How fungi make nutrients available to the world

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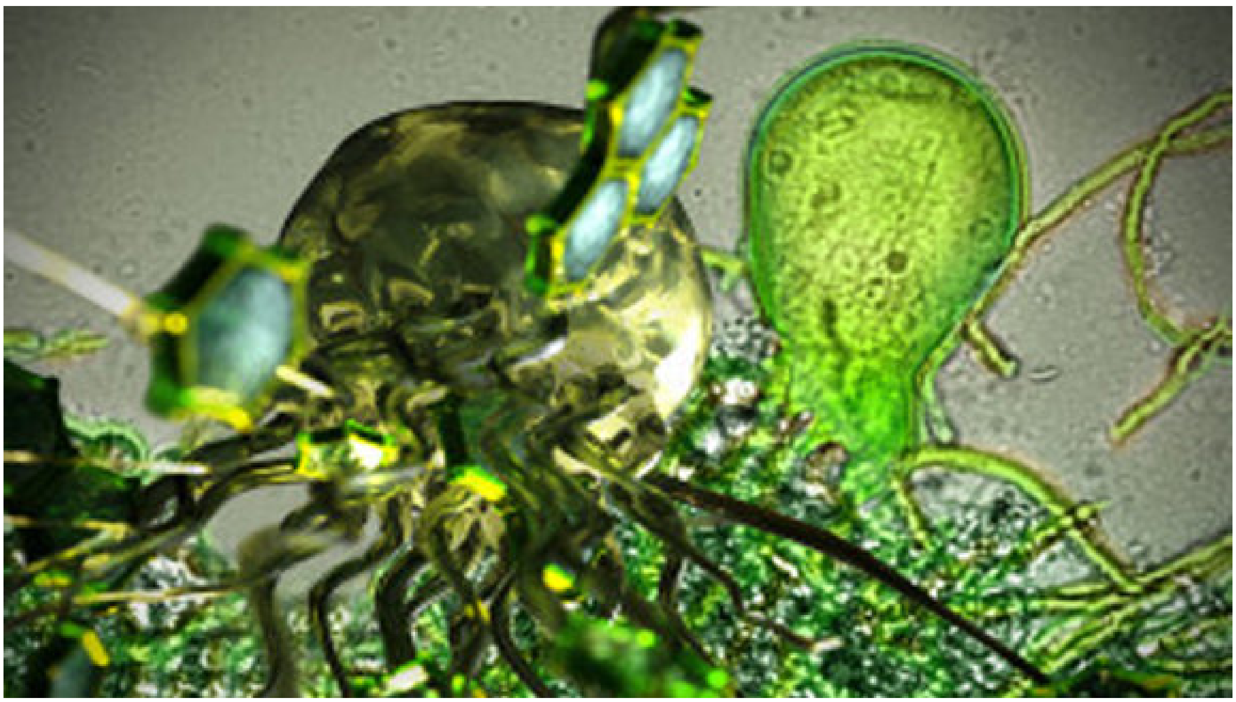

Anaerobic gut fungi colonize plant matter and release enzymes that break cell walls into simple sugars. Credit: US Department of Energy

Like most of us, trees don't want to be eaten alive.

To prevent this gruesome fate, they developed extremely tough cell walls around 400 million years ago. For millions of years, nothing could break down lignin, the strongest substance in those cell walls. When a tree died, it just sank into the swamp where it grew. When the fossil record started showing trees breaking down around 300 million years ago, most scientists assumed it was because the ubiquitous swamps of the time were drying up.

But biologist David Hibbett at Clark University suspected that wasn't the whole story. An alternative theory from researcher Jennifer Robinson intrigued him. She theorized that instead of ecosystem change alone, something else played a major role – something evolving the ability to break down lignin. Through evolutionary biology research supported by the Department of Energy's (DOE) Office of Science, Hibbett and his team confirmed her theory. They found that, just as she predicted, a group of fungi known as "white rot fungi" evolved the ability to break down lignin approximately the same time that coal formation drastically decreased. His research illustrated just how essential white rot fungi were to Earth's evolution.

Fungi are still indispensable. The short-order cooks of the natural world, they have an unheralded job making nutrients accessible to the rest of us. Just like cooking spinach makes it easier to digest, some fungi can break down plant cell walls, including lignin. That makes it easier for other organisms to use the carbon that is in those cell walls.

"We all live in the digestive tract of fungi," said Scott Baker, a biologist at DOE's Pacific Northwest National Laboratory. If we weren't surrounded by fungi that decay dead plant material, it would be much harder for plants to obtain the nutrients they need.

To understand fungi's role in the ecosystem and support biofuels research, scientists supported by DOE's Office of Science are studying how fungi have evolved to decompose wood and other plants.

The Special Skills of Fungi

Fungi face a tough task. Trees' cell walls contain lignin, which holds up trees and helps them resist rotting. Without lignin, California redwoods and Amazonian kapoks wouldn't be able to soar hundreds of feet into the air. Trees' cell walls also include cellulose, a similar compound that is more easily digested but still difficult to break down into simple sugars.

By co-evolving with trees, fungi managed to get around those defenses. Fungi are the only major organism that can break down or significantly modify lignin. They're also much better at breaking down cellulose than most other organisms.

In fact, fungi are even better at it than people and the machines we've developed. The bioenergy industry can't yet efficiently and affordably break down lignin, which is needed to transform non-food plants such as poplar trees into biofuels. Most current industrial processes burn the lignin or treat it with expensive and inefficient chemicals. Learning how fungi break down lignin and cellulose could make these processes more affordable and sustainable.

Tracing the Fungal Family Tree

While fungi live almost everywhere on Earth, advances in genetic and protein analysis now allow us to see how these short-order cooks work in their kitchen. Scientists can sample a fungus in the wild and analyze its genetic makeup in the laboratory.

By comparing genes in different types of fungi and how those fungi are evolutionarily related to each other, scientists can trace which genes fungi have gained or lost over time. They can also examine which genes an individual fungus has turned "on" or "off" at any one time.

By identifying a fungus's genes and the proteins it produces, scientists can match up which genes code for which proteins. A number of projects seeking to do this tap the resources of the Joint Genome Institute (JGI) and the Environmental Molecular Sciences Laboratory (EMSL), both Office of Science user facilities.

Understanding the Rot

Just as different chefs use different techniques, fungi have a variety of ways to break down lignin, cellulose, and other parts of wood's cell walls.

White Rot

Although fungi appeared millions of years earlier, the group of fungi known as white rot was the first type to break down lignin. That group is still a major player, leaving wood flaky and bleached-looking in the forest.

"White rot is amazing," said Hibbett.

To break down lignin, white rot fungi use strong enzymes, proteins that speed up chemical reactions. These enzymes split many of lignin's chemical bonds, turning it into simple sugars and releasing carbon dioxide into the air. White rot is still better at rending lignin than any other type of fungus.

Brown Rot

Compared to white rot's powerful effects, the scientific community long thought the group known as brown rot fungi was weak. That's because brown rot fungi can't fully break down lignin.

Recalling his college classes in the 1980s, Barry Goodell, a professor at the University of Massachusetts Amherst said, "Teachers at the time considered them these poor little things that were primitive."

Never underestimate a fungus. Even though brown rot fungi make up only 6 percent of the species that break down wood, they decompose 80 percent of the world's pine and other conifers. As scientists working with JGI in 2009 discovered, brown rot wasn't primitive compared to white rot. In fact, brown rot actually evolved from early white rot fungi. As the brown rot species evolved, they actually lost genes that code for lignin-destroying enzymes.

Like good cooks adjusting to a new kitchen, evolution led brown rot fungi to find a better way. Instead of unleashing the brute force of energy-intensive enzymes alone, they supplemented that enzyme action with the more efficient "chelator-mediated Fenton reaction" (CMF) process. This process breaks down wood cell walls by producing hydrogen peroxide and other chemicals. These chemicals react with iron naturally in the environment to break down the wood. Instead of fully breaking down the lignin, this process modifies it just enough for the fungus to reach the other chemicals in the cell wall.

There was just one problem with this discovery. In theory, the CMF chemical reaction is so strong it should break down both the fungus and the enzymes it relies on. "It would end up obliterating itself," said Jonathan Schilling, an associate professor at the University of Minnesota.

Scientists' main theory was that the fungus created a physical barrier between the reaction and the enzymes. To test that idea, Schilling and his team grew a brown rot fungus on very thin pieces of wood. As they watched the fungus work its way through the wood, they saw that the fungus was breaking up the process not in space, but in time. First, it expressed genes to produce the corrosive reaction. Two days later, it expressed genes to create enzymes. Considering fungi can take years or even decades to break down a log, 48 hours is a blip in time.

Scientists are still trying to figure out how much of a role the CMF process plays. Schilling and like-minded researchers think enzymes are still a major part of the process, while Goodell's research suggests that CMF reactions do most of the work. Goodell's team reported that CMF reactions could liquefy as much as 75 percent of a piece of pine wood.

Either way, the CMF process offers a great deal of potential for biorefineries. Using brown rot fungi's pretreatment could allow industry to use fewer expensive, energy-intensive enzymes.

Not all fungi stand alone. Many types live in symbiosis with animals, as the fungus and animal rely on each other for essential services.

Partnerships with Rumens

Cows and other animals that eat grass depend on gut fungi and other microorganisms to help break down lignin, cellulose, and other materials in wood's cell walls. While fungi only make up 8 percent of the gut microbes, they break down 50 percent of the biomass.

To figure out which enzymes the gut fungi produce, Michelle O'Malley and her team at the University of California, Santa Barbara grew several species of gut fungi on lignocellulose. They then fed them simple sugars. As the fungi "ate" the simple sugars, they stopped the hard work of breaking down the cell walls, like opting for take-out rather than cooking at home.

Depending on the food source, fungi "turned off" certain genes and shifted which enzymes they were producing. Scientists found that these fungi produced hundreds more enzymes than fungi used in industry can.

They also discovered that the enzymes worked together to be even more effective than industrial processes currently are.

"That was a huge diversity in enzymes that we had never seen," said O'Malley.

O'Malley's recent research shows that industry may be able to produce biofuels even more effectively by connecting groups of enzymes like those produced by gut fungi.

Termites as Fungus Farmers

Some fungi work outside the guts of animals, like those that partner with termites. Tropical termites are far more effective at breaking down wood than animals that eat grass or leaves, both of which are far easier to digest. Young termites first mix fungal spores with the wood in their own stomachs, then poop it out in a protected chamber. After 45 days of fungal decomposition, older termites eat this mix. By the end, the wood is almost completely digested.

"The cultivation of fungus for food [by termites] is one of the most remarkable forms of symbiosis on the planet," said Cameron Currie, a professor at the University of Wisconsin, Madison and researcher with the DOE's Great Lakes Bioenergy Research Center.

Scientists assumed that the majority of the decomposition occurred outside of the gut, discounting the work of the younger termites. But Hongji Li, a biologist at the University of Wisconsin, Madison, wondered if younger insects deserved more credit. He found that young workers' guts break down much of the lignin. In addition, the fungi involved don't use any of the typical enzymes white or brown rot fungi produce. Because the fungi and gut microbiota associated with termites have evolved more recently, this discovery may open the door to new innovations.

From the Lab to the Manufacturing Floor

From the forest floor to termite mounds, fungal decomposition could provide new tools for biofuels production. One route is for industry to directly produce the fungal and associated microbiota's enzymes and other chemicals. When they analyzed termite-fungi systems, scientists found hundreds of unique enzymes.

"We're trying to dig into the genes to discover some super enzyme to move into the industry level," said Li.

A more promising route may be for companies to transfer the genes that code for these enzymes into organisms they can already cultivate, like yeast or E. coli. An even more radical but potentially fruitful route is for industry to mimic natural fungal communities.

For millions of years, fungi have toiled as short-order cooks to break down wood and other plants. With a new understanding of their abilities, scientists are helping us comprehend how essential they are to Earth's past and future.

Provided by US Department of Energy

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