# Validation of Faecalibacterium prausnitzii with in vitro data

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Abstract: The gut bacterium *Faecalibacterium prausnitzii* is one of the most abundant species in the gut. To understand the role of this species in health and its involvement in gut communities, this species has been studied using metabolic modelling. A genome-scale metabolic model (GEM) has been refined for this species and is available. To validate this model, we would like to predict species growth in different media and compare the result with biological data.

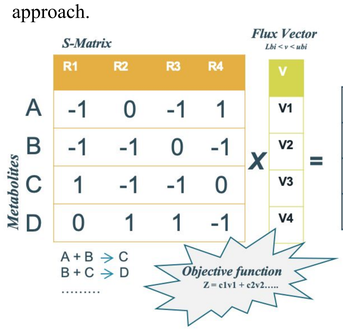
## Introduction

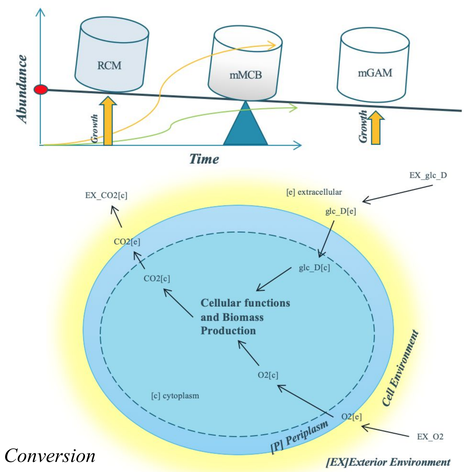
*1. Wet Lab process and In Silico Data*

Contains the Optical Density (OD) values for bacterial colony growth over the course of the experiment, which are converted to growth curves in R. Determining the optimized biomass production (objective function) in our *in silico* model, our expectation is that it will be comparable with *in vitro* growth values as a validation test.

*2. In-Silico Model*

The genome scale metabolic model was constructed from gene annotation and transcriptomics data. This is often referred to as a "bottom-up" approach.



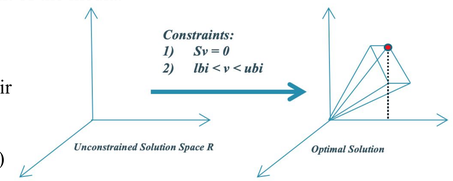




*3. Medium Definition & Unit Conversion*

*In silico* media used for FBA should be well defined in their metabolic composition. Unit conversion is necessary for calculating flux of each individual molecular component of the media.

*4. Flux Balance Analysis (FBA)*

All metabolites in matrix *S*, and their fluxes in vector *v*. Goal: maximize biomass production *Z* (objective function) given steady state ($Sv = 0$) and flux constraints ($lbi < v < ubi$).




## Methods

*Medium Composition*

Media from the *in vitro* experiments was reconstructed into an *in silico* media using BiGG metabolite IDs for the 3 media: 1 manually defined (mMCB) and 2 commercially available (mGAM, RCM). Several iterations of medium definition were necessary (Fig. 2). Lack of detailed media information led to using informed assumption for some metabolites.

*Flux Constraints*

For each metabolite in the in silico media, a flux constraint needed to be calculated based on its concentration. Metabolites in media are available for exchange reactions in the model (Fig. 1)

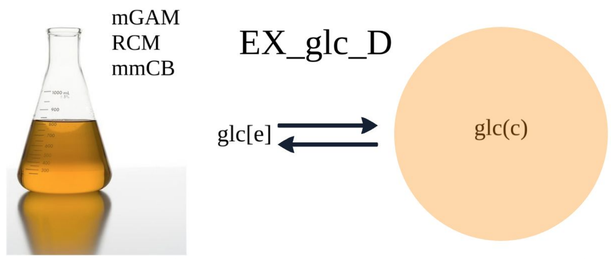



*Figure 1: Visual concept of exchange reactions in the metabolic model. From the perspective of the media, negative flux values represent consumption of metabolite by the cell from the media; positive values indicate production of the metabolite by the cell, excreted into the media.*

*FBA Pipeline in Python*

Using the provided metabolic model and interpolated media, a FBA pipeline was made using Python and COBRApy. (Fig. 3

*In vitro growth rate*

Growth rates of *in vitro* experiments determined in R based on OD at discrete time points throughout fermentation. (Fig. 4)

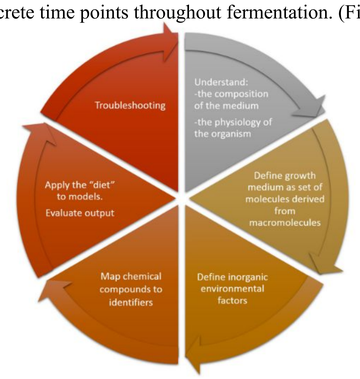



*Figure 2: High-level overview of medium definition taken from Marinos et al., 2020. The medium definition is an iterative process that also involves an component of trial and error.*

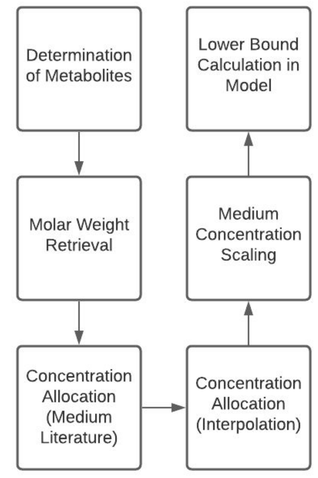
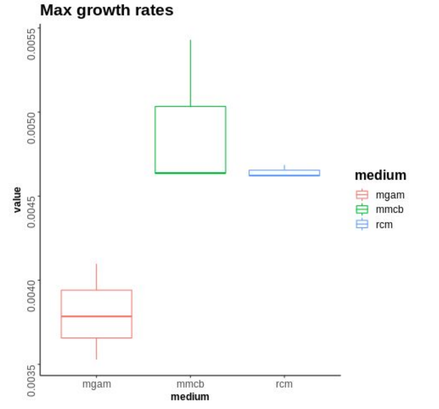



*Figure 3: Workflow of simulating in silico growth of F.prausnitzii in Python, beginning from media definition, covering ambiguous assumptions, and concluding in the calculation of a lower flux bound.*

## Results



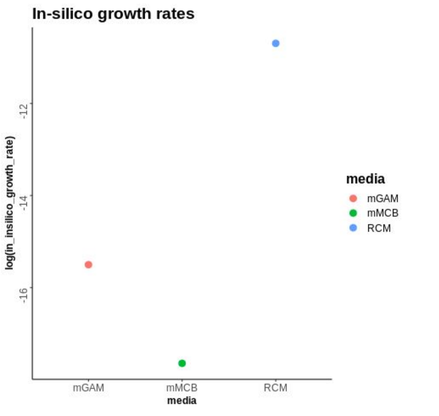
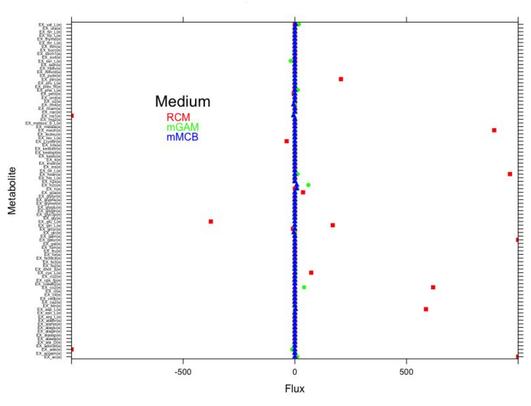




*Figure 4: [TOP] Growth rates of F.prausnitzii in vitro. mMCB and RCM media are within a comparable range of growth, considerably more suitable than mGAM.*
*[BOTTOM] In-silico growth rate obtained from the model designed by Heinken et al., in the environment we designed in this study. The growth rate is plotted on log-scale due to the large differences in the rates obtained.*



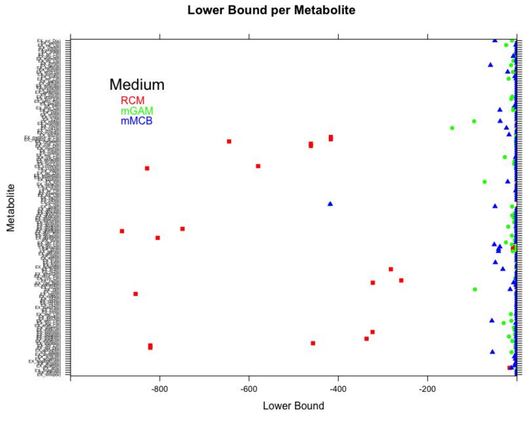




*Figure 5: [TOP] Resulting flux for each metabolite with a nonzero flux. RCM highly variable, mGAM much smaller, and mMCB much closer to 0 for all metabolites.*
*[BOTTOM] Calculated lower flux bound for each metabolite in the medium. Consistent with resulting flux values, RCM has much greater lower bounds, whereas mGAM and mMCB are much closer to 0.*

| RCM | |
|---|---|
| flux | BiGG_ID |
| 0.14771568 | glc(e) |
| 0.13707869 | glcur(e) |
| 1.04E-06 | ribflv(e) |
| | |
| **mMCB** | |
| flux | BiGG_ID |
| 0.68471129 | glc(e) |
| 0.6354053 | glcur(e) |
| 0.6354053 | pect(e) |
| 0.3247159 | ribflv(e) |
| | |
| **mGAM** | |
| flux | BiGG_ID |
| 0.01477157 | glc(e) |
| 0.01370787 | glcur(e) |

| Metabolite | Growth |
|---|---|
| Niacin | 7.88E-13 |
| L-Tryptophan | 6.21E-15 |
| Potassium | 3.82E-15 |
| Cobalt | 3.60E-15 |
| Copper | 3.60E-15 |
| Folate | 1.34E-15 |
| L-Serine | -2.87E-15 |
| Chlorine | -2.94E-15 |
| Biotin | -7.17E-15 |
| Calcium | -1.53E-14 |
| Pantothenate | -8.22E-14 |
| Sulphate | -8.60E-14 |
| Pyridoxal | -1.57E-13 |
| Magnesium | -1.71E-13 |

*Figure 6: [LEFT] Limiting metabolites within each medium. These metabolites have reached the maximum intake that would be theoretically allowed by the metabolic model based on the medium composition. [RIGHT] Essential metabolites that, if removed from a medium where all other metabolites in the metabolic model are available, limit bacterial growth to near-zero levels.*

## Improvements

The growth rate results in figure 4 and 5 indicate that the model created by Heinken et al., given the environment we estimated can be used to simulate growth of F.Prausnitzii. However, we could not reproduce the growth-rate results obtained in the wet-lab, neither qualitatively nor quantitatively. There are a number of factors that contribute to the deviating results:

1. Medium Composition - Complex metabolites
   a. Knowing the exact composition of the medium is critical to predict growth rate in-silico.
   b. Among the biggest factors of uncertainty are B-Vitamins of which many were required for growth and in the current solution
   c. Riboflavin was growth limiting for 2/3 media.

=> In order to improve on our model the concentration of B-vitamins in undefined medium components such as yeast/meat/liver extract could be investigated.

2. Estimation of unknown metabolite concentration.
   a. Metabolites found to be limiting growth if excluded from the medium got a small negative flux assigned to still allow for growth.
   b. Metabolites that were known to be present in the medium but at unknown concentration got the average flux value across all specific fluxes assigned.

=> Reducing the number of unknown metabolites in the medium will enhance the accuracy of the predicted growth rate.

3. Flux Calculation
   a. The exact dry-weight of F. prausnitzii was not known.

=> Possibly overestimating the weight of F. prausnitzii results in underestimating the fluxes.
=> The weight estimate could be improved by measuring the weight of the bacteria in addition to counting the number of the cells in in-vitro experiments.

4. Additional assumptions that increased the uncertainty of the outcome are the assumption of steady-state of the metabolites of the bacterium.

=> The steady state assumption would be justified when using a chemostat instead of a batch culture experiment to compare the in-silico results to.

5. Surprisingly, we detected consumption of acetate for the mGAM medium, which does not contain acetate. Upon investigating acetate was produced in all 3 media.

=> It could be interesting to investigate this further.

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