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Optimization of the quenching method for metabolomics analysis of *Lactobacillus bulgaricus*

Key words: Metabolomics, Quenching method, *Lactobacillus bulgaricus*, Leakage

Introduction

- ***Lactobacillus delbrueckii* subsp. *bulgaricus*** is an important lactobacillus commonly used for fermented milk products.

- *L. bulgaricus* metabolomics requires efficient and reliable quenching and extracting methods. Cold methanol-water quenching was considered as a general procedure to rapidly inactivate bacteria metabolism, which has been widely applied to *Escherichia coli*, *Staphylococcus aureus*, and yeast.

- This study aimed to examine different quenching solutions for the investigation of the L. bulgaricus metabolomics. The optimized quenching processing for *L. bulgaricus* would be applicable for other lactobacillus.

Methods

Optimization of the quenching method for metabolomics analysis of *Lactobacillus bulgaricus*

Bacterial strain:
L. bulgaricus ATCC 11842 was obtained from the American Type Culture Collection.

Evaluation of quenching:
1. Cell leakage test: OD-based recovery ratio; flow cytometry
2.Evaluation of quenching protocols by gaschromatography-mass spectrometry (GC-MS)

Statistical analysis:
1. Principal component analysis (PCA)
2. Orthogonal partial least squares-discriminant analysis (OPLS-DA)

Results and Conclusion

- The 80% Meth/gly effectively protected the cell membranes and reduced the ratio of PI labeling to 6.6%, which was significantly less than that in the Meth quenching ($P<0.05$).
- It was found that the OD_{660} recovery ratio of the cold 80% Meth/gly was higher than those of other quenching methods, which indicated that Meth/gly had a positive effect on preventing the *L. bulgaricus* cell lyses.
- It respectively found 807, 603, and 841 substances in the GC/MS TICs of 60% Meth, 80% Meth/gly, and 80% Meth samples, including ATP and its derivatives, amino acids, organic acids, sugars, and fatty acidS, the PCA score plot showed that the samples prepared by different methanols were clearly separated, although 80% Meth/gly and 80% Meth were grouped closely.
- The differences between the extracellular and intracellular metabolite samples were examined by OPLS-DA. The OPLS-DA score plot for the extracellular and intracellular substances of the 60% Meth, quenching group (one orthogonal and two predictive components, $R_2X=0.941$, $R_2Y=1$, $Q_2=0.999$) and the extracellular and intracellular metabolites of the 80% Meth quenching group ($R_2X=0.901$, $R_2Y=1$, $Q_2=0.998$) indicated that the two models were reliable and the metabolic samples had significant differences.
- In summary, quenching is a critical step in the evaluation of L. bulgaricus metabolomics. Compared to the general quenching in 60% cold methanol fluid, it is proposed that a 80% cold methanol or 80% cold methanol/glycerol solution was better for reducing the extent of leakage and examining higher concentrations of intracellular metabolites. The application of an optimized quenching process would contribute to identifying the specific biomarkers and also help to analyze the metabolic characteristics of L. bulgaricus.