Designing a Microbial Community for Production of Biofuel from Lignocellulose

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Project Goals: The goal of our research is to implement a high-throughput pipeline for the systematic, computationally-driven study and optimization of microbial interactions and their effect on lignocellulose degradation and biofuel production. We combine multiple approaches, including computer modeling of ecosystem-level microbial metabolism, mass spectrometry of metabolites, genetic engineering, and experimental evolution.

The microbial production of biofuels from lignocellulose is a complex, multi-step process. Microbial consortia are an ideal approach to consolidated bioprocessing: a community of microorganisms performs a wide variety of functions more efficiently and is more resilient to environmental perturbations than a microbial monoculture. Each organism we have chosen for this project addresses a specific challenge: lignin degradation (*Pseudomonas putida*); (hemi)cellulose degradation (*Cellulomonas fimi*); lignin degradation product demethoxylation (*Methylobacterium* spp); generation of biofuel lipid precursors (*Yarrowia lipolytica*). These organisms are genetically tractable, aerobic, and have been used in biotechnological applications.

In the past year, we have made significant experimental and computational progress both at the level of single organism characterization, and at the level of the assembly and observation of co-cultures.

In terms of individual organisms, we have analyzed the growth of *C. fimi*, *P. putida*, and *Y. lipolytica* in different environmental conditions. Experimentally, we have used mass spectrometry to characterize and measure the metabolic inputs and outputs of each of these consortium members, providing valuable information for model

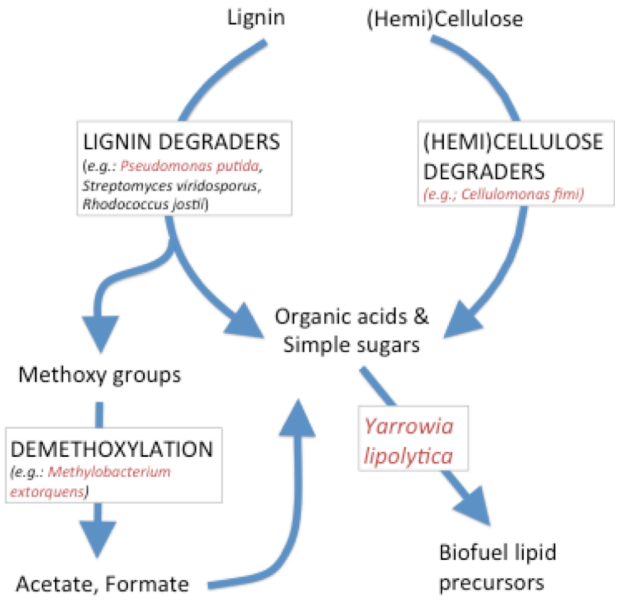

Figure 1. *Microbial community design.*

refinement, and for understanding possible metabolism-mediated interactions. On the computational side, we have implemented draft genome-scale models for all consortium members, based on KBase reconstructions and published information. Models have been imported into our platform for spatio-temporal dynamic flux balance modeling, COMETS (Computation Of Microbial Ecosystems in Time and Space). In parallel, we have started exploring the incorporation of extracellular enzymes in COMETS, in order to simulate more faithfully lignocellulose degradation processes in the community.

In addition to lignocellulose degradation, we have begun to address the challenge of removing the free formaldehyde produced by the demethoxylation of lignin monomers, which can otherwise inhibit microbial growth due to its toxicity. *M. extorquens* utilizes formaldehyde as a central metabolic intermediate, but cannot remove and detoxify the methoxy groups on lignin. We have identified soil-dwelling *Methylobacterium* species that can break down lignin degradation products, and are genetically engineering the well-studied *M. extorquens* so that it expresses these enzymes. We have also identified potential pathways to introduce the demethoxylation and cleavage of complex aromatics, with the goal to introduce these pathways into *M. extorquens*.

At the level of consortia – a key goal of this project - we have started studying how the lignocellulose degradation by *C. fimi* and *P. putida* can ensure a flow of saccharides to *Y. lipolytica* and of lignin degradation byproducts usable by *M. extorquens*. Using mass spectrometry, we have identified potential cross-feeding metabolites for synergistic growth of *C. fimi* with *Y. lipolytica* and *P. putida*, including metabolites released by *Y. lipolytica* and *P. putida* that are consumable by *C. fimi*. In order to develop an optimized defined medium for the biofuel consortium, we are developing minimal defined media for each individual consortium member that sustains growth and induces the production of cross-feeding metabolites.

Given the availability of draft genome-scale models, we have in parallel started performing COMETS simulations of metabolism in co-cultures of consortium members, yielding predictions of the metabolic activity of the whole community. Simulations will help us refine the optimal environmental conditions towards enabling the formation of a stable biofuel precursor-producing ecosystem.

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