

Microfluidic Device Used for Multigene Analysis of Individual Environmental Bacteria

December 1 2006

When it comes to digestive ability, termites have few rivals due to the gut activities that allow them to literally digest a two-by-four. But they do not digest wood by themselves--they are dependent on the 200 or so diverse microbial species that call termite guts home and are found nowhere else in nature.

Despite several successful attempts, the majority of these beneficial organisms have never been cultivated in the laboratory. This has made it difficult to determine precisely which species perform the numerous, varied functions relevant to converting woody plant biomass into a material that can be directly used as food and energy by their insect hosts.

Now, scientists using state-of-the-art microfluidic devices have come up with a new way of investigating microbial ecology. In the December 1 issue of the journal *Science*, California Institute of Technology associate professor of environmental microbiology Jared Leadbetter, biology graduate student Elizabeth Ottesen, and their colleagues announce a new and efficient way of revealing guild-species relationships in complex microbial communities. The approach allows them to discover connections between bacterial cells from natural samples, and the activities encoded by genes.

The results also reveal important insights into the relationship between

termites and key gut microbes called spirochetes, which aid them in the process of digesting wood.

"I think these results involve two pinnacles of novelty," says Leadbetter, "What we're showing are key results relevant to the symbiosis that occurs between termites and the bacteria involved in the conversion of wood fiber into a form of energy that can be used by the insect. But we're also revealing an approach that can lead to a better understanding of the many microbial processes that underlie the environments in which we all live."

According to Leadbetter, the techniques of gene amplification, cloning, and sequencing developed over the past two decades have already revolutionized microbial ecology. As a result, we now have a much greater appreciation of the vast diversity of microbial species occurring in nature, as well as the diversity of genes involved with processes that we know are mediated by as-yet unstudied microbes in the environment.

However, researchers have had difficulty in determining which subset of the species that have been inventoried actually encode these various key genes. The biggest problem has been the practice of extracting as one mass the composite genetic information of an entire, complex sample. This destroys the individual cells that are the source of the information, thus mixing together that which is encoded by hundreds if not thousands of unique species. As a result, the procedure inevitably dissolves the natural order underlying the organization of genetic information in the environment.

The approach of Leadbetter and his collaborators is to use microfluidic devices, in which thousands of individual cells harvested from the environment can be distributed into separate chambers prior to any gene-based analysis, so that each can be studied as an individual. If the cell reveals that it has a certain key gene of interest, then the researchers are

also able to determine the species identity of the cell, or whether it contains other key genes of interest.

The traditional approach involves removing the gut contents of individual termites, smashing the microbial cells, then extracting and pooling their DNA as one mass, with subsequent analysis of the genes found in the randomized mash. The genes are there, but assigning relationships between any two genes or to the organisms from which they are derived is complicated at best, and often just not possible.

"We're trying to move beyond investigating the jumbled information," says Leadbetter. "In the past, trying to study a microbial environment using gene-based techniques was often like studying the contents of several hundred books in a library after first having torn off their covers, ripped up all the pages into small pieces, and jumbled them together into a big pile. We would find sentences and paragraphs that we found extremely interesting and important, but then we were left frustrated. It was very difficult to determine what was in the rest of the book.

"But with this technique, we are suddenly able to read portions of the books without having first torn off their covers. We are still reading with a narrow penlight, but certainly, when we identify a sentence of interest, we can rapidly ascertain the title and author of the book that we are reading, and even move on to examine the other pages."

In the paper, the researchers describe an analysis of a complex, species-rich microbial community that allowed two genes of interest to be colocalized to the same environmental genome. An early result analyzing thousands of individual cells harvested straight from the gut environment reveals the species identity of a group of microbes resident in the California dampwood termite (*Zootermopsis*) that perform a key act in the nutritional symbiosis involved in wood decay.

The good news for nonscientists is that this provides a new path to reaching a better understanding of many diverse ecosystems. It also leads to a refined appreciation of certain details underlying the activities of a destructive pest, while shedding light on a key step involved in the conversion of plant biomass into useful products. Understanding that conversion in detail is critical to achieving a current societal need—the conversion of low-value lignocellulose materials into biofuels and other commodities of greater value.

Termites are extremely abundant and active in many tropical ecosystems, so the current work could also lead to a better understanding of several processes of global environmental relevance, Leadbetter adds.

"There are 2,600 different species of termites, and it is estimated that there are at least a million billion individual termites on Earth. It is thought that they emit two and four percent of the global carbon dioxide and methane budget, respectively—both mediated directly or indirectly by their microbes," he says. "Also, by extrapolation of what we understand from numerous studies of a few dozen termites species, we think that there could be millions of unique and novel microbial species found only in the hindguts of termites."

The other authors of the paper are Stephen Quake, professor of bioengineering at Stanford, and Jong Wook Hong, an assistant professor of materials engineering at Auburn University.

Source: Caltech

Citation: Microfluidic Device Used for Multigene Analysis of Individual Environmental Bacteria (2006, December 1) retrieved 16 August 2024 from <https://phys.org/news/2006-12-microfluidic-device-multigene-analysis-individual.html>

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