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Ab Initio Whole Cell Kinetic Model of Clostridioides difficile R20291 (cdlFN26)

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ABSTRACT

Clostridioides difficile, particularly hypervirulent and antibiotic-resistant strains such as R20291 poses a rising nosocomial threat due to metabolic adaptations that enhance toxin production, persistence, and recurrence. While existing genome-scale metabolic reconstructions have provided valuable information associating metabolic states with virulence levels, dynamic kinetic models that capture temporal metabolic behaviour remain limited. A whole cell kinetic model of C. difficile R20291 has not been developed. Hence, this study aimed to construct a whole-cell kinetic model of C. difficile R20291 using an ab initio approach by identifying enzymes from its reference genome. The resulting model, cdlFN26, comprises 880 metabolites, 386 enzymes with corresponding transcription and translation, and 1126 enzymatic reactions. The cdlFN26 model provides a foundational platform for future iterations to incorporate strain-specific kinetic parameters, nutrient-dependent gene expression, and regulatory layers such as riboswitch regulation, and increased enzyme annotations to improve biological realism and predictive capacity.

Keywords

Whole-cell model, Kinetic model, Pathogen, Anaerobe, Resistant spores, Differential equations, AdvanceSyn Toolkit.

Introduction

Clostridioides difficile R20291 is a Gram positive, spore-forming, strictly anaerobic bacterium capable of prolonged survival due to the formation of environmentally resistant spores [1]. Under anoxic and dysbiotic conditions in the colon, *C. difficile* R20291 primarily undergoes Stickland amino acid fermentation for anaerobic ATP production [2]. The central carbon metabolism utilises host-derived carbohydrate sources for rapid vegetative growth. Additionally, the Wood-Ljungdhal pathway enables cofactor regeneration in an energy-conserving state while short chain fatty acid metabolism supports energy and redox balance [3-5]. These metabolic processes directly regulate the expression of major virulence factors (TcdA, TcdB), facilitating vegetative expansion and sporulation in response to changing nutrient availability [6]. Despite extensive studies on virulence-associated metabolic pathways, current approaches remain correlative and endpoint-based [7,8], indicating a knowledge gap in mechanistic causality between intracellular metabolic pools and hypervirulence of *C. difficile* R20291.

Modelling can play a role in both the study of pathogenicity [9-11] and in metabolic engineering by allowing researchers to examine genetic interventions computationally before laboratory testing [12,13]. GSMs and KMs represent the two central modelling strategies [14,15]. GSMs excel in predicting fluxes but provide limited insight into product yields. KMs, on the other hand, deliver predictions for both yields and rates [16] and handle simulated gene knock-ins with greater flexibility [17]. These advantages make KMs particularly valuable tools for screening engineering ideas. In response, there has been increasing momentum behind

initiatives to build and refine kinetic models to strengthen their role in the design process [18,19].

However, there is no whole cell KM of *C. difficile* to-date. Hence, this study aims to construct a KM of *C. difficile* R20291 using *ab initio* approach by identifying enzymes from its published genome [20] and identifying the corresponding reaction from KEGG [21]. The result is a whole cell KM of *C. difficile* R20291, named as cdlFN26 using the nomenclature proposed by Cho and Ling [22], which consists of 880 metabolites, 386 enzymes with corresponding transcriptions and translations, and 1126 enzymatic reactions. This model may form part of a collation of kinetic models to predict metabolite levels of gut microbiome as recently proposed [23,24].

Materials and Methods

Identification of Reactome

The genome of *Clostridioides difficile* R20291 (*C. difficile* R20291 (NCBI RefSeq assembly GCF_000027105.1; NCBI GenBank Accession GCA_000027105.1) was used as source to identify enzymatic genes using the process previously described [17,25,26]. Briefly, each enzymatic gene was identified as a presence of complete Enzyme Commission (EC) number in the GenBank record and mapped into reaction IDs via KEGG Ligand Database for Enzyme Nomenclature [21]. 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 ordinary differential equation (ODE) formats described in Sim et al. [27]. Based on BioNumbers, a typical *Escherichia coli* cell contains about 3000 RNA polymerases (BioNumbers 106199) [28], but only one quarter are producing RNA at any moment (BioNumbers 111676) [29]. With a polymerization rate of 22 nucleotides per second (BioNumbers 104109) [30] and a nucleotide mass of about 339.5 Da, this equals about 5600 kDa of RNA synthesized each second. Converting to mass (9.3e-18 grams per second) and scaling to the 7e-16 litres cell volume [31] and 4225 genes (BioNumbers 105443) [32] yields 2.92 micromolar per gene per second. With an mRNA lifetime of about 108 seconds (BioNumbers 107666) [33], the decay rate is 0.0093 per second. Thus: d[mRNA]/dt = 0.00292 – 0.0093[mRNA]. Translation outputs 0.278 peptides/s/transcript (BioNumbers 106382) [34], while protein degradation follows 2.78e-6 per second (BioNumbers 109924) [35], giving: d[peptide]/dt = 0.278[mRNA] – 0.00000278[peptide]. The metabolic network was encoded as ODEs [25,36] using median values (kcat 13.7 per second, Km 1 mM) consistent with Bar-Even et al. [37] and structured using AdvanceSyn 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) C00008 (ADP), (VIII) C00009 (Phosphoric acid), (IX) C00010 (CoA), (X) C00013 (Diphosphoric acid), (XI) C00020 (AMP), (XII) C00022 (Pyruvate), (XIII) C00024 (Acetyl-CoA), (XIV) C00025 (L-Glutamate), (XV) C00031 (D-Glucose), (XVI) C00064 (L-Glutamine), (XVII) C00123 (L-Leucine), (XVIII) C00138 (Reduced ferredoxin), (XIX) C00139 (Oxidized ferredoxin), (XX) C00148 (L-Proline), (XXI) C00183 (L-Valine), (XXII) C00407 (L-Isoleucine). 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. The simulation results were sampled every 2 seconds.

Results and Discussion

The annotated genome of *C. difficile* R20291 consists of 3828 genes as of the latest annotation dated 28 February 2026, including 3678 protein-coding sequences. 386 unique EC numbers consisting of 1126 enzymatic reactions involving 880 metabolites were identified and developed into a model using AdvanceSyn Model Specification [38]. In addition, 772 ODEs acting as placeholders for transcription and translation were added.

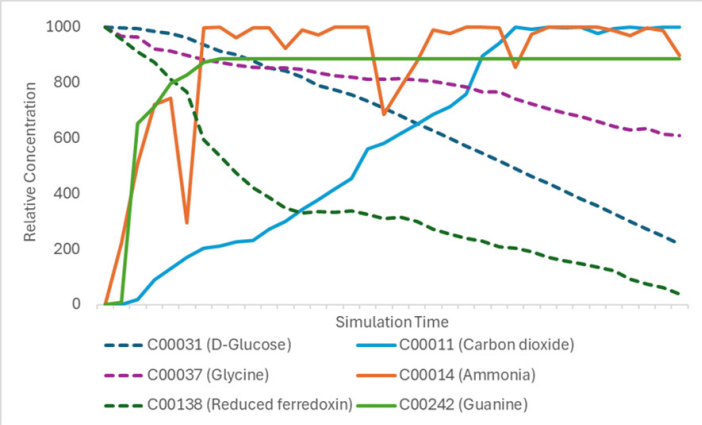

Figure 1: Selection of Simulation Results.

Simulating the cdlFN26 model with AdvanceSyn Toolkit [34] yielded stable, interpretable dynamics (Figure 1), indicating the model is syntactically correct and structurally consistent as argued in recent model constructions [17, 26, 41-45]. In the simulation, production of ammonia increases steadily but this may be an outcome tied to our use of median turnover number and Michaelis-Menten constant across all enzymatic reactions [33]. These homogenized kinetics mask enzyme-specific behaviour and bias relative fluxes despite showing substantial predictability [46]. Therefore, while the quantitative output should be treated with caution, the broader contribution of this work lies in delivering a working whole-cell kinetic model of *C. difficile* R20291. This baseline model can now be expanded, refined, or adapted to include more realistic kinetics, regulatory layers, or growth and resource-allocation processes [37-39]. This model may also be

incorporated into a collation of kinetic models, to be a meta-model for predicting metabolite levels produced by various interacting gut microorganisms as recently proposed [23,24].

Conclusion

In this study, we present an *ab initio* whole cell kinetic model of *Clostridioides difficile* R20291. The resulting kinetic model, cdlFN26; comprising of 880 metabolites, 386 enzymes with corresponding transcriptions and translations, and 1126 enzymatic reactions.

Supplementary Materials

Reaction descriptions and model can be download from https://bit.ly/cdlFN26.

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