217. Computational Algorithms for Metabolic Engineering Strain Design

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Project Goals: Computational models of biological systems can be used to explain observed behaviors, predict un-measurable quantities, and predict cellular behavior arising from environmental, genetic perturbations, or both. Models can be useful in engineering biofuel production strains, where challenges include finding bottlenecks in metabolic pathways and identifying appropriate perturbations to force a microorganism to produce or consume more of a compound of interest. Our research efforts have focused on developing and improving computational tools for designing strains, and applying these tools to models of *Escherichia coli* and *Saccharomyces cerevisiae*.

Metabolic engineering seeks to improve cellular production of valuable biochemicals, such as biofuels, by altering metabolic and regulatory pathways in a host strain. Computational tools are becoming increasingly available to design microbial strains using genome-scale *in silico* models of metabolism that predict the re-distribution of metabolic fluxes after genetic or environmental perturbations. In this poster, we describe some of our recent results involving computational and experimental approaches to improve biofuel production.

First, we developed and applied a new computational tool for identifying fluxes that must change to increase biofuel production or increase sugar utilization. The method determines how much to increase or decrease fluxes to improve production. This tool, which we named CosMos (for continuous modifications of fluxes), was able to determine strategies for up- and down- regulation of metabolic genes in *E. coli* and *S. cerevisiae* that lead to production of a variety of desired chemicals, including biofuels and amino acids. We have compared our results from CosMos to previous results in the experimental metabolic engineering literature, and we found a number of cases wherein our predictions with CosMos closely matched successful experimental strategies. We are currently using this approach to design *E. coli* and yeast strains for advanced biofuel production.

Second, we used our developed algorithm (OptORF) to identify gene deletions needed to improve pyruvate production in *E. coli*. OptORF identifies which metabolic and regulatory genes to delete or over express so that chemical production is coupled to cellular growth [1]. We engineered a number of strains and have achieved yields of more than 0.88 g pyruvate per g of glucose (~90% theoretical yield). To re-engineer the pyruvate strains to produce ethanol, pyruvate formate-lyase (PflB) was deleted and pyruvate decarboxylase (Pdc) and alcohol dehydrogenase II (AdhB) from *Zymomonas mobilis* were highly expressed. These re-engineered strains fermented glucose to ethanol with a yield of 0.35 g ethanol per g of glucose (~70% of theoretical yield). While higher ethanol yields have been

achieved, these results illustrate that pyruvate over-producing strains can serve as a platform to generate other biofuels derived from pyruvate.

Third, we used our previously developed algorithms (OptORF and RELATCH [2]) to identify gene deletions needed to improve xylose fermentation in *E. coli* when glucose is present. Knockout mutant strains were constructed and adaptively evolved under anaerobic conditions. Some of the resulting strains were able to co-utilize glucose and xylose when grown anaerobically in minimal medium and were able to consume xylose at an increased rate (albeit at the expense of glucose consumption). Together these three studies illustrate how computational tools can be used to facilitate the design of strains for converting lignocellulosic biomass into biofuels.

References

1. Kim, J. and J.L. Reed, *OptORF: Optimal metabolic and regulatory perturbations for metabolic engineering of microbial strains.* BMC Syst Biol, 2010. **4**: p. 53.
2. Kim, J. and J.L. Reed, *RELATCH: relative optimality in metabolic networks explains robust metabolic and regulatory responses to perturbations.* Genome Biol, 2012. **13**(9): p. R78.

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