To Make Better Biofuels, Scientists Mine Microbiomes

Microbes within pandas and other creatures may hold keys to cost-competitive cellulose-based biofuels

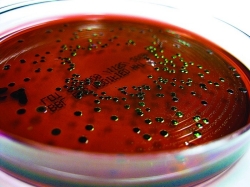
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**Ya Ya and Le Le** are a couple of giant pandas at the [Memphis Zoo](http://www.memphiszoo.org/) who don’t realize they are participating in an important science experiment. Once in a while a person will come into their space at the zoo, grab their poo, and scurry away. Ya Ya and Le Le shrug off the intrusions and go back to what they do best: eating bamboo.

The results coming from their backsides could benefit the pandas. Scientists studying the microbial community in the animal’s gastrointestinal tract are gaining a better understanding of panda nutrition, which could help improve the health and reproduction of the lovable and endangered creatures.

But people have still another motive for these panda poo investigations. They want to figure out which symbiotic microbes living in the panda gut are deconstructing the tough, fibrous [lignocellulose](http://pubs.acs.org/cen/coverstory/86/8649cover2.html) that makes up the plant cell walls of bamboo. By analyzing DNA they glean from the stool samples, scientists are working backward to identify the microbes and pinpoint genes that code for their cellulose-busting enzymes.

And researchers aren’t stopping at pandas: They are searching for such enzymes in a variety of vegetarian animals. Ultimately, they want to cut and paste the genetic blueprints for such enzymes into other microorganisms, giving the engineered microbes the ability to break apart biomass into soluble sugars that can be fermented into biofuels.

**PANDA BUGS**

Bacteria isolated from panda poo grow on an agar plate stained with eosin methylene blue, which lights up lactose-fermenting *Escherichia coli* colonies (green).

Credit: Candace Williams

Bacteria isolated from panda poo grow on an agar plate stained with eosin methylene blue, which lights up lactose-fermenting Escherichia coli colonies (green).

It remains to be seen whether such technologies will eventually lead to an economically viable cellulosic biofuel market. But those challenges aren’t stopping biochemist Ashli Brown and members of her Mississippi State University research group. They watch Ya Ya and Le Le eat and wait for them to poo. When the time comes, they alert Memphis zookeepers to move in for a search and recover operation.

And it’s not such a dirty job. “Panda pellets are fairly desiccated and fibrous,” Brown says. “They are like mini hay bales. For anyone with experience working with animal fecal material, I can assure you panda poo has a fairly pleasant smell and is probably the nicest to work with.”

To find useful enzymes in the pellets, Candace L. Williams in Brown’s group takes them, grinds them up, performs extractions, and then uses traditional anaerobic bacterial culture methods to [grow and identify bacterial colonies](http://dx.doi.org/10.1111/j.1439-0396.2012.01299.x). The microbes found clinging to incompletely digested bamboo in panda poo can be difficult to cultivate in a lab, like any microbe when it’s out of its element. Brown’s group inevitably misses a few of them.

To circumvent that problem, Brown’s team has added another tool to its arsenal: [metagenomics](http://www.youtube.com/watch?v=AA20Po5-M90). This relatively [new DNA sequencing approach](http://pubs.acs.org/isubscribe/journals/cen/87/i21/html/8721cover2.html) can reveal the diverse array of microbes that live in a given environment, such as the panda gut. With metagenomics, scientists can take a census of all of an environment’s microbes—collectively known as a microbiome—without having to culture them.

The researchers chemically dice up the DNA extracted from the panda poo into pieces of about 100 to 600 base pairs and sequence each piece, Williams explains. They then take the thousands of short sequences generated from the microbiome, align them to find overlapping regions, and piece them together to isolate the 16S ribo­somal RNA genes. This gene codes for a component of the ribosome, the machinery used to build all the proteins in a cell. It has a relatively short sequence—about 1,500 base pairs long—and each microbial species has a unique one.

**The Mississippi State** team is not alone in its quest. By using a combination of metagenomics, whole-genome sequencing, enzyme activity assays, and sugar and fatty acid composition analyses, scientists have been profiling the gut microbiomes of a variety of animals to begin to understand how they degrade plant biomass, says biophysicist [Susannah G. Tringe](http://www.jgi.doe.gov/whoweare/tringe.html), head of metagenomics at the Department of Energy’s [Joint Genome Institute](http://www.jgi.doe.gov/) (JGI).

“We now have a pretty standard approach to metagenomics projects,” Tringe says. “DNA is a common currency no matter where it comes from, allowing us to study microbiomes in [soil](http://www.earthmicrobiome.org/), water, [plants](http://www.cen-online.org/articles/90/i40/Plant-Microbiomes-Unfurl.html), or animals, including [humans](http://hmpdacc.org/).”

Animal metagenomics studies like that of the panda serve as a foundation for biofuel development, Tringe says. “The [Human Genome Project](http://www.genome.gov/10001772), for example, hasn’t actually cured any diseases itself. But it has given scientists a much faster way to understand the genetics of individual diseases and potential treatments. Now that we are getting cellulosic enzyme meta­genomics data, it’s creating opportunities for a variety of downstream studies.



**COW BUGS**

This fragment of switchgrass is in the process of being demolished by cow rumen microbes.

Credit: Damon Tighe/JGI

 Like pandas, cows are unwitting participants in these gut metagenomics projects. Enzymes in the cow’s rumen, the primary grass-digesting chamber in the cow’s multistomach GI tract, allow the ruminants to process massive amounts of grass. In 2011, Tringe was part of a multi-institution team that conducted a metagenomics study of the cow rumen (*Science,* DOI: [10.1126/science.1200387](http://dx.doi.org/10.1126/science.1200387)). The researchers took 268 billion bases they sequenced and whittled them down to 27,755 genes that encode for carbohydrate-active enzymes. They ultimately identified 51 enzymes with cellulose-degrading activity and are now testing them for their catalytic abilities.

Another animal with potentially useful cellulase enzymes is the [hoatzin](http://www.jgi.doe.gov/sequencing/why/hoatzin.html), a chicken-sized, reddish-brown bird with a spiky crest that hails from South America. The hoatzin is unusual among birds in that it primarily eats leaves. Like other birds it has a crop, a built-in storage bin that is part of the digestive system—a kind of prestomach. But in the hoatzin, the crop functions as a biomass fermenter, similar to the cow’s rumen. In fact, the hoatzin is nicknamed the stink bird because its droppings smell like cow manure.

This fragment of switchgrass is in the process of being demolished by cow rumen microbes.

Studies on the hoatzin microbiome, led by Filipa Godoy-Vitorino and María G. Domínguez-Bello of the University of Puerto Rico, Río Piedras, detected hundreds of microbes. The researchers also carried out a comparative analysis of the hoatzin and cow microbiomes.

“Despite being phylogenetically distant, adult hoatzins and pasturing cows share specific digestive microbes,” says Godoy-Vitorino, who is now at JGI. After classifying the microbial species, the researchers found seven that were unique to the hoatzin, she says. They are now classifying the hoatzin cellulose-degrading enzymes.

And then there are pandas. “If you can use a microbe that efficiently breaks down biomass naturally and as efficiently as pandas do, and convert them into sugars that could be converted to oils and other chemicals, production costs for alternative fuels would be cut tremendously,” Mississippi State’s Brown says. “That would be fantastic.”

Pandas have to be efficient at breaking down biomass—they eat as much as 30 lb of bamboo each day, and not much else, Brown notes. Bamboo makes up a whopping 99% of the giant panda’s diet in the wild. The creatures have unusual adaptations to accommodate, she says. For one, pandas have a pseudothumb that allows them to grab bamboo and strip away leaves and stems. They also have well-developed teeth, jaws, and chewing muscles, designed more for masticating like a cow than ripping meat like a carnivore.

But the panda’s digestive system isn’t built like a cow’s, Brown continues. Whereas cows and other ruminants typically have a long GI tract with a multichambered stomach system designed to digest grasses, she says, the panda sports a short GI tract and a single-chambered stomach. The combination of bamboo gluttony and short GI tract means the panda processes bamboo quickly, in only about four hours from front to rear; in cows, it’s a one- to three-day process.

Brown’s research has been guided by the panda genome, which was sequenced and reported in 2009 by an international consortium led by scientists in China (*Nature,* DOI: [10.1038/nature08696](http://dx.doi.org/10.1038/nature08696)). The Chinese team assembled the genome of Jingjing, a panda at the Beijing Zoo named after one of the mascots of the 2008 Olympic Games.

The study revealed a genetic basis for the panda’s love of bamboo. It turns out pandas have mutations resulting in loss of function of the gene that codes for umami taste receptors, which recognize glutamate, Brown notes. These mutations appear to have robbed pandas of the ability to savor the taste of meat and other high-protein foods, she explains, pushing them toward their diet of readily available bamboo.

As for biofuel prospects, among the hundreds of microbe species inside pandas, the Mississippi State researchers discovered 17 cellulose-degrading microbes. They are still trying to identify the exact species of them all, but some of them have also been found in other biomass-digesting animals. Brown’s team has done preliminary work culturing the newfound microbes, feeding them cellobiose, the primary disaccharide in cellulose. They are finding that some of the microbes convert the sugar to fatty acid methyl esters for energy storage.

“We see considerable cellobiose consumption and quite a mix of different triglycerides and free fatty acids forming,” she says. Those that yield primarily oils or alcohols are likely to be more amenable to producing biofuels, she adds. Her group is collaborating with Mississippi State chemical engineer Darrell Sparks Jr. and colleagues to explore the possibility of transplanting genes unearthed from the panda gut into the yeast *Rhodotorula glutinis* to enhance its oil-producing ability. For example, they are hoping to engineer yeast strains that can feed off waste lignin in paper mills or residual organic carbon in wastewater at treatment plants.

“There is now a ton of sequence data that’s been generated hinting at putative cellulolytic enzymes,” JGI’s Tringe says. “I am not sure how many more animal microbiomes we need to explore. The real need now is to understand enzyme functions better to move the data downstream to make them useful for industry.”

But even as hundreds of researchers and millions of research dollars have been dedicated to enzyme bioprospecting, there is one thing still missing: a viable market for cellulosic biofuels. If scientists like Brown come up with improved technologies for processing the biomass, a viable market will require more, including a practical and economical source for the biomass, companies to produce biofuels, and consumer demand.

Biomass supplies are ample, according to DOE estimates. It projects that by 2030 the U.S. could produce some [1.1 billion dry tons](https://bioenergykdf.net/content/billiontonupdate) of bioenergy crops or agricultural residues annually without upsetting food, animal feed, and fiber production—enough to replace about 30% of the country’s petroleum consumption.

With that in mind, enzyme producers, chemical and oil companies, and biofuel start-ups are forming partnerships as they pick and choose technology platforms to make cellulosic biofuels. But no companies have yet to achieve commercial-scale production ([C&EN, Oct. 8, page 22](http://cen.acs.org/signin.html?resource=/content/cen/articles/90/i41/Enzymes-Poised-Cellulosic-Success&e=2)).

Panda poo, sampled at the Memphis Zoo, is kept under anaerobic conditions while it is being mined for cellulose-degrading enzymes.

Scientists anticipate that the mounds of data coming out of the animal meta­genomics studies will provide replacement or supplemental genes needed to improve current biofuel production systems to make them cost-competitive. But so far, neither metagenomics researchers nor enzyme producers have reached out to each other to collaborate on the gut microbiome results.

The biofuel industry faces a formidable foe in fossil fuels that are cheaper and will remain readily available for years to come. Increased federal fuel-efficiency standards will put biofuels at a further disadvantage. And although the federal [Renewable Fuel Standard](http://cenblog.org/cleantech-chemistry/2012/11/advanced-biofuels-makers-thankful-for-rfs/), which gradually increases the amount of cellulosic biofuels required to be produced each year, continues to create market demand for new enzymes, the federal government has declined to mandate that carmakers produce vehicles that can accept a range of biofuel/fossil-fuel blends.

Emalfarb suggests the cellulosic biofuel industry needs to become more engaged in the outcome of the metagenomics studies to prevent the research on pandas, cows, hoatzins, and other creatures from sitting on a shelf collecting dust.

In Memphis, oblivious to these dilemmas, Ya Ya and Le Le keep chowing down on bamboo. Mississippi State’s Brown and her team, probing pellets for more enzymatic clues, are more aware of what’s at stake.

“Our work suggests that metagenomics is useful for enzyme bioprospecting,” Brown states. “Hopefully we are helping standardize DNA sequencing technology and high-throughput screening technology to make this approach more credible. And hopefully in doing that we can make a difference for the pandas and for biofuels.”

**GUT FUNCTIONS**

This NIMS assay pits cellulase enzymes derived from animal gut metagenomics studies against oligosaccharides.The goal: learn how enzymes break apart oligosaccharides into simpler sugars.

Credit: Courtesy of Trent Northen/JBEI