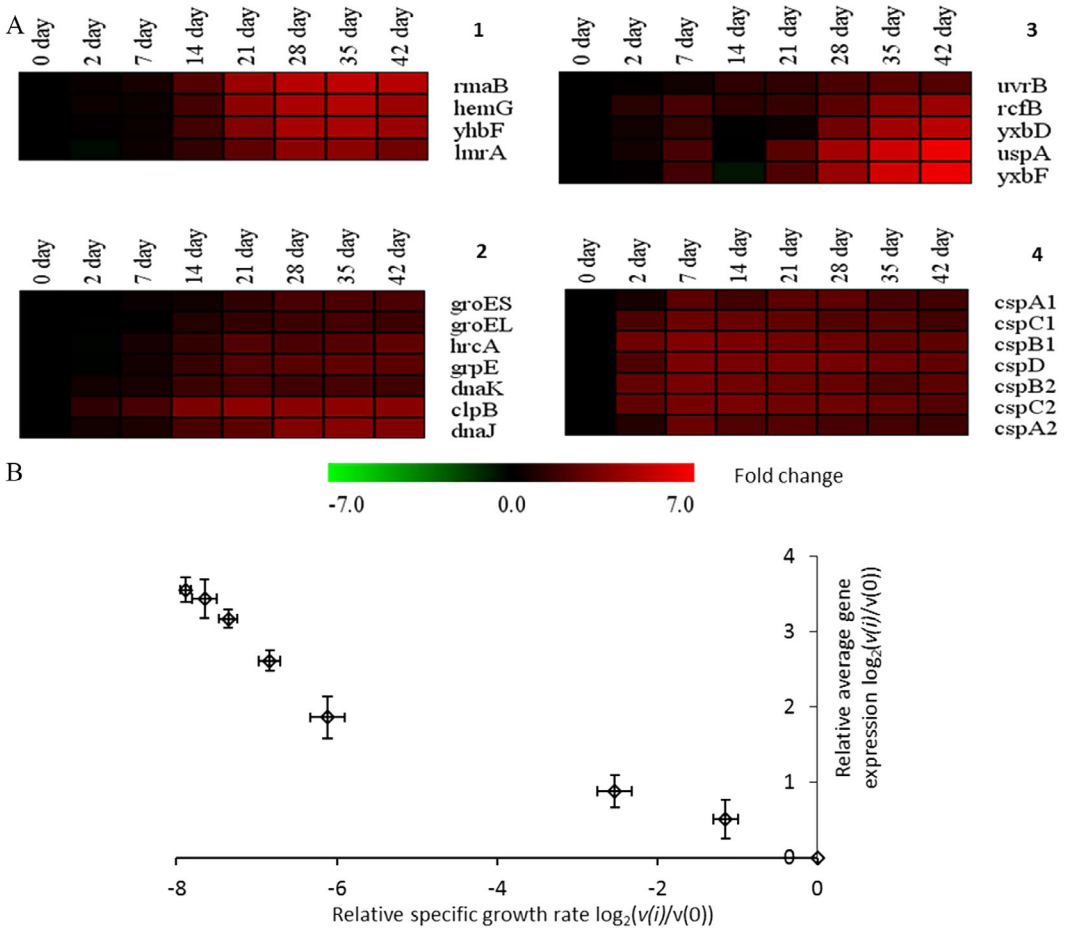

Fig. 1. Expression of stress-associated genes of *L. lactis* KF147 and correlation with specific growth rate during retentostat cultivation. Average gene-specific expression values (Ercan *et al*., 2015a) of *L. Lactis* KF147 stressome-associated genes during 2, 7, 14, 21, 28, 35 and 42 days of two independent retentostat cultivations are displayed relative (in $\log_2$-scale, corrected p-value ≤0.05) to their expression levels during chemostat growth (day 0) (panel A). The broad spectrum of stressome expression activation is apparent from the genes associated with cell membrane (A1), heat (A2), acid (A3) and low temperature (A4) stress responses. Panel B displays the relationship of the averaged differential expression of stressome genes in comparison to chemostat conditions (average-fold-change; $\log_2$-scale) and the relative growth rate during retentostat cultivation [$v(i)$] compared to chemostat cultivation [$v(0)$; specific growth rate 0.025 h^{-1}] (Ercan *et al*., 2013) in $\log_2$-scale. Data points represent average ± standard deviation of measurements of two independent retentostat cultures.

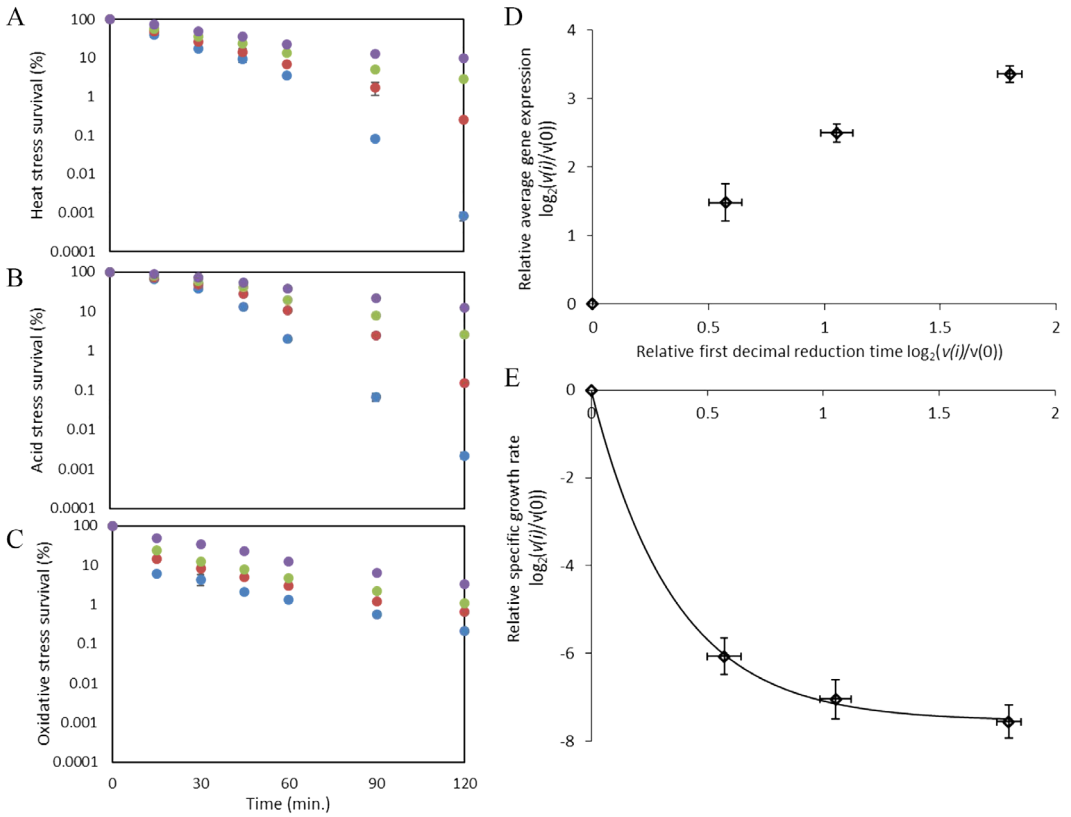

Fig. 2. Kinetics of loss of cultivability of chemostat- and retentostat-grown *L. lactis* KF147 cells during exposure to heat stress at 50°C (panel A), acid stress at a pH of 2.5 (panel B) and oxidative stress at 20 mM H_2O_2 (panel C). Remaining cultivability is displayed as the percentage (plotted in a log10 scale) of remaining cultivability relative to the original numbers of colonies obtained in the untreated samples (i.e. prior to stress exposure). The different culture samples included are chemostat-grown cells (blue); retentostat-grown cells on day 14 (red), 21 (green) and 29 (purple). Data points represent average ± standard deviation of measurements of two independent retentostat cultures. Notably, CFU enumerations did not decline for untreated and non-stressed cells (i.e. the initial 100% numbers of CFU) over a time span of 2 h. Panels D and E show the relationship between the average of the loss of cultivability kinetics [expressed as the average first decimal reduction time (δ) in $\log_2$-scale] and the averaged differential expression of stressome genes (see Fig. 1 for details) (panel D), or the relative growth rate during retentostat cultivation (see Fig. 1 for details) in $\log_2$-scale (panel E). The curve in panel E represents the best-fit model and data points represent average ± standard deviation of the measurements derived from two independent retentostat cultures.

broad panel of stressome associated genes is consistently and progressively induced, and increased expression is quantitatively correlated with reduction of the specific growth rate, providing a molecular mechanism that plausibly underlies the growth-survival trade-off in this species.

To evaluate whether increased stressome transcription indeed increases the stress resistance of these energy-restricted *L. lactis* KF147 cultures, stress tolerance was analysed in bacteria sampled after 14, 21 and 29 days of retentostat cultivation and compared to the tolerance levels measured for the initial chemostat culture (see supplementary methods and supplementary Fig. S2 for details). These sampling time-points for the retentostat culture were chosen to encompass the period of the most prominent change in stressome gene expression levels (Fig. 1A). Exposure to heat (50°C) and acid (pH 2.5) stress revealed a significantly increased survival capacity of retentostat- compared to chemostat-derived cells. Moreover, prolongation of retentostat cultivation led to consistent improvement of stress tolerance reaching maximum stress resistance levels in samples obtained after 29 days of retentostat cultivation (Fig. 2). Two hours of heat or acid stress exposure led to an almost 5-log reduction in cultivability in chemostat-derived bacteria (equivalent to approximately 0.001% survival), whereas the remaining cultivability was more than 10^2, 10^3 and 10^4-fold higher in the bacteria taken from the retentostat cultivation after 14, 21 and 29 days respectively (Fig. 2, panels A and B). Exposure to oxidative stress (20 mM H_2O_2) was characterized by a rapid loss of cultivability (more than 90% within the first 15 min) of the cells derived from the chemostat cultivation. This level of oxidative stress was also detrimental for the culture samples obtained from the retentostat, but after 15 min of exposure to H_2O_2 approximately 15%, 25% and 50% of the

cells remained culturable in samples obtained after 14, 21 and 29 days retentostat cultivation respectively (Fig. 2, panel C). Prolongation of oxidative stress exposure roughly sustained the magnitude of this initial difference.

Stress tolerance of the different *L. lactis* KF147 culture samples was quantified using the Weibull microbial survival model (den Besten *et al*., 2006), which showed acceptable model-fitting performance for most of the experimental conditions (Supplementary Table ST1). Stress tolerance is represented by the δ parameter in the Weibull model, which represents the first decimal reduction time (see Supplementary Methods). The δ values obtained for heat and acid stress exposure were increasing with prolongation of the retentostat cultivation, reaching a 2.5–3-fold higher δ after 29 days of retentostat cultivation relative to the value obtained from a chemostat-derived culture (Supplementary Table ST2). Analogously, model fitting of oxidative stress survival confirmed a higher δ value for the retentostat-derived samples than those from the chemostat cultivation (Supplementary Table ST2). Importantly, a strong correlation between the determined stress tolerance levels (as reflected by the δ value) and the stressome gene expression levels was observed, illustrating that the selected transcripts are adequate cellular indicators for bacterial stress tolerance (den Besten *et al*., 2010; Abee *et al*., 2011), even in these energy-restricted cells at near-zero growth rate (Fig. 2, panel D). Remarkably, a non-linear relationship was revealed between stress tolerance and growth rate, indicative of an exponential stress tolerance increase upon the adaptation towards near-zero growth rates (Fig. 2, panel E). Previous studies have argued that the shape of a trade-off curve between growth rate and stress survival determines the response of an ecosystem to competition (Maharjan *et al*., 2013; Abram *et al*., 2021). The non-linear shape of the growth-survival trade-off curve in *L. lactis* implies that *L. lactis* is evolutionarily specialized for survival at the cost of rapid growth under nutrient-poor conditions (Maharjan *et al*., 2013; Abram *et al*., 2021).

This quantitative study exemplifies that during the approximation of a zero-growth state, *L. lactis* employs intrinsic gene-regulatory networks for progressively increased stressome expression even though cells are not exposed to environmental stress conditions. This molecular adaptation protects the bacterial cells and ensures their sustained fitness and cultivability under conditions that do not support growth. The quantitative relationships between growth rate and the level of expression of stress-associated functions correspond with stress tolerance levels, exemplifying the hard-wiring of the growth-survival trade-off within the gene regulation network of *L. lactis*. In *Escherichia coli*, a role has been suggested for small regulatory RNA molecules (sRNAs) in the adjustment of gene regulation repertoires under nutrient-poor conditions and during biofilm formation (Andreassen *et al*., 2018; Abram *et al*., 2021). Intriguingly, in *L. lactis* a role of sRNAs has been described for the regulation of nutrient acquisition (van der Meulen *et al*., 2016), energy generation (van der Meulen *et al*., 2019) as well as stress response (van der Meulen *et al*., 2017; Wu *et al*., 2018; Tian *et al*., 2019), indicating that these sRNAs might play a role in several of the gene expression adjustments observed in *L. lactis* KF147 during prolonged retentostat cultivation.

Taken together, our study contributes to the understanding of the transcription and stress tolerance adaptation that underlie the growth-survival trade-off in *L. lactis*, using well-controlled retentostat cultivation to mimic environmentally prevalent oligotrophic conditions that induce non-growing states.

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References

Abee, T., Wels, M., de Been, M., and den Besten, H. (2011) From transcriptional landscapes to the identification of biomarkers for robustness. *Microb Cell Fact* **10**: S9.

Abram, F., Arcari, T., Guerreiro, D., and O'Byrne, C.P. (2021) Evolutionary trade-offs between growth and survival: the delicate balance between reproductive success and longevity in bacteria. *Adv Microb Physiol* **79**: 133–162.

Andreassen, P.R., Pettersen, J.S., Szczerba, M., Valentin-Hansen, P., Moller-Jensen, J., and Jorgensen, M.G. (2018) sRNA-dependent control of curli biosynthesis in *Escherichia coli*: McaS directs endonucleolytic cleavage of *csgD* mRNA. *Nucleic Acids Res* **46**: 6746–6760.

Biselli, E., Schink, S.J., and Gerland, U. (2020) Slower growth of *Escherichia coli* leads to longer survival in carbon starvation due to a decrease in the maintenance rate. *Mol Syst Biol* **16**: e9478.

Boutte, C.C., and Crosson, S. (2013) Bacterial lifestyle shapes stringent response activation. *Trends Microbiol* **21**: 174–180.

den Besten, H.M., Arvind, A., Gaballo, H.M., Moezelaar, R., Zwietering, M.H., and Abee, T. (2010) Short- and long-term biomarkers for bacterial robustness: a framework for quantifying correlations between cellular indicators and adaptive behavior. *PLoS One* **5**: e13746.

den Besten, H.M., Mataragas, M., Moezelaar, R., Abee, T., and Zwietering, M.H. (2006) Quantification of the effects of salt stress and physiological state on thermotolerance

of *Bacillus cereus* ATCC 10987 and ATCC 14579. *Appl Environ Microbiol* **72**: 5884–5894.

Dressaire, C., Redon, E., Milhem, H., Besse, P., Loubiere, P., and Cocaign-Bousquet, M. (2008) Growth rate regulated genes and their wide involvement in the *Lactococcus lactis* stress responses. *BMC Genomics* **9**: 343.

Egli, T. (2010) How to live at very low substrate concentration. *Water Res* **44**: 4826–4837.

Ercan, O., Bisschops, M.M., Overkamp, W., Jorgensen, T. R., Ram, A.F., Smid, E.J., *et al*. (2015b) Physiological and transcriptional responses of different industrial microbes at near-zero specific growth rates. *Appl Environ Microbiol* **81**: 5662–5670.

Ercan, O., Smid, E.J., and Kleerebezem, M. (2013) Quantitative physiology of *Lactococcus lactis* at extreme low-growth rates. *Environ Microbiol* **15**: 2319–2332.

Ercan, O., Wels, M., Smid, E.J., and Kleerebezem, M. (2015a) Molecular and metabolic adaptations of *Lactococcus lactis* at near-zero growth rates. *Appl Environ Microbiol* **81**: 320–331.

Ferenci, T. (2016) Trade-off mechanisms shaping the diversity of bacteria. *Trends Microbiol* **24**: 209–223.

Hallsworth, J.E. (2021) Water is a preservative of microbes. *J Microbial Biotechnol* **15**: 191–214.

Harrison, J.J., Ceri, H., and Turner, R.J. (2007) Multimetal resistance and tolerance in microbial biofilms. *Nat Rev Microbiol* **5**: 928–938.

Hoehler, T.M., and Jorgensen, B.B. (2013) Microbial life under extreme energy limitation. *Nat Rev Microbiol* **11**: 83–94.

Kleerebezem, M., Bachmann, H., van Pelt-KleinJan, E., Douwenga, S., Smid, E.J., Teusink, B., and van Mastrigt, O. (2020) Lifestyle, metabolism and environmental adaptation in *Lactococcus lactis*. *FEMS Microbiol Rev* **44**: 804–820.

Lennon, J.T., and Jones, S.E. (2011) Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nat Rev Microbiol* **9**: 119–130.

Lopez-Maury, L., Marguerat, S., and Bahler, J. (2008) Tuning gene expression to changing environments: from rapid responses to evolutionary adaptation. *Nat Rev Genet* **9**: 583–593.

Lu, C., Brauer, M.J., and Botstein, D. (2009) Slow growth induces heat-shock resistance in normal and respiratory-deficient yeast. *Mol Biol Cell* **20**: 891–903.

Maharjan, R., Nilsson, S., Sung, J., Haynes, K., Beardmore, R.E., Hurst, L.D., *et al*. (2013) The form of a trade-off determines the response to competition. *Ecol Lett* **16**: 1267–1276.

Navarro Llorens, J.M., Tormo, A., and Martinez-Garcia, E. (2010) Stationary phase in gram-negative bacteria. *FEMS Microbiol Rev* **34**: 476–495.

Papadimitriou, K., Alegria, A., Bron, P.A., de Angelis, M., Gobbetti, M., Kleerebezem, M., *et al*. (2016) Stress physiology of lactic acid bacteria. *Microbiol Mol Biol Rev* **80**: 837–890.

Tian, K., Li, Y., Wang, B., Wu, H., Caiyin, Q., Zhang, Z., and Qiao, J. (2019) The genome and transcriptome of *Lactococcus lactis* ssp. *lactis* F44 and G423: insights into adaptation to the acidic environment. *J Dairy Sci* **102**: 1044–1058.

van der Meulen, S.B., de Jong, A., and Kok, J. (2016) Transcriptome landscape of *Lactococcus lactis* reveals many novel RNAs including a small regulatory RNA involved in carbon uptake and metabolism. *RNA Biol* **13**: 353–366.

van der Meulen, S.B., de Jong, A., and Kok, J. (2017) Early transcriptome response of *Lactococcus lactis* to environmental stresses reveals differentially expressed small regulatory RNAs and tRNAs. *Front Microbiol* **8**: 1704.

van der Meulen, S.B., Hesseling-Meinders, A., de Jong, A., and Kok, J. (2019) The protein regulator ArgR and the sRNA derived from the 3'-UTR region of its gene, ArgX, both regulate the arginine deiminase pathway in *Lactococcus lactis*. *PLoS One* **14**: e0218508.

van Mastrigt, O., Abee, T., Lillevang, S.K., and Smid, E.J. (2018) Quantitative physiology and aroma formation of a dairy *Lactococcus lactis* at near-zero growth rates. *Food Microbiol* **73**: 216–226.

van Mastrigt, O., Egas, R.A., Lillevang, S.K., Abee, T., and Smid, E.J. (2019) Application of a partial cell recycling chemostat for continuous production of aroma compounds at near-zero growth rates. *BMC Res Notes* **12**: 173.

van Tatenhove-Pel, R.J., Zwering, E., Solopova, A., Kuipers, O.P., and Bachmann, H. (2019) Ampicillin-treated *Lactococcus lactis* MG1363 populations contain persisters as well as viable but non-culturable cells. *Sci Rep* **9**: 9867.

Wu, H., Song, S., Tian, K., Zhou, D., Wang, B., Liu, J., *et al*. (2018) A novel small RNA S042 increases acid tolerance in *Lactococcus lactis* F44. *Biochem Biophys Res Commun* **500**: 544–549.

Zakrzewska, A., van Eikenhorst, G., Burggraaff, J.E., Vis, D. J., Hoefsloot, H., Delneri, D., *et al*. (2011) Genome-wide analysis of yeast stress survival and tolerance acquisition to analyze the central trade-off between growth rate and cellular robustness. *Mol Biol Cell* **22**: 4435–4446.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Supporting Information.
Appendix S2. Supporting Information.