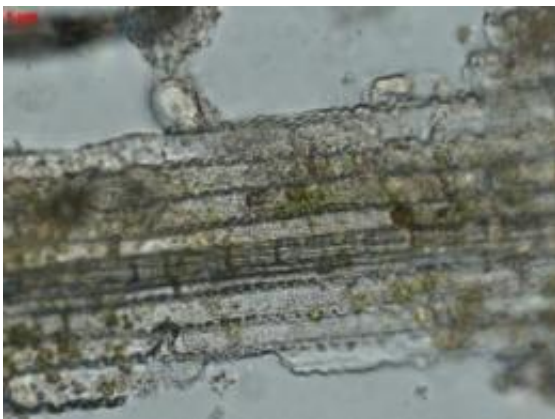


How now, inside the cow: Nearly 30,000 novel enzymes for biofuel production improvements

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Here, a fragment of switchgrass is decomposing in contact cow rumen microbes.
Credit: Damon Tighe, DOE JGI

Cows eat grass -- this has been observed for eons. From this fibrous diet consisting mainly of the tough to degrade plant cell wall materials cellulose and hemicellulose, substances of no nutritional value to most animals, ruminants manage to extract all they need to nourish themselves, their progeny and their keepers. And now, the cow, or rather the network of organisms working unseen in the cow's forestomach or rumen, is providing researchers with vital information that may someday accelerate the large-scale deployment of biofuels. This will offer a window into a major category of microbes that has long resisted the

attempts of scientists to grow and study.

Through massive-scale DNA sequencing, researchers at the U.S. Department of Energy (DOE) Joint Genome Institute (JGI) and the Energy Biosciences Institute (EBI), have characterized the genes and genomes of plant-digesting microbes isolated from the cow rumen and reported their results in a study published January 28 in the journal *Science*. One of the challenges associated with developing alternative fuels from plants has been the high cost and energy inputs that are associated with the processing plant biomass so that it can be more easily converted into sugars and from there into biofuels to address our transportation needs.

"Microbes have evolved over millions of years to efficiently degrade recalcitrant biomass," said Eddy Rubin, Director of the JGI and a lead on this study. "Communities of these organisms can be found in diverse ecosystems, such as in the rumen of cows, the guts of termites, in compost piles, as well as covering the forest floor. Microbes have solved this challenge, overcoming the plant's protective armor to secure nutrients, the rich energy source that enables them and the cow to thrive."

Bovines are thought to have first appeared on the landscape millions of years ago and were domesticated by humans about 10,000 years ago. Rumen microbes evolved to produce molecular machines in the form of enzymes able to efficiently deconstruct plant cell wall polysaccharides such as cellulose and hemicellulose into their constituent small sugar molecules. Another way of looking at it is that in exchange for housing in the cow rumen, these microbes pay rent by efficiently converting fiber that the cow cannot utilize into small sugar molecules that serve as substrates for fermentation into end-products that provide energy for the cow. This study has enabled the JGI investigators and collaborators to mine, at a scale thousands of fold greater than in any prior work, the

enzymatic capabilities encoded in the genomes of previously uncharacterized rumen microbes. This has deepened our understanding of nearly 30,000 genes generating enzymes that may possess powerful capabilities for degrading biomass into simple sugars, the essential first step in cellulosic biofuel production.

"Industry is seeking better ways to break down biomass to use as the starting material for a new generation of renewable biofuels," said JGI Director and project lead Eddy Rubin. "Together with our collaborators, we are examining the molecular machinery used by microbes in the cow to break down plant material."

Only about one percent of the planet's microbial species can be readily grown in the laboratory; the vast majority—in the soil, water, and residing in the other larger life forms such as in cows—cannot be cultured in a lab. "Metagenomic" studies such as this provide an alternative method of analysis.

Rubin's postdoctoral fellows Matthias Hess and Alex Sczyrba used one of the most promising large-scale bioenergy crops — switchgrass (*Panicum virgatum*) – and let the cows' microbial symbionts located in the foregut perform their magic. To better control the process, Hess and his colleagues at the University of Illinois worked with the fistulated cow model that, for scientific research purposes, allows direct access through a tube into the foregut, which can be considered as a fermentation chamber in which oxygen is absent. Instead of feeding the grasses directly to the cows, the switchgrass samples were placed in nylon bags and then inserted into the cow rumen, where they were left to be digested. After 72 hours, the bags were removed, and the DNAs from the microbes involved in digesting the material that were adherent to the switchgrass were isolated and then sequenced.

The amount of data generated for this study of rumen microbes, 270

billion letters of the DNA code, was enormous; about 100-fold greater than the number of letters in the entire human genome. Generating the data was not the most challenging part of this project, Hess noted. "The real challenge was to analyze the vast amount of data for which no reference genome was available and to identify and produce full-size functional enzymes based solely on information obtained from billions and billions of short snippets of DNA sequences," he said.

To analyze the information, the researchers developed a genome assembly strategy that could handle the vast amount of data while making sure to avoid misassemblies that would have led to chimeras – artificial genes not present in the microbial community. "Imagine someone mixing hundreds of jigsaw puzzles with millions of pieces each into a big pile," said Sczyrba. "We tried to put as many of these back together, making as few mistakes as possible. It is not an easy problem. You need a good strategy and a lot of computational resources to solve this problem.

By employing different filters Sczyrba whittled down the number of the more than two million predicted genes to 27,755 candidate genes that encoded a specific category of enzymes called carbohydrate-active enzymes (CAZymes) that can break down plant polysaccharides (e.g. cellulose) into small sugars. Hess identified the most promising candidates, tested a subset of 90 candidate genes for functionality and found that more than 50 percent of the tested candidates had cellulose-degrading activity with almost 20 percent able to break down the "real-world" biofuel crop switchgrass. This made it clear to the team that a significant fraction of the 30,000 genes identified are indeed active against plant material and would be a treasure trove of novel enzymes for biofuel researchers. Hess said that the discovery of these novel enzymes from this one study significantly increased the number of enzymes believed to act on carbohydrates by nearly a third compared to numerous previous studies carried out over decades.

Besides the identification of genes encoding enzymes that might play a major role in future processes for the industrial production of lignocellulosic biofuel, Hess and Sczyrba wanted to assemble not only genes but the entire genomes of organisms involved in biomass breakdown from the rumen. Using various computational puzzle-solving approaches, they were able to build 15 genomes for the rumen microbes, none of which matched anything that had previously been described.

To confirm their computational results the team turned to another way of examining the genomes of microbes that also did not require a culturing step: single cell genomics involving the study of the genome of a single uncultured microbial cell. They were able to isolate a single rumen microbe using a cell sorter, and without having it grow, they generated the genome of this uncultured microbe using single cell sequencing technology. When the DNA sequences derived from the single genome were mapped to the 15 computationally assembled genomes, the researchers found that more than 98 percent of the data matched to one single genome that had been assembled in silico. "The single cell data made us confident that what we saw was real," Hess said. "Otherwise we'd have computational data only, which would have made our work much, much less convincing."

This pioneering work, led by the JGI and its collaborators, illustrates that with large scale sequencing capabilities combined with large scale data analysis and computational capabilities and state-of-the-art next generation single cell genomics, researchers can now explore a vast array of cellulosic degrading enzymes from new microbes not tied to the ability to grow them in culture. This may therefore reduce the vast space of "microbial dark matter" that has up to now been difficult to explore. Additionally, the approach used here could be readily extended to searches for other microbial enzyme capabilities of relevance to other DOE missions.

More information: "Metagenomic Discovery of Biomass-Degrading Genes and Genomes From Cow Rumen," *Science*.

Provided by DOE/Joint Genome Institute

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