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Review Article

Ab Initio Whole Cell Kinetic Model of *Bifidobacterium bifidum* BGN4 (bbfMA24)

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Abstract

Bifidobacterium bifidum is a common probiotic in human gut and has been shown to be beneficial in many disorders. B. bifidum BGN4 is recognised by US FDA as "generally recognised as safe" for use in infant formulations among other food applications, leading to potential engineered probiotics applications. Mathematical kinetic models provide time-course profile of modelled metabolites, which can be used to guide metabolic engineering approaches. However, there is no kinetic model of *B. bifidum* to-date. In this study, we present a whole cell simulatable kinetic model of *B. bifidum* BGN4, bbfMA24, constructed using ab initio approach by identifying enzymes from its published genome. The resulting model consists of 236 metabolites, 68 enzymes with corresponding transcriptions and translations, and 162 enzymatic reactions; which 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: *Bifidobacterium bifidum*; bbfMA24

Introduction

Bifidobacterium bifidum is a Gram-positive, anaerobic, probiotic bacterium in human gut [1,2]. As a probiotic, it has been shown to reduce irritable bowel syndrome [3,4], eczema [5], non-alcoholic fatty liver disease [6], neuropsychiatric disorders [7], and neurodegenerative disorders [8]. In 2019, US FDA has classified *B. bifidum* BGN4 to be "generally recognised as safe" for use in infant formulations among other food applications (GRAS GRN No. 814). Recently, Kang., *et al*. [9] engineered *B. bifidum* BGN4 to produce superoxide dismutase, catalase, and interleukin-10 as probiotics to remedy irritable bowel syndrome; suggesting potential engineered probiotics applications [10-12].

Mathematical modelling is an important aspect in both metabolic engineering and synthetic biology [13] as it can predict biological phenotypes under metabolic perturbations, which can be used to guide engineering approaches [14,15]. Kinetic models (KMs) use ordinary differential equations (ODE) to define the rate of change of concentrations of the metabolites involved [16], which offers a transient dynamic approach as it provides specific solutions in time for steady-state fluxes from the initial concentration of the substrates [17]. This allows KMs to address the relationships between flux, enzyme expression, metabolite levels, and regulation; and provide time-course profile of modelled metabolites [18-20].

However, there is no KM of *B. bifidum* to-date. As such, this study aims to construct a KM of *B. bifidum* BGN4 using *ab initio* approach by identifying enzymes from its published genome [21], and identifying the corresponding reaction from KEGG [22]. The result is a whole cell KM of *B. bifidum* BGN4, named as bbfMA24 using the nomenclature proposed by Cho and Ling [18], which consists of 236 metabolites, 68 enzymes with corresponding transcriptions and translations, and 162 enzymatic reactions

Materials and Methods

Identification of reactome

The genome of *Bifidobacterium bifidum* BGN4 (Accession number NC_017999.1) [21] was used as source to identify enzymatic genes using the process described in Kwan., *et al*. [23]. 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 Nomen-

clature [22]. For example, EC 1.1.1.23 (https://www.genome.jp/entry/1.1.1.23) catalyses reactions R01158, R01163, and R03012; where the substrates and products of each rection can be identified.

Model development

Given that the number of RNA polymerase per *Escherichia coli* cell is 3000 (BioNumbers 106199) [24] where about 25% of the RNA polymerases are active (BioNumbers 111676) [25] with the polymerization rate of 22 ribonucleotides per second (BioNumbers 104109) [26], 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. 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 [27] or 7e-16 litres with 4225 protein-coding genes (BioNumbers 105443) [28], the total mRNA synthesis rate can be estimated at 2.92 uM per protein-coding genes per second. The average lifespan estimated from 11 *E. coli* mRNA transcripts is 1.79 minutes (BioNumbers 107666) [29] or 107.56 seconds; therefore, 0.93% degraded per second. 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) [30], which equates to 0.278 peptides per mRNA transcripts per second; and the average protein degradation rate for E. coli is about 1 percent per hour (BioNumbers 109924) [31], 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.

The reactome was modelled as a set of ordinary differential equations (ODEs) where each ODE represented one metabolite concentration as previously described [23,32]. 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*. [33]. The model was written in accordance to AdvanceSyn Model Specification [34].

Model simulation

The constructed model was tested for simulatability using AdvanceSyn Toolkit [34]. 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) C00008 (ADP), (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-asparate), (xvi) C00064 (L-glutamine), (xvii) C00065 (L-serine), (xviii) C00073 (L-methionine), (xix) C00097 (L-cysteine), (xx) C00133 (D-alanine), (xxi) C00135 (L-histidine), and (xxii) C00148 (L-proline). The model was simulated using the fourth-order Runge-Kutta method [35,36] 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 *B. bifidum* BGN4 [21] consists of 1854 genes, including 1729 protein coding sequences. 463 EC numbers; of which, 68 are unique with identifiable reactions from KEGG [22]. From these 68 unique EC numbers, 162 enzymatic reactions involving 236 metabolites were identified and developed into a model based on AdvanceSyn Model Specification [34]. In addition, 136 ODEs acting as placeholder for enzyme transcriptions and translations were added.

The resulting model, denoted as bbfMA24, was simulated using AdvanceSyn Toolkit [34]. 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. Although our simulation results show that production of hydrogen sulfide is higher than that of total protein pool, this cannot be taken at face value as all enzyme kinetics (turnover number and Michaelis-Menten constant) are kept the median levels [33]. Hence, we present a simulatable whole cell KM of *B. bifidum* BGN4, which can be a base template for incorporating other cellular and growth processes [37-39] or as a system to examine cellular resource allocations [40-43].

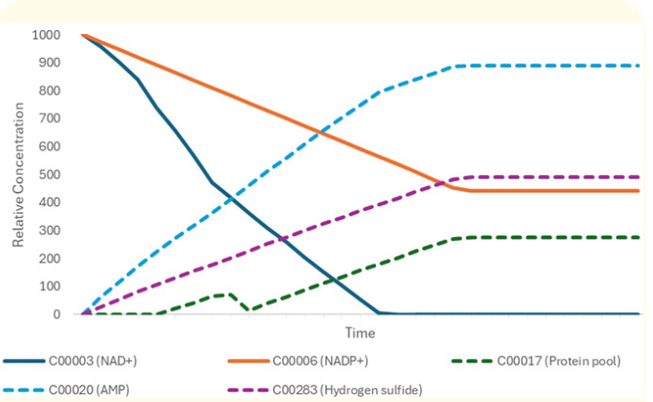

Figure 1: Selection of Simulation Results.

Conclusion

In this study, we present an *ab initio* whole cell kinetic model of *Bifidobacterium bifidum* built from the enzymes found in the genomic sequence of *B. bifidum* BGN4. The resulting kinetic model, bbfMA24; comprising of 236 metabolites, 68 enzymes with corresponding transcriptions and translations, and 162 enzymatic reactions.

Supplementary Materials

Reaction descriptions and model can be download from https://bit.ly/bbfMA24.

Conflict of Interest

The authors declare no conflict of interest.

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