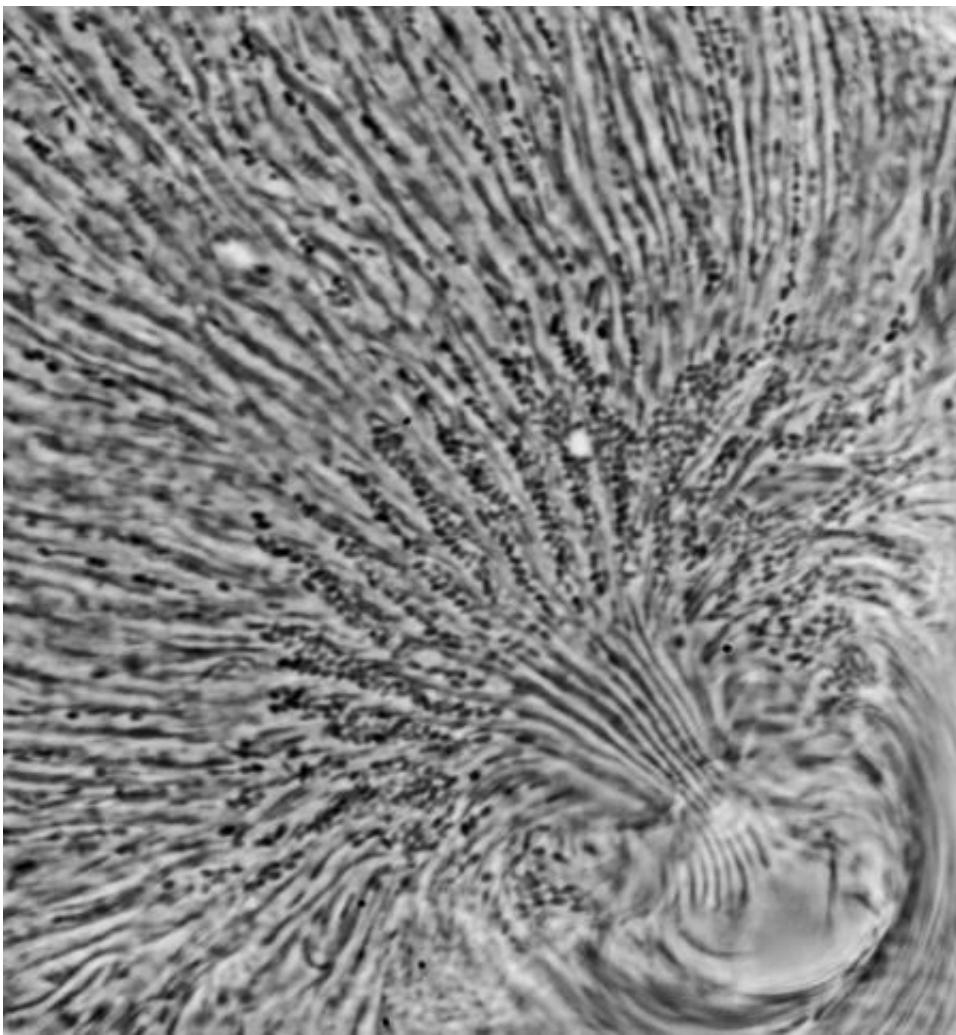


New gut bacterium discovered in termite's digestion of wood

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Phase contrast photomicrograph of a termite gut protozoan and attached symbiotic bacteria (small dark dots). Caltech researchers describe details of this association in the sept. 16 edition of *PNAS* online. Credit: Jared R. Leadbetter

When termites munch on wood, the small bits are delivered to feed a community of unique microbes living in their guts, and in a complex process involving multiple steps, these microbes turn the hard, fibrous material into a nutritious meal for the termite host. One key step uses hydrogen to convert carbon dioxide into organic carbon—a process called acetogenesis—but little is known about which gut bacteria play specific roles in the process. Utilizing a variety of experimental techniques, researchers from the California Institute of Technology (Caltech) have now discovered a previously unidentified bacterium—living on the surface of a larger microorganism in the termite gut—that may be responsible for most gut acetogenesis.

"In the termite gut, you have several hundred different species of [microbes](#) that live within a millimeter of one another. We know certain microbes are present in the gut, and we know microbes are responsible for certain functions, but until now, we didn't have a good way of knowing which microbes are doing what," says Jared Leadbetter, professor of [environmental microbiology](#) at Caltech, in whose laboratory much of the research was performed. He is also an author of a paper about the work published the week of September 16 in the online issue of the *Proceedings of the National Academy of Sciences (PNAS)*.

Acetogenesis is the production of acetate (a source of nutrition for [termites](#)) from the carbon dioxide and hydrogen generated by gut [protozoa](#) as they break down decaying wood. In their study of "who is doing what and where," Leadbetter and his colleagues searched the entire pool of termite gut microbes to identify specific genes from organisms responsible for acetogenesis.

The researchers began by sifting through the microbes' RNA—[genetic information](#) that can provide a snapshot of the genes active at a certain point in time. Using RNA from the total pool of termite gut microbes, they searched for actively transcribed formate dehydrogenase (FDH)

genes, known to encode a protein necessary for acetogenesis. Next, using a method called multiplex microfluidic digital polymerase chain reaction (digital PCR), the researchers sequestered the previously unstudied individual microbes into tiny compartments to identify the actual microbial species carrying each of the FDH genes. Some of the FDH genes were found in types of bacteria known as spirochetes—a previously predicted source of acetogenesis. Yet it appeared that these spirochetes alone could not account for all of the acetate produced in the termite gut.

Initially, the Caltech researchers were unable to identify the microorganism expressing the single most active FDH gene in the gut. However, the first authors on the study, Adam Rosenthal, a postdoctoral scholar in biology at Caltech, and Xinning Zhang (PhD '10, Environmental Science and Engineering), noticed that this gene was more abundant in the portion of the gut extract containing wood chunks and larger microbes, like protozoans. After analyzing the chunkier gut extract, they discovered that the single most active FDH gene was encoded by a previously unstudied species from a group of microbes known as the *deltaproteobacteria*. This was the first evidence that a substantial amount of acetate in the gut may be produced by a non-spirochete.

Because the genes from this *deltaproteobacterium* were found in the chunky particulate matter of the termite gut, the researchers thought that perhaps the newly identified microbe attaches to the surface of one of the chunks. To test this hypothesis, the researchers used a color-coded visualization method called hybridization chain reaction-fluorescent *in situ* hybridization, or HCR-FISH.

The technique—developed in the laboratory of Niles Pierce, professor of applied and computational mathematics and bioengineering at Caltech, and a coauthor on the *PNAS* study—allowed the researchers to

simultaneously "paint" cells expressing both the active FDH gene and a gene identifying the deltoproteobacterium with different fluorescent colors simultaneously. "The microfluidics experiment suggested that the two colors should be expressed in the same location and in the same tiny cell," Leadbetter says. And, indeed, they were. "Through this approach, we were able to actually see where the new deltaproteobacterium resided. As it turns out, the cells live on the surface of a very particular hydrogen-producing protozoan."

This association between the two organisms makes sense based on what is known about the complex food web of the termite gut, Leadbetter says. "Here you have a large eukaryotic single cell—a protozoan—which is making hydrogen as it degrades wood, and you have these much smaller hydrogen-consuming deltaproteobacteria attached to its surface," he says. "So, this new acetogenic bacterium is snuggled up to its source of hydrogen just as close as it can get."

This intimate relationship, Leadbetter says, might never have been discovered relying on phylogenetic inference—the standard method for matching a function to a specific organism. "Using phylogenetic inference, we say, 'We know a lot about this hypothetical organism's relatives, so without ever seeing the organism, we're going to make guesses about who it is related to,'" he says. "But with the techniques in this study, we found that our initial prediction was wrong. Importantly, we have been able to determine the specific organism responsible and a location of the mystery organism, both of which appear to be extremely important in the consumption of hydrogen and turning it into a product the insect can use." These results not only identify a new source for acetogenesis in the termite gut—they also reveal the limitations of making predictions based exclusively on phylogenetic relationships.

The paper is titled "Localizing transcripts to single cells suggests an important role of uncultured deltaproteobacteria in the termite gut

hydrogen economy."

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