

## **Multi-omics Approach Unveil Microbial Transformations of Lignocellulose in the Gut of the Wood-Feeding Beetle *Odontotaenius disjunctus***

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<https://envmicro.wordpress.com/research-projects/passalid-beetles-natures-efficient-lignocellulosic-biorefineries/>

**Project Goals: Our research aims 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 and computational analyses, to understand carbon and nutrient transformation relevant to biofuel production in complex microbial communities. A comprehensive understanding of such communities may help in the development of efficient, industrial-scale processes for lignocellulose degradation.**

*Odontotaenius disjunctus* is a wood feeding beetle that processes large amounts of hardwoods and plays an important role in forest carbon cycling. In its gut, plant material is transformed into simple molecules by sequential processing during passage through the insect's digestive system. Fourier Transformed Infrared Spectroscopy – Attenuated Total Reflectance (FTIR-ATR) demonstrated the sequential transformation of cellulose, xylan and lignin, and the accumulation of reduced nitrogen through the beetle digestive system. To identify the organisms and pathways contributing to these processes, we used multiple 'omics approaches to analyze the distribution of the different symbiotic communities and their specific functions in lignocellulose deconstruction within the insect's gut.

Fosmid clones were selected and sequenced from a pool of clones based on their expression of plant polymer degrading enzymes, allowing the identification of a wide range of carbohydrate degrading enzymes from different microorganisms associated with the beetle. Comparison of metagenomes from four gut regions demonstrated that lignin-degrading genes were more abundant in the first two gut sections, the foregut and midgut. Cellulose, starch, and xylan degradation genes were more abundant in the midgut and posterior hindgut. Genes for hydrogenotrophic methanogenesis and for nitrogen fixation were more abundant in the anterior hindgut. Assembled scaffolds were binned into 127 genome bins representing Bacteria, Archaea, Fungi, and Nematoda. Eleven nearly complete genomes were reconstructed, allowing us to identify linked functions/traits, including organisms with cellulosomes, and a combined potential for cellulose, xylan, starch hydrolysis and nitrogen fixation. A mixed eukaryotic bin containing different lignin peroxidases, catalase peroxidases and lacasses of fungal origin was also identified.

A metaproteomic study was conducted to determine the expression of these pathways. Preliminary analyses suggest enrichment of pathways related to hemicellulosic degradation in the midgut and anterior hindgut. A complete xylan degradation pathway was reconstructed and complementary GC-MS/MS based metabolomics identified xylobiose and xylose as major metabolite pools. To test 'omic generated hypotheses of *in situ* metabolism in the beetle gut, we used Chip-SIP stable isotope tracing, to analyze the isotopic composition of microbial RNA from beetles fed  $^{13}\text{C}$ -cellulose. Multiple bacterial groups (including the Spirochaetaceae, Ruminococcaceae, Rhodospirillaceae, Thermotogaceae, and Promicromonosporaceae) were  $^{13}\text{C}$  enriched, mainly in the midgut. Ongoing cultivation studies are focusing on these community members.

Our combined multi-omics and analytical chemistry approach demonstrates the continuous transformation of lignocellulosic materials through the beetle gut and the contribution of organisms belonging to multi-trophic levels to the different metabolic processes.

## References

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