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A study of the community relationships between methanotrophs and their satellites using constraint-based modeling approaches

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Motivation and Aim: Methanotrophs are a group of microorganisms that use methane as their primary source of carbon and energy, and they are actively utilized in modern biotechnology [1]. One of the most studied methanotrophs is *Methylococcus capsulatus*, whose growth in monoculture is hindered by the accumulation of repressing byproducts. One of the solutions to this problem is co-cultivation of the methanotroph with other satellite microorganisms, which consume byproducts as substrates for their growth [2]. However, metabolic interactions within such microbial communities remain poorly understood. To assess metabolic interactions in the community, a new systems biology approach is currently applied – the reconstruction of constraint-based models of microbial communities, which allows for the assessment of strain interactions during co-cultivation [3]. Therefore, the aim of this study is to investigate the community model of *Methylococcus capsulatus* and its satellites, using as examples *Escherichia coli* and *Cupriavidus necator*.
Methods and Algorithms: To reconstruct community models, published genome-scale metabolic models of the bacteria *Methylococcus capsulatus* (*i*McBath) [4], *Cupriavidus necator* (*i*CN1361) [5], and two *Escherichia coli* models from the BIGG Models database were used: e_coli_core (http://bigg.ucsd.edu/models/e_coli_core) for the community model development and *i*AF1260 (http://bigg.ucsd.edu/models/iAF1260) for the community model with *M. capsulatus*. Prior to the community model construction, the quality of the *M. capsulatus* and *C. necator* models was assessed using the MEMOTE toolbox [6]. To consider metabolic interactions between two bacterial strains in the community model, transport, exchange reactions and external metabolites for them were added to both models using the Cobrapy library [7]. In the *C. necator* model, reaction and metabolite identifiers were replaced with corresponding BIGG database identifiers to facilitate interactions with the *M. capsulatus* model. The CommunityModel package from the Mewpy library [8] was employed to build the constraint-based community model of the methanotroph with its satellites. The package includes several widely used modules for analyzing microbial community flux balance models. The study was conducted in the BioUML platform [9], and the original program code is presented within a Jupyter Notebook framework (https://shorturl.at/eEOTW).
Results:
Evaluation of the models M. capsulatus, C. necator
Prior to the microbial community model reconstruction, it was necessary to assess the quality of the original models. MEMOTE tool was used for this purpose. The models demonstrated good quality and did not require improvement for further investigation

(detailed reports are available at https://shorturl.at/orFIL, https://shorturl.at/oIOR0 for *i*McBath and *i*CN1361 respectively). At the next step, transport and exchange reactions for acetate were added to the iMcBath model, and the methane uptake rate was increased from 18.46 to 28.1 mmol/gDCW/h^{-1} (gDCW – gram dry cell weight) [10], resulting in an increase in growth rate from 0.178 to 0.27 h^{-1}. Acetate secretion was optimized by selecting acetate exchange reaction as the objective function, with a minimum biomass condition of 0.9 times the maximum. Thus, the acetate exchange rate in the model was amounted to 0.702 mmol/gDCW/h^{-1}.

Pipeline validation for the community model reconstruction

The pipeline for construction of the community model for M. capsulatus and its satellite was tested with selected tools on two combined and simplified (e_coli_core) models of *E. coli*. Acetate was chosen as the main metabolite for interaction between two *E. coli* strains (acetate producer and consumer), as it presumably inhibited the growth rate of *M. capsulatus*, but satellites in mixed culture can use it as a source of carbon. Optimization of acetate secretion by one strain was achieved by adjusting the system to microaerobic cultivation conditions, while for the second strain, the system was adjusted to cultivation conditions with acetate as the carbon source. The community model was constructed using the CommunityModel module from the Mewpy library. As a result, the model yielded the following community metabolites: the interaction between strains occurred via acetate (18.4 mmol/gDCW/h^{-1}), water (17.7 mmol/gDCW/h^{-1}), and hydrogen ions (10.7 mmol/gDCW/h^{-1}). The growth rates of the acetate producer and acetate consumer *E. coli* strains become 0.82 and 0.33 h^{-1}, respectively. This difference can be attributed to the use of different substrates for bacterial growth within the community – acetate being a less efficient substrate compared to glucose that was a carbon source for the acetate producer.

Reconstruction of the community model for Methylococcus capsulatus and Escherichia coli

Following the validation of the pipeline on simplified models, the next step involved constructing a model for the interaction between *M. capsulatus* and *E. coli*. The cultivation medium was chosen based on the microelements required for the models, which are included in the biomass equations. Also, methane was used as a single carbon source for *M. capsulatus* growth. For the *E. coli* model, fluxes were blocked for reactions not present in the community medium, and constraints were set for acetate uptake rate, resulting in a growth rate of 0.46 h^{-1}. Analysis of the resulting community revealed that in the co-cultivation, *M. capsulatus* produced 2.79 mmol/gDCW/h^{-1} of acetate, which was entirely consumed by *E. coli*. Other cross-feeding metabolites are included formaldehyde (1.24 mmol/gDCW/h^{-1}), glycerol (0.0005 mmol/gDCW/h^{-1}), and threonine (1.27 mmol/gDCW/h^{-1}), produced by the methanotroph, and serine (1.27 mmol/gDCW/h^{-1}) produced by *E. coli* (see the Fig. 1).

The growth rates in the community were 0.15 h^{-1} for *M. capsulatus* and 0.077 h^{-1} for *E. coli*. The decrease in the growth rate for the *M. capsulatus* model is attributed to higher acetate production compared to the original model, while the lower growth rate of *E. coli* is associated with the reduced availability of acetate compared to the original model.

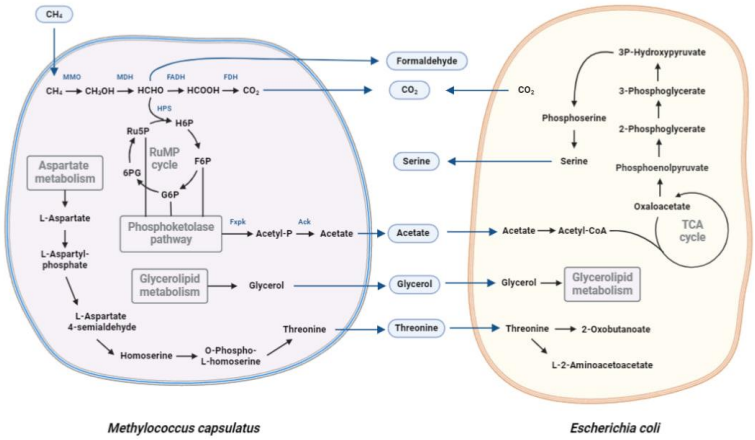

Fig. 1. Interaction between models of *M. capsulatus* (*on the left*) and *E. coli* (*on the right*) after reconstruction of a community model

Conclusion: Using simplified models of *Escherichia coli* as a basis, a pipeline for reconstructed constraint-based metabolic models describing microbial communities was developed. Based on this pipeline was reconstructed a community model for *Methylococcus capsulatus* and *E. coli*, demonstrating their interaction through acetate utilization by satellite strain, the secretion of metabolites such as formaldehyde and serine by the community. Quality assessment of the *M. capsulatus* and *Cupriavidus necator* models was conducted for subsequent model construction and analysis of their interactions.

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