226. Engineering Synthetic Anaerobic Consortia for Sustainable Chemical Production from Biomass

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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, native to the digestive tracts of large herbivores, are capable of breaking down lignin-rich biomass. This is accomplished through invasive growth of mycelial root structures and the secretion of lignocellulolytic enzymes and complexes. Thus far, very little is known about these fungi on a genomic and metabolic level compared to cellulolytic bacteria and aerobic fungi. We have used next generation sequencing to identify biomass degrading enzymes through sequence homology and orthology analyses as well as through regulation patterns during growth on substrates of varying complexity. The enzymes contained within anaerobic fungi make them a valuable resource to improve bioprocessing methods. Based upon the ability of these fungi to degrade a variety of materials ranging in complexity, we have examined several co-culture systems to determine how these abilities may be enhanced and how they may be used for bio- based production.

Natively, gut fungi maintain a syntrophic relationship with archaeal methanogens. Fungi degrade the plant material and produce both CO2 and hydrogen that is then converted to methane gas by the methanogens. We have isolated cultures of anaerobic fungi with their dependent methanogens from herbivore fecal materials. These co-cultures establish a simplified system to model cooperative actions of the anaerobes and are stable in culture for > 60 weeks. (e.g. Methanocorpusculum) during biomass breakdown shows that fungal-methanogen syntrophy drastically accelerates cellulose and lignocellulose breakdown by the fungi. Furthermore, an excess of sugar hydrolysates and metabolites produced by anaerobic fungi enable metabolic linkage of non-native, facultative anaerobes to the consortium. Strains of S. cerevisiae and E. coli have been tested for their use in synthetic consortia with gut fungi, by which they consume the excess sugars (~5 g/L) left over from fungal cellulose breakdown. As anaerobic fungi are not yet genetically tractable, our strategy offers a path forward to make value-added products directly from crude lignocellulose.