Constraint-Based Analysis of Microbial Metabolism and Regulation for Improving Biofuel Production

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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 and/or genetic perturbations. Models can be useful in engineering biofuel production strains, where some of the challenges are finding bottlenecks in metabolic pathways and suggesting the appropriate perturbations to force a microorganism to produce more of a compound of interest. Our research efforts over this past year have focused on developing and improving computational tools for designing strains, refining models, integrating omics datasets, and estimating kinetic parameters from experimental data. We have developed a number of methods and applied them to *Escherichia coli* and *Saccharomyces cerevisiae*.

Metabolic engineering seeks to improve cellular production of valuable biochemicals, such as biofuels. Computational tools are becoming increasingly available to design microbial strains using *in silico* models of metabolism that predict the re-distribution of metabolic fluxes after genetic perturbations. In this poster, we describe results from four recent projects. First, we analyzed fermentation results generated by our collaborators at GLBRC, including biomass and extracellular metabolite concentration data, and made predictions about metabolic states during fermentative growth in AFEX-treated corn stover hydrolysate by *Saccharomyces cerevisiae* and *Escherichia coli*. Second, we developed a constraint-based kinetic model by integrating multi-omics datasets and used it to identify potential reactions limiting flux through central metabolic pathways. Third, we developed a new approach (RELATCH) that shows significantly improved predictions of intracellular flux distributions in response to genetic and environmental perturbations. Fourth, we applied some of our recently developed methods (OptORF, BiMOMA, SimOptStrain) to propose metabolic engineering strategies to improve chemical production using constraint-based models. The genetic strategies identified can include deletion of transcription factors and metabolic genes, as well as over-expression of native and non-native metabolic genes. Using these approaches we have identified strategies for improving ethanol production in *Escherichia coli* and *Saccharomyces cerevisiae*. These approaches can be applied to improve production of a wide variety of compounds in other biological systems by proposing beneficial metabolic and/or regulatory changes.

Publications

1. Dipak Barua, Joonhoon Kim, Jennifer L. Reed, "An Automated Phenotype-Driven Approach (*Gene*Force) for Refining Metabolic and Regulatory Models," PLoS Computational Biology, 2010, vol. 6, issue 10.

2. Joonhoon Kim and Jennifer L. Reed, "OptORF: Optimal metabolic and regulatory perturbations for metabolic engineering of microbial strains," BMC Systems Biology, 2010, vol 4, issue 53.
3. Joonhoon Kim, Jennifer L. Reed, Christos T Maravelias, "Large-Scale Bi-Level Strain Design Approaches and Mixed-Integer Programming Solution Methods," PLoS ONE, 6(9): e24162 2011.
4. Cameron Cotten and Jennifer L. Reed, "Analysis of Multiomics Datasets to Generate Kinetic Parameters for Constraint-Based Metabolic Models," Submitted.
5. Michael S. Schwalbach, David H. Keating, Mary Tremaine, Wesley D. Marner, Yaoping Zhang, William Bothfeld, Alan Higbee, Jeffrey A. Grass, Cameron Cotten, Jennifer L. Reed, Leonardo da Costa Sousa, Mingjie Jin, Venkatesh Balan, James Ellinger, Bruce Dale, Patricia J. Kiley, and Robert Landick, "Complex physiology and compound stress responses during fermentation of alkaline-pretreated corn stover hydrolysate by an *Escherichia coli* ethanologen." Under review.