# Flow cytometric analysis reveals culture-condition dependent variations in phenotypic heterogeneity of Limosilactobacillus reuteri

S. Rao, N.

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# Flow cytometric analysis reveals culture-condition dependent variations in phenotypic heterogeneity of *Limosilactobacillus reuteri*


**N. S. Rao[1], L. Lundberg[2,3], S. Palmkron[4], S. Håkansson[1,5], B. Bergenståhl[4], M. Carlquist[1]**

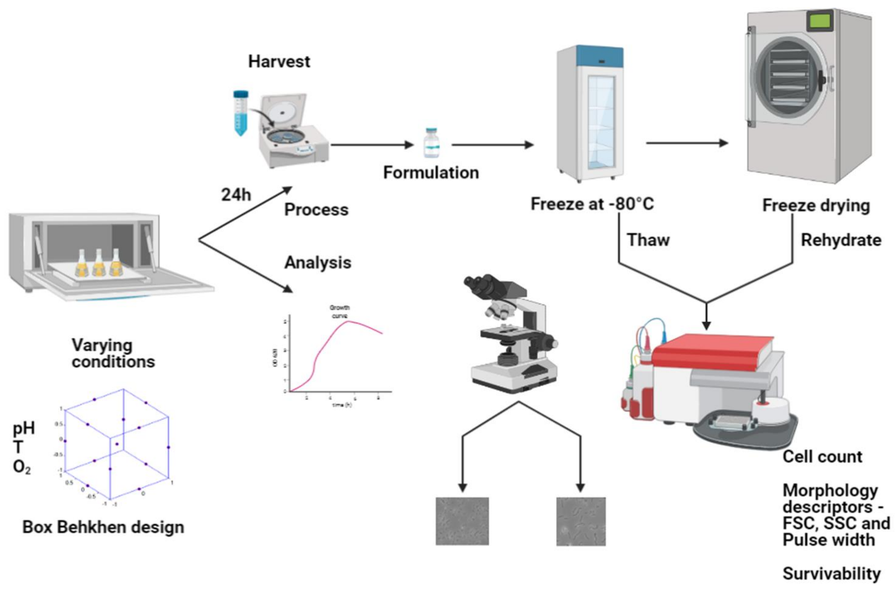
[1]Division of Applied Microbiology, Lund University, Lund, Sweden, [2]The Department of Molecular Sciences, Uppsala BioCenter, Swedish University of Agricultural Sciences, Uppsala, Sweden, [3]BioGaia, Stockholm, Sweden, [4]Department of Food Technology, Engineering and Nutrition, Lund University, Lund, Sweden, [5]BioGaia, Eslöv, Sweden

E-mail: nikhil.seshagiri_rao@tmb.lth.se


## INTRODUCTION

Optimization of cultivation conditions in the industrial production of probiotics is crucial to reach a high-quality product with retained probiotic functionality[1,2]. In the current study, the effects of temperature, pH and oxygen levels on cell growth, size distributions and freeze-drying (FD) tolerance of *L. reuteri* DSM 17938 were measured using flow cytometry (FCM). A pleomorphic behaviour was evident from the measurement of light scatter and pulse width distributions[3,4]. The fact that *L. reuteri* morphology varies depending on cultivation conditions suggests that it can be used as marker for estimating physiological fitness and responses to its environment.

## METHOD

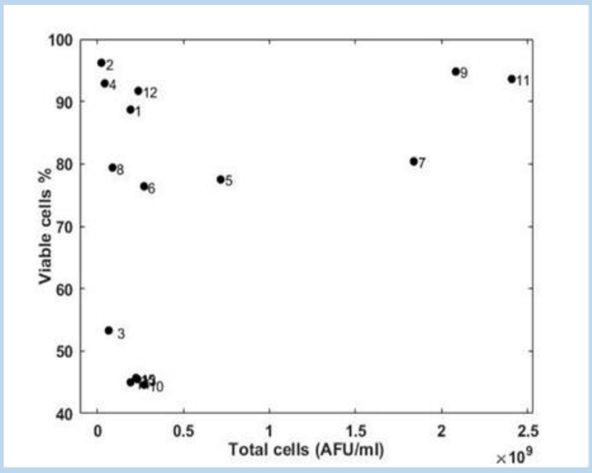


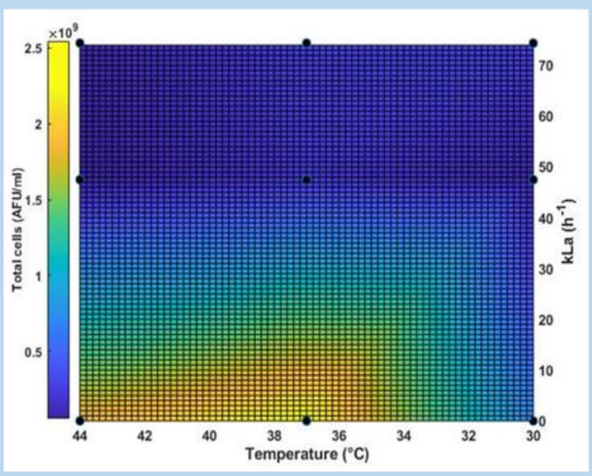

## RESULTS

### Design of experiment

| Condition | Temperature (T) (°C) | pH | $k_La$ ($h^{-1}$) |
|---|---|---|---|
| 1 | 30 | 5 | 47.5 |
| 2 | 30 | 7 | 47.5 |
| 3 | 44 | 5 | 47.5 |
| 4 | 44 | 7 | 47.5 |
| 5 | 30 | 6 | 0 |
| 6 | 30 | 6 | 74.4 |
| 7 | 44 | 6 | 0 |
| 8 | 44 | 6 | 74.4 |
| 9 | 37 | 5 | 0 |
| 10 | 37 | 5 | 74.4 |
| 11 | 37 | 7 | 0 |
| 12 | 37 | 7 | 74.4 |
| 13 | 37 | 6 | 47.5 |
| 14 | 37 | 6 | 47.5 |
| 15 | 37 | 6 | 47.5 |

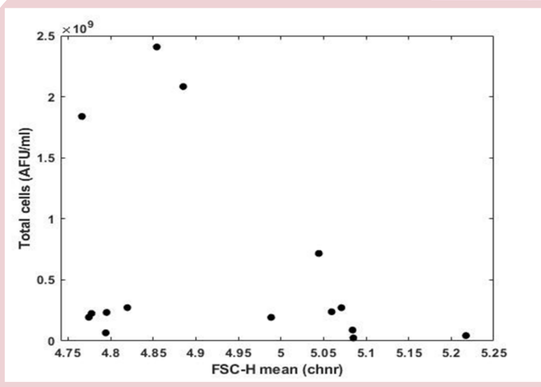
### Growth

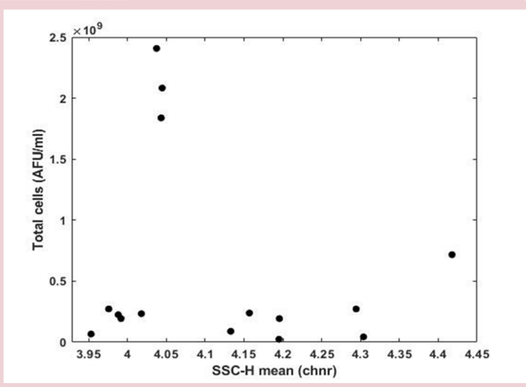


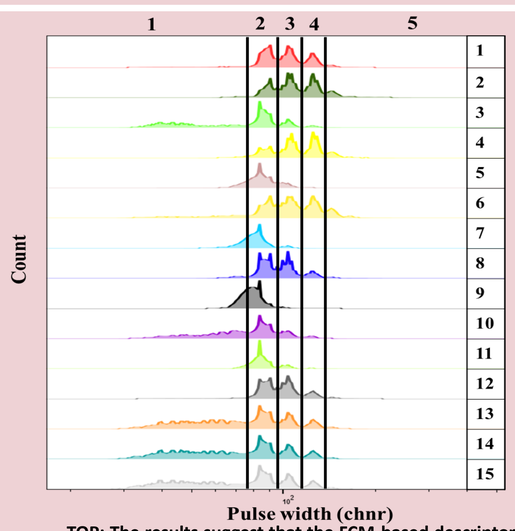



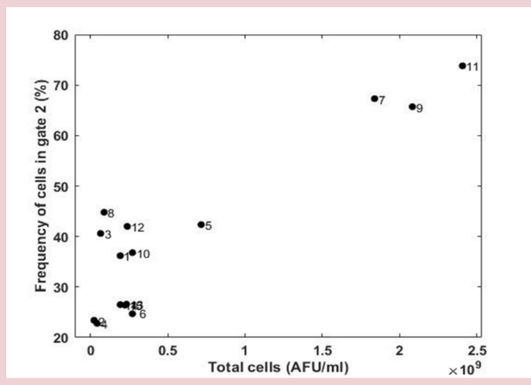

TOP: High variation in growth behaviour and viability. A considerable number of cultures yielded low cell concentrations, although this was not correlated to poor viability. BOTTOM: No oxygen and T between 30°C and 44 °C aided in growth.

### Morphology



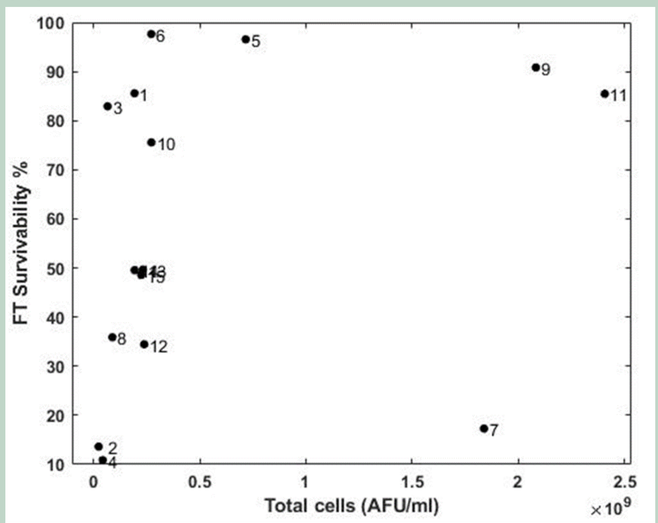



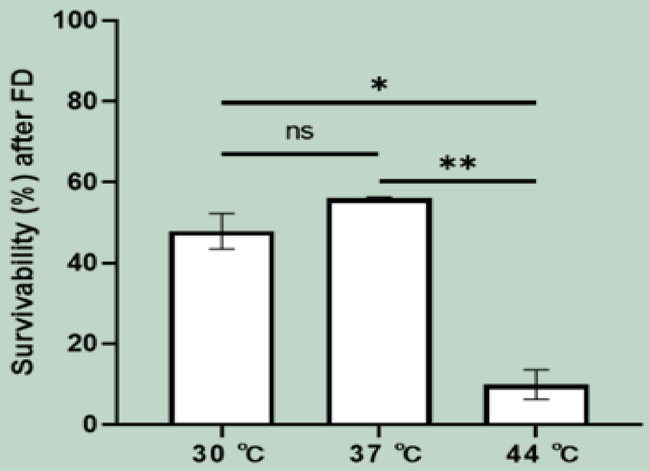




TOP: The results suggest that the FCM-based descriptors (means of FSC-H and SSC-H) were able to capture subtle differences amongst the cultures. Large mean cell size did not coincide with high cell counts under any condition evaluated, which suggests that a high frequency of their occurrence is a sign of poor growth. BOTTOM: Population was divided into 5 gates based on peaks of pulse width parameter. The frequency of total cells in gate 2 correlated well with the bacterial growth suggesting that high growth is conducive when there is less heterogeneous population. FSC-H= Forward scatter height and SSC-H= Side scatter height.

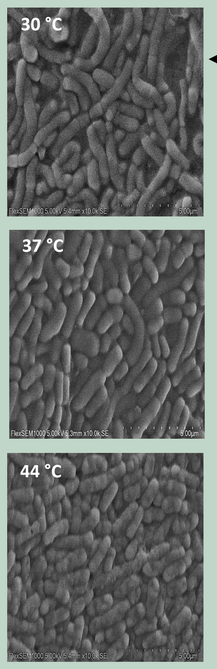
### Freeze-thaw (FT) and FD tolerance

A large variation in FT survivability was observed. There was no clear correlation to cell growth. The results suggested that viable but non-growing cells are not robust to freeze-thaw stress; instead, the specific combination of environmental factors play the dominant role.

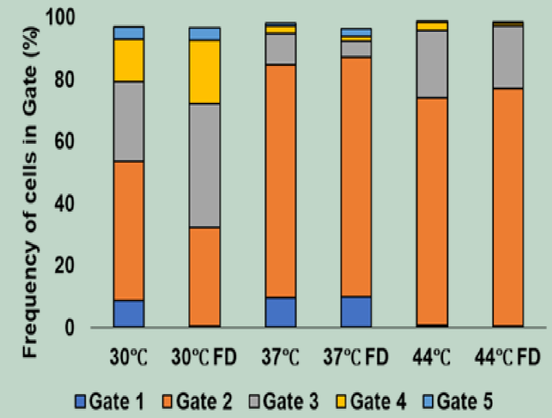



Bacteria were cultured under three different conditions found to cause variation in size distributions and at the same time enable growth (T=30, 37 and 44 °C pH 6, kLa [O2] 0 h-1). freeze-drying survivability was higher for cells grown at 37 °C and 30 °C than for cells grown at 44 °C. ns = not significant, * indicates p-value < 0.05, ** indicates p-value < 0.01.




The SEM analysis revealed that the larger bacteria obtained from the 30 °C cultivation indeed consisted of chains with two or more cells attached together, with clear septa. Cultures grown at 37 °C and 44 °C had smaller cell size when examined carefully by visual inspection.




For bacteria grown at 30 °C there was a relatively even distribution of viable cells in pulse width gates 2-4. This was different from bacteria grown at 37 °C and 44 °C where most of the cells were found in gate 2. Freeze-drying treatment did not change the pulse width distributions to a significant degree.

## CONCLUSION

- A FCM pipeline for analysing and correlating between environmental factors and cell morphology of *L. reuteri* DSM 17938 during cultivation and subsequent FD processing has been established. The pulse width distribution parameter can be used a Process Analytical Tool (PAT) in process control of morphology during fermentation.

Video


## ACKNOWLEDGEMENTS

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