

Enzymes from horse feces could hold secrets to streamlining biofuel production

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Stepping into unexplored territory in efforts to use corn stalks, grass and other non-food plants to make biofuels, scientists today described the discovery of a potential treasure-trove of candidate enzymes in fungi thriving in the feces and intestinal tracts of horses.

They reported on these enzymes—the key to [economical production](#) of biofuels from non-food plant material—at the 245th National Meeting & Exposition of the American Chemical Society (ACS).

Michelle A. O'Malley, Ph.D., explained that [cellulose](#) is the raw material for making biofuels from non-food plant materials. Cellulose, however, is sealed away inside a tough network of lignin within the cell walls of plants. To produce biofuels from these materials, lignin must be removed through an expensive pretreatment process. Then, a collection of enzymes breaks cellulose down into sugars. Finally, in a process much like production of beer or wine, those sugars become food for microbes to ferment into alcohol for fuel, ingredients for plastics and other materials.

"Nature has made it very difficult and expensive to access the cellulose in plants. Additionally, we need to find the best enzyme mixture to convert that cellulose into sugar," O'Malley said. "We have discovered a fungus from the digestive tract of a horse that addresses both issues—it thrives on lignin-rich plants and converts these materials into sugars for the animal. It is a potential [treasure trove](#) of enzymes for solving this problem and reducing the cost of biofuels."

The digestive tracts of large herbivores like cows and [horses](#), which can digest lignin-rich grasses, have been a well-trodden path for scientists seeking such enzymes. But in the past, their focus has been mainly on enzymes in bacteria, rather than [fungi](#), which include yeasts and molds. The goal: Take the genes that produce such enzymes from gut fungi and genetically engineer them into yeasts. Yeasts already are used in time-tested processes on an industrial scale to produce huge quantities of antibiotics, foods and other products. That proven production technology would mean clear sailing for commercial production of biofuels.

O'Malley explained that several genes from gut fungi are unique compared to bacteria, since the fungi grow invasively into [plant material](#). Also, they secrete powerful enzyme complexes that work together to break down cellulose. Until now, however, fungi have largely been ignored in the search for new biofuel enzymes—and for good reason.

"There was relatively little scientific knowledge about fungi in the digestive tracts of these large animals," O'Malley explained. "They are there, but in very low numbers, making it difficult to study. The low concentrations also fostered a misconception that fungi must be unimportant in digestion of cellulose. And it is extremely difficult to isolate and grow these fungi to study their enzymes."

O'Malley's research group at the University of California, Santa Barbara, collaborated with researchers at the Broad Institute of the Massachusetts Institute of Technology and Harvard University. They worked with a gut fungus isolated from horse feces and identified all the genetic material that the fungus uses to manufacture enzymes and other proteins. This collection of protein-encoding material—the fungus's so-called "transcriptome"—led to the identification of literally hundreds of enzymes capable of breaking through that tough lignin in plant cell walls and the cellulose within. The team now is shifting through that bounty to identify the most active [enzyme](#) and working on methods for

transferring the genetic machinery for its production into the yeast currently used in industrial processes.

More information: Abstract

Anaerobic gut fungi are attractive lignocellulose-degrading microbes, yet the enzymatic mechanisms responsible for fungal hydrolysis remain unknown. To discover novel biomass-degrading enzymes and characterize their coordinated expression in fungi, we have implemented methods to sustain an anaerobic fungus in batch culture and analyze its transcriptome via RNAseq under several growth conditions. A new species of gut fungus from the *Piromyces* genus was isolated from the digestive tract of a horse, and its proliferation was monitored via fermentation gas production. Fungi exhibited high enzymatic reactivity against cellulosic and lignocellulosic substrates (filter paper, reed canary grass), which was repressed in the presence of simple sugars. Through strand-specific RNAseq and use of the TRINITY assembly platform, we were able to assemble novel cellulase genes de novo from >27,000 transcripts without the need for genomic sequence information. We will discuss the coordinated regulation patterns observed for important enzyme families under catabolic regulatory conditions.

Provided by American Chemical Society

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