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Research Article

Four Ab *Initio* Whole Cell Kinetic Models of *Bacillus subtilis* 168 (bsuLL25) 6051-HGW (bshSM25), N33 (bsuN33SS25), FUA2231 (bsuGR25)

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Abstract

Bacillus subtilis has been used in synthetic biology and metabolic engineering due to its generally recognized as safe status, its tolerance to harsh conditions and high protein expression and secretion abilities. Whole cell kinetic models are useful for in silico screening and evaluation of engineering approaches prior to experimental manipulations. However, there is no whole cell KM of *B. subtilis* to date. In this study, we present four whole cell KMs of *B. subtilis* - one KM for each strain of *B. subtilis*; namely, 168, 6051-HGW, N33 and FUA2231; as models bsuLL25, bshSM25, bsuN33SS25 and bsuGR25 respectively. Each of these four KMs contain between 1300 and 1565 reactions, catalyzes by between 571 and 679 enzyme types; which uses between 1053 and 1681 metabolites. These models can be a baseline models for incorporating other cellular and growth processes or as a system to examine cellular resource allocations necessary for engineering.

Keywords: *Bacillus subtilis;* Gram-Negative Microorganism*; E. coli*

Introduction

While *Escherichia coli* is a model organism of Gram-negative microorganism [1], *Bacillus subtilis* is a model organism of Gram-positive microorganism [2]. Similar to *E. coli*, *B. subtilis* is a common cell factory for many applications [3-5] including probiotics [6] and space microbiology [7] such as potential survivability in Mars [8]. Hence, *B. subtilis* is often a candidate for applications in harsh environments [9]. Furthermore, *B. subtilis* is a Generally Recognized As Safe (GRAS) organism [10] as it has been used in food fermentation [11] and is employed in synthetic biology and metabolic engineering due to its protein expression and secretion abilities [12].

Mathematical modelling is an important tool in metabolic engineering [13] as it can predict biological phenotypes under metabolic perturbations, which can be used to guide engineering approaches [14]. Genome-Scale Models (GSMs, also known as constraint-based models) and Kinetic Models (KMs) are the two main modelling approaches [15]. Transgenes can be added into KMs easier than GSMs [16]. Furthermore, KMs can predict both rates and yield of metabolites [17] while GSMs are primarily for rates. This makes KMs a more suitable tool for in silico screening and evaluation of engineering approaches prior to experimental manipulations compared to GSMs.

Although there are GSMs of *B. subtilis*, there is no whole cell KM of *B. subtilis* to date. Hence, this study aims to construct whole cell KMs of *B. subtilis* [18-20]. Here, we present four whole cell KM of *B. subtilis* based on four strains of *B. subtilis*; namely, 168, 6051-HGW, N33, FUA2231.

Materials and Methods

Model Development

The reactomes of *B. subtilis* 168, 6051-HGW, N33, FUA2231 were identified from its genomes; Accession numbers NC_000964.3 [21, 22], NC_020507.1 [23], NZ_CP163458.1 and NZ_CP154918.1, respectively; via identification of enzymatic genes using the process previously described [24-27]. 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 [16, 27]. Briefly, the production of each enzyme was modelled as a pair of ODEs [28] - an ODE for transcription and an ODE for translation. Based on BioNumbers 106199 [29] and 111676 [30], *E. coli* cell is has about 750 active units of RNA polymerase capable of polymerizing 22 ribonucleotides per second (BioNumbers 104109) [31]. At 339.5 Daltons per ribonucleotide, the total mRNA synthesis rate at 5600 kDa per second or 9.3e-18 grams per second. An *E. coli* cell is about 0.7 cubic micrometres [32] or 7e-16 litres with 4225 protein-coding genes (BioNumbers 105443) [33], the total mRNA synthesis rate is estimated at 2.92 uM per protein-coding genes per second. The average lifespan of mRNA transcripts is 1.79 minutes (BioNumbers 107666) [34] 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. Similarly, the median protein synthesis in mammalian cell culture is 1000 peptides per mRNA transcript per hour (BioNumbers 106382) [35], 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) [36], 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 [28, 37]. The turnover number of enzyme (Kcat) and Michaelis-Menten constant (Km) were set at 13.7 per second and 1 millimolar respectively; which were the median values estimated by Bar-Even, et al. [38]. The model was written in AdvanceSyn Model Specification [39].

Model Simulation

The constructed model was tested for simulatability using AdvanceSyn Toolkit [39]. 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) C00009 (Orthophosphate), (x) C00010 (Coenzyme A), (xi) C00011 (Carbon Dioxide), (xii) C00013 (Diphosphate), (xiii) C00014 (Ammonia), (xiv) C00015 (UDP), (xv) C00016 (FAD), (xvi) C00019 (SAM), (xvii) C00020 (AMP), (xviii) C00021 (S-Adenosyl-L-Homocysteine), (xix) C00022 (Pyruvate), (xx) C00024 (Acetyl-CoA), (xxi) C00025 (L-Glutamate), (xxii) C00029 (UDP-glucose), (xxiii) C00031 (D-Glucose), (xxiv) C00035 (GDP), (xxv) C00037 (Glycine), (xxvi) C00041 (L-Alanine), (xxvii) C00047 (L-Lysine), (xxviii) C00049 (L-Aspartate), (xxix) C00062 (L-Arginine), (xxx) C00064 (L-Glutamine), (xxxi) C00065 (L-Serine), (xxxii) C00078 (L-Tryptophan), (xxxiii) C00079 (L-Phenylalanine), (xxxiv) C00080 (H+), (xxxv) C00082 (L-Tyrosine), (xxxvi) C00097 (L-Cysteine), (xxxvii) C00147 (Adenine), (xxxviii) C00148 (L-Proline) and (xxxix) C00183 (L-Valine). The model was simulated using the fourth-order Runge-Kutta method [40, 41] 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

In this study, we develop four whole cell KMs of *B. subtilis* - one model for each of the four strains (Table 1); namely, 168 (as model bsuLL25), 6051-HGW (as model bshSM25), N33 (as model bsuN33SS25) and FUA2231 (as model bsuGR25). Based on their corresponding GenBank records, the number of protein coding genes ranged from 4243 to 4634. Of which, we are able to identify between 571 to 679 unique EC numbers, which catalyzes between 1300 to 1565 reactions. These reactions used between 1053 to 1681 types of metabolites. The total number of unique EC numbers is 812 (Fig. 2); of which, 506 (62%) are common across all four strains. Notably, *B. subtilis* 168 has the most strain-specific EC numbers (n = 151 or 18.6%). This is not surprising as *B. subtilis* 168 is the first *B. subtilis* strain to be sequenced. Hence, likely to be the best annotated [42].

The four resulting models were simulated using AdvanceSyn Toolkit [39]. Our simulation results (Fig. 2) suggest that the model is free from syntax error as the presence of simulation results suggests that the constructed models can be simulated. At the same time, our simulation results show that time course concentrations of six metabolites; namely, water (C00001), coenzyme A (C00010), D-glucose (C00031), L-lysine (C00047), L-arginine (C00062) and L-glutamine (C00064); show various degrees of oscillations, suggesting that the constructed models are able to show time course differences which are characteristics of kinetic models. However, the metabolite concentrations over time 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 four simulatable whole cell KM of *B. subtitis*, which can be a base template for incorporating other cellular and growth processes [43-45] or as a system to examine cellular resource allocations [46-49].

	***Bacillus subtilis* Strains**			
	168	**6051-HGW**	**N33**	**FUA2231**
Number of Protein Coding Genes	4243	4254	4542	4634
Number of Reactions	1565	1369	1305	1300
Number of Unique EC Numbers	679	649	571	650
Number of Metabolites	1554	1681	1388	1053
Model Name	bsuLL25	bshSM25	bsuN33SS25	bsuGR25

Table 1: Statistics of kinetic models.

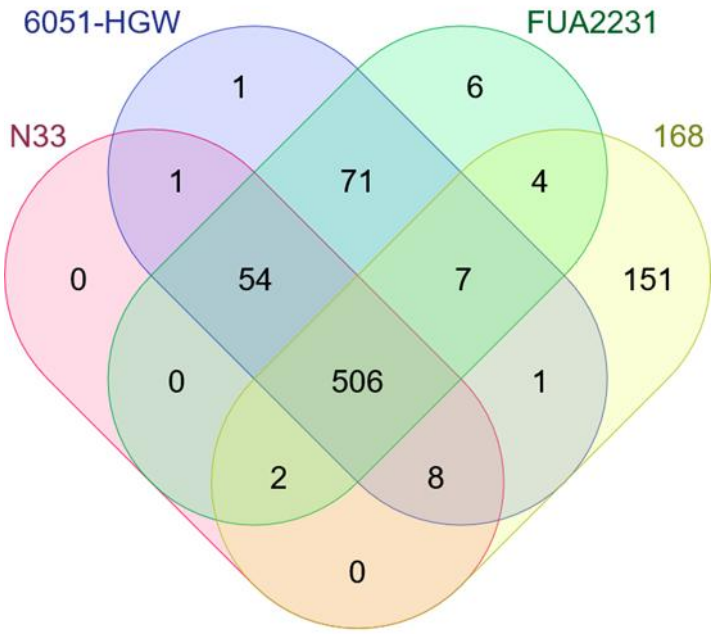

Figure 1: EC Number Commonalities. Among the 812 unique EC numbers across all 4 strains, 506 (62%) are common across all 4 strains.

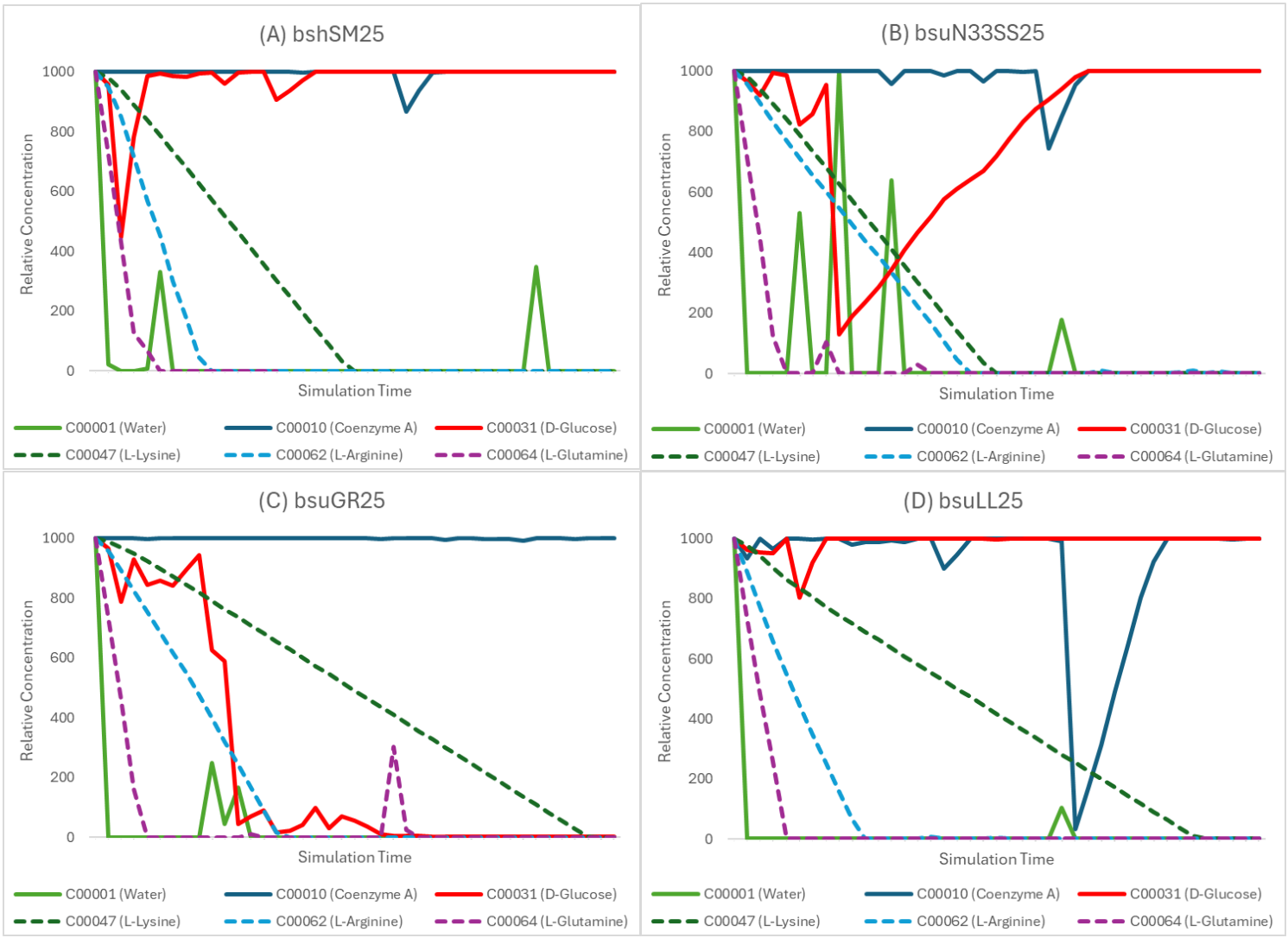

Figure 2: Selection of Simulation Results. The time course concentrations of six metabolites; namely, water (C00001), coenzyme A (C00010), D-glucose (C00031), L-lysine (C00047), L-arginine (C00062) and L-glutamine (C00064); are graphed for each of the four KMs. Panel A shows *B. subtilis* 6051-HGW (bshSM25). Panel B shows *B. subtilis* N33 (bsuN33SS25). Panel C shows *B. subtilis* FUA2231 (bsuGR25). Panel D shows *B. subtilis* 168 (bsuLL25).

Conclusion

In this study, we present four whole cell KMs of *B. subtilis* - one KM for each strain of *B. subtilis*; namely, 168 (as model bsuLL25), 6051-HGW (as model bshSM25), N33 (as model bsuN33SS25) and FUA2231 (as model bsuGR25); respectively.

Supplementary Materials

Reaction descriptions and model can be download from the following: (i) https://bit.ly/bshSM25 (for bshSM25), (ii) https://bit.ly/bsuN33SS25 (for bsuN33SS25), (iii) https://bit.ly/bsuGR25 (for bsuGR25) and (iv) https://bit.ly/bsuLL25 (for bsuLL25).

Note: Sriinithi Maiyappan constructed bshSM25. Shannon SY Sim constructed bsuN33SS25. Geeta Ramesh constructed bsuGR25. Lingxin Low constructed bsuLL25. Hence, Sriinithi Maiyappan, Shannon SY Sim, Geeta Ramesh and Lingxin Low; contributed equally to this work and should be recognized as joint first authors.

Conflict of Interest

The authors have declared no conflict of interest.

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