Defining the Minimal Set of Microbial Genes Required for Valorization of Lignin Biomass

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Project Goals: Lignin is the second most abundant biopolymer on earth and represents a critically underutilized biomass resource for hydrocarbon feedstocks. Despite substantial effort, there is still no efficient process to convert lignin to useable carbon-based platform chemicals and materials. The goal of this project is identify a minimal set of microbial enzymes necessary for lignin breakdown and sufficient for the synthesis of valuable chemical intermediates from lignin isolated as a byproduct of lignocellulosic ethanol production. These genes will be then used to engineer functional whole cell biocatalysts for tunable lignin metabolism.

To date, although a number of enzymes have been associated with lignin degradation, most have been tested in isolation (as individual enzymes) and on drastically different substrates -- often dyes that are not related to lignin. In contrast, lignin utilization in nature likely occurs by microbial consortia with multiple enzymes acting synergistically. We propose to examine two separate stages of lignin breakdown carried out by the microbes that do it best: (1) early breakdown of native polymeric lignin into soluble fragments by a set of sequenced wood-rotting fungal species, and (2) downstream metabolism of these soluble lignin fragments to useful chemical intermediates by a panel of sequenced soil saprophytes. Our approach involves testing sets of genes that will be assayed combinatorially in the context of a heterologous expression host. The resulting engineered strains will be systematically assayed using soluble lignin fragments, synthetic defined polymeric lignin, and finally lignin directly sourced from lignocellulosic processing streams. In addition to resulting in a functional whole cell biocatalyst for lignin utilization, we anticipate that this approach will allow us to address key unanswered questions about lignin metabolism in nature, including: (1) Why does the *Trametes versicolor* genome contain 25 different class II peroxidases? (2) What is the role of laccases in lignin metabolism? Why do some aggressive lignin degraders have many laccases (e.g. >7 in *T. versicolor*) while others have none (e.g. *P. chrysosporium*)? (3) How is peroxide provided in a controlled manner to drive peroxidase activity without causing the enzyme inhibition that is so often observed in vitro? (4) What strategies do microbial lignin degraders use to avoid the problem of repolymerization during active lignin degradation? and (5) Can microbial lignin metabolism be diverted for high level production of defined aromatics? A final critical question is whether combining key minimal sets of enzymes from a wide range of organisms will result in engineered strains capable of highly efficient, streamlined pathways for lignin utilization that can be tuned for a specific carbon output. This effort will leverage DOE investments in microbial genome sequencing, and secure a critical channel for lignin biomass utilization that will also help to render lignocellulosic a viable feedstock for the production of renewable liquid biofuels.

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