

Computational Analysis of Microbial Metabolism for Fuel and Chemical Production

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Project Goals: Cyanobacteria offer a promising route for directly converting solar energy and CO₂ into biofuels. The objectives of this research are to integrate modeling and experimental approaches to guide development of a butanol producing cyanobacterium, *Synechococcus* sp. PCC 7002. New computational approaches will be developed to facilitate these efforts which will (1) design experiments and analyze their results, and (2) identify genetic engineering strategies for improving butanol production in *S. 7002*. Experiments will subsequently be performed to construct and analyze *Synechococcus 7002* strains engineered for butanol production. The developed approaches will be systematically applied to suggest genetic engineering strategies for improving production of a variety of biofuels in five other microorganisms. This research will support the U.S. Department of Energy's mission for developing renewable ways of producing advanced biofuels.

Renewable sources of transportation fuels and chemicals are needed to reduce the amount of oil used to satisfy transportation energy needs in the U.S. and to alleviate our dependence on foreign sources of oil. Microbes can be used to produce a wide variety of liquid biofuels including: ethanol, butanol, isobutanol, isoprene, hydrogen, and alkanes. Cyanobacteria offer an alternative route for converting solar energy and CO₂ into biofuels, without the need for using lignocellulosic biomass as an intermediate. The biofuel production capabilities of microbes can be improved through metabolic engineering, where metabolic and regulatory processes are adjusted using targeted genetic manipulations. Traditionally, metabolic engineering strategies are found through manual inspection of metabolic pathways, where enzymes involved in biosynthesis are overexpressed or added, competing pathways are eliminated, and the performance of resulting strains are evaluated. However, such approaches cannot predict the effects that these changes will have on other parts of metabolism, and generally will not suggest alterations to more distant pathways. Computational models of cellular metabolic and regulatory networks can be used instead to guide and accelerate these metabolic engineering efforts by integrating and analyzing experimental data, and identifying genetic manipulations that would increase product yields.

We systematically evaluated what potential products could be produced by *Escherichia coli* (using both native and heterologous pathways) and then evaluated their distance (in terms of metabolic reactions steps) from central metabolic precursors [1]. Using a genome-scale metabolic model of *E. coli* and a set of potential heterologous reactions (from the KEGG database), ~1,800 non-native products could potentially be produced in *E. coli* using heterologous enzymes, with ~300 having commercial applications. Subsequent analysis found

that pyruvate was the closest central metabolic precursor to the most non-native commercial products. Since pyruvate has industrial applications and can be converted into valuable products, we sought to develop a strain of *E. coli* that could produce pyruvate at high yields. Guided by a genome-scale metabolic model of *E. coli*, we then identified different strategies for enhancing production of pyruvate from glucose. We constructed a number of strains which achieved yields up to 0.92 g pyruvate per g substrate (~95% theoretical yield) and which can be used to produce other biofuels and biochemicals (Zhang and Reed, in preparation). These results illustrate how computational models can be used to prioritize precursor-based strategies and identify genetic modifications to enhance precursor production.

Current efforts are extending these analyses to evaluate chemical production in the cyanobacterium, *Synechococcus sp.* PCC 7002. Strain designs for enhancing butanol (and other chemicals) production in *Synechococcus sp.* PCC 7002 have been made using computational models and are currently being constructed [2].

References

1. Zhang X, Tervo CJ, and JL Reed. Metabolic Assessment of *E. coli* as a Biofactory for Commercial Products. *Metabolic Engineering*. Accepted (2016).
2. Vu TT, Hill EA, Kucek LA, Konopka AE, Beliaev AS, and JL Reed. Computational evaluation of *Synechococcus sp.* PCC 7002 metabolism for chemical production. *Biotechnology Journal*, 8(5):619-30 (2013).

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