Decrease in Transcriptomic Liberality during Batch Cultivation of *Streptococcus salivarius* ssp. *thermophilus* in Milk: A Comparative Observation with CHO Cells

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Abstract

Batch cultivation imposes physiological constraints on cells, often resulting in characteristic transcriptomic changes. In Chinese hamster ovary (CHO) cells, transcriptomic **liberality**, defined as the α-diversity of intracellular transcriptomes, has been shown to decrease during batch culture. To investigate whether this phenomenon also occurs in prokaryotes, we analyzed RNA-seq data from *Streptococcus salivarius* ssp. *thermophilus* cultured under standard milk fermentation conditions. Samples were collected at four phases of fermentation corresponding to distinct pH milestones (lag phase at 2 h, pH 6.0; mid-exponential at 5 h, pH 5.1; late-exponential at 7 h, pH 4.5; stabilization phase). Raw counts obtained from a published dataset were used to calculate liberality. We observed a progressive decrease in liberality across fermentation phases, consistent with previous findings in CHO cells. These results suggest that the decline in transcriptomic liberality represents a general feature of batch cultivation across both prokaryotic and eukaryotic systems.

Introduction

Batch culture is a widely used system in both industrial biotechnology and fundamental research. In mammalian cell cultures such as Chinese hamster ovary (CHO) cells, transcriptomic properties have been reported to change dynamically throughout cultivation. In particular, transcriptomic **liberality**, an index defined as the α-diversity of intracellular transcriptomes, was found to decrease during batch culture of CHO cells (Matsuda, 2021).

Lactic acid bacteria, such as *Streptococcus salivarius* ssp. *thermophilus* (commonly used in yogurt production), are also cultivated under batch conditions for dairy fermentation. While numerous studies have investigated growth kinetics, acidification, and metabolite production in *S. thermophilus*, little is known about the dynamics of transcriptomic liberality during fermentation. Investigating whether liberality decreases in this prokaryotic system would allow us to determine whether the phenomenon observed in CHO cells is a general consequence of batch cultivation.

In this study, we analyzed RNA-seq data obtained from *S. thermophilus* cultured in milk under standard fermentation conditions (Snew fermentation). Samples were collected at four stages of fermentation defined by pH milestones (2 h, 5 h, 7 h, and stabilization), representing the lag, mid-exponential, late-exponential, and stabilization phases, respectively. Using raw counts provided in a published dataset (Wang et al., 2025), we calculated liberality and compared its trajectory to that previously reported in CHO cells.

Materials and Methods

Dataset and cultivation conditions

RNA-seq raw count data of *S. thermophilus* were obtained from the supplemental materials of the published study (Wang et al., 2025). The bacteria were cultured in milk under standard Snew fermentation conditions. Sampling was designed to capture transcriptomic changes across fermentation pH milestones: Lag phase (2 h, pH 6.0), Mid-exponential phase (5 h, pH 5.1) and Late-exponential phase (7 h, pH 4.5).

Calculation of liberality

Liberality was measured as previously described (Ogata, 2022; Ogata et al., 2015; Ogata & Hosaka, 2022), defined as the α-diversity of intracellular transcriptomes (Martínez & Reyes-Valdés, 2008). Raw counts were used as input, and diversity indices were calculated across expressed transcripts.

Comparative reference

For contextual comparison, we referred to previous studies on CHO cells reporting decreased liberality during batch culture (Matsuda, 2021).

Results and Discussion

Liberality values of *S. thermophilus* decreased progressively across fermentation phases. This trend was consistent with the pattern previously reported in CHO cells, where batch culture similarly led to a reduction in transcriptomic liberality (Figure 1).

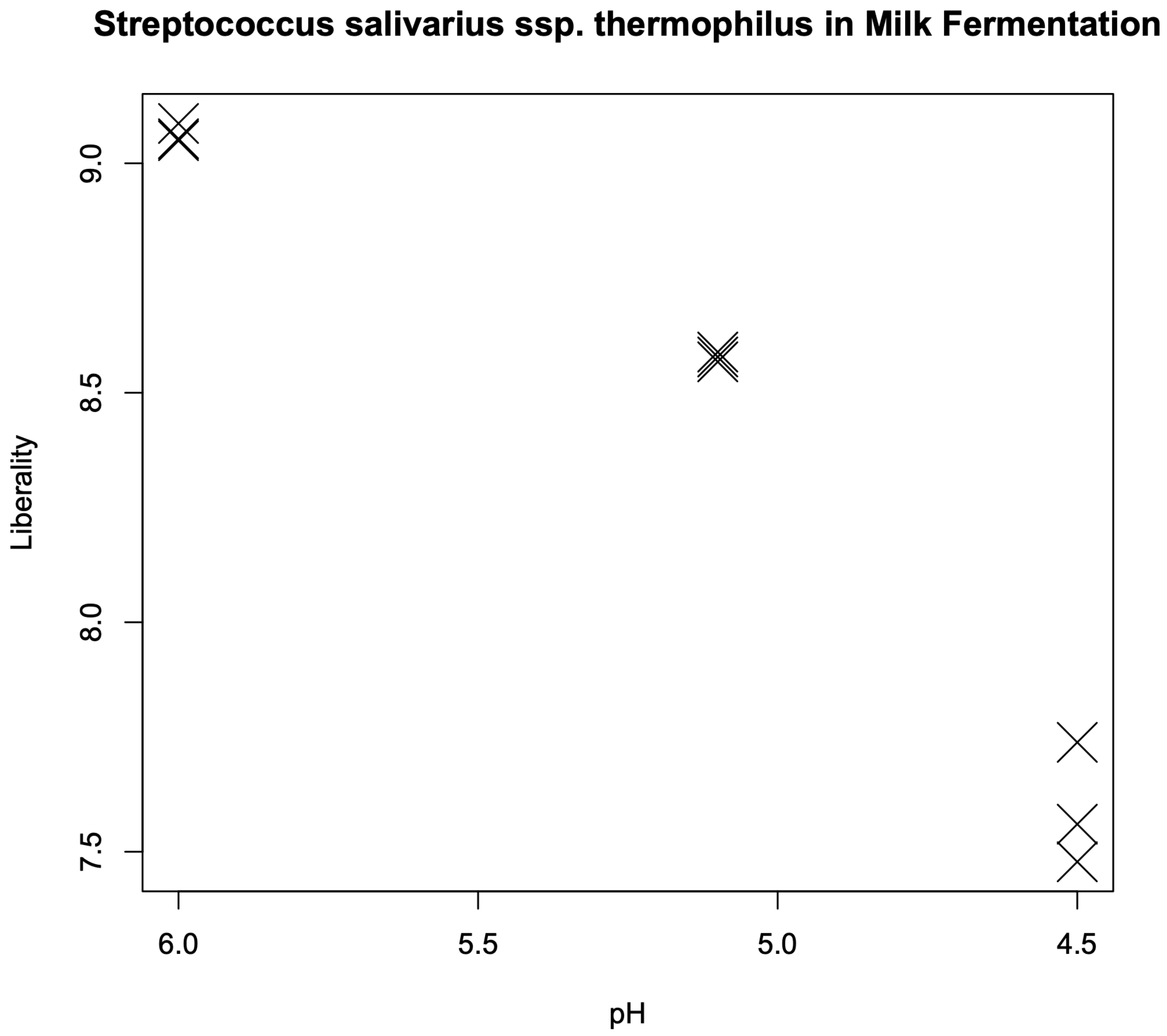

Figure 1. Correlation between pH of culture conditions and liberality

The similarity between bacterial and mammalian systems suggests that decreased transcriptomic α-diversity is a general feature of closed cultivation systems. In both cases, the decline may reflect reduced transcriptional plasticity due to environmental constraints such as nutrient depletion, acidification, and accumulation of metabolic byproducts.

While the absolute values of liberality differ between *S. thermophilus* and CHO cells, likely due to fundamental differences in transcriptome complexity between prokaryotes and eukaryotes, the shared trend highlights liberality as a universal index for monitoring culture dynamics. These findings extend the relevance of transcriptomic liberality beyond mammalian cell biotechnology to industrial microbial fermentation processes.

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