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Research Article

Four *Ab Initio* Whole Cell Kinetic Models of *Bacillus cereus* ATCC 14579 (bceDT26), E33L (bczDT26), F837/76 (bcfKN26) and G9842 (bcgLPT26)

Dinis Toh[1,2], Lay Ping Tan[1,2], Divya Thirunavukarasu[1,2], Kowsalya Natarajan[1,2], Wira Bin Ambel[1,2] and Maurice HT Ling[2,3,4]*

[1]*School of Health & Life Sciences, Teesside University, UK*

[2]*Management Development Institute of Singapore, Singapore*

[3]*Newcastle Australia Institute of Higher Education, University of Newcastle, Australia*

[4]*HOHY PTE LTD, Singapore*

***Corresponding Author:** Maurice HT Ling, Management Development Institute of Singapore, Singapore.

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Abstract

Bacillus cereus is a bacterium with tolerance to diverse environmental stresses and demonstrated ability to enhance plant resilience to abiotic stressors, making it a promising candidate for soil conditioning, aquaculture probiotics, and metabolic engineering applications. 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. cereus* to date. Here, we present four simulatable whole cell KMs based on four strains of *B. cereus*; namely, (i) model bceDT26 for ATCC 14579 strain, (ii) model bczDT26 for E33L strain, (iii) model bcfKN26 for F837/76 strain, and (iv) model bcgLPT26 for G9842 strain. 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 cereus*; Whole-Cell Kinetic Model; Ordinary Differential Equations; AdvanceSyn Toolkit

Introduction

Bacillus cereus is a rod-shaped, Gram-positive bacterium initially isolated by Grace Frankland and Percy Frankland in 1887 [1], with tolerance to various environmental conditions; including, heavy metals [2], alkaline [3], and heat [4]. More importantly, when found in soil, several strains of *B. cereus* have been shown to improve salt [5], drought [6], and heavy metal tolerance [7] of other plants [8]. This makes *B. cereus* a potential candidate for soil conditioning. Interestingly, probiotic potential of *B. cereus* has also been explored in aquaculture [9,10]. Hence, *B. cereus* is a versatile microorganism and not surprisingly, been considered for a wide range of industrial applications [11–13]. This gives rise to metabolic engineering of *B. cereus* [14,15].

Metabolic engineering strategies are often guided by mathematical modelling [16,17]. Genome-scale models (GSMs, also known as constraint-based models) and kinetic models (KMs) are the two main modelling approaches [18,19]. *In silico* gene knock-ins are easier in KMs compared to GSMs [20]. Yet, KMs can predict both rates and yield of metabolites [21] while GSMs are primarily for rates. This makes KMs a more suitable tool over GSMs for *in silico* screening and evaluation of competing engineering approaches prior to experimental manipulations. As such, there is

a call for building KMs [22,23]. However, there is no whole cell KM of *B. cereus* to date. Hence, this study aims to construct whole cell KMs of *B. cereus*. Here, we present four whole cell KM of *B. cereus* based on four strains of *B. cereus*; namely, ATCC 14579, E33L, F837/76, and G9842.

Materials and Methods

Identification of reactomes

Complete genomic records of *B. cereus* ATCC 14579 (Assembly GCF_045287585.1; Accession CP138336.1), E33L (Assembly GCF_000011625.1; Accession CP000001.1), F837/76 (Assembly GCF_000239195.1; Accession CP003187.1), and G9842 (Assembly GCF_000021305.1; Accession CP001186.1); were obtained. These genomic records were sources to identify enzymatic genes using the process described in Kwan., *et al*. [24]. 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 [25]. 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

The model was developed using the principles described in Sim., *et al*. [26]. Parameters pertaining to transcription and translation were used from *Escherichia coli* as it is likely to be the best prokaryote studied. In addition, these parameters were used in *Bacillus subtilis* draft kinetic models [27]. From BioNumbers, an *E. coli* cell carries around 3000 RNA polymerases (BioNumbers 106199) [28], with approximately 25% actively elongating transcripts (BioNumbers 111676) [29]. At a polymerization rate of 22 nucleotides per second (BioNumbers 104109) [30] and an average nucleotide mass of 339.5 Da, the cell synthesizes roughly 5600 kDa of RNA per second. When this is normalized to a cellular volume of about 0.7 cubic micrometres [31] and 4225 protein-coding genes (BioNumbers 105443) [32], the estimated mRNA production rate becomes 2.92 micromolar per gene per second. Using an average transcript lifespan of 107.56 seconds (BioNumbers 107666) [33] implying a decay of 0.93% per second, the governing equation is: d[mRNA]/dt = 0.00292 – 0.0093[mRNA]. For proteins, mammalian estimates suggest about 1000 peptides produced per transcript per hour (BioNumbers 106382) [34], or 0.278 peptides per second. Protein turnover in *E. coli* averages about 1% per hour (2.78e-6 of mRNA pool per second) (BioNumbers 109924) [35]. Thus, translation rate law is d[peptide]/dt = 0.278[mRNA] – 0.00000278[peptide]. The complete reactome was framed as a system of ODEs [24,36] using median kinetic constants (kcat = 13.7 per second; Km = 1 millimolar), in line with Bar-Even., *et al*. enzyme survey [37] and structured according to AdvanceSyn's modelling conventions [38].

Model simulation

The constructed model was tested for simulatability using AdvanceSyn Toolkit [38]. 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 (Phosphoric Acid), (X) C00010 (CoA), (XI) C00011 (Carbon Dioxide), (XII) C00013 (Diphosphoric Acid), (XIII) C00014 (Ammonia), (XIV) C00015 (UDP), (XV) C00016 (FAD), (XVI) C00018 (Pyridoxal Phosphate), (XVII) C00019 (SAM), (XVIII) C00020 (AMP), (XIX) C00022 (Pyruvate), (XX) C00024 (Acetyl-CoA), (XXI) C00025 (L-Glutamate), (XXII) C00026 (2-Oxoglutarate), (XXIII) C00031 (D-Glucose), (XXIV) C00033 (Acetate), (XXV) C00036 (Oxaloacetate), (XXVI) C00037 (Glycine), (XXVII) C00041 (L-Alanine), (XXVIII) C00042 (Succinate), (XXIX) C00044 (GTP), (XXX) C00047 (L-Lysine), (XXXI) C00049 (L-Aspartate), (XXXII) C00062 (L-Arginine), (XXXIII) C00063 (CTP), (XXXIV) C00064 (L-Glutamine), (XXXV) C00065 (L-Serine), (XXXVI) C00073 (L-Methionine), (XXXVII) C00074 (Phosphoenolpyruvate), (XXXVIII) C00075 (UTP), (XXXIX) C00077 (L-Ornithine), (XL) C00078 (L-Tryptophan), (XLI) C00079 (L-Phenylalanine), (XLII) C00080 (H+), (XLIII) C00082 (L-Tyrosine), (XLIV) C00086 (Urea), (XLV) C00088 (Nitrite), (XLVI) C00091 (Succinyl-CoA), (XLVII) C00092 (D-Glucose 6-phosphate), (XLVIII) C00097 (L-Cysteine), (XLIX) C00100 (Propanoyl-CoA), (L) C00103 (D-Glucose 1-phosphate), (LI) C00118 (D-Glyceraldehyde 3-phosphate), (LII) C00122 (Fumarate), (LIII) C00123 (L-Leucine), (LIV) C00135 (L-Histidine), (LV) C00149 ((S)-Malate), (LVI) C00158 (Citrate), (LVII) C00183 (L-Valine), (LVIII) C00188 (L-Threonine), (LIX) C00221 (beta-D-Glucose), (LX) C00232 (Succinate semialdehyde), (LXI) C00244 (Nitrate), (LXII) C00253 (Nicotinate), (LXIII) C00255 (Riboflavin), (LXIV) C00267 (alpha-D-Glucose), (LXV) C00311 (Isocitrate), (LXVI) C00345 (6-Phospho-D-gluconate), (LXVII) C00350 (Phosphatidylethanolamine), (LXVIII)

C00378 (Thiamine), (LXIX) C00407 (L-Isoleucine), (LXX) C00417 (cis-Aconitate), (LXXI) C00504 (Folate), (LXXII) C00533 (Nitric oxide), (LXXIII) C00631 (2-Phospho-D-glycerate), (LXXIV) C00864 (Pantothenate), (LXXV) C01172 (beta-D-Glucose 6-phosphate), (LXXVI) C05345 (beta-D-Fructose 6-phosphate), (LXXVII) C14818 (Fe2+). The model was simulated using the fourth-order Runge-Kutta method [39,40] 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 as a simple method for mass-balance constraints [41]. The simulation results were sampled every 2 seconds.

Results and Discussion

In this study, we develop four whole cell KMs of *B. cereus* – one model for each of the four strains (Table 1); namely, ATCC 14579 (as model bceDT26), E33L (as model bczDT26), F837/76 (as model bcfKN26) and G9842 (as model bcgLPT26). Based on their corresponding GenBank records, the number of protein coding genes ranged from 5259 to 5857 (Table 1). Of which, we are able to identify between 412 to 545 unique EC numbers, which catalyzes between 824 to 2418 reactions. These reactions used between 802 to 1230 types of metabolites. Of which, 682 (51.1%) of the metabolites are common across all four strains (Figure 1). In terms of unique EC numbers, the total number of unique EC numbers is 812 (Figure 2); of which, 298 (38.8%) are common across all four strains. This is substantially lower than 62% of common unique EC numbers within four strains from the clade of *Bacillus subtilis* [27], which is supported by a study suggesting that the Cereus clade has higher enzyme diversity than Subtilis clade [42].

	***Bacillus cereus* Strains**			
	ATCC 14579	**E33L**	**F837/76**	**G9842**
Number of Protein Coding Genes	5259	5641	5468	5857
Number of Reactions	1073	1765	2418	824
Number of Unique EC Numbers	545	514	529	412
Number of Metabolites	1208	1230	802	919
Model Name	bceDT26	bczDT26	bcfKN26	bcgLPT26

Table 1: Statistics of Kinetic Models.

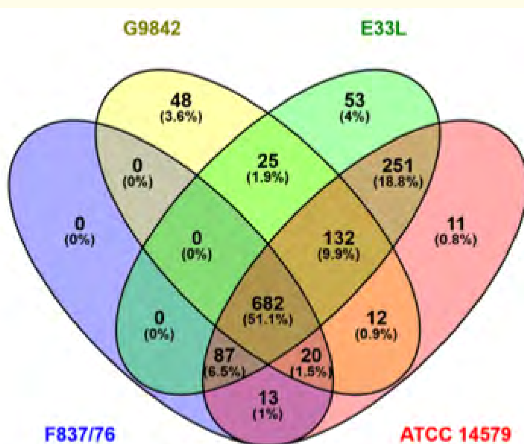


Figure 1: Metabolite Commonalities. Among the 1334 unique metabolites across all 4 strains, 682 (51.1%) are common across all 4 strains.

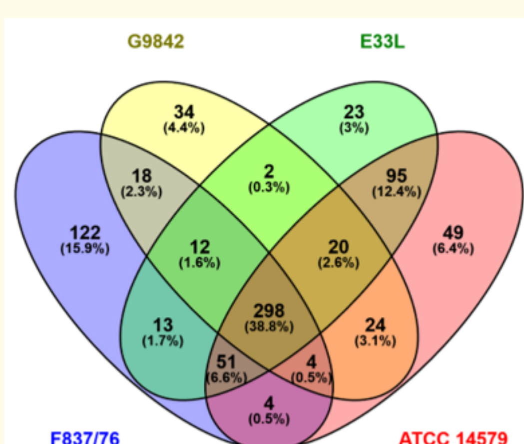
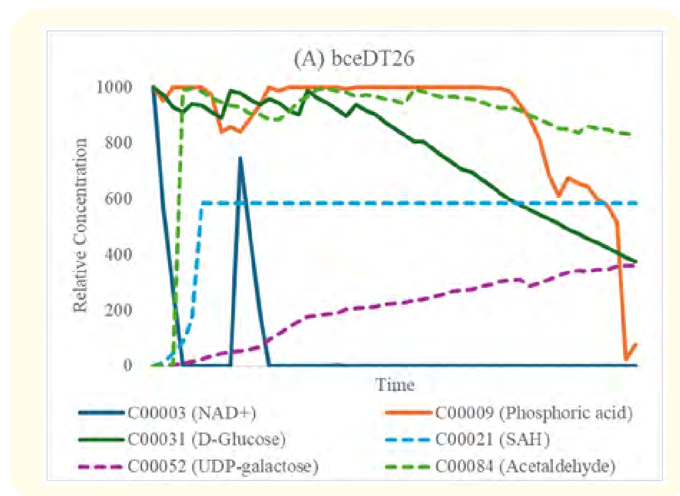


Figure 2: EC Number Commonalities. Among the 769 unique EC numbers across all 4 strains, 298 (38.8%) are common across all 4 strains.

We tested the models using the AdvanceSyn Toolkit [34], and Figure 3 demonstrates that all 4 model runs cleanly without syntax or structural errors. Considering the complexity of a whole-cell kinetic formulation, the ability to simulate it end-to-end already establishes a baseline level of correctness as previously argued [20,27,43-47] – this is defined as (a) model simulatability by the fact of completing the simulation process without errors leading to the generation of simulation results, and (b) the presence of fluctuations in simulated concentrations of metabolites; namely, (i) nicotinamide adenine dinucleotide (NAD+; C00003), (ii) phosphoric acid (C00009), (iii) D-glucose (C00031), (iv) S-adenosyl-L-homocysteine (SAH; C00021), (v) UDP-galactose (C00052), and (vi) acetaldehyde (C00084), as shown in Figure 3. Differences in simulated concentrations across the four strains; for example, phosphoric acid concentration remains high in bczDT26 but drops at different time points in the other three models; also suggests variations between different strains of *B. cereus*.

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], which removes enzyme-specific tuning and compresses biological diversity. Due of this, such trends should not be interpreted as physiological predictions. Rather, the value of this work lies in establishing four simulatable whole-cell kinetic models for *B. cereus* that future efforts can extend by adding metabolic, regulatory, or growth modules, or by incorporating more accurate organism-level kinetics [48-50] or as a system to examine cellular resource allocations [51-54]. As this study presents the 4 B. cereus KMs as draft structural but simulatable models; hence, sensitivity analysis may be carried out as part of future model refinements.



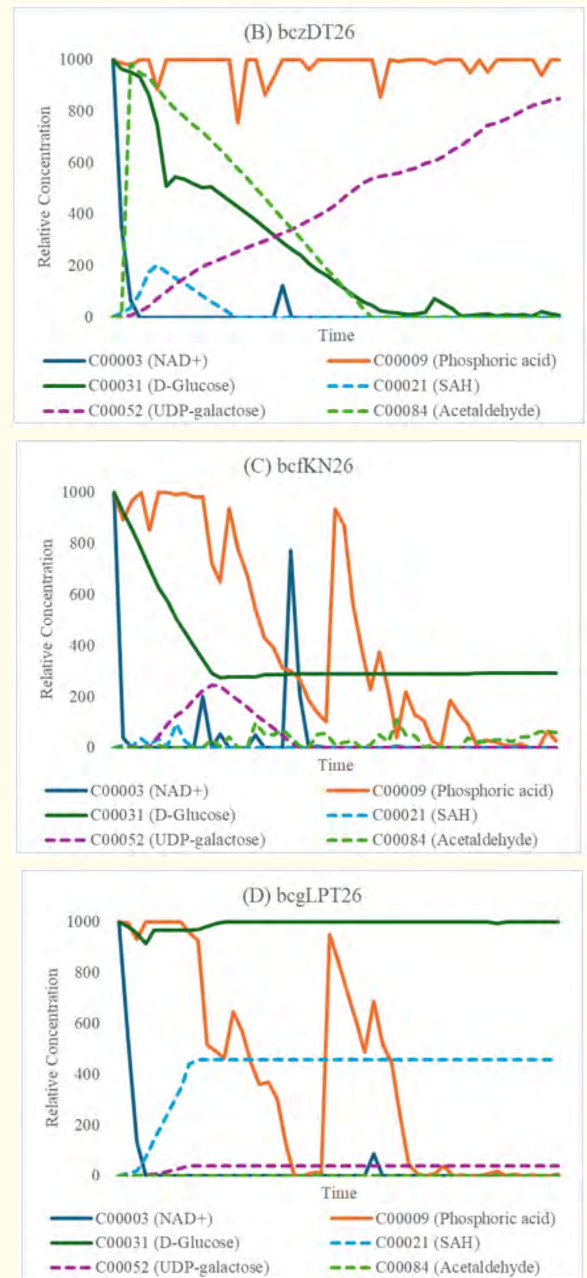


Figure 3: Selection of Simulation Results. The time course concentrations of six metabolites; namely, nicotinamide adenine dinucleotide (NAD+; C00003), phosphoric acid (C00009), D-glucose (C00031), S-adenosyl-L-homocysteine (SAH; C00021), UDP-galactose (C00052), and acetaldehyde (C00084); are graphed for each of the four KMs. Panel A shows B. cereus ATCC 14579 (bceDT26). Panel B shows B. cereus E33L (bczDT26). Panel C shows B. cereus F837/76 (bcfKN26). Panel D shows B. cereus G9842 (bcgLPT26).

Conclusion

In this study, we present four whole cell KMs of *B. cereus* – one KM for each strain of *B. cereus*; namely, ATCC 14579 (as model bceDT26), E33L (as model bczDT26), F837/76 (as model bcfKN26), and G9842 (as model bcgLPT26), respectively.

Supplementary Materials

Reaction descriptions and model can be download from the following: (i) https://bit.ly/bceDT26 (for bceDT26), (ii) https://bit.ly/bczDT26 (for bczDT26), (iii) https://bit.ly/bcfKN26 (for bcfKN26), and (iv) https://bit.ly/bcgLPT26 (for bcgLPT26).

Note

Dinis Toh constructed bceDT26. Lay Ping Tan constructed bcgLPT26. Divya Thirunavukarasu constructed bczDT26. Kowsalya Natarajan constructed bcfKN26. Hence; Dinis Toh, Lay Ping Tan, Divya Thirunavukarasu, and Kowsalya Natarajan; should be recognized as joint first authors.

Conflict of Interest

The authors declare no conflict of interest.

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Conflict of Interest

The authors declare no conflict of interest.

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