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Research Article

Ab Initio Whole Cell Kinetic Model of *Lactobacillus acidophilus* NCFM (lacAS24)

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Abstract

Lactobacillus acidophilus is a commonly used probiotic that offers numerous health benefits in the human gut, particularly in addressing various disorders. *L. acidophilus* North Carolina Food Microbiology (NCFM), a specific and well-characterized strain, has been classified by the US FDA as "Generally Recognized As Safe" (GRAS) for inclusion in dairy fermentation and probiotic formulations, highlighting its potential for engineered probiotic applications. Mathematical kinetic models allow for the development of time-course profiles for the metabolites produced by these bacteria, which can be used in future metabolic engineering or synthetic biology projects but a whole cell kinetic model *L. acidophilus* NCFM has not yet been established. In this study, a whole-cell simulatable model of *L. acidophilus* NCFM (lacAS24) was developed using an *ab initio* approach, identifying enzymes based on its published genome. The resulting model encompasses 580 metabolites, 231 enzymes with 581 enzymatic reactions. This preliminary model provides a basis for further incorporating additional cellular functions or novel growth mechanisms supporting future advances in biotechnology.

Keywords: *Lactobacillus acidophilus*; lacAS24; North Carolina Food Microbiology; Kinetic Model; Differential Equations; AdvanceSyn Toolkit

Introduction

Lactobacillus acidophilus NCFM was isolated from the human gastrointestinal tract in the late 1970s as part of a study aimed at identifying probiotic bacteria capable of surviving gastric conditions, colonizing the gut and providing health benefits [1,2]. Functional studies have revealed several strain-specific genetic determinants that contribute to its hallmark characteristics; such as, enhanced adhesion intestinal mucosa, high acid and bile tolerance and immunomodulatory effects [3-6]. As a result, US FDA has granted it "Generally Recognized as Safe" (GRAS, GRN No. 357) status for use in probiotic formulations and dairy fermentation. This renders *L. acidophilus* NCFM as a promising candidate for the mucosal delivery of vaccines and biotherapeutics [7].

Mathematical modelling is an important aspect in both metabolic engineering and synthetic biology as it can predict biological phenotypes under metabolic perturbations, which can be used to guide engineering approaches [8-10] including biotherapeutic engineering [11]. Kinetic Models (KMs) use Ordinary Differential Equations (ODE) to define the rate of change of concentrations of the metabolites involved, which offers a transient dynamic approach as it provides specific solutions in time for steady-state fluxes from the initial concentration of the substrates [12,13]. This allows KMs to address the relationships between flux, enzyme expression, metabolite levels and regulation; and provide time-course profile of modelled metabolites [14-16].

However, there is no KM of *L. acidophilus* to-date. As such, this study aims to construct a KM of *L. acidophilus* NCFM using *ab initio* approach by identifying enzymes from its published genome [5] and identifying the corresponding reaction from KEGG [17]. The result is a whole cell KM of *L. acidophilus* NCFM, named as lacAS24 using the nomenclature proposed by Cho and Ling, which consists of 580 metabolites, 231 enzymes with corresponding transcriptions and translations and 581 enzymatic reactions [14].

Material and Methods

Identification of Reactome

The genome of *Lactobacillus acidophilus* NCFM (Accession number CP000033.3) [18] was used as source to identify enzymatic genes using the process described in Kwan, et al., [19]. Briefly, each enzymatic gene was identified as a presence of complete Enzyme Commission (EC) number in the GenBank record or via the coding sequence's protein ID or locus tag. Each EC number is then mapped into reaction IDs via KEGG Ligand Database for Enzyme Nomenclature [17]. For example, lactate dehydrogenase (EC 1.1.1.27) maps to https://www.genome.jp/entry/1.1.1.27, showing three reactions: R00703 (C00186 and C0003 to produce C00022, C00004 and C00080), R001000 (C05984 and C00003 to produce C00109, C00004 and C00080) and R03104 (C05823 and C00003 to produce C00957, C00004 and C00080).

Model Development

Given that the number of RNA polymerase per *Escherichia coli* cell is 3000 (BioNumbers 106199) [20] where about 25% of the RNA polymerases are active (BioNumbers 111676) with the polymerization rate of 22 ribonucleotides per second (BioNumbers 104109) and the average ribonucleotide is 339.5 Daltons, the total mRNA synthesis rate per *E. coli* cell can be estimated as 5600 kDa per second [21,22]. One Dalton is 1.66054e-24 gram; hence 5600 kDa per second is 9.3e-18 grams per second. Given that the volume of one *E. coli* cell is about 0.7 cubic micrometres or 7e-16 litres with 4225 protein-coding genes (BioNumbers 105443), the total mRNA synthesis rate can be estimated at 2.92 uM per protein-coding genes per second [23,24]. The average lifespan estimated from 11 *E. coli* mRNA transcripts is 1.79 minutes (BioNumbers 107666) or 107.56 seconds; therefore, 0.93% degraded per second [25]. Therefore, the rate law for mRNA concentration can be written as d[mRNA]/dt = (0.00292 - 0.0093[mRNA]) mM per second.

Given that the median protein synthesis in mammalian cell culture is 1000 peptides per mRNA transcript per hour (BioNumbers 106382), which equates to 0.278 peptides per mRNA transcripts per second and the average protein degradation rate for *E. coli* is about 1% per hour (BioNumbers 109924), which equates to 0.00000278 per second; the rate law for peptide concentration can be written as d[peptide]/dt = (0.278[mRNA] - 0.00000278[peptide]) uM per second [26,27].

The reactome was modelled as a set of Ordinary Differential Equations (ODEs) where each ODE represented one metabolite concentration as previously described [19,28]. The k_{cat} and K_m were set at 13.7 per second and 1 millimolar, respectively; which were the median values from a survey of more than 1000 enzymes by Bar-Even, et al., [29]. The model was written in accordance to AdvanceSyn Model Specification [30].

Model Simulation

The constructed model was tested for simulatability using AdvanceSyn Toolkit [30]. 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) C00011 (Carbon Dioxide), (ix) C00014 (Ammonia), (x) C00025 (L-Glutamate), (xi) C00031 (D-Glucose), (xii) C00037 (Glycine), (xiii) C00041 (L-Alanine), (xiv) C00047 (L-Lysine), (xv) C00049 (L-Aspartate), (xvi) C00064 (L-Glutamine), (xvii) C00065 (L-Serine), (xviii) C00073 (L-Methionine), (xix) C00097 (L-Cysteine), (xx) C00133 (D-Alanine), (xxi) C00148 (L-Proline). The model was simulated using the fourth-order Runge-Kutta method 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 [31,32]. The simulation results were sampled every 2 seconds.

Results and Discussion

The annotated genome of *L. acidophilus* NCFM consists of 1928 genes, including 1775 protein coding sequences [18]. 772 EC numbers; of which, 581 are unique with identifiable reactions from KEGG [17]. From these 581 unique EC numbers, 231

enzymatic reactions involving 580 metabolites were identified and developed into a model based on AdvanceSyn Model Specification [30]. In addition, 260 ODEs acting as placeholder for enzyme transcriptions and translations were added.

The resulting model, denoted as lacAS24, was simulated using AdvanceSyn Toolkit [30]. Our simulation results (Fig. 1) suggest that the model is free from syntax error as the simulation results illustrate the fluctuations in metabolite concentrations over time, which are indicative of dynamic enzyme-substrate interactions within the kinetic model. These fluctuations highlight the complex biochemical processes occurring in the system. Specifically, the concentration of NAD+ (C00003) decreases over time, suggesting that it is being consumed or utilised in reactions as it functions as an electron carrier in redox reaction such as glycolysis or even during fermentation. Thus, monitoring the changes in the concentration of NAD+ over time using the kinetic model presented in this study can allow researchers to infer that the organism is actively metabolising releasing energy [33]. If NAD+ is being rapidly consumed, it can point towards increased cellular growth or stress responses. In contrast, the concentration of phosphoric acid (C00009) exhibits noticeable fluctuations, reflecting its involvement in various biochemical reactions within the organism, possibly as part of phosphate homeostasis which is a common physiological process in many bacterial species including *L. acidophilus* [34]. Fluctuations in the phosphate pool in the organism could also be attributable to increased demand for phosphates which are required for nucleic acid synthesis by organisms experiencing rapid cell division. These patterns suggest that the metabolic network is actively adjusting to maintain homeostasis, with metabolites being continuously cycled, utilized and replenished as part of the organism's ongoing metabolic processes.

However, these simulation results cannot be taken at face value as all enzyme kinetics (turnover number and Michaelis-Menten constant) are kept the median levels [29]. Hence, we present a simulatable whole cell KM of *L. acidophilus* NCFM, which can be a base template for incorporating other cellular and growth processes or as a system to examine cellular resource allocations [35-41]. At the same time, it can also be used to simulate different combinations of probiotics with supplement to identify possible synergistic effects as Kim, et al., suggest a synergistic effect between *L. acidophilus* and prebiotic Curcuma Longa Rhizome Extract (CLE) [42].

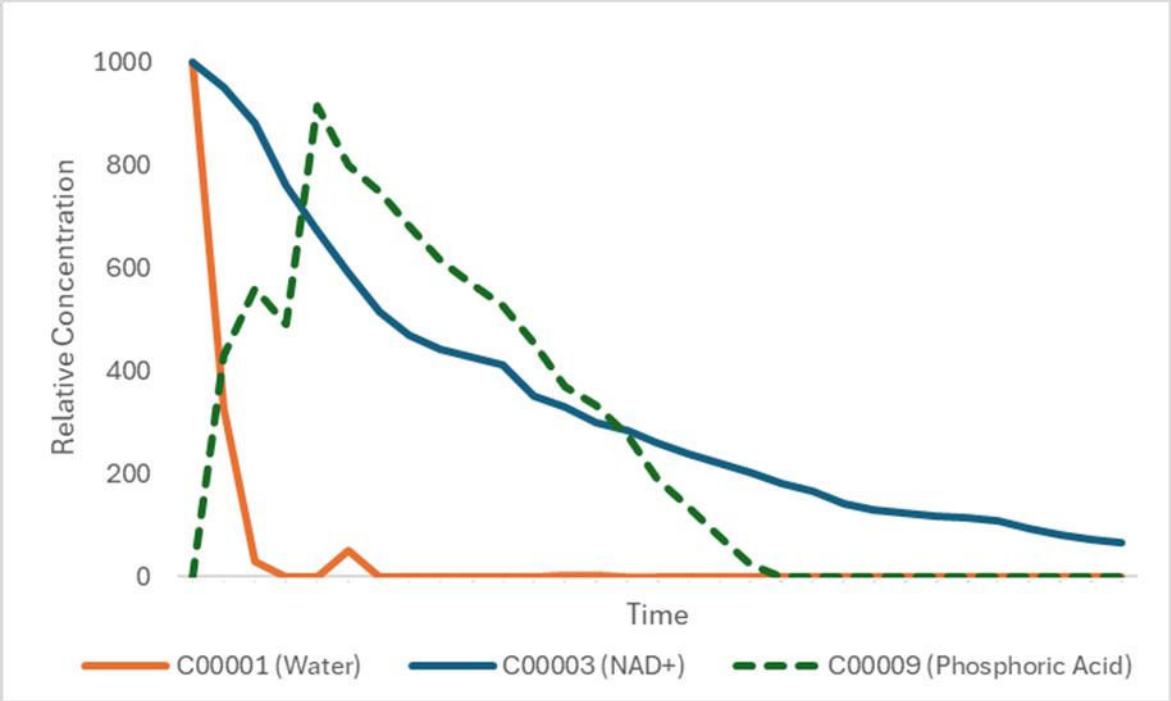

Figure 1: Selection of simulation results.

Conclusion

In this study, we present an Ab initio whole cell kinetic model of *Lactobacillus acidophilus* NCFM built from the enzymes from its genomic sequence. The resulting kinetic model, lacAS24; comprising of 580 metabolites, 231 enzymes with corresponding transcriptions and translations and 581 enzymatic reactions

Supplementary Materials

Reaction descriptions and model can be download from https://bit.ly/lacAS24

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

All authors have contributed equally to the final manuscript.

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