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Research Article

Ab Initio Whole Cell Kinetic Model of *Stutzerimonas balearica* DSM 6083 (pbmKZJ23)

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Abstract

Stutzerimonas balearica (formerly, known as *Pseudomonas balearica*) is an environmentally tolerant bacterium with denitrification and bioremediation capabilities. Hence, it has been studied for industrial applications; such as, high-value chemical production using metabolic engineering or synthetic biology approaches. Mathematical modelling has the potential to predict biological phenotypes under metabolic perturbations, which can be used to guide engineering approaches. However, there is no mathematical model of *S. balearica* to-date. In this study, we present a whole cell simulatable kinetic model of *S. balearica* DSM 6083, pbmKZJ23, constructed using *ab initio* approach by identifying enzymes from its published genome. The resulting model consists of 737 metabolites, 533 enzymes, and 802 reactions; which can be a baseline model for incorporating other cellular and growth processes, or as a system to examine cellular resource allocations necessary for engineering.

Keywords: *Stutzerimonas balearica; Pseudomonas balearica; ab initio*

Introduction

Stutzerimonas balearica [1] (formerly, known as *Pseudomonas balearica* [2]) is an environmental Gram-negative bacilliform bacterium found in diverse environments with denitrifying capabilities [3,4] and the ability to degrade several organic compounds; such as, naphthalene [2] and thiosulfate [5]; suggesting potential applications in bioremediation [6]. Due to its presence and tolerance in diverse and stressful environments [7], such as heavy metal stress [8]; *S. balearica* has been studied for biosurfactant production [9], bioleaching of electronic waste [10], enzyme production [11], and novel chemical production [12].

It is plausible that potential applications of *S. balearica* may capitalize on its diverse environmental tolerance for novel or high-value chemical production using metabolic engineering or synthetic biology approaches [13,14]. Mathematical modelling is an important aspect in both metabolic engineering and synthetic biology [15] as it can predict biological phenotypes under metabolic perturbations, which can be used to guide engineering approaches [16]. The two main paradigms [17] are kinetic models (KMs) and constraint-based models (also known as genome-scale metabolic models - GSMs or GSMMs). GSMs are based on reaction stoichiometries and reversibilities to provide steady-state production rates of metabolites [18]. Kinetic models (KMs) generally use ordinary differential equations (ODE) that defines the rate of change of concentrations of the substrates involved [19], which offers a transient dynamic approach as it provides specific solutions in time for steady-state fluxes from the initial concentration of the substrates [20]. KMs and GSMs have their own advantages and disadvantages [21] - more specifically, KMs can address relationship between flux, enzyme expression, metabolite levels, and regulation; and this enables KMs to provide time-course profile of modelled metabolites [22] which are notx` possible in GSMs. However, KMs require higher accuracy for parameters than GSMs [20]; hence, more demanding and as a result, there are fewer large-scale KMs than GSMs [23]. Therefore, tools to draft a KM from existing GSM has emerged [24].

However, there is no GSM of *S. balearica* to-date. As such, this study aims to construct a KM of *S. balearica* DSM 6083 using *ab initio* approach by identifying enzymes from its published genome [25], and identifying the corresponding reaction from KEGG [26]. The result is a whole cell KM of *S. balearica* DSM 6083, named as pbmKZJ23 using the nomenclature proposed by Cho and Ling [22], which consists of 737 metabolites, 533 enzymes, and 802 reactions.

Materials and Methods

Identification of reactome

The genome of *Stutzerimonas balearica* DSM 6083 (Accession number NZ_CP007511.1) [25] was used as source to identify enzymatic genes. Each enzymatic gene was identified as a presence of complete Enzyme Commission (EC) number. Each complete

EC number was matched to the corresponding entry in Enzyme nomenclature database (https://enzyme.expasy.org/) [27], and linked to the corresponding entry in KEGG Ligand Database for Enzyme Nomenclature [26]; which served as an intermediate to link to the corresponding KEGG reaction entry or entries. From each KEGG reaction entry, substrate(s) and product(s) for the reaction can be identified. For example, adhP gene (protein ID WP_041107839.1) corresponded to alcohol dehydrogenase with EC number of 1.1.1.1, which can be used to match against as Enzyme nomenclature database https://enzyme.expasy.org/EC/1.1.1.1. Hence, the corresponding entry in KEGG Ligand Database for Enzyme Nomenclature was https://www.genome.jp/dbget-bin/www_bget?ec:1.1.1.1, showing two reactions for this enzyme (R00623 and R00624). From KEGG Reaction R00623; metabolites C00226, and C00003 were substrates while metabolites C00071, C00004, and C00080 were products (See Figure 1 for illustration of steps). From KEGG Reaction R00624; metabolites C01612, and C00003 were substrates while metabolites C01450, C00004, and C00080 were products.

Model development

The reactome was modelled as a set of ordinary differential equations (ODEs) where each ODE represented one metabolite concentration as previously described [23] (Figure 1). Briefly, an ODE was in the form of $\frac{d[metabolite]}{dt} = \sum_{i=1}^{N} production_i - \sum_{i=1}^{N} usage_i$, where production represents a formation or synthesis of the metabolite, and usage represents a usage of the metabolite to form another metabolite. As production and usage terms are in pairs, they can be modelled as a Michaelis-Menten expression, $\frac{k_{cat}[enzyme](\prod_{i=1}^{N}[substrate_i])}{K_m+(\prod_{i=1}^{N}[substrate_i])}$, where k_{cat} is the turnover number (per second) of the enzyme, K_m is the Michaelis-Menten constant, [enzyme] and [substrate] are the concentrations (in molar) of the enzyme and substrates respectively and N represents the number of molecules. The concentrations of metabolites and enzymes were set at 1 micromolar and 32 micromolar, respectively. The k_{cat} and K_m were set at 13.7 per second and 1 millimolar, respectively; which were the median values from a survey of more than 1000 enzymes by Bar-Even., *et al.* [28]. The model was written in accordance to AdvanceSyn Model Specification [29].

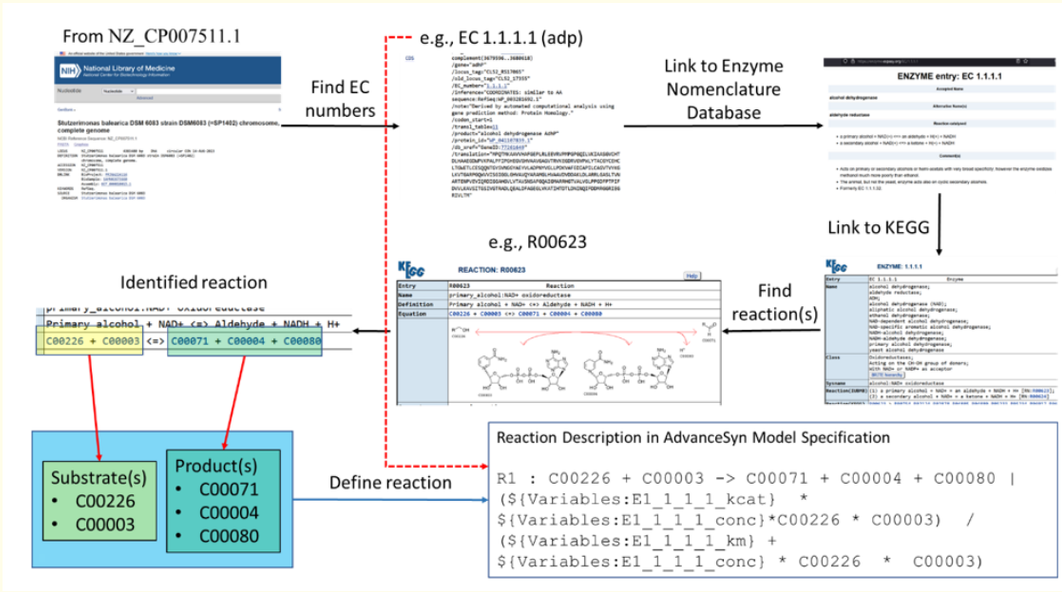

Figure 1: Illustration of steps of reaction identification from genome to reaction definition in AdvanceSyn Model Specification [29].

Model simulation

The constructed model was tested for simulatability using AdvanceSyn Toolkit [29]. Initial concentrations of ammonia (KEGG ID C00014), d-glucose (C00031), triphosphate (C00536), and sulfur donor (C17023) were set to 92600, 22000, 349100, 2000 micromolar, respectively; to resemble concentrations in M9 media. The model was simulated using the fourth-order Runge-Kutta method [30,31] from time zero to 1000 seconds with timestep of one second, and the concentrations of metabolites were bounded between 0 millimolar and 1 millimolar. The simulation results were sampled every 10 seconds.

Results and Discussion

The genome of *Stutzerimonas balearica* DSM 6083 is 4,383,480 basepairs; consisting of 4004 protein coding genes and 68 pseudogenes [25]. Of the 4004 coding sequences, 1044 contains EC numbers. Of which, 199 (19.06%) contains incomplete EC numbers, and 533 (51.05%) contains complete EC number that can be successfully matched to KEGG reactions [26] with substrate(s) and product(s) in KEGG compound IDs. These 533 successfully matched enzymes catalyze 802 reactions involving 737 metabolites (See supplementary materials for description of enzymes, metabolites, and reactions). The number of reactions and metabolites are within the range listed in Microbial Metabolites Database (MiMeDB) [32] for *Pseudomonas putida* KT2440 (MMDBm0002392; 454 enzymes with 385 metabolites), and *Pseudomonas aeruginosa* PAO1 (MMDBm0002391; 737 enzymes with 591 metabolites).

The 802 reactions involving 533 enzymes and 737 metabolites were developed into a model based on AdvanceSyn Model Specification [29]. The resulting model, denoted as pbmZJK23, was

simulated using AdvanceSyn Toolkit [29]. Our simulation results (Figure 2) suggests that the model is free from syntax error as the presence of simulation results suggests that the constructed model can be simulated. In this case, our simulation suggests that coproporphyrinogen III (C03263) is being produced while alpha-ketoglutaric acid (C00026) and 5,6,7,8-tetrahydrofolate (C00101) are being used. Hence, we present a simulatable whole cell KM of *S. balearica* DSM 6083, which can be a base template for incorporating other cellular and growth processes as demonstrated by Ahn-Horst., *et al*. [33] or as a system to examine cellular resource allocations as demonstrated by Thornburg., *et al*. [34] and Bianchi., *et al*. [35].

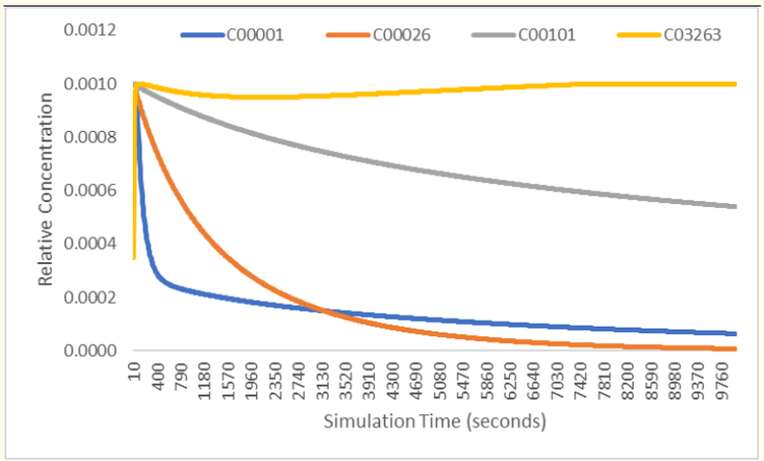

Figure 2: Selection of simulation results. KEGG compounds C00001, C00026, C00101, and C03263 are water, alpha-ketoglutaric acid, 5,6,7,8-tetrahydrofolate, and coproporphyrinogen III, respectively.

Conclusion

In this study, we present an *ab initio* whole cell kinetic model of *Stutzerimonas balearica* built from the enzymes found in the genomic sequence of *S. balearica* DSM 6083. The resulting kinetic model, pbmZJK23, comprising of 737 metabolites, 533 enzymes, and 802 reactions.

Supplementary Materials

Reaction descriptions and model can be download from https://bit.ly/pbmKZJ23.

Conflict of Interest

The authors declare no conflict of interest.

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