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Ab Initio Whole Cell Kinetic Model of *Streptococcus equi subsp. zooepidemicus* SEZ13 (seqSA26)

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Abstract

Streptococcus equi subsp. zooepidemicus (SEZ) is a Gram-positive opportunistic pathogen that is recognised for its natural capacity to produce hyaluronic acid through fermentation; hence, may be a candidate for metabolic engineering. Although both genome scale metabolic models (GSMs) and kinetic models (KMs) inform metabolic engineering strategies, KMs are more advantageous than GSMs as it captures dynamic concentration changes. While a GSM of SEZ has been recently published, there is no KM of SEZ to-date. This study reports the ab initio construction of a whole-cell kinetic model for SEZ strain SEZ13 by identifying enzymatic genes from the annotated genome and mapping them into reactions in KEGG database. The resultant model, seqSA26, comprises 580 metabolites, 325 enzymes and 861 enzymatic reactions. Our results suggest that seqSA26 can be simulated and exhibit dynamic fluctuations of metabolite concentrations rather than flat lines, indicating a coherent network structure; therefore, establishing a draft model for future refinements, including growth coupling and improved resource allocation simulations.

Keywords: Kinetic model; Whole cell model; Ab initio modelling; Ordinary differential equations, AdvanceSyn Toolkit

Introduction

Streptococcus equi subsp. zooepidemicus (SEZ; commonly known as *Streptococcus zooepidemicus*) is a Gram-positive, encapsulated, beta-haemolytic bacterium [1] categorised as Lancefield group C streptococci [2]. SEZ is primarily recognised in veterinary medicine as an opportunistic pathogen [3] but it has recently garnered significant industrial interest because of its innate ability to produce hyaluronic acid (HA) [4]. HA is essential in specialised medical procedures like ocular surgery (e.g., cataract extraction and corneal transplantation), viscosupplementation for osteoarthritis, and accelerated wound healing, along with it being a main constituent in dermal fillers in cosmetic plastic surgery procedures due to its biocompatibility and hygroscopic properties [4]. Traditionally, HA was extracted from animal-derived sources, such as rooster combs or bovine vitreous humour; there is a risk of cross-species contamination and allergenicity [5]. Therefore, microbial fermentation using SEZ offers a highly pure, non-animal-derived, scalable source of HA, reducing the risk of pathogenic transmission and providing consistent product quality in the biotechnology industry [6]. Recently, type I-C CRISPR-Cas system may potentially allow improved production of HA through the knockout of the hyaluronidase gene to prevent HA product degradation [7]; thus, opening potential novel metabolic engineering of SEZ.

The design of metabolic engineering strategies typically begins with mathematical modelling to identify promising directions [8, 9]. Two frameworks are most widely used [10, 11]: GSMs and KMs. Although GSMs have shaped much of the field, their predictions emphasise rates rather than yields. KMs overcome this limitation by modelling both aspects [12], and enabling easier in silico gene knock-ins [13]. As a result, KMs offer a more complete platform for evaluating engineering strategies computationally. This has fuelled a growing movement within the field to invest more effort into constructing comprehensive kinetic models that can better support decision-making [14, 15].

Although a GSM of SEZ has been recently constructed specifically to study the production of HA (iZN522 [16]), there is no KM of SEZ to-date. As such, this study aims to construct a KM of *S. zooepidemicus*

SEZ13 using *ab initio* approach by identifying enzymes from its annotated genome, and identifying the corresponding reaction from KEGG [17]. The result is a whole cell KM of *S. zooepidemicus* SEZ13, named as seqSA26 using the nomenclature proposed by Cho and Ling [18], which consists of 580 metabolites, 325 enzymes with corresponding transcriptions and translations, and 861 enzymatic reactions.

Materials and Methods

Identification of Reactome. The genome of *Streptococcus zooepidemicus* strain SEZ13 (NCBI RefSeq assembly GCF_015689395.1; NCBI GenBank Accession NZ_CP065054.1) was used as source to identify enzymatic genes using the process previously described [13, 19, 20]. 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 [17]. 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 reaction can be identified.

Model Development. The model was developed using the ordinary differential equation (ODE) formats described in Sim et al. [21]. BioNumbers show that *Escherichia coli* harbours roughly 3000 RNA polymerases (BioNumbers 106199) [22], 25% of which actively elongate transcripts (BioNumbers 111676) [23] at 22 nucleotides per second (BioNumbers 104109) [24]. The average ribonucleotide weighs 339.5 Da, allowing an estimate of ~5600 kDa per second of mRNA produced, or 9.3e-18 grams per second. Factoring in a cellular volume of 7e-16 litres [25] and 4225 protein-coding genes (BioNumbers 105443) [26] gives an approximate synthesis rate of 2.92 micromolar per gene per second. Given a transcript lifespan of 107.56 s (BioNumbers 107666) [27] (0.93% decay per second), we arrive at: d[mRNA]/dt = 0.00292 – 0.0093[mRNA]. Protein synthesis proceeds at 0.278 peptides per transcript per second (BioNumbers 106382) [28], and protein turnover operates at about 2.78e-1 per second (BioNumbers 109924) [29], giving: d[peptide]/dt = 0.278[mRNA] – 0.00000278[peptide]. The biochemical reactome was represented as ODEs [19, 30] with enzyme parameters taken from Bar-Even et al.'s median survey [31] and formatted according to AdvanceSyn Model Specification [32].

Model Simulation. The constructed model was tested for simulatability using AdvanceSyn Toolkit [32]. 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) 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-Aspartate), (XVI) C00064 (L-Glutamine), (XVII) C00065 (L-Serine), (XVIII) C00073 (L-Methionine), (XIX) C00097 (L-Cysteine), (XX) C00133 (D-Alanine), (XXI) C00148 (L-Proline). The model was simulated using the fourth-order Runge-Kutta method [33, 34] 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 NCBI Prokaryotic Genome Annotation Pipeline (PGAP) predicts that SEZ has 2030 genes, where only 1933 genes are coding for proteins. 325 unique EC numbers consisting of 861 enzymatic reactions involving 580 metabolites were identified and developed into a model based on AdvanceSyn Model Specification [32]. In addition, 650 ODEs acting as placeholder for enzyme transcriptions and translations were added.

We ran seqSA26 through AdvanceSyn Toolkit [32], and the resulting simulation (Figure 1) confirms that the model executes correctly, with no evident syntax or compilation errors; as (a) the successful generation of simulation results demonstrates that the model can be simulated, and (b) the presence of fluctuations in simulated metabolite concentrations (rather than straight lines) implies that the metabolites respond to each other's concentrations. Given the intricacy of whole-cell models, successful runs are essential to demonstrate that the network structure is coherent. The simulation result is driven by the use of median kinetic constants [33] across all enzymes, a deliberate simplification to validate structure before parameter refinement. Accordingly, these outcomes should be regarded only as structural tests as argued in recent model constructions rather than direct representation within the cell [13, 20, 35–39]. Therefore, the key deliverable is a functioning whole-cell kinetic model for *S. zooepidemicus* SEZ13, serving as a flexible template for further expansion, such as adding growth coupling, variable enzyme kinetics, or resource allocation simulations [40–42].

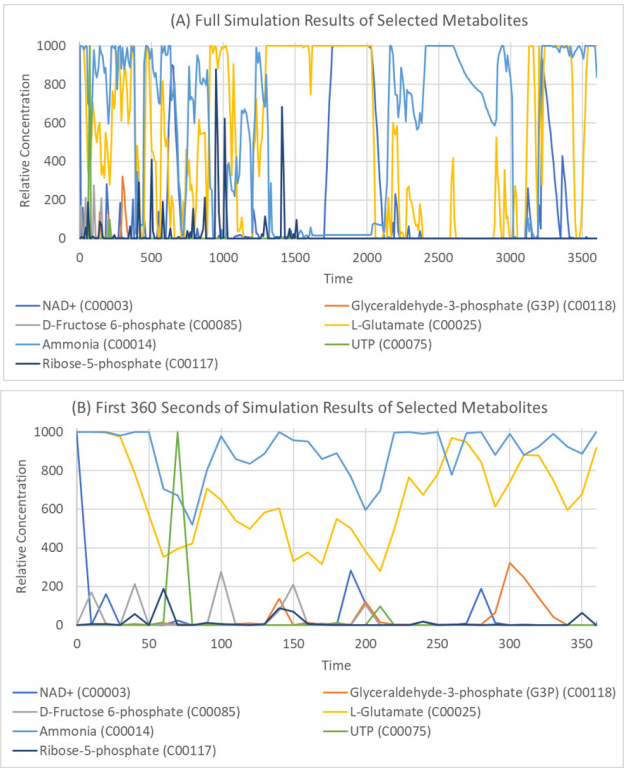

Figure 1. Selection of Simulation Results.

Panel A shows the full simulation results (3600 seconds) of seven metabolites while Panel B zooms into the first 360 seconds.

Conclusion

In this study, we present an *ab initio* whole cell kinetic model of *Streptococcus zooepidemicus* strain SEZ13. The resulting kinetic model, seqSA26; comprising of 580 metabolites, 325 enzymes with corresponding transcriptions and translations, and 861 enzymatic reactions.

Supplementary Materials

Reaction descriptions and model can be downloaded from https://bit.ly/seqSA26.

Conflict of Interest

The authors declare no conflict of interest.

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