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Research Article

Adaptation of Whole Cell Kinetic Model Template, UniKin1, to *Escherichia coli* Whole Cell Kinetic Model, ecoJC20

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Abstract

Mathematical modelling can be used to study metabolism involving thousands of biochemical reactions and kinetic models (KMs) of metabolism enable the time-course analysis of metabolic changes. Recently, a universal whole cell KM of central metabolism, UniKin1, has been presented. Here, we adapt UniKin1, into an *Escherichia coli* specific model by modifying the initial concentrations for 48.7% (n = 149) of the metabolites and 25.2% (n = 78) of the enzymes into *E. coli* specific concentrations, and term our model as ecoJC20. Our simulation results suggest that ecoJC20 is substantially different from UniKin1. We also demonstrate the potential of ecoJC20 to evaluate the effects of transgenic nitrogen fixation pathways on the central metabolism; thus, underpinning the potential applications of kinetic models as an experimental design tool.

Keywords: *Whole Cell Kinetic Model; Simulation; Escherichia coli; Computer-Aided Design (CAD)*

Introduction

Mathematical models are widely used to research natural phenomenon or design complex system scientifically [1]. One of the advantages of utilizing mathematical model is that the complex information of natural phenomenon can be expressed in a concise format by abstraction [2]. For example, Michaelis-Menten equation allows to express enzyme reaction by using two parameters: inflexion point (Km) and turnover number (kcat). Likewise, mathematical models are important in biotechnology field [3] as it can be a tool to aid experimental design [4].

Among many mathematical models, KMs and GSMs are widely used in describing metabolic process [5]. GSMs contain metabolic stoichiometries, allowing us to predict the metabolic difference caused by genetic variation [6]; thus, observing the relationship between genotype and metabolism. However, metabolite concentration can also be affected by are also affected by kinetic parameters of enzymes [7], which are generally not captured in GSMs but is fundamental to KMs. This allow KMs to provide time-course profile of modelled metabolites.

Madhu., *et al*. [8] presented a universal kinetic model template, UniKin1, which can be a base model for adaptation into an organism-specific model. In this study, we adapt UniKin1 into an *E. coli* specific kinetic model, ecoJC20 and demonstrate its utility in predicting the effects of transgenic nitrogen fixation pathways on the overall metabolism of *E. coli*.

Materials and Methods

Kinetic model adaptation: The kinetic model, UniKin1 [8], which was a non-species specific model, was used as a template for adaptation into an *E. coli* model by amending default metabolite and enzyme concentrations to *E. coli* specific concentrations. This is carried out in four steps with the overarching principle to maintain relative concentrations within metabolites and enzymes separately. Firstly, the relative fluxes from *E. coli* genome-scale model, iAF1260 [9], obtained from flux balance analysis using Cameo [10] via AdvanceSyn Toolkit [11] were used to vary the initial concentrations where the average flux was deemed as default concentrations. Secondly, intracellular metabolite concentrations from Bennett., *et al*. [12] of *E. coli* cultured in glucose were used as initial metabolite concentrations. Thirdly, intracellular metabolite concentrations from Park., *et al*. [13] were used as initial metabolite concentrations. Fourthly, the measured intracellular metabolite concentrations from Tepper., *et al*. [14] of *E. coli* cultured in glucose were used as initial metabolite concentrations and the enzyme concentrations from Taniguchi., *et al*. [15] were used to transform the enzyme concentrations. Taniguchi., *et al*. [15] reported enzyme concentrations as milligrams of enzyme per gram dry weight of cell. The average was 194.7 mg enzyme per gram dry weight of cell, which was used to calibrate enzyme concentrations to the average of 0.1M [the default enzyme concentration in Madhu., *et al*. [8]]. Hence, the new enzyme concentration in the *E. coli* kinetic model can be calibrated as the enzyme concentration from Taniguchi., *et al*. [15] divided by 1947. Finally, the intracellular metabolite concentrations of *E. coli* from Park., *et al*. [16] were used as initial metabolite concentrations. In these steps, an amended metabolite or enzyme concentration in an earlier step can be re-amended in a later step.

Model simulation and analysis: Model simulation was performed using AdvanceSyn Toolkit [11]. The model was simulated using the fourth-order Runge-Kutta method [17,18] from time zero to 21600 seconds with timestep of one second, and the concentrations of metabolites were bounded between 0 millimolar and 100 millimolar. The simulation results were sampled every 600 seconds. Simulation results were analysed using end-point analysis and mean squared error (MSE). End-point analysis examined the metabolite concentration differences between the same metabolites from 2 different simulation results at 21600 seconds of simulation time. MSE examined the mean squared differences of metabolite concentrations across the time-points between the same metabolites from 2 different simulation results. Hence, end-point analysis provided steady-state differences while MSE provide profile differences.

Results and Discussion

From universal kinetic model to *Escherichia coli* model: The universal kinetic model of core metabolism, UniKin1 [8], consist 309 metabolites and 310 enzymes (Table 1). Here, we adapted UniKin1 into an *E. coli* specific kinetic model, ecoJC20 ("eco" is the KEGG organism code, "JC" is the initial of the first author, and "20" is the year when this work started), by changing default metabolite and enzyme concentrations to *E. coli* specific concentrations. 149 out of 306 (48.7%) metabolite and 78 out of 310 (25.2%) enzyme concentrations were changed into *E. coli* specific concentrations. Several studies examining intracellular metabolite concentrations show different intracellular metabolite concentrations between species [16,19,20] while conserved in central metabolic steps [21-24]; thereby, suggesting that the differences in central metabolism between different species can be elucidated by differences in intracellular metabolite and enzyme concentrations and enzyme kinetics. This further supports the theoretical basis that a template kinetic model of core metabolism can be used for developing species-specific whole cell kinetic models [8].

Stages of Adaptation	Number of [Metabolite]	Number of [Enzyme]
Stage 0: UniKin1	306 (100%)	310 (100%)
Stage 1: iAF1260	5 (1.6%)	62 (20.0%)
Unchanged after Stage 1	301 (98.3%)	248 (80.0%)
Stage 2: Bennett., *et al.* [12]	54 (17.6%)	0 (0.0%)
Unchanged after Stage 2	251 (82.0%)	248 (80.0%)
Stage 3: Park., *et al.* [13]	4 (1.3%)	0 (0.0%)
Unchanged after Stage 3	249 (81.4%)	248 (80.0%)
Stage 4: Tepper., *et al.* [14] and Taniguchi., *et al.* [15]	134 (43.8%)	29 (9.4%)
Unchanged after Stage 4	159 (52.0%)	232 (74.8%)
Stage 5: Park., *et al.* [16]	65 (21.2%)	0 (0.0%)
Unchanged after Stage 5	157 (51.3%)	232 (74.8%)
Total Adapted	149 (48.7%)	78 (25.2%)
Total Un-Adapted	157 (51.3%)	232 (74.8%)

***Table 1:** Summary of stepwise model adaptation.*

By simulating UniKin1 [8] and ecoJC20 and comparing the simulation end-points (21600 seconds); our results show that 44 metabolites decreased in concentration while 28 metabolites increased in concentration (Figure 1); thus, 72 out of 306 (23.5%) of the metabolites changed in end-point concentrations. Moreover, 184 (60.1%) metabolites show non-zero MSE across simulation time between UniKin1 and ecoJC20, suggesting that ecoJC20 is substantially different from UniKin1.

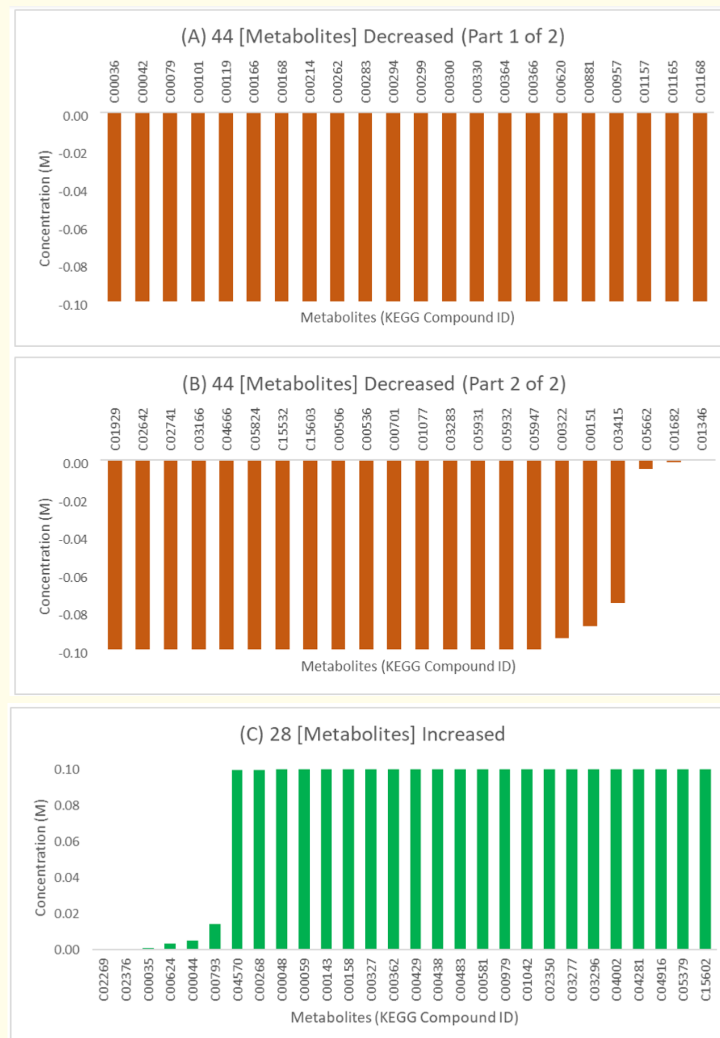

***Figure 1:** 72 metabolites changed in concentration at simulation end-points (21600 Seconds) between UniKin1 (8) and ecoJC20.*

Use case: Perturbation from nitrogen fixations: We examine the effects of two nitrogen fixation pathways (Figure 2) on *E. coli* central metabolism using ecoJC20. In the first pathway (N1), nitrogen (KEGG C00697) is fixed into ammonia (KEGG C00014) using 2 enzymes [nitrogenase molybdenum-iron protein alpha chain (EC 1.18.6.1) and vanadium-dependent nitrogenase alpha chain (EC 1.18.6.2)]. Hence, nitrogen, nitrogenase molybdenum-iron protein alpha chain (EC 1.18.6.1), and vanadium-dependent nitrogenase alpha chain (EC 1.18.6.2) are added into ecoJC1, forming ecoJC20_N1 as ammonia is native to ecoJC20. In the second pathway (N2), 4 metabolites [nitrogen (KEGG C00697), dihydro-orotate (KEGG C00337), orotate (KEGG C00295), and orotidine 5'-phosphate (KEGG C01103)] and 6 enzymes [nitrogenase molybdenum-iron protein alpha chain (EC 1.18.6.1), vanadium-dependent nitrogenase alpha chain (EC 1.18.6.2), dihydroorotate dehydrogenase (NAD^+) catalytic subunit (EC 1.3.1.14), dihydroorotate dehydrogenase (fumarate) (EC 1.3.98.1), dihydroorotate dehydrogenase (EC 1.3.5.2), and orotidylic phosphorylase (EC 2.4.2.10)] were added to ecoJC20 forming ecoJC20_N2. The concentrations of added metabolites and enzymes are 1 uM and 100 mM respectively. The turnover numbers (kcat) and Michaelis-Menten constants (Km) of added enzymes are set to 10 per second and 0.001M respectively.

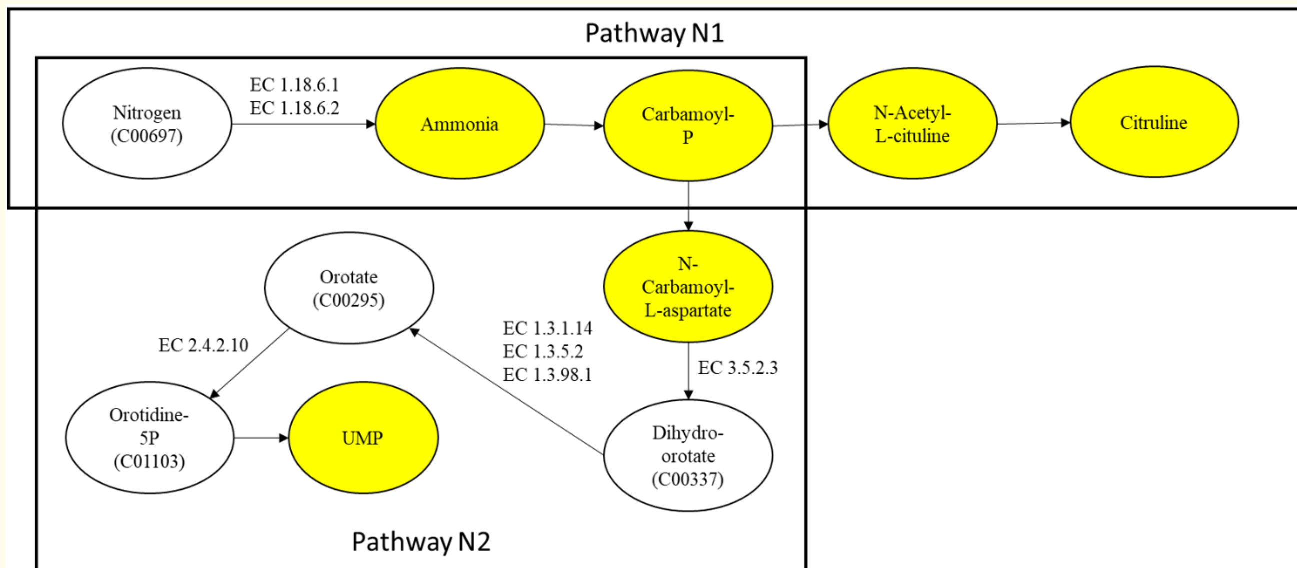

Figure 2: *Modifications to ecoJC20 to ecoJC20_N1 and ecoJC20_N2. The shaded metabolites represent metabolites native to ecoJC20 while the unshaded metabolites represent metabolites added into ecoJC20.*

Our simulation results (Figure 3 and 4) show that of the 10 metabolites decreased in concentration in N1, only 5 (C00322, C00624, C00956, C00979, and C04002) are common in the decreased metabolite list of N2. In terms of common metabolites with increased concentration, only 2 (C00151, and C05662) are common. This suggests the two nitrogen fixation pathways have different impact on the central metabolism of *E. coli* when compared to results from wild-type model, ecoJC20; thereby, underpinning the potential applications of kinetic models as an experimental design tool [25-27] for multiple applications [28].

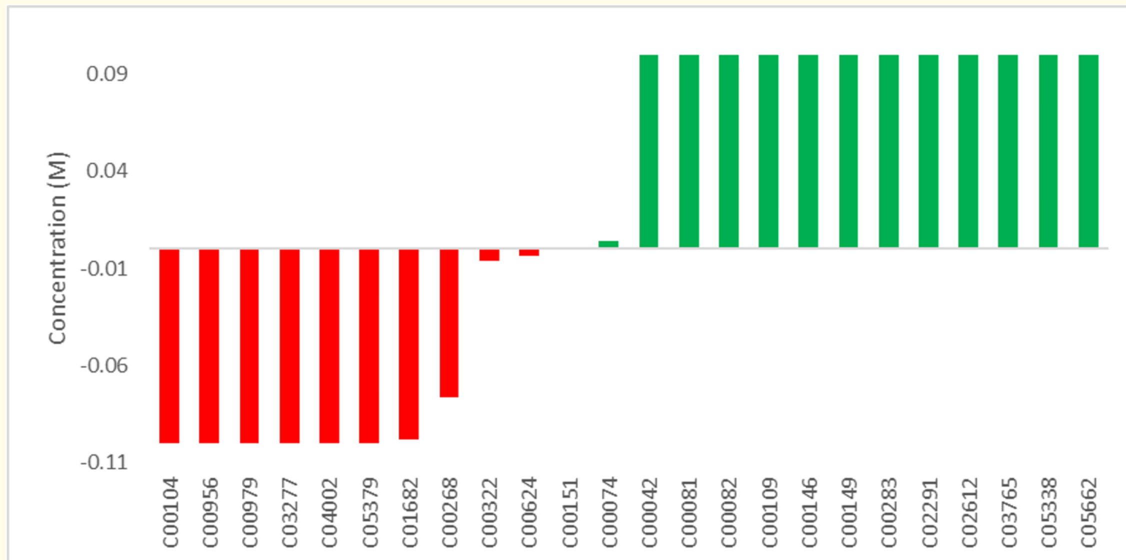

Figure 3: *24 metabolites changed in concentration at simulation end-points (21600 seconds) between ecoJC20 and ecoJC20_N1. 10 metabolites decreased in concentration (red) while 14 metabolites increased in concentration (green).*

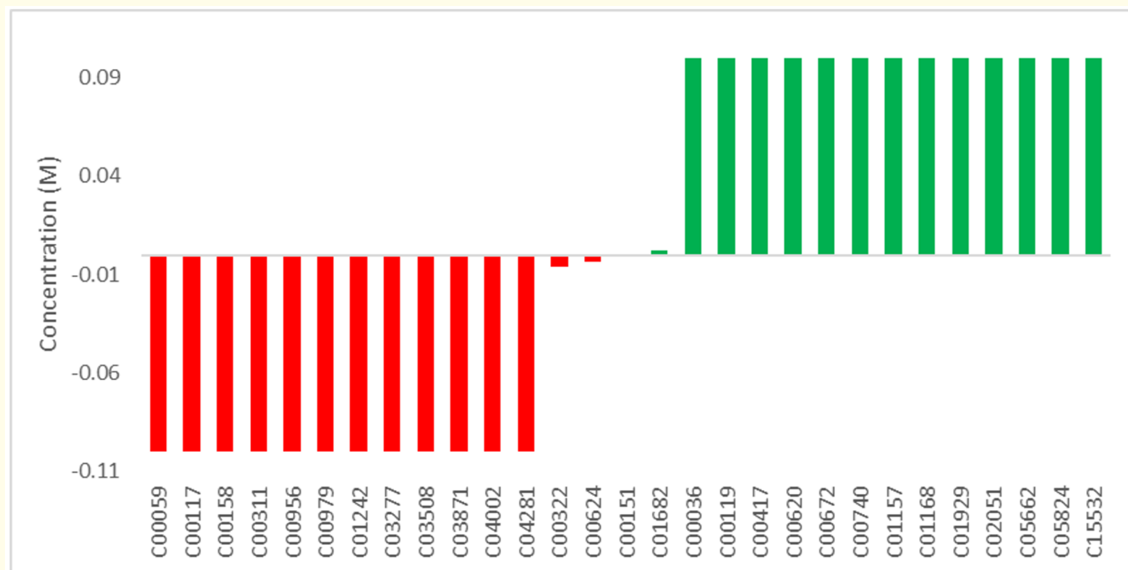

Figure 4: *29 metabolites changed in concentration at simulation end-points (21600 seconds) between ecoJC20 and ecoJC20_N2. 14 metabolites decreased in concentration (red) while 15 metabolites increased in concentration (green).*

Conclusion

In this study, we adapt a previously published universal (non-species specific) whole cell kinetic model, UniKin1, into an *E. coli* specific whole cell kinetic model, ecoJC20. Our simulation results suggest that ecoJC20 is substantially different from UniKin1.

Supplementary Materials

Data files for this study can be downloaded from http://bit.ly/ecoJC20.

Conflict of Interest

The authors declare no conflict of interest.

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