Metabolomics of Clostridial Biofuel Production

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Project Goals:

The production of biofuels from cellulosic biomass holds promise as a source of renewable clean energy. Members of the genus Clostridium collectively have the ideal set of the metabolic capabilities for biofuel production from cellulosic biomass: C. acetobutylicum rapidly ferments glucose to biofuels (butanol, hydrogen) and C. cellulolyticum effectively degrades cellulose. Here we aim to integrate metabolomics, genomics and genetic engineering to dramatically advance understanding of metabolism in C. acetobutylicum and C. cellulolyticum. In so doing, we will lay basic science groundwork for engineering of an organism that cost-effectively converts cellulose into solvents and/or hydrogen gas.

Results and Plans:

The fermentation carried out by *C. acetobutylicum* is characterized by production of acids during exponential growth ("acidogenesis"), followed by production of solvents as growth slows down ("solventogenesis"). We previously used metabolomics and quantitative flux modeling to map the metabolic changes associated with this acidogenic-solventogenic transition. This effort revealed a marked reorganization of central metabolism that involved both large changes in intracellular metabolite concentrations and metabolic fluxes. Solventogenesis was characterized by a dramatic down regulation of fluxes through pyruvate carboxylase, the reductive TCA cycle, and amino acid biosynthesis. These flux changes favor build-up of pyruvate and reducing power, the substrates of solventogenesis. Recently, we have knocked out pyruvate carboxylase, and find that this facilitates transition into solventogenesis, improves acid re-assimilation, and slightly increases final solvent yields.

Another key aspect in biofuel production from cellulose is hemicellulosic sugar catabolism. We previously mapped the pathways of glucose metabolism in *C. acetobutylicum* by following the dynamics of ^{13}C-labeled glucose assimilation. Here we are conducting similar isotope tracer experiments to explore the simultaneous utilization of glucose and hemicelluloses sugars (xylose, arabinose, mannose, and galactose). Our initial experiments show that while galactose is not assimilated in the presence of glucose, the organism simultaneously and non-discriminantly catabolizes both glucose and mannose. Similarly, both xylose and arabinose are assimilated in the presence of glucose not only into the pentose phosphate pathway, but also lower glycolysis. There is no assimilation into upper glycolysis, however. This suggests that, in the presence of pentose sugars, there is minimal activity of the transaldolase reaction, which yields fructose-6-phosphate. Follow-up experiments with different types of ^{13}C-labeled glucose and xylose are underway to confirm this hypothesis. In addition, we are working to model quantitatively the associated fluxes.

Finally we aim to understand *C. cellulolyticum's* catabolic limitations. While an impressive cellulose degrader, *C. cellulolyticum* is a sluggish fermenter (20-fold slower than *C. acetobutylicum*). We will apply metabolomics and genomics to investigate the reasons for its slow sugar catabolism.

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