107. Engineering Anaerobic Gut Fungi for Lignocellulose Breakdown

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Project Goals: The goal of this project is to engineer anaerobic gut fungi as novel platform organisms for biofuel production from plant material. To accomplish this goal, a panel of anaerobic fungi will be isolated from different herbivores and screened for their ability to degrade lignocellulose. The basic metabolic networks that govern lignocellulose hydrolysis within anaerobic fungi will also be determined, and models will be generated to describe how important enzyme groups are coordinated during breakdown. Using this information, genetic transformation strategies to manipulate gut fungi will be developed, which would endow them with enhanced functionality against a range of industrially relevant substrates. Collectively, this information will establish the molecular framework for anaerobic fungal hydrolysis, and will guide in the development of lignocellulosic biofuels.

Anaerobic fungi are the primary colonizers of biomass within the digestive tract of large herbivores, where they have evolved unique abilities to break down lignin-rich cellulosic biomass through invasive, filamentous growth and the secretion of powerful lignocellulolytic enzymes and enzyme complexes (fungal cellulosomes). Despite these attractive abilities, considerably less genomic and metabolic data exists for gut fungi compared to well-studied anaerobic bacteria and aerobic fungi that hydrolyze cellulose. This presents a significant knowledge gap in understanding gut fungal function, substrate utilization, and metabolic flux, which has prohibited the genetic and functional modification of gut fungi. Recently, however, advances in sequencing technologies have made it possible to explore the dynamic metabolic networks within gut fungi for the first time. Our approach combines next-generation sequencing with physiological characterization to establish the critical knowledge base to understand lignocellulose breakdown by gut fungi. This project will (1) enable exploration of novel isolates for desirable enzymatic properties, (2) construct metabolic models to describe biomass degradation, and (3) develop new methods to metabolically engineer gut fungi for bioprocessing.

To initiate this project, we isolated a panel of novel gut fungi from sheep, goat, giraffe, and elephants at the Santa Barbara Zoo. To date, four unique strains from the *Piromyces, Neocallimastix, and Anaeromyces* genera have been obtained through roll tube isolation. Proliferation of the fungal isolates was monitored via fermentation gas production, and cellulosomes from each species were isolated through cellulose-precipitation. All of these isolates exhibited high enzymatic reactivity against a range of cellulosic and lignocellulosic substrates (filter paper, Avicel, reed canary grass), which was repressed in the presence of simple sugars. Within isolated cellulosomes, striking similarities are observed for certain dockerin-fused glycosyl hydrolases, and these proteins are not secreted from fungi when simple sugars are present, supporting the hypothesis that these enzymes are catabolically regulated.

Our subsequent goal is to enumerate novel biomass-degrading enzymes within these isolates and characterize their coordinated expression during biomass breakdown. Towards this goal, we have analyzed the transcriptome of the fungal isolate *Piromyces sp finn* via RNAseq under several growth conditions. This isolate exhibited high enzymatic reactivity against a range of cellulosic and lignocellulosic substrates, which was repressed in the presence of simple sugars. Through strand-specific RNAseq and use of the TRINITY assembly platform, we were able to assemble hundreds of novel cellulase genes *de novo* from >27,000 transcripts without the need for reference genomic sequence information. The *Piromyces sp finn* transcriptome is particularly rich in GH6 and GH43 enzymes, and we find that 27 of 54 diverse glycosyl hydrolase families are transcriptionally repressed during growth on glucose relative to reed canary grass. Within the majority of these transcripts, dockerin-tagged elements of fungal cellulosomes are abundant, and 30% of dockerin-containing transcripts are repressed in the presence of glucose. This suggests that catalytic components of fungal cellulosomes are highly regulated in response to simple sugars, which also agrees with proteomic data. We will further discuss the transcriptional regulation patterns observed for important enzyme families under catabolic regulatory conditions, and connect these regulation patterns to protein expression and lignocellulosic degradation. Additionally, we are collaborating with researchers at the DOE-JGI and PNNL EMSL to sequence the genomes/transcriptomes for other isolates, as well as investigating the dynamics of cellulosome secretion as part of the 2014 Community Science Program.

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