Untargeted Screening Based on UHPLC-HRMS of Total Folates Produced by Lactic Acid Bacteria in Fermented Milk and During Yogurt Shelf Life

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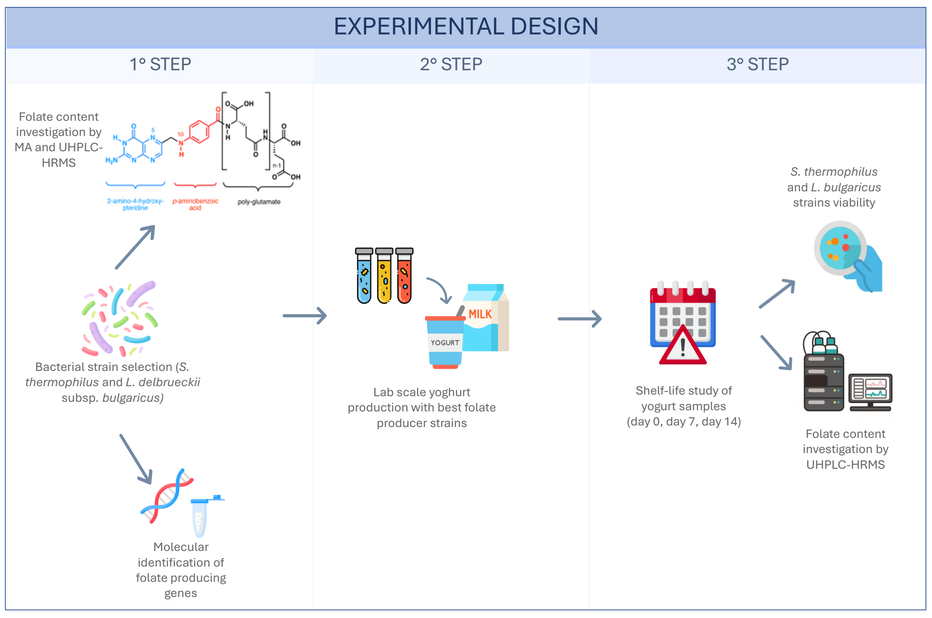
INTRODUCTION

Folate, the natural form of vitamin B9, plays a role in several biological processes, including DNA synthesis, repair and methylation, as well as erythropoiesis and critical growth phases such as pregnancy and infancy. This vitamin is naturally present in many foods and can not be synthesised by animal cells, making dietary intake crucial for maintaining adequate levels. Folate deficiencies are common and can lead to different health problems such as neural tube defects and megaloblastic anaemia.
In recent years, there has been a growing focus on the development of functional foods with enhanced nutritional value. Biofortification has emerged as a promising strategy to increase the natural folate content of foods, particularly when applied to fermented foods.

The objective of this study was to selecte and evaluate *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* strains with enhanced folate production in dairy matrices using UHPLC-HRMS analysis.



RESULTS

1. Strain selection

Initially, 36 strains of *S. thermophilus* (27) and *L. delbrueckii* subsp. *bulgaricus* (9) from traditional fermented milks were screened for folate production with a microbiological assay (MA).
PCR analysis revealed the presence of four genes involved in folate in all the tested strains: *folA* (dihydrofolate reductase), *folC* (folate synthetase/folyl polyglutamate synthetase), *folK* (2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase) and *folP* (dihydropteroate synthase).
To overcome MA's limitations, an untargeted and semi-quantitative method combining ultra-high-performance liquid chromatography (UHPLC) with high-resolution mass spectrometry (HRMS) was developed.

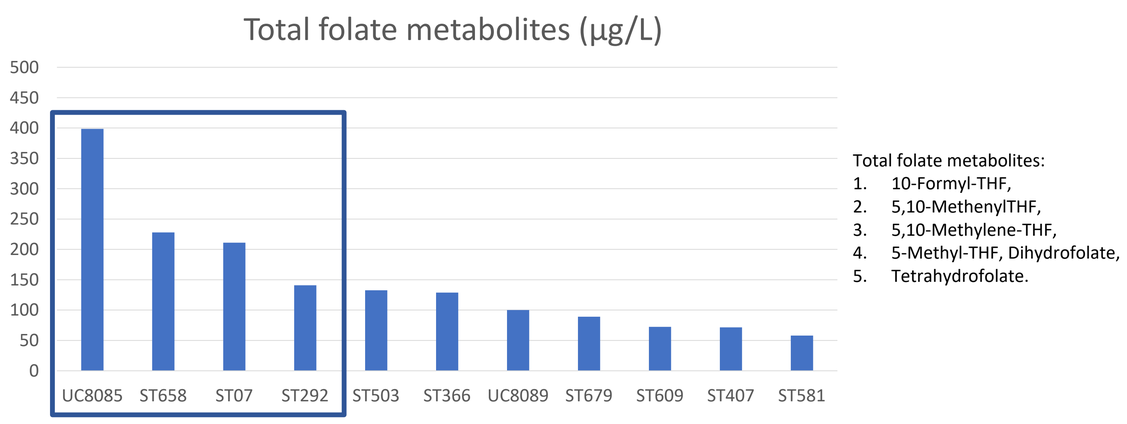


Fig. 1 - Semi-quantification of six folate metobolites produced by *S. thermophilus* and *L. bulgaricus* strains in milk samples.

Six key folate metabolites were identified with UHPLC-HRMS approach and grouped as total folate metabolites. (Fig. 1). Semi-quantification was performed using the 5-MTHF calibration curve.

3. Folates quantification in yoghurt

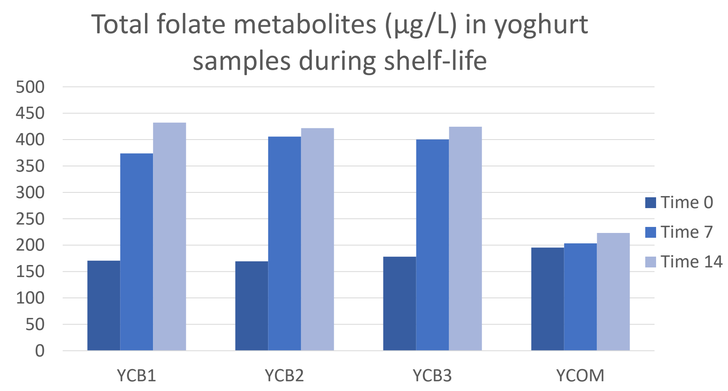


Fig. 3 - Folates content in cow yoghurt during shelf-life.

The folate levels in yoghurt samples (YCB1, YCB2, YCB3) increased over the shelf-life period respect to the commercial yogurt (YCOM). The blend YCB1, representing the optimal combination of bacterial species, showed the highest folate content at 432.08 µg/L (Fig. 3).

2. Yoghurt preparation and bacterial viability during the shelf-life

Name	*S. thermophilus*	*L. bulgaricus*	Ratio	% inoculated
YCB1	ST07	UC8085	2:1	8%:4%
YCB2	ST292	UC8085	2:1	8%:4%
YCB3	ST658	UC8085	2:1	8%:4%

Tab. 1 - Three distinct blends of the most effective strains of *S. thermophilus* and *L. bulgaricus* used to create the different yoghurts.

Three blends (Tab. 1) were inoculated into cow's milk, with an initial bacterial count of 8 log CFU/mL.
Over time, bacterial levels in yoghurt samples stayed approximately constant (Fig. 2). After 14 days of storage, the yogurt blend YCB3 showed the highest bacterial counts, with *S. thermophilus* (8.76 log CFU/mL) exceeding *L. bulgaricus* (8.20 log CFU/mL).

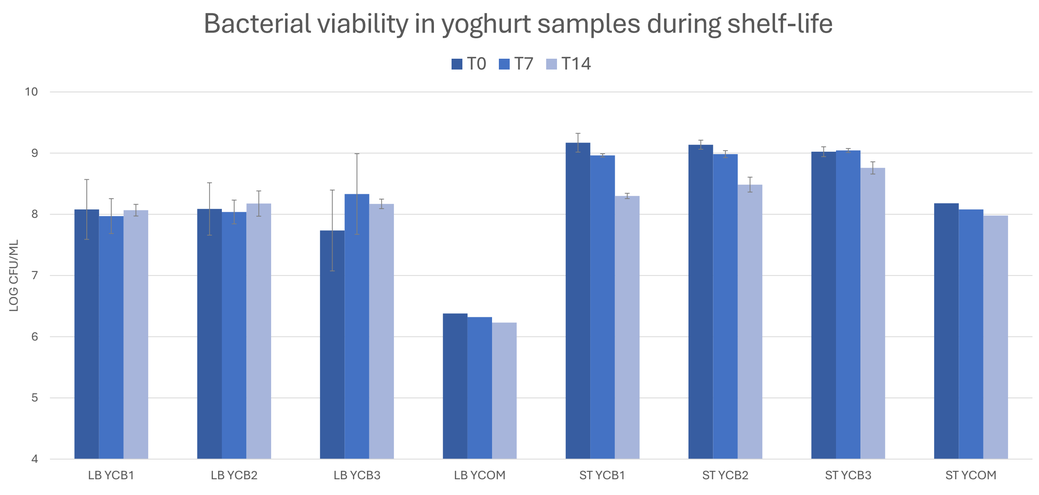


Fig. 2 – Concentration of *S. thermophilus* (ST) and *L. bulgaricus* (LB) strains in yoghurt samples.

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CONCLUSIONS

- *S. thermophilus* strains ST07, ST292 and ST658, and *L. bulgaricus* UC8085 were identified as the most efficient producers of folate and were selected as starter cultures for yoghurt production.
- Lab-scale yogurts exhibited a steady increase in folate content throughout shelf life, unlike the commercial control.
- The study has demonstrated that the folate content of yoghurt can be enhanced through the biofortification of lactic acid bacteria. Further investigations are recommended to optimise this potential.

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