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

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**INTRODUCTION**

Optimization of cultivation conditions in the industrial production of probiotics is crucial to reach a high-quality product with retained probiotic functionality¹,². 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 distributions³,⁴. 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.

**RESULTS**

### DESIGN OF EXPERIMENT

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</table>

**GROWTH**

- **Temperature (°C)**: The results suggest that high temperatures and high pH values can be used to grow *L. reuteri* DSM 17938. However, at 37°C and pH 7, the growth was significantly lower compared to other conditions.
- **Size Distribution**: The size of the bacteria increased with temperature and pH, indicating a change in bacterial morphology.

**MORPHOLOGY**

- **Pulse Width (µm)**: The results suggest that FCM-based descriptors (e.g., FSC-H and SSC-H) were able to capture subtle differences amongst the cultures. Large cell size did not correlate with high cell counts under any condition evaluated, which suggests that a high frequency of their occurrence is a sign of poor growth.

**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 reliant on freeze-thaw stress; instead, the specific combination of environmental factors play the dominant role.

**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 as a Process Analytical Tool (PAT) in process control of morphology during fermentation.

**REFERENCES**

3. Vollmer, B. et al. (2011) FEMS One 1, 1–6

**ACKNOWLEDGEMENTS**

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