

Using a Metabolic and Gene-Expression Model to Predict and Analyze the Phenotypic Response of Acetogen *Clostridium ljungdahlii*

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Project Goals: We have reconstructed a metabolic and gene-expression model (ME-model) for the acetogen *Clostridium ljungdahlii*. This model details the organism's interconnectivity of metabolism, energy conservation, and macromolecular synthesis in a computable format, which substantially enhances our knowledge about acetogens. We are now using the model to explore the potential for biocommodity production from inexpensive sources through alterations to media composition, genetic manipulation, and more.

The acetogen *Clostridium ljungdahlii* has emerged as a potential chassis for strain designed chemical production for not only can it grow heterotrophically on a diverse set of sugars, but it can also grow autotrophically on carbon monoxide (CO), carbon dioxide (CO₂) and hydrogen (H₂), or a mixture of all three gases (i.e. syngas). When grown autotrophically, *C. ljungdahlii* metabolizes the gases into multi-carbon organics, an ability that can be redirected and engineered to produce biocommodities from low cost substrates.

To advance towards this goal, a constraint-based modelling method was used to systematize the biochemical, genetic, and genomic knowledge of *C. ljungdahlii* into a computable mathematical framework. This metabolic and gene expression model (ME-model) accounts for 944 ORFs that are responsible for the production of transcriptional units, functional RNAs (e.g., tRNAs, rRNAs), prosthetic groups, cofactors, and protein complexes that are necessary for all of the major central metabolic, amino acid, nucleotide, and lipid biosynthesis pathways. This ME-model is able to compute the molecular constitution (i.e. transcriptome, proteome, and fluxome) of *C. ljungdahlii* as a function of genetic and environmental parameters, and is able to do so accurately, as the ME-model's *in silico* transcriptome reflects *in vivo* subsystem expression under CO and fructose growth ($r_{CO} = 0.887$, $r_{fructose} = 0.906$, $p < 0.001$).

Not only does the ME-model recapitulate results from standard laboratory growth conditions, but it can also calculate *C. ljungdahlii*'s phenotypic responses to gene knockouts, alternate carbon sources, and even changes in metal availability. For example, a simulated carbon monoxide dehydrogenase; acetyl-CoA synthesis knockout in the ME-model predicts that *C. ljungdahlii* will stop acetate production, increase ethanol production, reduce CO uptake, and reduce CO₂ production, similar to the findings in Liew *et al.* 2016. Additionally, the ME-model, unlike a metabolic model, can predict electron overflow resulting in ethanol secretion and, for certain carbon sources, glycerol production, which was validated through HPLC. Finally, the ME-model provides a systems biology approach to analyze unmetabolized media components in a constraint-based manner, which was validated with the effects of nickel and manganese availability on both heterotrophic and autotrophic growth rates and secretion profiles.

Thus, with this ME-model, we have a foundation for predicting and understanding the phenotype of *C. ljungdahlii* for multiple situations, which is vital for effective strain design.

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