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Maps for when the living gets tough: Maneuvering through a hostile energy landscape

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Abstract: With genome sequencing of thousands of organisms, a scaffold has become available for data integration: molecular information can now be organized by attaching it to the genes and their gene-expression products. It is however, the genome that is selfish not the gene, making it necessary to organize the information into maps that enable functional interpretation of the fitness of the genome. Using flux balance analysis one can calculate the theoretical capabilities of the living organism. Here we examine whether according to this genome organized information, organisms such as the ones present when life on Earth began, are able to assimilate the Gibbs energy and carbon that life needs for its reproduction and maintenance, from a relatively poor Gibbs-energy environment. We shall address how *Clostridium ljungdahlii* may use at least two special features and one special pathway to this end: gear-shifting, electron bifurcation and the Wood-Ljungdahl pathway. Additionally, we examined whether the *C. ljungdahlii* map can also help solve the problem of waste management. We find that there is a definite effect of the choices of redox equivalents in the Wood-Ljungdahl pathway and the hydrogenase on the yield of interesting products like hydroxybutyrate. We provide a drawing of a subset of the metabolic network that may be utilized to project flux distributions onto by the community in future works. Furthermore, we make all the code leading to the results discussed here publicly available for the benefit of future work.

Keywords: Wood-Ljungdahl pathway, *Clostridium ljungdahlii*, genome-wide metabolic map, flux-balance analysis, electron bifurcation, gear-shifting, syngas, biodegradable plastics

1. INTRODUCTION

The question of how life got started in the early periods of our planet is still very much open. In the early soup of chemicals, some billion years ago, no organic carbon compounds may have been abundant, there was no oxygen and the question of how life got going is therefore intriguing. A critical question in this regard is how the early organisms could produce their ATP (Schuchmann and Müller, 2014) and grow autotrophically on the available mixtures of CO, CO_2 and H_2.

Schuchmann and Müller (2014) highlighted the Wood-Ljungdahl pathway (WLP) as a network feature enabling to overcome the difficulties faced by early colonizers of Earth. Since the WLP is ATP-neutral, an additional 'trick' is also required so as to be able to harvest the limited amount of Gibbs free energy made available by the WLP. This has been identified as electron bifurcation. Electron bifurcating enzymes consist of multiple subunits coupling an endergonic to an exergonic redox reaction, thereby achieving what would otherwise be impossible without ATP input or chemiosmotic coupling of the endergonic reaction.

Schuchmann and Müller (2014) described how WLP and electron bifurcation could lead to the production of ATP from ADP and phosphate provided the use of two more transmembrane enzyme complexes is made, i.e. the H^+-ATPase and the Rnf complex. They did not show whether other solutions to the ATP synthesis problem may be possible, and whether what they proposed was in immediate concordance with the knowledge integrated through the genome-wide metabolic maps of the organisms (Nagarajan, 2013).

In the present paper, we wish to compare the analysis by Schuchmann and Müller with the predictions of maximal ATP synthesis emanating from the genome-wide metabolic reconstruction of the model acetogen *Clostridium ljungdahlii* through flux-balance analysis (FBA) (Orth, 2010). Some analysis on the effect of redox equivalents on growth and product synthesis was already present in (Nagarajan, 2013). Specifically, it was shown that the genome-wide map predicts the possibility of growth on CO_2/H_2 and CO and the effect of various options in redox equivalents were analyzed under the knockout of acetate kinase. However, we hope to extend that analysis here by including various alternative reactions that were considered in the treatment by Schuchmann and Müller. Specifically, we will investigate alternatives in the electron

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donors/acceptors for various enzymes and their effect on ATP yield coupled to acetogenesis. Additionally, we will focus on the importance of the Wood-Ljungdahl *pathway* as opposed to single enzymes, the need for electron bifurcation and the Nfn complex, the concept of gear-shifting, the requirement of low gear and advantages of high gear operation, and how much product yield might be attained when engineering *C. Ljungdahlii* with two additional genes for producing poly-hydroxybutyrate (PHB) under various redox alterations.

2. METHODS

2.1 Genome-scale reconstruction of Clostridium ljungdahlii

Our starting point was the previously published genome-scale metabolic reconstruction of *Clostridium ljungdahlii* (Nagarajan, 2013). We obtained the SBML file of the reconstruction from the BiGG database (King et al., 2016) through http://bigg.ucsd.edu/models/iHN637 on August 25th 2016. This reconstruction covers 698 metabolites, in 785 reactions, encoded by 637 genes. The map was shown to recover experimentally measured growth rates on media of various compositions (Nagarajan, 2013).

2.2 Extending the Genome-wide metabolic model with alternative reactions considered in Schuchmann et al.

In this work we are concerned with reproducing and understanding in more detail the analysis provided by Schuchmann and Müller (2014) for *C. ljungdahlii* with its genome-wide metabolic reconstruction. To that end we made sure all reactions considered in that study also came to exist in a (slightly) enhanced version of the the genome-wide metabolic map (GeMM) (which we shall call GeMM*), adding reactions where necessary, and we set the simulation conditions appropriately for the acetogenesis problem.

We adjusted the *in-silico* medium to be a mixture of CO_2 and H_2 in a 2:4 ratio by setting the lower bounds on the CO_2 and H_2 exchange reactions to -2 and -4 respectively, where the negative direction indicates uptake of the metabolites. We set the ATP maintenance reaction (ATP -> ADP + phosphate) as the objective function with a lower bound of zero, thereby asking with which flux distribution the network could make the ATP synthesis reaction as high as possible. Additionally, we forced the flux through the exchange reaction of acetate to be equal to 1 flux unit in the outward direction. Note that this does force the organism to make full use of both CO_2 and H_2 in the medium. If simulation conditions deviate from those described here, we will explicitly highlight the new conditions.

Several reactions described in Schuchmann and Müller (2014) are essential and were added to the metabolic reconstruction in order to enable the simulations in the scenarios considered, see Table 2. In traditional biochemistry considering a single compartment, it is of no importance to keep clear track of protons across reactions since the pH-buffer of the medium is large enough to assuage any problems. When chemiosmotic coupling plays a role however, one needs to keep track of protons that move across the membrane and because membrane potential is often the more important component of the proton motive force, contribute to the membrane potential. Thereby other charged species that move across that membrane should also be taken into account. However, because flux-balance analysis requires all species, i.e. also the protons, to be balanced, even one wrongly annotated proton can lead to problems including inaccurate bioenergetics/ATP synthesis. The modeler must account for each of these protons i.e. perform accurate bookkeeping of protons and do so while taking into account the protonation of the metabolites already existing in the reconstruction. Alternatively, one should become explicit in transmembrane charge movement, which is not customary in existing flux balance analysis. We listed the reactions (Table 1) that were manually added to the reconstruction but that are slightly different in terms of protons from those considered in Schuchmann and Müller (2014).

In the reconstruction downloaded from the BiGG database, the ACACT1r (Acetyl-CoA C-acetyltransferase) and HACD1 (3-hydroxyacyl-CoA dehydrogenase) reactions had been blocked, i.e. both the lower and upper bound had been set to zero, for unknown reasons. We unblocked these so as to allow flux into the beta-hydroxybutyrate synthesis pathway. Finally, we added a demand reaction for removing (S)-3-hydroxybutanoyl-CoA (while recycling the CoA) from the cell, so that we may predict its maximal production flux for various network perturbations.

Reaction ID	Formula
FDHH2	$CO_2 + H_2 \rightarrow Formate^- + H^+$
FDHFDNADPH	$2\,CO_2 + Fd^{2-} + NADPH + H^+ \rightarrow Fd + 2\,Formate^- + NADP^+$
MTHFD_alt	$Methenyl-THF^{2-} + NADH + Methylene-THF^{3-} + NAD^+$
MTHFR5_alt	$Methylene-THF^{3-} + NADH + 2\,H^+ \rightarrow Methyl-THF^{2-} + NAD^+$

Table 1. Reactions that were added to the genome scale reconstruction ('GeMM') from (Nagarajan, 2013). The genome-wide metabolic reconstruction with these reactions added will be referred to as GeMM*. Electric charges of compounds are important and subject to standards set by the existing GeMM. They present an issue to be elaborated upon elsewhere.

2.3 Flux balance analysis

In all simulations in this paper we apply the computational technique of FBA (Orth, 2010). Briefly, this technique concerns the following linear programming problem:

$$\text{maximize or minimize } Z = c^T v, \text{such that for all k}$$
$$Sv = 0$$
$$\alpha_k \leq v_k \leq \beta_k.$$