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Research Article

Ab Initio Whole Cell Kinetic Model of *Limosilactobacillus fermentum* EFEL6800 (lfeTS24)

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Abstract

Limosilactobacillus fermentum is found in the gastrointestinal tract of various animals, including human, with potential probiotic properties. A recent study shows that kale juice fermented with *L. fermentum* EFEL6800 has high kaempferol and quercetin levels, suggesting it a potential candidate for further metabolic engineering for probiotic applications. Kinetic model (KM) is an important tool to guide metabolic engineering but there is no KM of *L. fermentum* to-date. In this study, we present a whole cell simulatable KM for *L. fermentum* EFEL6800, IfeTS24, constructed using *ab initio* approach by identifying enzymes from its genome. The resulting model consists of 931 metabolites, 302 enzymes with corresponding transcriptions and translations, and 853 enzymatic reactions. This can be a baseline model for incorporating other cellular and growth processes, or as a system to examine cellular resource allocations necessary for engineering.

Keywords: *Limosilactobacillus fermentum EFEL6800 (lfeTS24); Kinetic Model (KM); Ab Initio*

Introduction

Limosilactobacillus fermentum is a Gram-positive, heterofermentative bacterium with potential probiotic properties [1]. It is found in the gastrointestinal tract of birds, pigs, and humans [2]. It is also found to have potential immune modulation capacity in the gut [3]; with studies demonstrating its potential capacity in modulating gut microbiome [4] to improve gut health [5], displacing *Helicobacter pylori* (the main causative pathogen for gastric ulcers) [6], displaying anti-fungal [7] and anti-inflammatory [8] properties. Specifically, kale juice fermented with *L. fermentum* EFEL6800 has high kaempferol and quercetin levels [9] - both have been shown to have antioxidant, anti-inflammatory, and anticancer properties [10,11]. This makes *L. fermentum* EFEL6800 a potential candidate for further metabolic engineering for probiotic applications.

Mathematical modelling using kinetic models (KMs) is an important tool to guide metabolic engineering approaches [12,13]. KMs use ordinary differential equations (ODE) to define the rate of metabolite concentration changes [14]; thereby, provide time-course profile of modelled metabolites [15-17]. However, there is no KM of *L. fermentum* to-date. As such, this study aims to construct a KM of *L. fermentum* EFEL6800 using *ab initio* approach by identifying enzymes from its genome, and identifying the corresponding reaction from KEGG [18]. The result is a whole cell KM of *L. fermentum* EFEL6800, named as lfeTS24 using the nomenclature proposed by Cho and Ling [15], which consists of 931 metabolites, 302 enzymes with corresponding transcriptions and translations, and 853 enzymatic reactions.

Methods

The reactome of *L. fermentum* EFEL6800 was identified from its genome (Accession number NC_017999.1) via identification of enzymatic genes using the process previously described [19,20]. The end result was a list of enzymes, a list of substrates and products of each enzymatic reactions, and a list of metabolites deduced from the substrates and products. The production of each enzyme was modelled as the production of mRNA and peptide as previously described [20]. The model was constructed in accordance to AdvanceSyn Model Specification [21], and tested for simulatability.

Initial concentrations of all mRNA and enzymes were set to 0 mM. Initial concentrations of all metabolites were set to 1 mM except the following which were set to 1000 mM: (i) C00001 (Water), (ii) C00002 (ATP), (iii) C00003 (NAD+), (iv) C00004 (NADH), (v) C00005 (NADPH), (vi) C00006 (NADP+), (vii) C00007 (Oxygen), (viii) C00008 (ADP), (ix) C00011 (carbon dioxide), (x) C00014 (Ammonia), (xi) C00025 (L-Glutamate), (xii) C00031 (D-Glucose), (xiii) C00037 (Glycine), (xiv) C00041 (L-Alanine), (xv) C00047 (L-Lysine), (xvi) C00049 (L-Aspartate), (xvii) C00064 (L-Glutamine), (xviii) C00065 (L-Serine), (xix) C00073 (L-Methionine), (xx) C00078 (L-Tryptophan), (xxi) C00079 (L-Phenylalanine), (xxii) C00082 (L-Tyrosine), (xxiii) C00123 (L-Leucine), (xxiv) C00133 (D-Alanine), (xxv) C00135 (L-Histidine), (xxvi) C00148 (L-Proline), (xxvii) C00221 (beta-D-glucose), and (xxvii) C00267 (alpha-D-Glucose). The model was simulated using the fourth-order Runge-Kutta method [22,23] from time zero to 3600 seconds with timestep of 0.1 second, and the concentrations of metabolites were bounded between 0 millimolar and 1000 millimolar. The simulation results were sampled every 2 seconds.

Results and Discussion

The annotated genome of *L. fermentum* EFEL6800 consists of 2149 genes. 302 unique EC with identifiable reactions from KEGG [18] were identified from the 2149 genes. From these 302 unique EC numbers, 853 enzymatic reactions involving 931 metabolites were identified and developed into a model based on AdvanceSyn Model Specification [21]. In addition, 604 ODEs acting as placeholder for enzyme transcriptions and translations were added.

The resulting model, denoted as lfeTS24, was simulated using AdvanceSyn Toolkit [21]. Our simulation results (Figure 1) suggests that the model is free from syntax error as the presence of simulation results suggests that the constructed model can be simulated. Hence, we present a simulatable whole cell KM of *L. fermentum* EFEL6800, which can be a base template for incorporating other cellular and growth processes [24-26], or as a system to examine cellular resource allocations [27-30].

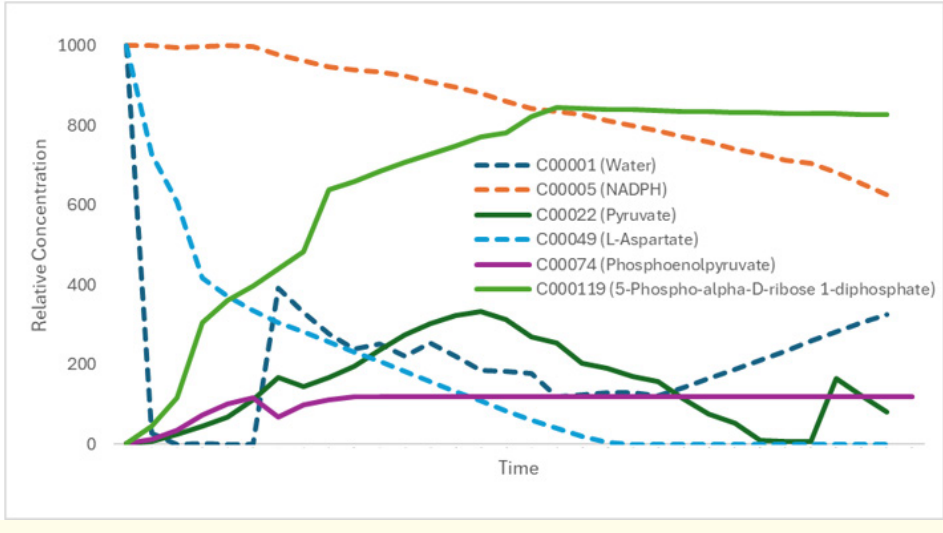

Figure 1: *Selection of simulation results.*

Conclusion

In this study, we present an *ab initio* whole cell kinetic model of *L. fermentum* EFEL6800, lfeTS24; comprising of 931 metabolites, 302 enzymes with corresponding transcriptions and translations, and 853 enzymatic reactions.

Supplementary Materials

Reaction descriptions and model can be download from https://bit.ly/lfeTS24.

Conflict of Interest

The authors declare no conflict of interest.

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