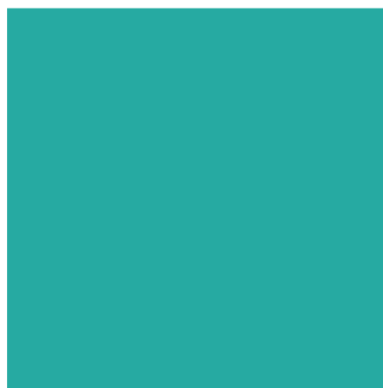


BOOK OF ABSTRACTS

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Inside Out: Quantification of Intracellular Lactic Acid in *Lactococcus lactis* in dynamic conditions

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Themes

Fermentation and Metabolism, including protein transition

Abstract

Lactococcus lactis is recognized for its rapid production of lactic acid and acidification, which is an important feature in dairy fermentations. The acidification not only improves the taste but also helps to extend the shelf life of the product. Despite the vital role of lactic acid in the physiology of lactic acid bacteria, little is known about the dynamics of intracellular lactic acid concentrations in growing cells. Quantification of intracellular lactic acid pools under dynamic conditions is a challenging task because of i) high extracellular volumes that greatly exceed intracellular volumes, ii) high extracellular lactic acid concentrations, iii) fast metabolite turnover times and iv) potentially low and changing cell densities, which renders NMR unsuitable as a method. Therefore, we developed a novel method to monitor intracellular lactic acid concentrations in *L. lactis* during fermentation, without the need for high cell densities. First, cells are quenched using cold (-20°C) glycerol-saline, to halt metabolism immediately without compromising the cell membrane, and washed with glycerol-saline to remove extracellular lactic acid. Subsequently, an optimized cell lysis step allows efficient extraction of lactic acid, which is quantified using high-performance liquid chromatography. Finally, we developed an image analysis pipeline that enables the estimation of the intracellular volume, allowing to accurately calculate concentrations. Overall, our approach provides a reliable way to quantify intracellular lactic acid concentrations, which can deepen our understanding of cellular physiology and may support the design and optimization of industrial fermentation processes.

Keywords

intracellular lactic acid, metabolomics, extraction, fermentation