Characterization of H_2 Producing Microbial Communities of the Wood-Feeding Beetle *Odontotaenius disjunctus* by Multi-Scale Measurement of Metabolic Function

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Project Goals: Our research aims to develop an integrated analysis of energy flow in complex microbial communities. We are combining biogeochemical, stable isotope probing, metatranscriptomic/metagenomic, and computational approaches, to understand nutrient cycling and biofuel (H_2) production in complex microbial communities. A comprehensive understanding of such communities is needed to develop 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.

One of our model systems is represented by the hindgut of the passalid beetle *Odontotaenius disjunctus* (Fig. 1A). This wood-feeding beetle is able to survive on a low nutrient diet through symbiotic interaction with its gut microbiome, which provides nutrients from plant polymer decomposition and nitrogen through fixation. Both of these processes result in the production of hydrogen gas.

The digestive system of the passalid beetle is compartmentalized in 4 morphologically differentiated regions (Fig. 1D); each has been characterized with our integrated approach, which consists of:

(1) Culture independent techniques used to assess the diversity and composition of the microbial communities inhabiting each gut region by using the PhyloChip, together with analysis of community gene expression by qPCR, and sequencing of enriched cDNA libraries.
(2) Use of Chip-SIP, a high-sensitivity, high-throughput stable isotope probing (SIP) method performed on a phylogenetic array to directly measure functional roles of uncultivated microorganisms.
(3) Construction of Fosmid libraries for enzymatic assays and sequencing to search for the genes involved in the transformation of specific substrates. We have optimized HMW-DNA extraction and library construction protocols for their application to the beetle gut microbiome.
(4) Micron-scale measurement of physicochemical gradients (O_2, H_2, pH) on the distinct gut regions of the passalid beetle using microelectrodes (Fig 1C).
(5) Characterization of changes in the chemical composition of digested/undigested substrate (oak wood) using Fourier Transform Infrared Spectroscopy (FTIR).

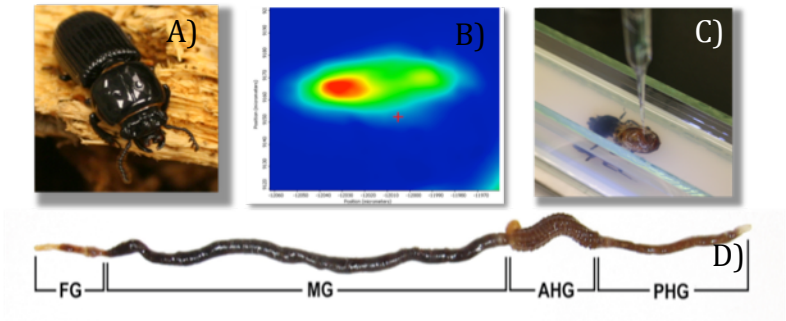

Fig 1. A) The passalid beetle, B) SIR image of undigested oak wood, c) arrangement of the dissected beetle and H_2 microsensor, D) the compartmentalized gut of the passalid beetle, F=Foregut, MG = Midgut, AHG= Anterior hindgut, and PHG =Posterior Hindgut.

By combining these complementary approaches we are attempting to determine the primary processes and organisms contributing to energy flow in this natural system. We have characterized the microbial communities of the passalid beetle, obtaining the highest levels of diversity in the Foregut (FG), followed by Posterior Hindgut (PHG), Midgut (MG), and Anterior Hindgut (AHG). *nifH* expression analysis showed that N_2 fixation was highest in the AHG and sequencing of *nifH* genes identified the dominant N_2 fixing group as members of the Porphyromonadacea (Clostridiales). Concentrations of O_2 and H_2 varied across the different gut compartments; the highest nifH gene expression and lowest bacterial diversity spatially correspond to regions with high concentrations of H_2 production and O_2 is at its minimum.

Using FTIR, we have also characterized changes in the composition of the beetle's substrate (Oak wood); results suggest cellulose and hemicellulose content declines as the substrate passes through the sequential gut regions. We are currently screening fosmid libraries, and performing analysis of enriched cDNA libraries and Chip-SIP microarrays.

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