INNER WORKINGS

Mapping the microbiome location helps elucidate its role

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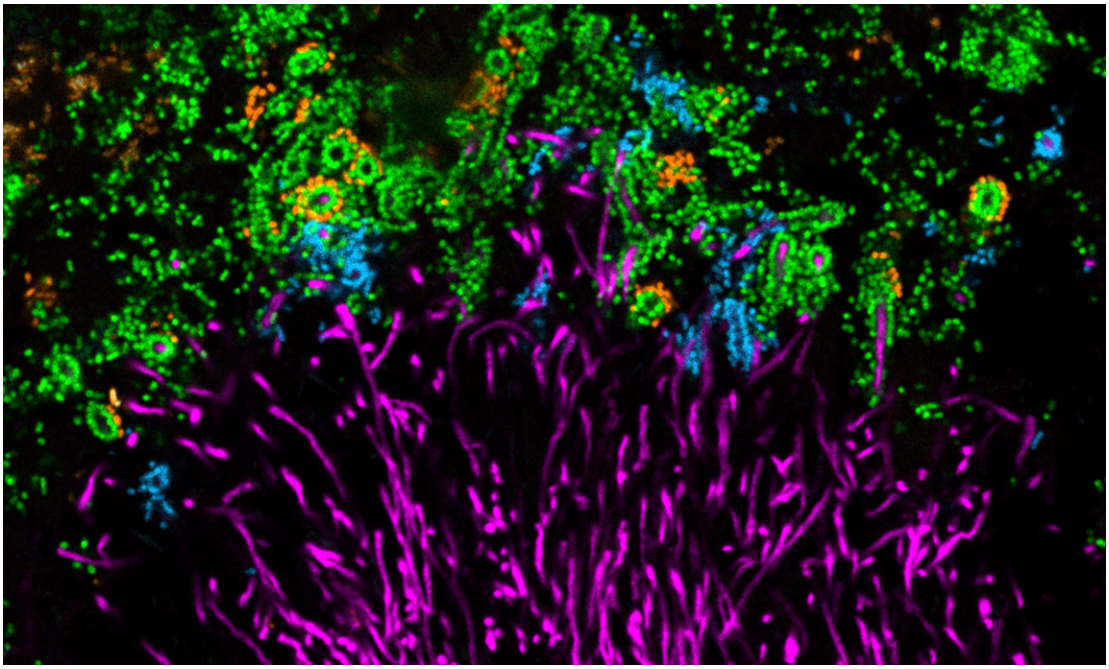
For a microbe, location is everything. Factors such as temperature, as well as access to water and nutrients, determine what kinds of microbes can thrive in a particular place. By sequencing microbial DNA, researchers have discovered vast and previously unrecognized microscopic worlds, identifying the bugs that live in locales ranging from soils and subways to showerheads and sourdoughs. Researchers have cataloged the microbes residing on and in the human body, whether in the microbial desert of the back of the hand, the lush microbial forest of the armpit, or the microbial jungle of the mouth.

But sequencing goes only so far. It may enable researchers to compare the denizens of a "rainforest" with, say, those of a "desert," but it won't reveal where each microbe resides within an ecosystem. Knowing location details can reveal with whom those microbes interact and how. Like any frog or fern, a microbe's physical place elucidates its ecological role.

Over the past few years, thanks to advances in imaging technology, researchers have gotten the first glimpse of where exactly individual microbes live. They are taking snapshots of entire microbiomes—inside the mouth, for example—with detail down to the single-cell level, revealing communities that arrange themselves with remarkable order. "In the oral microbiome, we're finding an astonishing amount of spatial structure," says Jessica Mark-Welch, a biologist at the Marine Biological Laboratory (MBL) in Woods Hole, MA. "These bacterial communities are much more highly organized than we had anticipated."

Precise microbiome mapping is still a new area of research, but such snapshots are already empowering researchers to ask and answer new questions about



Researchers are finding ample spatial structure in the oral microbiome. This dental plaque structure, dubbed "hedgehog" for its appearance, shows several genera of bacteria: *Corynebacterium* (magenta), *Porphyromonas* (cyan), *Streptococcus* (green), and *Haemophilus*/*Aggregatibacter* (orange). Reprinted with permission from ref. 1.

www.pnas.org/cgi/doi/10.1073/pnas.1816174115

how microbes interact and work together, not just to better understand the microbial universe, but potentially to improve human health and the environment.

Architectural Interest

The microbe map crafted by Mark-Welch and colleagues resembles a piece of art. Magenta streaks branch outward, punctuated with dabs of green at the ends. Strokes of cyan and orange mingle in between. Each color represents a different genus of bacteria, together forming the microbial community of dental plaque, a 3D structure that Mark-Welch and Gary Borisy, a biologist with the Forsyth Institute in Cambridge, MA, have dubbed a "hedgehog" for its likeness to the bristled animal (1).

The hedgehog image contains several color-coded genera of bacteria. No one knows all the details of how these microbes interact and why. What's clear is their arrangement is no accident. For example, anaerobic microbes, which live without oxygen, congregate in the center of the microbe community, whereas aerobic genera are near the exterior, where there's more access to oxygen. Bacteria that produce compounds such as lactate are near others that consume the lactate.

The discovery of this surprising pattern, published in 2016, was the success of a technique called CLASI-FISH, which Borisy helped develop over several years while director of the MBL (2). The method is based on a well-established technique called fluorescence in situ hybridization (FISH), which researchers have used in the past to tag a specific bacterium with a fluorescent dye by targeting RNA.

In conventional FISH, researchers use color filters to identify microbes that glow red, blue, or green. But with the "combinatorial labeling and spectral imaging" of CLASI-FISH, Borisy and Mark-Welch instead use a detector to measure the whole spectrum of the fluorescent dye. That gives them the ability to distinguish multiple shades of red, for instance, providing a broader palette to see more types of bacteria at once. Using a powerful microscope, Borisy and Mark-Welch can identify about a dozen genera of bacteria in a single image. "It's amazing how much is in the mouth that has not been seen," Mark-Welch says. "It's really because only now we have the technology to see it."

More recently, the researchers have looked at the human tongue and the interior of the cheek, results which they have yet to publish. The tongue images revealed bacterial species and microbial configurations different from what's in dental plaque. But these species were just as organized—so much so that the microbes looked like a single cohesive entity, as if each bacterial cell were organized the way cells are in a liver or pancreas. This cohesive group of multiple cell types suggests that a microbial community can "act in a concerted manner to contribute to the normal physiology of its host," according to Borisy. "You would think you were looking at an organ of the body," he says.

Last year, the researchers started taking images of sterile mouse guts that had been populated with 15 bacterial species to mimic the human gut (3). Gut microbiota have been linked to a variety of diseases, from inflammatory bowel syndrome to colorectal cancer. Unlike the mouth, however, the gut microbiome doesn't seem to organize itself as much, instead showing more intermingling among bacterial types—perhaps because there are no permanent surfaces like teeth and tongues to cling onto, Mark-Welch says.

But that doesn't mean the gut microbiome is a random bacterial blend. Microbes are still organized according to where along the gut they reside. For example, the communities in the small intestines are more adapted to a metabolically faster and more transient life than those in the large intestines. In the colon, microbes vary radially outward from the center in what's called the lumen toward the mucus-lined inner walls. Some bacteria actually consume the mucus.

Justin Sonnenburg, a biologist at Stanford University, has shown using FISH-based methods that these bacteria and their proximity to the gut's mucosal lining could be important for understanding human health (4). Many of the microbes in the humans lower gut munch on the fiber that your body can't digest. But if you starve those microbes by consuming too little fiber, they'll start eating the mucus. The microbes deplete the mucosal lining, exposing the epithelial layer in the inner wall of the gut, which can trigger inflammation and disease. "The spatial organization between the lumen and the mucosal surface is of prime importance to understanding this process," Sonnenburg says.

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Sonnenburg's team is also focusing on tiny cavities along the inner wall of the gut called crypts, which house stem cells (5). The crypt also shelters a microbial community. "Displacing these microbes may be incredibly difficult once they're embedded there," Sonnenburg says. Whatever microbes colonize the crypt, then, may dictate the entire gut's microbiome. In a study this year involving mouse guts, however, his team showed that it may be possible to replace one key microbial player in the crypt community by introducing a different, more desirable strain of the same bug, while selectively feeding the new strain's preferred foods (they used a type of seaweed) and, thus, potentially reprogram a gut microbiome to treat disease (6).

Microbiomes at the Movies

Although FISH-based methods have provided unprecedented images of the microbiome, they only offer a snapshot in time. Raghuveer Parthasarathy, a biophysicist at the University of Oregon, instead uses a technique called light-sheet microscopy (7), which allows him to watch how a microbiome changes in space and time within a living gut.

Specifically, Parthasarathy looks at the living gut of zebrafish through the animal's transparent body. The

researchers populate a zebrafish with microbes engineered to have fluorescent proteins. They then use lasers to form a sheet of light that excites the proteins, producing a 2D image of the glowing microbes. Or, by scanning through the gut, they can create a 3D image. The method is quick enough that researchers can record how the microbes shift around the gut.

To study the basic principles of how microbes interact with one another and the host, Parthasarathy's team populates a sterile zebrafish gut with only a couple species. For example, in a study published earlier this year, they showed how the cholera virus might infect its host (8). After colonizing an otherwise sterile zebrafish gut with a bacterium called *Aeromonas veronii*, the researchers introduced the cholera virus, discovering that the virus induces the gut to contract, which expels the bacterium and enables the virus to take over.

Researchers have just started to explore how microbes are arranged in space and time and are eager to find out where new techniques such as CLASI-FISH might take them. Even basic systems such as thin bacterial films or the microbiomes in worms may spark new hypotheses and ideas. "There are these systems that are dying to be studied," Parthasarathy says. "We already have the tools to study them, we just need to dive in and do it."

Indeed, these microbiome-investigating tools are applicable to all sorts of environments. "The goal is not to be too singularly focused on the gut, but to use it as a template," says KC Huang, a biophysicist at Stanford University.

Mark-Welch is now using CLASI-FISH to map the microbial structure on marine plastic and kelp. With so much plastic in the oceans, the microbial communities that live on the plastic might affect the marine environment and the animals that swallow the plastic, she says. Microbes also come into play as a possible way to break down the refuse. Understanding how microbes are organized on plastic, then, could be useful.

Kelp, meanwhile, has been proposed as a source of biofuels, and insights into how it interacts with its microbiome could help keep farmed seaweed healthy and make such experimental ventures more viable. Mark-Welch and her collaborators have started taking images of the microbiome on plastic and kelp. Although it's too early to know whether such work will be fruitful, having the bacterial communities literally in sight is an important first step. "We know remarkably little about spatial organization in these multispecies microbial systems," Parthasarathy says. "We barely know what questions we should be asking. It's a great time to be exploring."

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