Synthetic microbial ecology for biofuel production 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 conversion of lignocellulose to biofuels is a complex process composed of many steps. Instead of engineering one microorganism to perform every function, our project assigns microbes to specific functions: (hemi)cellulose degradation, lignin degradation, removal and detoxification of methoxy groups on lignin, and the production of biofuel precursors (Figure 1). In order to test and predict the capacity of organisms to grow together and perform their specific tasks, we are using an array of tools: **growth experiments** to determine the best medium components, **metabolomics** to measure metabolite production and consumption, **flux balance analysis** (FBA) to model metabolite exchange between community members, and **genetic engineering and experimental evolution** to develop strains with desired traits.
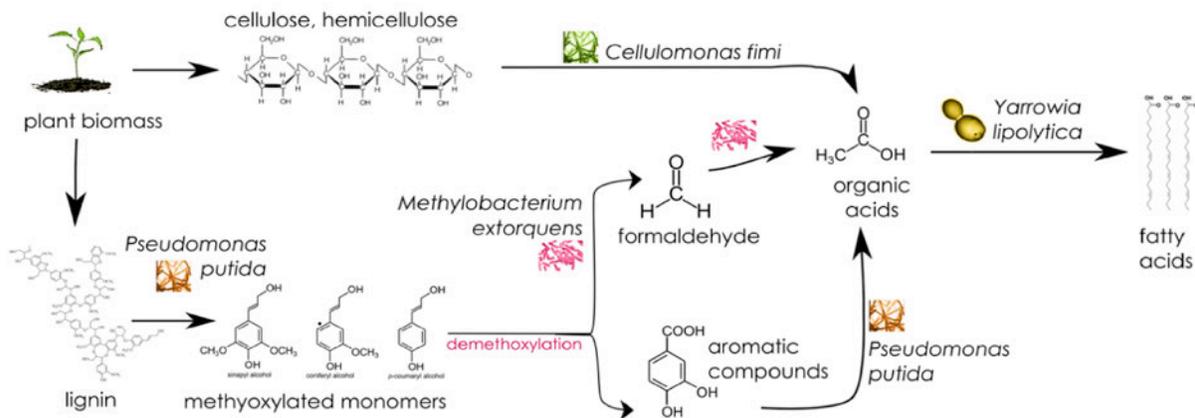

Figure 1. Synthetic microbial community design.

We chose each organism based on its metabolic capacity, oxygen requirement, pathogenicity, and genome availability. All community members grow aerobically, are not pathogenic, and have had their genome sequenced. ***Cellulomonas fimi*** is a soil-dwelling bacterium that degrades (hemi)cellulose, ***Pseudomonas putida*** is a soil-dwelling bacterium that degrades lignin, ***Methylobacterium extorquens*** is a soil-dwelling bacterium that can grow on C1 compounds such as formaldehyde, and ***Yarrowia lipolytica*** is an oleaginous yeast that can produce high yields of lipids. *C. fimi* is the only microbe that lacks a published metabolic model[1–3]; however, we built and gap-filled a draft model using KBase and have manually added reactions based on our experimental observations. Since lignocellulose is very complex and the models lack many of the carbon sources found in lignocellulose, we are using Biolog plates with our own protocol to scan for growth and utilization of additional carbon sources to be added to the models.

Free formaldehyde produced by the demethoxylation of lignin monomers can inhibit microbial growth due to its toxicity. Although *M. extorquens* can utilize formaldehyde as a central metabolic intermediate, it cannot naturally remove and detoxify the methoxy groups on lignin. In order to demethoyxlate and cleave complex aromatics, we have genetically engineered *M. extorquens* to express the *vanABK* operon from *Methylobacterium nodulans*. We have tested he capacity of several species to grow together, and obtained preliminary data on potential interactions between community members as a function of environmental conditions. We observed the growth of *C. fimi*, *P. putida*, and *M. extorquens* on vanillic acid and cellobiose, which serve as model compounds for lignin and cellulose, respectively. *C. fimi* grows very poorly in the presence of *P. putida*, but grows slightly better and consumes cellobiose faster in the presence of *M. extorquens* (irrespective of whether *P. putida* is present). *M. extorquens* grows better in the presence of *C. fimi*. This is probably because *C. fimi* provides a growth substrate for *M. extorquens* (since *M. extorquens* cannot use vanillic acid or cellobiose as a carbon source). The growth of *P. putida* was not affected by the presence of *C. fimi* or *M. extorquens*. However, the presence of *M. extorquens* decreases the amount of formaldehyde produced by *P. putida* growing on vanillic acid. We also observed the growth of *C. fimi*, *P. putida*, and *Y. lipolytica* on ionic liquid treated switchgrass, with slightly different patterns of interactions between specific organism pairs. Ongoing work is aimed at processing growth data, metabolic measurements and model predictions to converge towards a functioning prototype of the complete consortium.

References

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