

# New Insight into the Unseen World

**MICROORGANISMS**—bacteria, fungi, microscopic algae such as plankton, and other tiny life forms—live all around us but are invisible to the naked eye. The oceans worldwide are teeming with microbial life. Microbes can be found virtually everywhere: in soil and hot springs, on and beneath the ocean floor, high in the atmosphere, and deep inside rocks within Earth's crust. Even our skin is fruitful territory for bacteria of all shapes and sizes.

Despite their small size, microbes play many important roles on Earth, including breaking down organic materials. They are also expert chemists and excel at finding solutions to their environmental problems so they can survive and prosper. At Livermore, environmental microbiologist Jennifer Pett-Ridge, marine microbiologist Xavier Mayali, and Peter Weber, Paul Hoepflich, and Shalini Mabery of the Physical and Life Sciences Directorate have taken a significant step forward in answering one of the “Holy Grail” questions in microbiology: what jobs do the hundreds of thousands of microorganisms perform in a given environment? “If that can be determined, we might be able to predict which organisms can best help with bioremediation of polluted sites,” Pett-Ridge says. “Microbes also play a critical role in the production of biofuels, and they help turn atmospheric carbon dioxide into usable carbon at a rate of about 50 gigatons a year. The more we know about them, the better we understand their essential roles in our environmental challenges.”

## Who Does What, and How Much?

With early funding support from the Laboratory Directed Research and Development Program, the Livermore team has developed Chip-SIP, a high-throughput, high-sensitivity technique for linking the activities of microbes to their identity. The Chip-SIP method combines several technologies unique to Livermore and takes its name from the combined use of a microarray slide (the “chip”) and an analytical method commonly used by microbial ecologists called stable isotope probing (SIP). Pett-Ridge says, “In the microbiology community, many researchers hypothesize that

if organisms are closely related in taxonomy, they probably do the same job in the environment. However, in experiments using the Chip-SIP technique, we have shown that identity and function are not always related.”

Chip-SIP is based on the same premise as traditional SIP, a suite of techniques used to link the identity and functional role of microorganisms in environmental samples. Researchers expose microbial communities to food sources labeled with rare isotopes, such as carbon-13 and nitrogen-15, which are not naturally



Xavier Mayali (left) and Jennifer Pett-Ridge (right) work with Chip-SIP (stable isotope probing) microarrays to link microbes' activity to their identity.

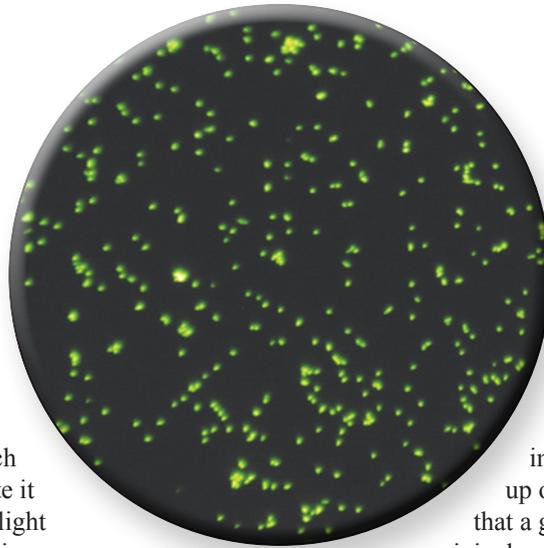
abundant. Active organisms in the system “eat” this food and incorporate the isotopic tracer into their cells, specifically into their DNA and ribosomal ribonucleic acid (rRNA).

Mayali says, “Traditional SIP looks at activity, measured by food source consumption, and asks questions about who’s doing what. However, processing rRNA samples using the SIP approach can take weeks.” Researchers have to collect all the DNA and rRNA from a sample, which contains a variety of organisms, and separate it based on density into isotopically heavy or light bands of nucleic acids that contain information on the organisms’ genetic identity. The assumption is that the denser samples are enriched with carbon-13 or nitrogen-15. “But because SIP is not quantitative, it’s hard to know how much of the food each microbe is taking up. With Chip-SIP, we’ve essentially reversed the process, by first separating nucleic acids by organism and then determining how enriched in the rare isotope it is,” Mayali says.

In the Livermore Microarray Center (LMAC), the team uses devices called microarrays to help sort the extracted nucleic acids and identify the dominant active organisms in a sample. LMAC provides onsite access to state-of-the-art microarray equipment for analyzing DNA and proteins. The microarrays are made of glass, nylon, or silicon slides with special Livermore-developed coatings on which tiny amounts of DNA from known organisms are printed in a regular pattern of spots.

### Match Game

The specific region of DNA that provides the most information about identity and evolutionary relatedness is the 16S rRNA gene. This gene is present in all bacteria, and related forms occur in other cell types. LMAC microarray slides can be spotted with up to 300,000 unique probe spots of 16S rRNA, representing tens of thousands of different sequences. Pett-Ridge says, “We design these probes to complement gene sequences of one or more organisms, called taxa, that we’re interested in studying.” When



A drop of seawater treated with a fluorescent stain that binds to DNA shows microbes as green spots. A single liter of seawater, once thought to contain about 100,000 microorganisms, can actually hold more than 1 billion.

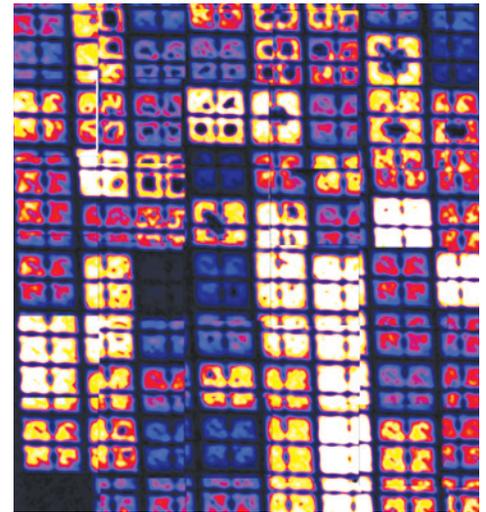
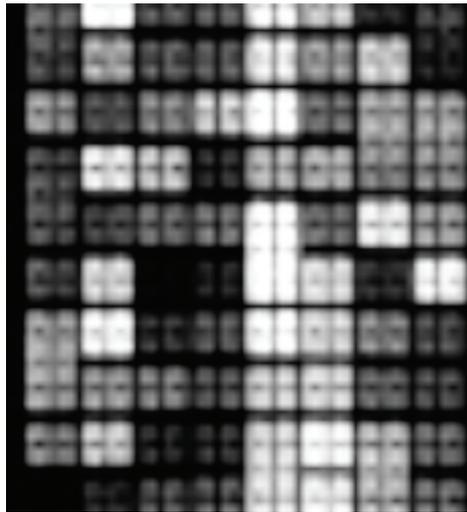
fluorescently labeled strands from the extracted nucleic acids of a sample bind, or hybridize, to matching counterparts immobilized on the slide, the match shows up on a fluorescence scan and indicates that a given taxa was present and active in the original sample.

Chip-SIP then combines these data with a scan of the same microarray by a nanometer-scale secondary-ion mass spectrometer (NanoSIMS), an ultrahigh-resolution imaging ion microprobe. The NanoSIMS beam scans across the surface of the microarray and creates an image that looks like a checkerboard, with each bright square depicting a probe spot. (See the figure on p. 26.) Chip-SIP researchers look for places where the ratio of carbon-13 to carbon-12 is higher than the normal terrestrial value, indicating that a microorganism took up the carbon-13 in its food. A darker square indicates the normal ratio, called natural abundance. The brighter a square is, the higher above normal the ratio is, and the more active the microorganism has been in feeding on the isotopically labeled food. Chip-SIP thus provides quantitative isotopic data not available through traditional methods.

The team validated the Chip-SIP method for the first time with aquatic samples from the San Francisco Bay. The seawater was incubated with carbon-13, enriched with mixed amino acids, fatty acids, ammonium, glucose, and nucleic acids. In a fraction of the time it would have taken with older methods, the researchers tested about 100 combinations of organisms and food sources from a complex microbial community. “Because we could identify organisms by their 16S sequence and determine which ones had consumed the labeled food sources, we could relate their taxonomic relationships to what role they play in the

(left) A microarray fluorescence image shows RNA hybridization to DNA probe spots. Bright spots indicate the highest signal.

(right) A nanometer-scale secondary-ion mass spectrometer montage of carbon-13 enrichment shows the same area of the array providing quantitative isotopic data.



environment,” Mayali says. A series of such experiments yielded a substrate-use diagram, or a food web, providing even more insight.

Traditional SIP is not only a more time-consuming analytical technique than Chip-SIP, it is also not as sensitive, requiring an isotopic enrichment of 20 to 40 percent to produce a strong enough signal for detection. “If we add a huge amount of food, we are actually fertilizing and changing the community,” Pett-Ridge says. Chip-SIP can detect an enrichment of just 1 percent. Another advantage of Chip-SIP is that it can simultaneously analyze both carbon and nitrogen, which are intimately linked in the environment.

### Bacterial Buddy System?

Microorganisms have been historically difficult to study because 99 percent of them are not easily cultivated in a laboratory environment. According to Pett-Ridge, this difficulty is partially because scientists do not completely understand what makes microorganisms tick, and therefore they do not have a way to duplicate their surroundings perfectly. “Millions of different taxa exist, yet we haven’t identified the majority of them,” she says. “We also don’t know what makes some of them thrive in the field, or which ‘buddies’ they need to grow in a laboratory environment.”

The Department of Energy’s Genomic Science Program is funding the development and use of Chip-SIP in two very different environments. By examining the hindgut of beetles that eat

wood, the team will seek to understand which microbial enzymes efficiently degrade cellulose and produce acetate and hydrogen gas, important information for biofuels research. In another study, the team hopes to learn more about the enzymes and energy web of microbial mats, films of layered bacterial communities that develop in intertidal, hypersaline regions and produce copious quantities of hydrogen, a potentially important biofuel. If the mats have figured out a better way to quickly produce hydrogen, the organisms or their enzymes might be used to bioengineer more tractable microbes, such as *Escherichia coli*, to produce large quantities in a semi-industrial manner.

Pett-Ridge says, “We can also foresee Chip-SIP being useful in a number of other settings, such as in the medical field, where it could help identify the organisms that perform particular tasks in the human gut. There’s still a great deal to learn about microbes’ role in health and disease.”

—Kris Fury

**Key Words:** biofuel, bioremediation, Chip-SIP, Livermore Microarray Center (LMAC), microarray, microorganism, nanometer-scale secondary-ion mass spectrometer (NanoSIMS), nucleic acids, stable isotope probing (SIP), taxa, taxonomy.

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