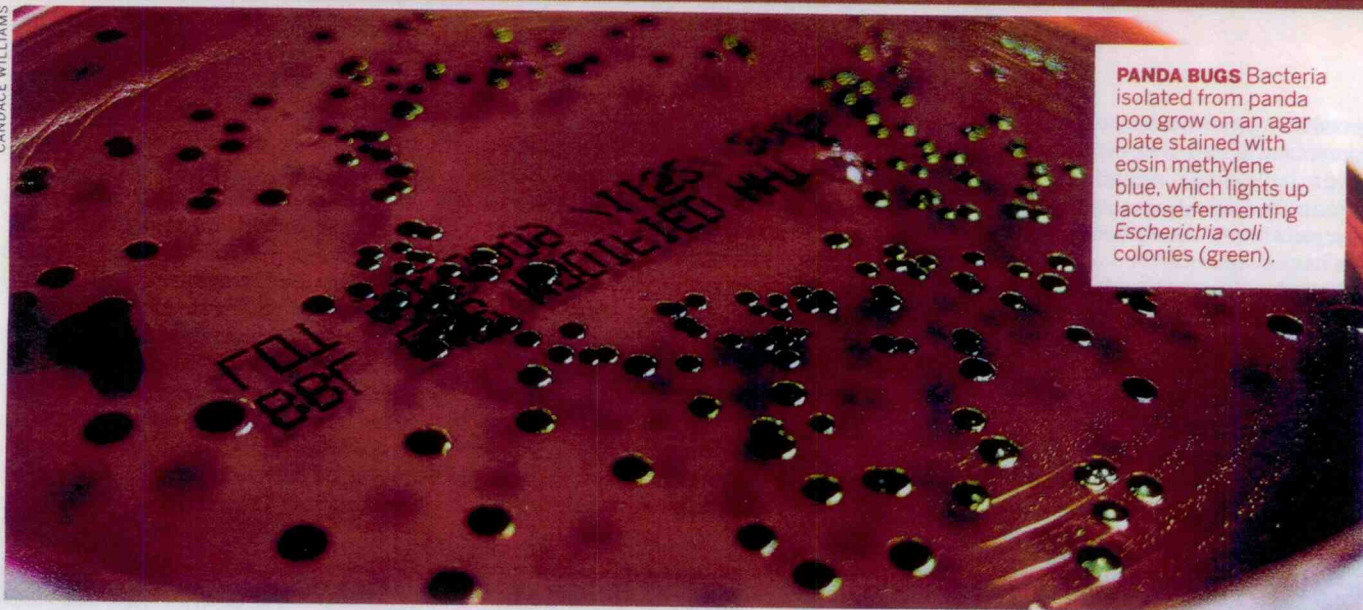


CANDACE WILLIAMS



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).

MICROBIOME MINING

Microbes within pandas and other creatures may hold keys to cost-competitive **CELLULOSE-BASED BIOFUELS**

STEPHEN K. RITTER, C&EN WASHINGTON

YA YA AND LE LE are a couple of giant pandas at the Memphis Zoo 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 back-sides 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 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 blue-

prints 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.

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 anaero-

bic bacterial culture methods to grow and identify bacterial colonies. 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. This relatively new DNA sequencing approach 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 ribo-

"DNA is a common currency no matter where it comes from."

somal 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 team then turns to computational algorithms to sort through the billions of base pairs sequenced to match the 16S rRNA and all the other genes in the microbiome to the individual microbes. They also use publicly available bioinformatics database tools to compare microbial diversity be-



Creature: Panda
Eats: Bamboo
Microbial enzymes allow pandas, like Le Le at the Memphis Zoo, to rapidly digest large amounts of bamboo.

SYMBIOTIC ZOO In search of cellulose-degrading enzymes to make biofuels, scientists mine the gut microbial communities of a menagerie of creatures. They use a combination of metagenomics, whole-genome sequencing, enzyme activity assays, and sugar and fatty acid composition analyses.

tween multiple microbiomes to determine what all the different microbes in the panda gut do and which enzymes they produce.

In the end, because the sequencing process and data analysis are not 100% accurate, the Mississippi State team is validating its metagenomics data by using the polymerase chain reaction to amplify the microbial cellulase genes they discover in panda poo.

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, head of metagenomics at the Department of Energy's Joint Genome Institute (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, water, plants, or animals, including humans."

Animal metagenomics studies like that of the panda serve as a foundation for biofuel development, Tringe says. "The Hu-

man Genome Project, 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."

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 multi-stomach GI tract, allow the

rumen. In fact, the hoatzin is nicknamed the stink bird because its droppings smell like cow manure.

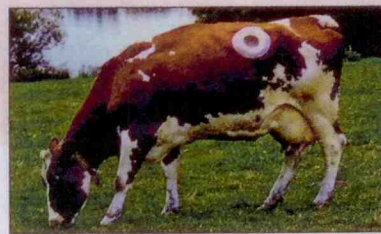
Studies on the hoatzin microbiome, led by Filipa Godoy-Vitorino and María G. Domínguez-Bello of the University of Puerto Rico, Río Piedras, detected hundreds of microbes. The researchers also carried out a comparative analysis of the hoatzin and cow microbiomes.

"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

Creature: Cow

Eats: Grasses and grains

This cow, fitted with a surgical port, participated in a study to identify enzymes in its rumen that break down switchgrass to oligosaccharides.



Creature: Hoatzin

Eats: Leaves

The South American hoatzin has a digestive tract similar to a cow's, loaded with cellulose-degrading microbes that aid in digesting its leafy diet.



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). 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, 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

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

"You can't design enzymes or biosynthetic pathways like a computer chip and have them work."

