Proceedings of the 10th IFAC Conference on Foundations of Systems Biology in Engineering, Corfu Island, Greece, September 8-11, 2024



# Decoding *Lactobacillus bulgaricus* Metabolism: Insights from a Dynamic Genome-Scale Model

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**Abstract:** *Lactobacillus delbrueckii subsp. bulgaricus* is one of the two indispensable bacteria constituting the yoghurt consortium. This work delves deeper into the dynamics of its metabolism in fermentation conditions. Our study combines a kinetic and a genome-scale model into a dynamic flux balance analysis to recover extracellular metabolite dynamics and pH effects on biomass growth, substrate uptake and by-product secretion. The dynamic model is based on experimental data and provides valuable insights into casein degradation and uptake by the cells.

*Keywords:* Kinetic model, Fermentation process, *Lactobacillus bulgaricus*, Model selection & optimization, Genome-Scale Metabolic Model, Microbial interactions

## 1. INTRODUCTION

The study of the interaction mechanisms between *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* is essential to understanding yoghurt production and designing novel fermented foods (Sieuwerts, 2016).

*S. thermophilus* usually is weakly or non-proteolytic, and *L. bulgaricus* is responsible for the degradation of casein - the most prolific protein in milk - into peptides, thereby enabling the uptake of nitrogen sources for both species. It can also release free amino acids in the media, providing more nutrients (Ulmer et al., 2022).

In this work, we delve deeper into the dynamics of its metabolism by formulating a dynamic genome-scale model in fermentation conditions. The model combines a kinetic dynamic model, describing the dynamics of external fluxes, with a metabolic reconstruction (Mendoza Farías, 2024) embedded into a dynamic flux balance analysis approach (dFBA, Orth et al (2010); Mahadevan et al (2002)) scheme.

The dynamic model was able to efficiently recover the available experimental data. It captures the pH effect on metabolite uptake, secretion and biomass growth. In addition, the model brought novel insights into the dynamics of metabolism of *L. bulgaricus*, into casein degradation and uptake.

By predicting bacterial growth, casein degradation and uptake, uptake of sugars and lactic acid production, this dynamic genome-scale modelling might aid in designing and controlling the fermentation process, leading to improved yoghurt production strategies.

## 2. METHODS

### *2.1 Experimental data*

Data used to formulate the model were taken from Ulmer et al. (2022). The bacteria were grown in a minimal synthetic medium for both species, where acidification was unconstrained and correlated with the concentration of Lactic acid. The medium, in opposition to milk media, enabled the analysis of biomass growth and the study of the proteolytic activity of the strains.

Time-series data of biomass ($gDW/L$) in mono-culture were measured using flow cytometry while quantities of fermentation products and substrates (sugars, organic acids and amino acids) ($g/L$) were determined through High-Performance Liquid Chromatography (HPLC) analysis.

### *2.2 Kinetic model selection and optimization*

Once the kinetic model is formulated as a set of ordinary differential equations (ODE), optimizing the model comes down to computing the model structure and performing parameter estimation. This means choosing the set of parameters minimizing the distance between the simulation and the experimental data. The estimation, formulated as a non-linear optimization problem, is automated here using the AMIGO2 Toolbox (Balsa-Canto et al., 2016).

The model-building process required several formulations to improve the quality of predictions while guaranteeing the identifiability of the parameters. Model selection was achieved by comparing the R-squared values to assess the goodness of fit and the Akaike criterion to select the most parsimonious model.

### 2.3 Dynamic genome-scale model

The ordinary differential equations are solved using a MATLAB-based ODE solver as implemented in AMIGO2. For each time point, the fluxes are computed and used to constrain inputs and outputs of the genome-scale metabolic reconstruction of *L. bulgaricus*. Parsimonious FBA (pFBA) is then used to compute the dynamics of the intracellular fluxes using the COBRA Toolbox (Henriques et al., 2021; Schellenberger et al, 2011).

## 3. RESULTS

The dynamic model is based on Monod-type and mass action law equations. The growth of biomass ($gDW/L/h$), in addition to the predicted pH dependency, rely on the uptake of the assumed limiting substrates: lactose and casein peptides ($g/L$). In parallel, the cells can consume and produce glucose and release free amino acids and lactic acid. Figure 1 presents a schematic representation of the mechanisms included in the final model.

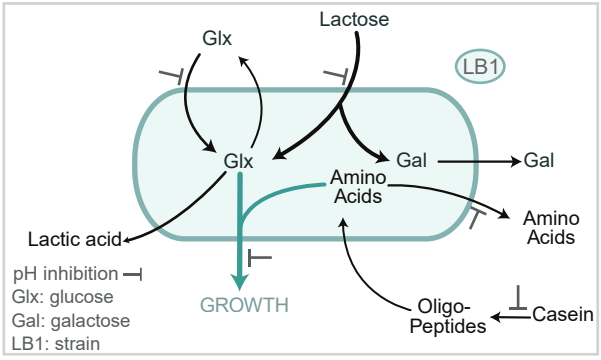


Fig. 1. Schematic representation of the model mechanisms.

The selected model successfully recovers the dynamics observed in the experiments with an R-squared value of $R^2 = 0.976$ (see Figure 2). This result gives us confidence that the mechanisms finally incorporated in the model are close to the real behaviour of the system.

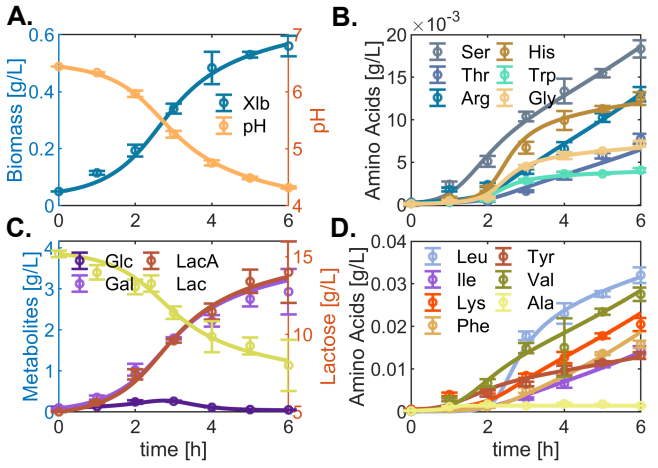


Fig. 2. Model simulations vs experimental data. The dynamics of **A.** Biomass, pH and **C.** glucose, galactose, Lactic acid, lactose are displayed. Plots **B.** and **D.** show amino acids dynamics.

The dFBA enabled us to estimate the flux of casein being degraded then consumed and its composition in amino acid proportions. Note that the casein composition resulting from the dFBA agrees with previously published results and available databases (see Figure 3).

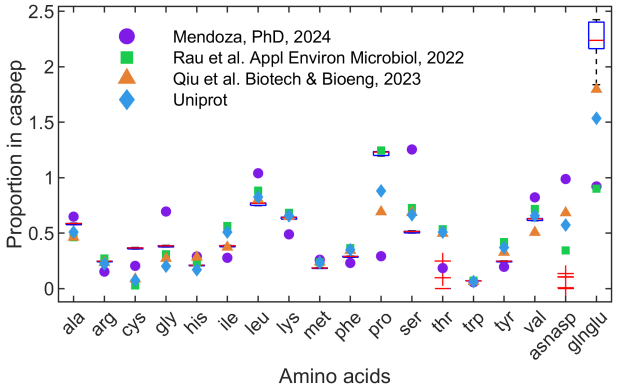


Fig. 3. Casein composition: comparison with other sources of data. The red lines correspond to the results obtained in this work.

Importantly, this information was used as a reference to introduce a description of the peptide dynamics in the dynamic model. This brings the possibility to formulate a dynamic model describing the co-culture with *S. thermophilus*.


## ACKNOWLEDGEMENTS

This work was funded by e-MUSE MSCA-ITN-2020 European Training Network under the Marie Sklodowska-Curie Grant Agreement No. 956126 and GAIN–Xunta de Galicia Grant IN607B 2023/04 and Contrato Programa.


## REFERENCES


Balsa-Canto, E., et al. (2016). AMIGO2, a toolbox for dynamic modeling, optimization and control in systems biology. *Bioinformatics*, 32(21), 3357–3359.

Sieuwerts, S. (2016). Microbial Interactions in the Yoghurt Consortium: Current Status and Product Implications. *SOJMID*,4(2), 1–5.

Ulmer, A., et al.(2022) Differential Amino Acid Uptake and Depletion in Mono-Cultures and Co-Cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* in a Novel Semi-Synthetic Medium. *Microorganisms*, 10(9), 1771.

Henriques, D., et al.(2021) A Multiphase Multiobjective Dynamic Genome-Scale Model Shows Different Redox Balancing among Yeast Species of the *Saccharomyces* Genus in Fermentation. *mSystems*, 6(4), 00260–21.

Mendoza Farías, S.N.(2024) Metabolic modeling of microorganisms and dynamics of microbial communities in the food industry. PhD, Vrije Universiteit Amsterdam.

Schellenberger, J., et al.(2011) Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox v2. 0 *Nat. Protoc.*, 6(9), 1290.

Orth, J.D., et al.(2010) What is flux balance analysis? *Nat. Biotechnol.*, 28(3), 245–248.

Mahadevan, R., et al(2002) Dynamic flux balance analysis of diauxic growth in *Escherichia coli*. *Biophys. J.*, 83(3), 1331–1340.