45. A Systems Biology Characterization of the Biotechnological Potential Stored in the Wood-Feeding Beetle Odontotaenius disjunctus

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Project Goals: We seek to develop an integrated analysis of energy flow in complex microbial communities by combining multi-scale approaches including biogeochemical, stable isotope probing, metagenomic/transcriptomic, proteomic/metabolomic and computational analyses, to understand nutrient cycling and biofuel production. A comprehensive understanding of such communities may help in the development of efficient, industrial-scale processes for microbial H_2 production and lignocellulose degradation. Our ultimate goal is the development of multi-scale models that can predict ecological and biochemical relationships within multi-trophic microbial systems.

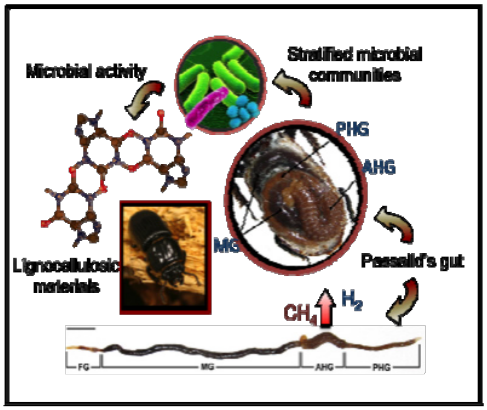

Fig 1. The passalid beetle and its gut. The associated microbial communities aid in the transformation of complex polymers.

The passalid beetle (*Odontotaenius disjunctus*) is a subsocial insect that survives on a low-nutrient diet by feeding on large amounts of decaying wood. The morphologically differentiated gut regions of these insects represent a complex of subunits with stratified microbial communities that degrade lignocellulosic materials. Our goal is to characterize the potential stored in the microbiome of the passalid beetle for the optimization of lignocellulosic-dependent energy production processes (Fig. 1).

We tested the ability of the passalid beetle to transform lignocellulosic materials by measuring the fermentation products of plant polymer decomposition (H_2 and CH_4) using microelectrodes and gas chromatography- isotope ratio mass spectrometry (GC-IRMS). Transformations of lignin after its passage through the gut were determined by ^{13}C-labeled tetramethylammonium hydroxide thermochemolysis. Using fosmid libraries constructed from DNA from different beetle gut regions we have done high-throughput screening for lignin, cellulose, and hemicellulose degrading activity. Illumina-baed metagenomic libraries were also prepared, sequenced and annotated along with the positive clones from our fosmid libraries. Proteomics and metabolomics have been used to detect expressed proteins and produced metabolites in each gut region. Finally, we have screened beetle gut microbiota for assimilation of ^{13}C-labeled cellulose using Chip-SIP isotope arrays and NanoSIMS imaging.

Lignin side chain oxidation was confirmed by thermochemolysis which show acid/aldehyde ratios increasing in the beetle frass. Hydrogen gradients, were measured using microelectrodes, and indicate concentrations as high as 140 µmol/L in the anterior hindgut (AHG). GC-IRMS analyses of C and H

stable isotope fractionation indicated that the produced CH_4 was primarily hydrogenotrophic. Fosmid library screening yielded a high number of clones with activity for the decomposition of cellulose, hemicellulose, and lignin – with the highest potential detected in the AHG. The annotation of metagenomic libraries has allowed us to identify the likely contributors to cellulose, xylose and lignin modification. Notably lignocellulosic organisms related to the Clostridiales, Bacillales, Actinobacteria were abundant. We also identified the presence of fungal laccases and peroxidases in addition to bacterial peroxidases that may be involved in the process of lignin degradation. Sequences from hydrogenotrophic methanogenic archaea were more abundant in the anterior hindgut, confirming our previous phylogenetic studies of compartmentalization in the passalid beetle gut. A filtered isolate database and predicted protein sequences from the metagenomes were used to search peptide spectra for proteome reconstruction – preliminary results indicate a variation in the protein expression patterns among the different gut segments potentially indicating a compartmentalization of function. ChipSIP analyses to identify consumers of ^{13}C-cellulose are ongoing.

Our multi-scale approach demonstrates that the passalid beetle harbors and expresses the functional potential to deconstruct lignocellulosic materials and produce H_2, CH_4 and potentially other biofuels. Identifying the microbial contributors to polymer deconstruction and fermentation, and determining their spatial arrangement in the beetle gut will improve our understanding of the ecology of these beetles and inform the design of lignocellulosic fuel production processes.