Engineering Synthetic Systems Inspired by Anaerobic Gut Fungi

Sean P. Gilmore[1*] (SeanGilmore@umail.ucsb.edu), Charles H. Haitjema,[1] John K. Henske,[1] Jessica A. Sexton,[1] Kevin V. Solomon,[1] Heather M. Brewer,[2] Samuel O. Purvine,[2] Aaron T. Wright,[2] Alan Kuo,[3] Stephen Mondo,[3] Asaf Salamov,[3] Kurt LaButti,[3] Michael K. Theodorou,[4] Igor Grigoriev,[3] and **Michelle A. O'Malley**[1]

[1]Department of Chemical Engineering, University of California, Santa Barbara; [2]Enivronmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA 99352; [3]US DOE Joint Genome Institute, 2800 Mitchell Dr., Walnut Creek, CA 94598; and [4]Animal Production, Welfare and Veterinary Sciences, Harper Adams University, Newport, Shropshire, TF10 8NB, United Kingdom

http://omalleylab.weebly.com

Project Goals: The overall goal of this project is to engineer novel anaerobes as platform organisms for biofuel production from plant material. To accomplish this goal, anaerobic consortia were isolated from different herbivores and screened for their ability to degrade lignocellulose. From these native consortia, we seek to assemble a "parts list" that comprise multi-enzyme fungal cellulosomes – this includes identification of the fungal scaffolding system, and cohesin domain, which bind to fungal dockerin-fused enzymes. Additionally, we aim to characterize native fungal-containing microbial consortia to "mix-and-match" fungi with other anaerobes to enhance biomass hydrolysis and tune bioproduction.

Anaerobic fungi in the hindgut of large herbivores are among the most robust organisms at degrading crude lignocellulose. Their remarkable cellulolytic capabilities have great potential for use in biomass breakdown and biofuel processing. Anaerobic fungi achieve cellulolytic efficiency through the production of large, multi-enzyme complexes called fungal cellulosomes. In isolation, anaerobic fungi metabolize some of the released sugars and convert them into fermentation products. In nature, however, they exist in a community with archaea, bacteria, and protozoa, which drastically alter the behavior of the fungi. By elucidating the parts responsible for efficient biomass degradation at both the protein and cellular level, we seek to replicate this efficiency in synthetic systems.

Fungal cellulosomes are similar to bacterial cellulosomes in that the protein-protein interactions are mediated through parts termed the dockerin and cohesin. However, many differences exist. The dockerin domains exist in tandem repeats and bear no species specificity like those in the bacterial systems. Furthermore, the exact sequence for the cohesin module has yet to be established. Through a combination of –OMICs approaches and traditional biochemical assays, a large putative scaffoldin molecule was identified. The scaffoldin was heterologously expressed and screened for interaction with recombinant dockerin through an ELISA. The K_D^{app} was determined using Equilibrium Surface Plasmon Resonance. A transcriptomic survey of dockerin domain-containing proteins revealed some degree of conservation in dockerin location on classes

of CAZymes. Using this observation, the dockerin domains were adapted to thermostable cellulases, demonstrating its applicability as a novel protein scaffolding systems and suggesting the possibility of synthetic cellulosomes for biomass degradation.

Additionally, anaerobic fungi have been shown to interact closely with methane producing archaea (methanogens). The methanogens siphon hydrogen and other metabolites from the fungi, allowing the fungi to more efficiently produce energy by increasing the flux through their hydrogenosomes. To further investigate this mechanism, native fungal/methanogen consortia were isolated from herbivore fecal materials. Consortia were maintained together and also separated into monocultures for comparison. Genomic sequencing revealed the presence of one fungus, two methanogens, and one bacterium in one consortium, which was stable under continuous passage for over 20 months. The consortium demonstrated faster and more complete degradation of cellulosic substrates, as well as a wider range of utilized substrates compared to the monocultured fungus alone. By introducing the methanogens into cultures of other well-characterized anaerobic fungi, stable synthetic co-cultures were established. These stable synthetic consortia demonstrated similar efficiency, and suggest a promising option for conversion of crude biomass into sustainable chemicals.

This Project is supported by the Office of Biological and Environmental Research through the DOE Office of Science (Early Career Program)