



Quantitative physiology of *Lactococcus lactis* cultivated under lactic acid limitation

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Background

Lactococcus lactis is widely used in starter cultures to make fermented dairy products, such as cheese and buttermilk. In these fermentations, *L. lactis* produces lactic acid causing acidification of the fermented product and thereby prolonging its shelf life. However, during the production of these starter cultures the lactic acid inhibits bacterial growth despite control of the pH.

This growth inhibition by lactic acid has never been studied quantitatively, and predictive models for the growth of LAB during starter culture production rely on growth parameters that has been obtained in either pH-uncontrolled batch fermentations or substrate-limited chemostat cultivations, which both do not reflect lactic acid-limited conditions.

Objective

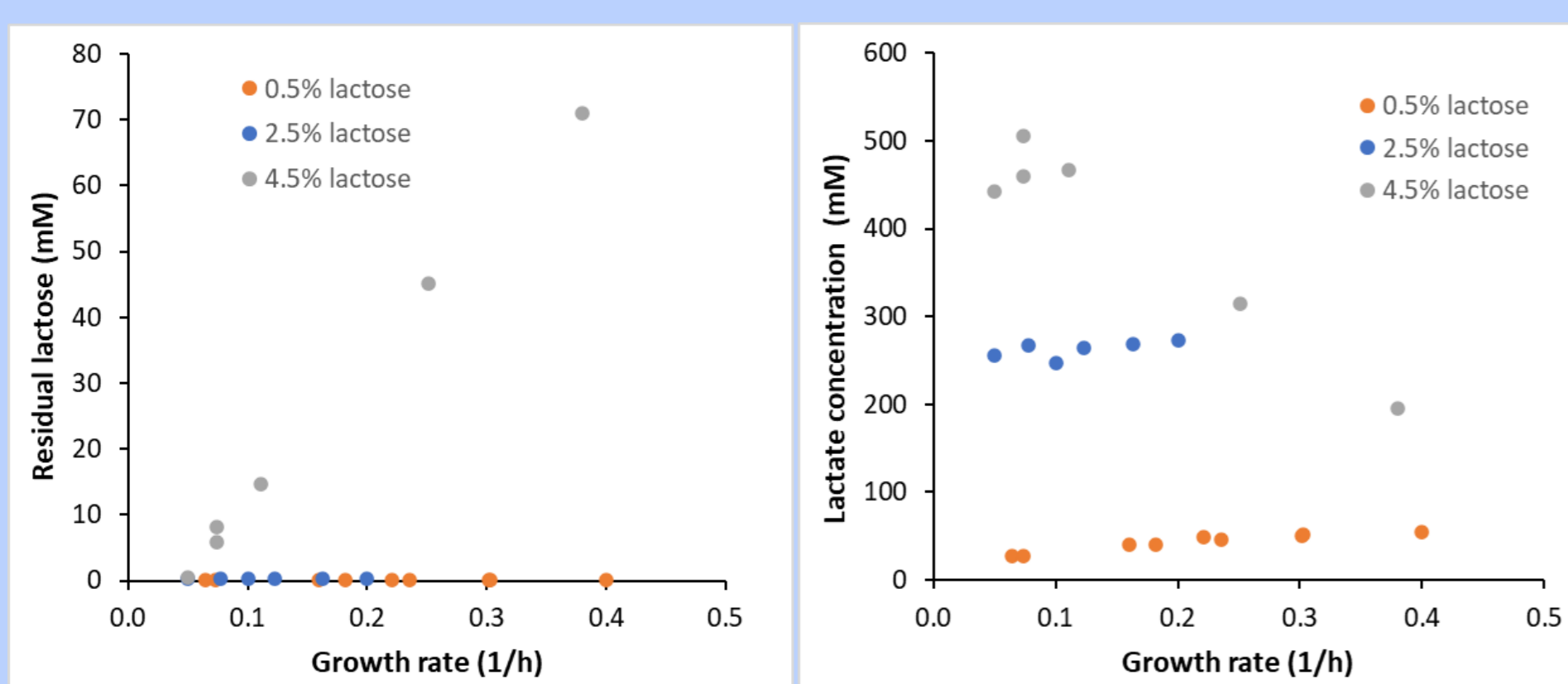
This project aims to determine the quantitative physiology of *L. lactis* cultivated under lactic acid-limited conditions at pH 6.5.

Method

L. lactis subsp. *lactis* biovar diacetylactis FM03-V1 was grown in chemostats ($D=0.05-0.4 \text{ h}^{-1}$) under lactose and lactic acid limitation by varying the lactose and tryptone content of the media. Lactic acid limited conditions were obtained by supplying all nutrients in surplus.

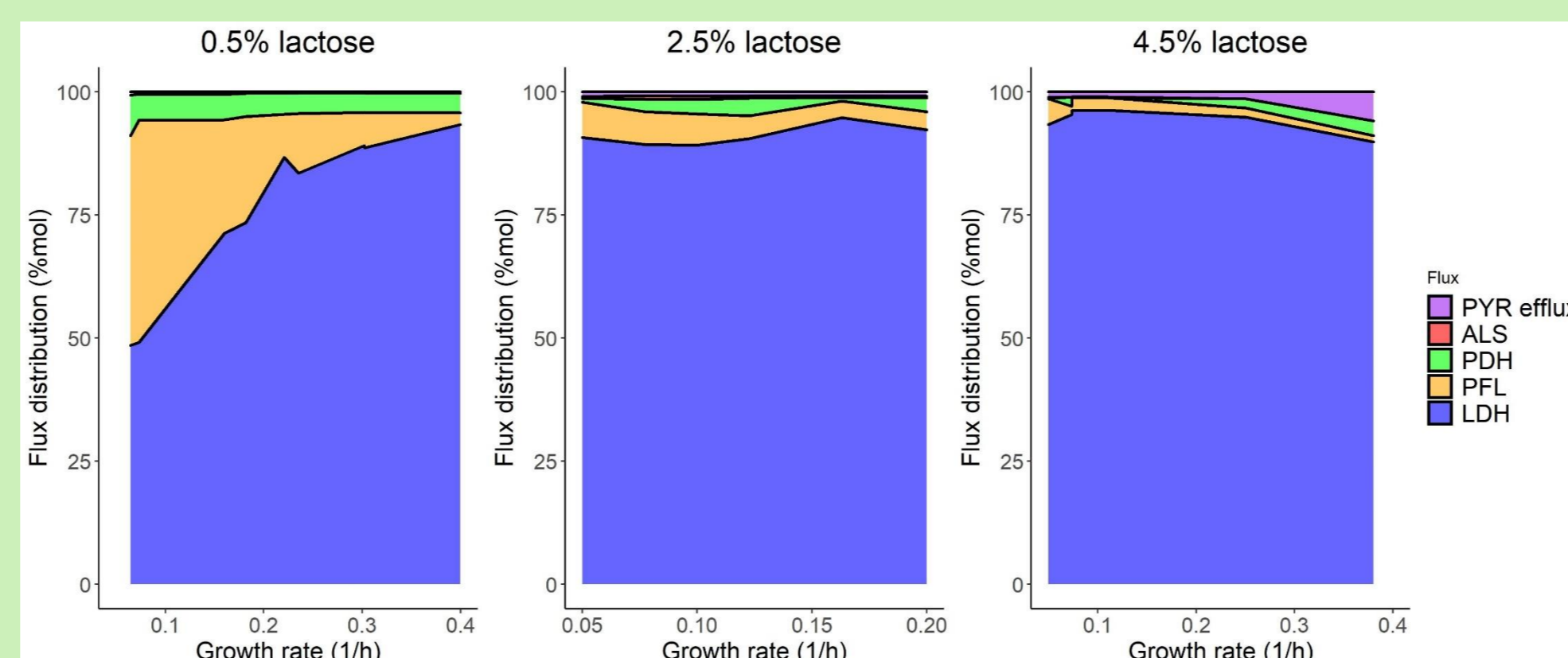
Results

Growth rate vs lactic acid



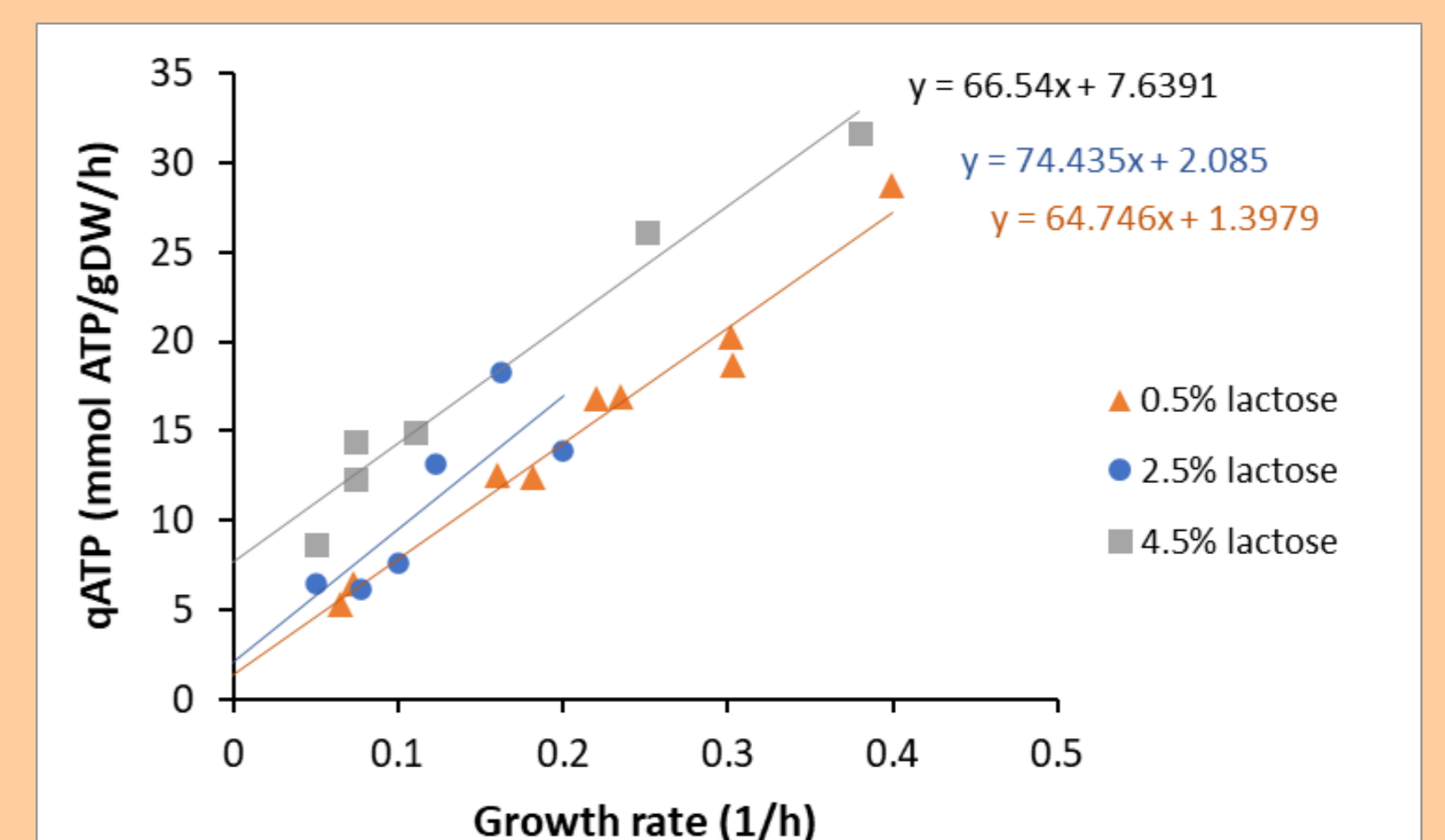
- At 0.5% and 2.5% lactose, no residual lactose indicated growth was lactose limited.
- At 4.5% lactose, the residual lactose concentration increased linearly with growth rate indicating lactic acid limitation.
- Correspondingly, the lactic acid concentration decreased linearly with growth rate at 4.5% lactose.

Metabolism



- At 0.5% lactose, *L. lactis* switches from homolactic (lactic acid) to mixed acid fermentation (acetate, ethanol and formate) at low growth rates.
- *L. lactis* did not switch under lactic acid limitation (4.5% lactose)
- Surprisingly, also under lactose limitation with 2.5% lactose, only a minor switch was found.
- At 4.5% lactose, pyruvate was produced at high growth rates.

Biomass yield and maintenance



- The maximum biomass yield on ATP (1/slope) was not significantly different between the tested conditions.
- The maintenance requirements (intercept) increased ~5-fold under lactic acid limitation
- The maintenance requirement did not increase with high lactic acid concentrations under lactose-limited conditions (2.5% lactose).

Conclusions

- Increasing the substrate content in the medium resulted in lactic acid-limited growth of *L. lactis* in chemostats.
- Growth rates decreased linearly with the lactic acid concentration
- Growth remained homolactic under lactic acid limitation, even at low growth rates
- The maintenance requirement increased approximately 5-fold under lactic acid limitation

Acknowledgements

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