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Genomically and biochemically accurate metabolic reconstruction of Methanosarcina barkeri Fusaro, iMG746

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Supplementary material

Flux balance analysis

The S matrix is a sparse matrix that contains the information on the coefficients for the metabolic reactions and it is the mathematical representation of the reconstructed metabolic reaction network. The S matrix is an m by n matrix, where m is the number of metabolites in the model, and n is the number of reactions. The S_{ij} entry in the matrix corresponds to the coefficient of the i^{th} metabolite in the j^{th} reaction.

Flux balance analysis is a well-characterized linear programming technique in which the principal constraints are mass balance constraints [1]. Simulations are typically performed under steady state conditions due to lack of metabolite accumulation data. Under these conditions, the mass balance for the metabolic network is

$$Sv = 0 \quad \text{(S1)}$$

where v is an n by 1 vector that contains the reaction rates ("fluxes") and lies in the null space of S. Due to limited availability of reaction rates and the number of reactions being larger than the number of metabolites, the system is underdetermined. Additional constraints are added based on reaction direction,

$$lb \leq v \leq ub \quad (2)$$

where *lb* is the lower bound of the reaction (-1000 for reversible, 0 for irreversible reactions) and *ub* is the upper bound of the reaction (1000). When information such as enzyme concentrations and turnover numbers are available, reaction bounds may be constrained further.

To pick a particular set of reaction rates, flux balance analysis requires the assignment of an objective function. All simulation results in this manuscript were calculated under the assumption of maximal growth, which has been shown to accurately predict metabolic states for prokaryotes. The final form of the FBA problem then becomes:

$$\text{Max } v_{BOF} \quad (3)$$

Subject to:

$$Sv = 0$$

$$lb \leq v \leq ub$$

Details of stoichiometry determination

As part of our development of the genome-scale reconstruction of *Methanosarcina barkeri*, we performed a thorough study to identify the most likely proton-pumping stoichiometry of Ech hydrogenase, sodium-pumping stoichiometry of Mtr, the $Na^+\backslash H^+$ ratio of the sodium-proton

antiporter, and the ion specificity of the ATP synthase, all of which are not completely characterized experimentally. All referenced data is available in the supplemental excel sheets.

Ech hydrogenase is an important part of the electron transport chain, but the exact number of protons or sodium ions pumped across the membrane has not been experimentally validated. However, it is known to generate an ion gradient [2]. Increasing the number of protons pumped by Ech was predicted to reduce the growth rate on H_2/CO_2 by consuming more of the proton gradient. On the other hand, when grown on methanol, the Ech hydrogenase operates in the opposite direction from that when grown on H_2/CO_2, since methanol metabolism produces excess reduced ferredoxin [3]. Therefore, the increased number of protons pumped by Ech produces a larger proton gradient, thus increasing the growth yield on methanol. Based on the data available when iAF692 was published, simulations suggested that only one proton was pumped per hydrogen produced [4]. After compiling additional growth data using H_2/CO_2 as a growth substrate [5], the analysis was repeated with iMG746. The results favor an Ech hydrogenase that pumps two protons out of the cell per hydrogen produced, which differs from the iAF692 model (Figure 2) [5-7]. Using either empirical formulas and pH gradients [8] or estimates based on ATP synthesis requirements [9], the energy associated with the translocation of one proton into the cell is estimated to be approximately -8 kJ/mol, which is reasonable given the reversibility of the Ech hydrogenase reaction and the estimated Gibbs energy of reaction of -16 kJ/mol H_2 produced (under standard conditions, H_2 pressure 1 bar) [9].

A recent study on ATP synthesis in *Methanosarcina acetivorans* suggested that under certain conditions, ATP synthase can utilize either Na^+ or H^+ to generate ATP [10]. Simulations using iMG746 predicted that if the Na^+/H^+ antiporter has a stoichiometry of 1 to 1, then the ionic specificity of the ATP synthase has no impact on the known phenotype. However, the antiporter stoichiometry is unknown and thus the Na^+ or H^+ preference for the ATP synthesis may have a significant impact on growth rates and yields, particularly if the ratio is not 1:1.

We found that there was no combination of ATP synthesis reactions that utilized both Na^+ and H^+ that matched the experimental growth yields for *M. barkeri*. If both a proton-driven and a sodium-driven ATP synthesis reaction were included in the model, FBA always selected the combination that generated the greatest ATP yield based on the Na^+/H^+ antiporter ratio. This often led to growth rates that are significantly higher than those found experimentally. Based on the simulations, we hypothesize that while the ATP synthesis reaction may have the capability to use both Na^+ and H^+, the selectivity for Na^+ is low and thus H^+ is preferred under physiological conditions.

While examining the stoichiometry of the other methanogenesis reactions, we found that an Ech proton pumping stoichiometry of 2 H^+/H_2 best matched growth rate data during growth on H_2/CO_2. We also found two combinations of ion-pumping stoichiometry for Mtr and the Na^+/H^+ antiporter that yield reasonable growth rate predictions. One combination suggests that Mtr pumps only 1 Na^+ per mole of MH4SPT and the Na^+/H^+ pumping stoichiometry is 1:1. However, this combination does not agree with experimental results in *M. mazei*, which suggest that Mtr has a pumping stoichiometry greater than 1 [11]**.** A BLASTP between the Mtr proteins of *M. barkeri* and *M. mazei* shows near perfect identity between the Mtr proteins of the two

methanogens, so it is unlikely that the stoichiometry is different between them. The other experimentally-consistent possibility is that Mtr pumps 2 Na^+ and the $Na^+\backslash H^+$ antiporter stoichiometry is 2 Na^+ per 1 H^+. Since this combination yields the most consistent predictions compared with experimental data, we incorporated these ratios into the model.

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