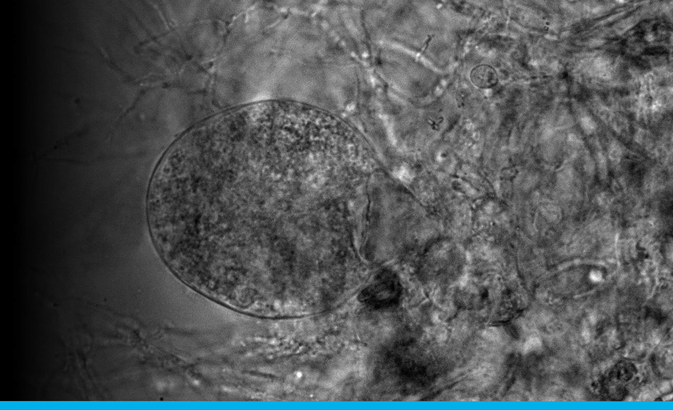
Studying Anaerobic Fungi for use in Biofuel Applications



Chris Eachus | Allan Hancock College | Mentor: Charles Haitjema, Ph.D.

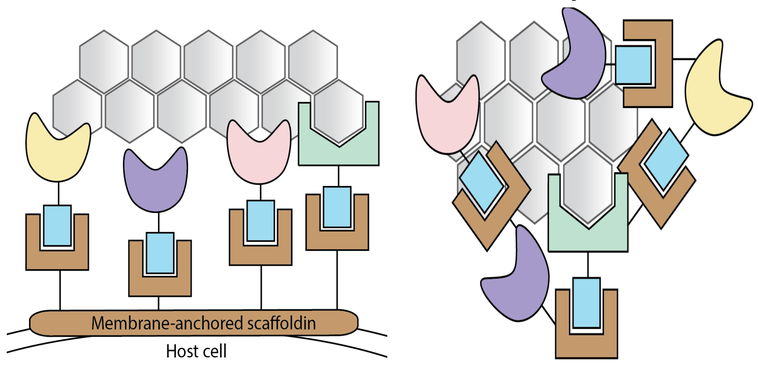
Abstract

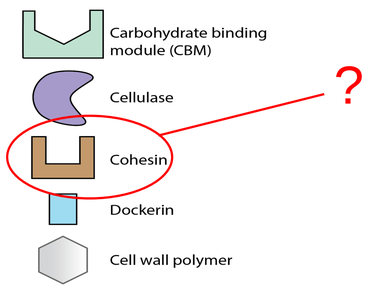
Anaerobic fungi found in the guts of herbivorous mammals provide perhaps the most promising mechanism to break down plant biomass for conversion to biofuels and other chemical commodities. However, very little is known about these organisms and the details of their molecular structure. This research focuses on the characterization of anaerobic gut fungi and how they mediate the breakdown of cellulose, the predominate component of plant biomass, into sugars. While many of the enzymes that provide the cellulolytic capability of these organisms have been identified, there are few details on how these enzymes assemble to form large complex structures, and this is thought to contribute significantly to plant biomass break down. In anaerobic bacteria, cellulases assemble by non-catalytic domains called dockerins. Dockerins bind to cohesin domains encoded on a membrane-anchored scaffoldin. In anaerobic fungi, dockerins have been identified, but the dockerin-binding cohesin and scaffoldin molecules have remained elusive, and this has been a major lack of understanding in these systems. To identify dockerin-binding cohesin molecules, we used a chemical crosslinker to trap protein-protein interactions between a purified dockerin and its binding partners. This method revealed a putative cohesin molecule with a molecular weight of approximately 75 kDa. The identity of this cohesin is currently being obtained by mass spectrometry. This new information on the molecular assembly of cellulose-degrading complexes may be exploited for use in the conversion of agricultural waste into biofuel and other value-added chemicals.

Hypothesized Models for Enzyme Complexes

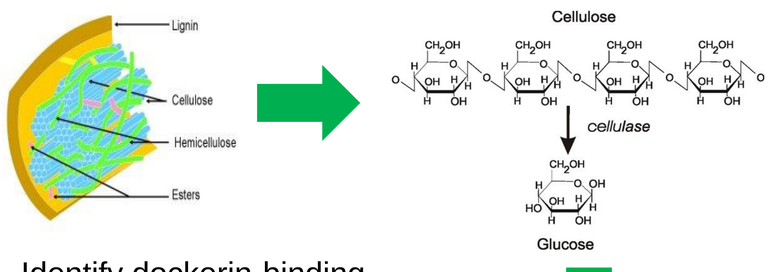
Model A: Enzymes are anchored

Model B: Enzymes run freely





Research Goals

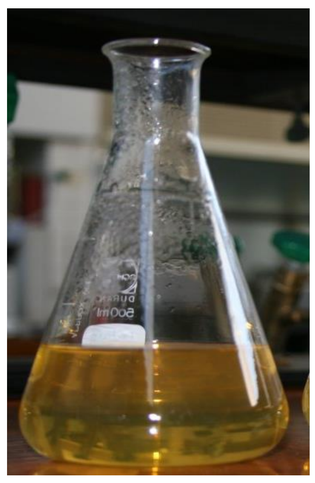


- Identify dockerin-binding cohesin molecules
- Fully Characterize the enzyme complex of anaerobic fungi
- Use this knowledge to engineer microbes to break down plant waste efficiently and synthesize valuable products such as fuels, pharmaceuticals, and many other valuable chemicals

Experimental Procedures

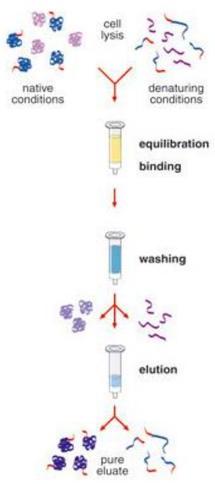
Protein Purification

Isolate test tube culture



Upscale: Cells multiply exponentially

Centrifuge/Break open cells

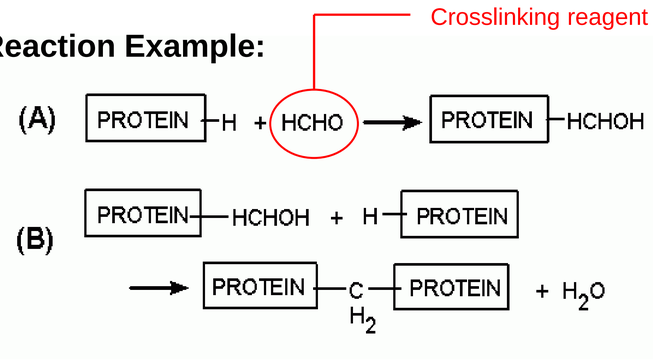


Run supernatant through nickel column to filter out impurities

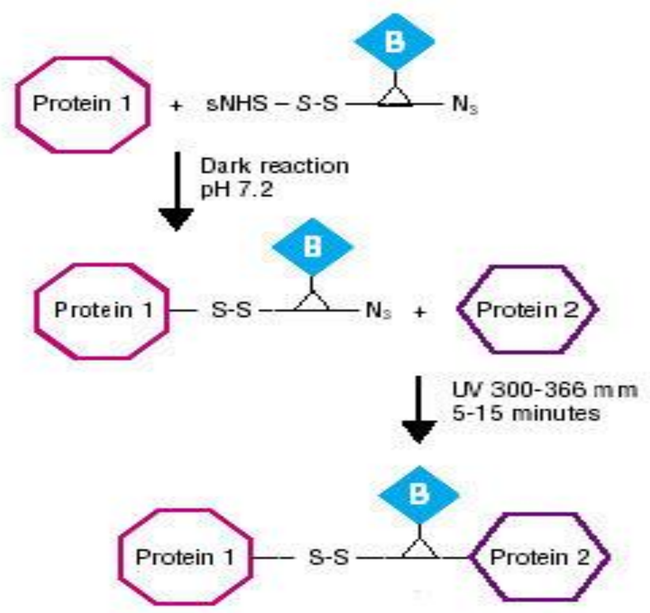
Crosslinking experiments

Pure proteins are used in crosslinking experiments to observe interactions with other proteins

Reaction Example:



Crosslinking reagent used: Sulfo-SBED

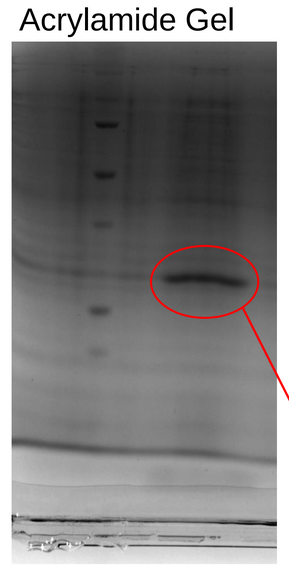


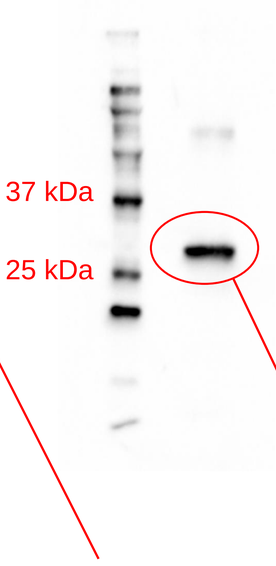
Data/Observations

Pure Proteins

Acrylamide Gel

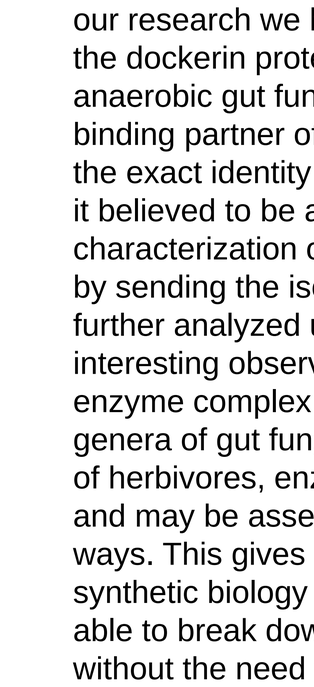
Western Blot





Purified Dockerin Protein

Crosslinking experiments reveal a binding partner with a mass of ~75 kDa



Conclusions

The details of the anaerobic fungal enzyme structure are becoming less elusive over time. In our research we have been able to observe that the dockerin protein that is present in the anaerobic gut fungal enzyme complex has a binding partner of approximately 75 kDa. Although the exact identity of this molecule is still unknown, it believed to be a cellulase. Future characterization of this protein will be carried out by sending the isolated binding partner off to be further analyzed using mass spectrometry. Another interesting observation is that the assembly of enzyme complex is conserved across different genera of gut fungi, indicating that within the guts of herbivores, enzyme complexes are malleable and may be assembled in a variety of different ways. This gives much flexibility in the use of synthetic biology to engineer microbes that are able to break down plant biomass efficiently, without the need for costly pretreatment that is currently used.