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INVESTIGATION OF MICROBIAL METABOLISM USING CONSTRAINT-BASED GENOME METABOLIC MODELS

Biotechnology

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KEYWORDS

INTRODUCTION

Metabolic Engineering is a field at the intersection of biology and engineering involving the study of molecular processes. It focuses on optimising genetic and regulatory approaches to improve the cell's yield of the target metabolite. It seeks to mathematically model the cell's metabolic network and investigate which parts can be modified to achieve better results.

A mathematical model of an organism's metabolic network is called a genome-scale metabolic model or GEM. These GEMs have several important uses:

- Inconsistencies exist between various databases, which can be systematically verified by implementing these models.
- Comparisons between different organisms can be performed concerning a particular metabolite.
- Models can be used in Metabolic Engineering to suggest pathways and changes for improved yields.

Genome-scale metabolic models, also known as GEMs, make it possible to predict metabolic phenotypes using only limited data on uptake and secretion fixes. They do this by defining the space of all the solutions that are feasible while excluding behaviours that are physiochemically and biologically implausible. There is a possibility that the accuracy of phenotypic prediction could be improved with the incorporation of additional biological information into genome-scale models, such as transcriptomic or proteomic profiles. This is especially significant for applications involving metabolic engineering, because more precise model predictions can lead to more dependable model-based strain design.

Constraining the models by using additional data improves the accuracy of these models. We can constrain the model using methods like Enzymatic Constraints using GECKO, TFA and 13C MFA. GECKO uses enzymatic data as a new constraint for each metabolic flux by gathering protein abundance and turnover number data. By integrating quantitative metabolomics data, TFA in metabolic models helps study effects on the network's net-flux directionality of reactions. At the same time, 13C MFA provides knowledge about how carbons are redistributed on central carbon metabolism. Such techniques are currently being explored to improve the predictive capabilities and accuracy of these models for GEMs of various organisms.

MATERIAL AND METHODS :

Selection of organism:

B. subtilis is one of the best-characterized Gram-positive bacteria and a model organism. Its genome has been sequenced, and there are well-established microbiological, molecular, and genetic techniques for its cultivation and manipulation. B. subtilis has a practical utility in synthetic biology because it is able to grow efficiently using inexpensive carbon and nitrogen supplies, integrate chromosomes easily, and secrete products efficiently. Its innate competence, ease of chromosomal integration, and ability to secrete enzymes and vitamins efficiently without engineering make it excellent for synthetic biology applications.

K. xylinus is a gram-negative, strictly aerobic, acetic acid bacteria that transforms glucose and other organic substrates into bacterial cellulose. Bacterial cellulose is a promising material for many medical and pharmaceutical applications, filtration and separation, dietary supplements, biosensors and nanocomposites

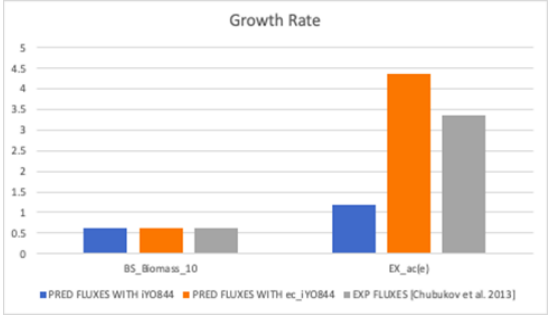
Enzymatic Constraining of B. subtilis

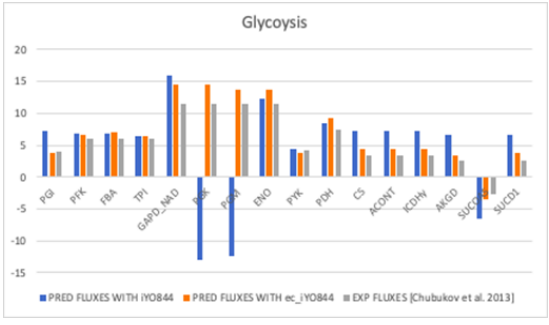
Initially, our first step involved studying techniques to help us build pre-studied models and predictions for K. xylinus. For this, we used B. subtilis as our model organism as it has been extensively studied and has many constrained models available. The reported enzymatic data was integrated into the iYO844 model, consisting of 1020 reactions, to obtain an enzyme-constrained model. This can be easily used with metabolic engineering design tools as it adds additional constraints to a subset of model reactions while retaining the linear structure of the original model. Kcat values and specific activities of 45 selected central carbon metabolism enzymes and other closely related reactions were integrated into the iYO844 model. GECKO approach was used with an additional constraint, that the metabolic flux through the jth reaction (Rj) does not exceed its maximum capacity (Vmax), corresponding to the product between the kcat value of the enzyme Ej and its abundance [Ej].

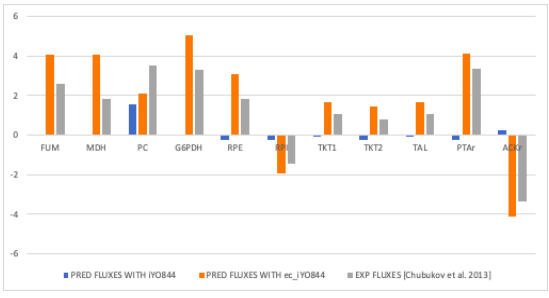
$$vj \leq kjcat \cdot Ej \text{ for } j = 1 \ldots 17$$

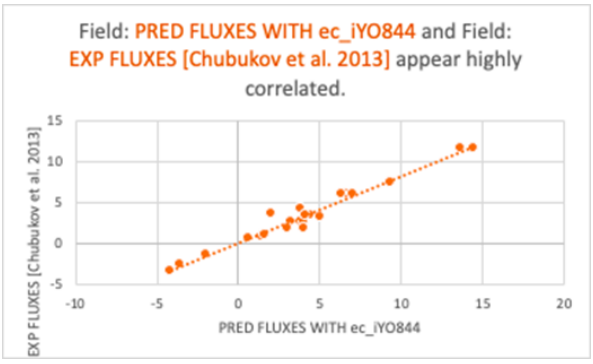
The iYO844 model was changed into an irreversible model, and the constraint for glucose uptake rate was removed. The model's stoichiometric matrix and upper bound vector were enlarged by using the kcat values and the known protein abundances. All strains were simulated using both the iYO844 model and the new enzyme-constrained model, ec_iYO844

The computations were implemented via MathWorks MATLAB R2021a and run with the COBRA toolbox using the Gurobi solver.



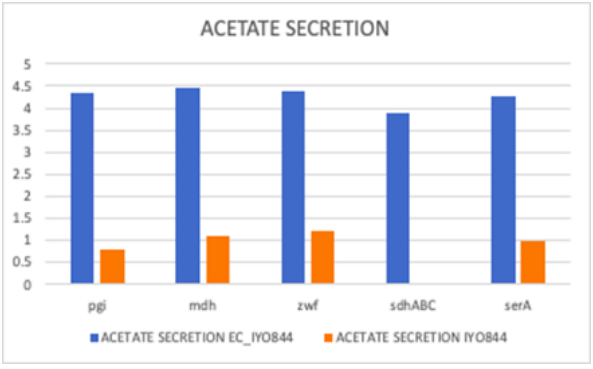


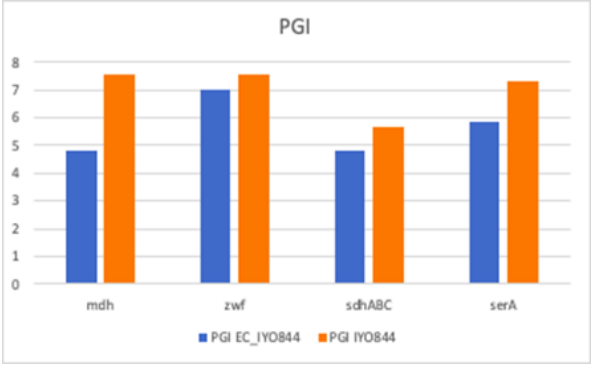


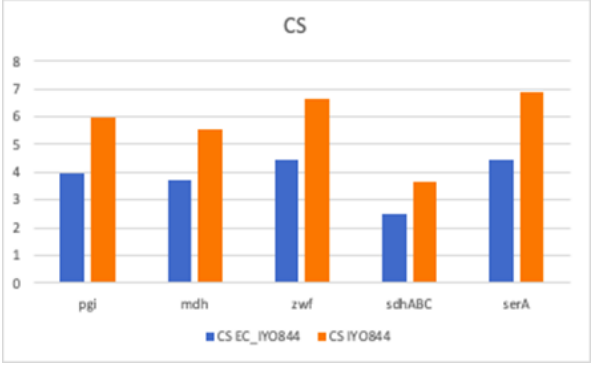


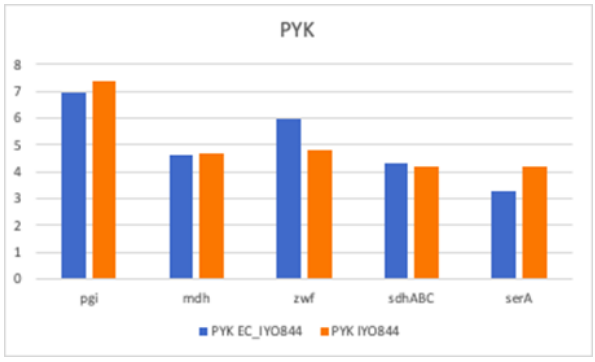
A comparison of the predicted and the available experimental fixes in the literature showed that the iYO844 model could accurately predict growth rate and fluxes through the glycolytic pathway. An integration of enzymatic data in ec_iYO844 for pentose phosphate pathway (PPP), TCA cycle and acetate secretion fluxes were significantly improved. The glucose uptake rate was also consistent with the experimental values.

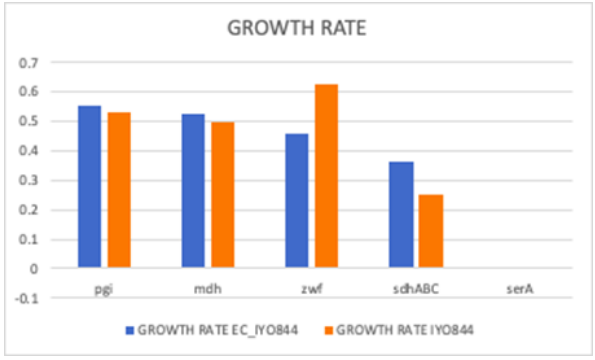
We further evaluated the performance of the ec iYO844 model by simulating the metabolic behaviours of five single-gene/ operon deletion strains: Δpgi, Δmdh, Δzwf, ΔsdhABC and ΔserA, for which the PGI, MDH, G6PDH, SUCD1 and PGCDr. The experimental fluxes of four central carbon processes [PGI, G6PDH, pyruvate kinase (PYK), and CS], growth rate, and acetate generation rate were matched with the two models' anticipated values.

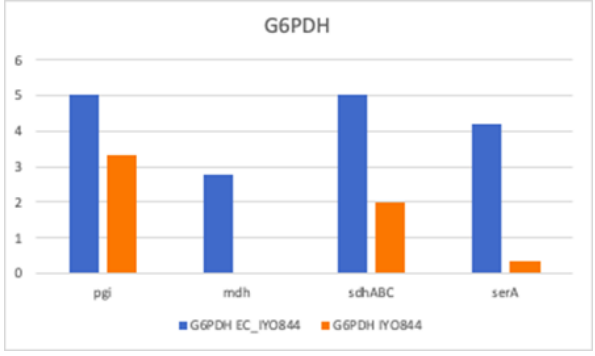












For the mutant strains, results give an overall improvement in prediction accuracy when enzymatic data are integrated. The mutant strain showed an overall decrease but not all mutant strains, so individual flux predictions were improved.

Upon incorporating enzymatic data into the model, the projected acetate secretion rates for Δmdh and ΔsdhABC strains became more comparable with the observed values. Nonetheless, the model was unable to predict the measured rates for Δzwf and ΔsdhABC strains. Additionally, a general improvement was noted for G6PDH response. The results demonstrated that the new model is also superior in predicting the mutant strain flux.
The decrease in prediction error by 29 percent confirms that the enzyme-constrained model improves overall prediction accuracy.

DISCUSSION

We combined enzymatic and proteomic data for 17 central carbon-related pathways in a current genome-scale metabolic model of B. subtilis. We pointed out that the resulting model (ec_iYO844) presents a more accurate prediction of metabolic data than the original non-enzyme constrained model (iYO844). All errors pertaining to the original model iYO844 were resolved in the ec_iYO844 model. The new model does not require fixing the glucose uptake rate to an experimentally determined value compared to the original model.

The enzyme-constrained one provided a correct prediction for more strains than the non-enzyme constrained model for the essential genes we used to compare the accuracy. This constraining was sufficient to demonstrate the potential of this approach with the experimental data used and how accuracy can be increased by employing these methods.

Although poor availability of high quality enzyme kinetic data has

been identified as a major problem for developing improved computational models of cellular metabolism in general.

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