

119. Analysis of Three Ruminal-associated Bacteria Reveal Different Mechanisms for Cellulose Degradation

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Project Goals: The goals of this project are to uncover the mechanism through which three ruminal associated bacteria degrade cellulose. These include *Cellulomonas gilvus*, *Fibrobacter succinogenes*, and *Ruminococcus albus*, all of which cellulose degradation strategies different from canonical approaches such as cellulosomes that are used by other well-understood microbes. We use a combination of cellulose-degradation assays, whole-genome sequencing, RNA-seq, and proteomics to demonstrate their ability to degrade cellulose using entirely different strategies. These data are important for advancing our understanding of cellulose degradation in natural systems and will have impact for enhancing the production of bioenergy products such as ethanol. Given the importance of these bacteria in their natural system (i.e. in ruminants such as cows), understanding their biology will also aid in efforts to improve digestion and optimize animal production.

The ability of ruminants such as cows to degrade and utilize plant biomass is predicated on the symbiotic relationship they have with their gut microbes. Through human domestication efforts, the ruminal microbial ecosystem has been selected for optimal fermentation of plant polysaccharides. This digestive process involves two steps: the degradation of plant polysaccharides such as cellulose and hemicelluloses into simple sugars, followed by their fermentation into short chain fatty acids like acetate, butyrate, and propionate. This model can thus be useful for informing on our ability to produce biofuels as many of the microbes may possess novel enzymes of interest or serve as a chassis for the direct fermentation of products from plant polysaccharides. We have been studying the cellulolytic and fermentative properties of three ruminal-associated microbes: *Cellulomonas gilvus*, *Fibrobacter succinogenes*, and *Ruminococcus albus*. Our first step was to sequence their genomes and determine their cellulolytic potential. Importantly, while all bacteria have cellulases and other carbohydrate-degrading genes, none possessed scaffoldins and dockerins characteristic of cellulosome-utilizing bacteria. This suggested that all three bacteria do not use cellulosomes and must use other strategies to degrade cellulose. We tested this for each bacterium using a combination of cellulase assays, fermentation assays, RNA-seq, and proteomics.

Cellulomonas gilvus ATCC13127. This bacterium is unique in that it is the only reported bacterium capable of degrading cellulose both aerobically and anaerobically. Analysis of this bacterium under both conditions revealed that it is capable of fermenting small amounts of ethanol. Since all of the predicted cellulases in its genome have signal peptides, we posited that it excretes its cellulases when degrading cellulose under both oxic and anoxic conditions. An analysis of the proteins from spent media confirmed this hypothesis and further revealed a specific set of cellulases excreted based on condition. Our preliminary RNA-seq data also confirm these findings, suggesting that this bacterium may utilize an aerobic cellulose-degrading strategy regardless of oxygen availability.

Fibrobacter succinogenes S85. As one of only two species that define an entire phylum, *F. succinogenes* is a unique cellulose-degrading bacterium best known for its presence in ruminants and the hindgut of termites. Like other strictly anaerobic cellulose degraders, it requires attachment to cellulose fibers for degradation. An analysis of its genome shows atypical cellulases, as none contain carbohydrate-binding modules in families 1, 2, or 3, which are associated with strict anaerobes. Moreover, while this bacterium contains genes for the degradation of hemicelluloses like xylan, it cannot utilize the degraded byproducts. To gain a better understanding of its cellulose degrading mechanism, we conducted RNA-seq experiments on *F. succinogenes* grown in a chemostat on either cellulose or cellobiose. We found a number of highly expressed cellulases and a unique set of Fibro-slime proteins that have been implicated in cellulose-binding. A second set of Fibro-slime proteins were expressed under both conditions, suggesting that these may play other roles in its physiology. These data were further confirmed by proteomics of both spent media and cell pellets. These data indicate that this bacterium utilizes a cell-surface attached approach to degrade cellulose that is different from other anaerobic cellulose degrading bacteria.

Ruminococcus albus 7. This entire family of bacterium is well-known for its cellulolytic abilities, particularly within the rumen ecosystem. *Ruminococcus albus* 7 is no exception in that it is highly cellulolytic with the ability of producing ethanol *in vitro*. An analysis of its genome shows that it does not contain the cellulosome machinery characteristic of other species in this genus like scaffoldins and dockerins. We performed an RNA-seq analysis of its cellulolytic capabilities in a chemostat on both cellulose and cellobiose. Our analysis revealed a number of highly-expressed cellulases along with many CBM37s, which are specific to this bacterium and may be involved in cell-surface attachment to cellulose fibers. Surprisingly, we found that the tryptophan operon was the most highly expressed set of genes when grown on cellulose. This is similar to what has been reported in the proteome of *Clostridium phytofermentens*, a related bacterium that also does not utilize a cellulosome. These data suggest that this bacterium thus utilizes a completely novel cellulose degradation approach likely involving CBM37s that may be embedded in a glycocalyx-like structure on its cell membrane which it uses to attach to cellulose fibers.

Taken together, these analyses are beginning to shed light on our understanding of cellulose degradation in nature. Moreover, this diversity of approaches suggests that the use of cellulosomes is not the necessarily widespread and does not represent the full extent through which microbes degrade cellulose. Finally, given that these microbes are fermenters, capable of generating ethanol in many cases, they may be useful as models for understanding the fermentation of cellulose to ethanol.

This work was funded by the US Department of Energy's (DOE) Office of Biological and Environmental Research (OBER) Early Career Research Program Award DE-SC0008104 to GS. Metaproteomics analysis were supported by the U.S. DOE OBER Pan-omics program at Pacific Northwest National Laboratory (PNNL) and performed in the Environmental Molecular Sciences Laboratory, a U.S. DOE OBER national scientific user facility on the PNNL campus. PNNL is a multiprogram national laboratory operated by Battelle for the DOE under contract DEAC05-76RL01830.