CHRISTOPHER LEAMAN

The ichip allows researchers to incubate pure cultures of microorganisms in soil.

ADVENTURES IN MICROBIOLOGY

Dogged researchers are designing technologies to find and grow microbes that have never before survived in the lab. **By Amber Dance**

Every researcher who enters Yoichi Kamagata's laboratory in the hope of growing interesting microorganisms undergoes an initiation: they try to culture *Oscillospira guilliermondii*, a bacterium found in the guts of cows and sheep, but never grown under lab conditions. Kamagata, a microbiologist at the National Institute of Advanced Industrial Science and Technology in Tsukuba, Japan, has been fascinated with the rod-shaped microbes — ten or more times the size of the well-known gut denizen *Escherichia coli* — for more than a decade, because they seem to thrive only in animals that feast on fresh grass.

"So far, no one's been successful," laments Masaru Nobu, an engineer and microbiologist in Kamagata's group.

Oscillospira guilliermondii is hardly unique; the vast majority of microbial diversity remains uncultured. This microbial 'dark matter' could hold useful enzymes, new antimicrobials and other therapeutics. Modern metagenomics, which involves sequencing the DNA of all the microbes in a community at once, has revealed the microbial make-up of diverse environments, but it doesn't allow researchers to answer fundamental questions about microbes, such as what do they eat? What metabolites do they produce? And how do they interact with others in their environment? To find the answers, microbiologists must first isolate, then culture, the organisms in the lab.

It can be a tricky business. Some microbes grow very slowly, have finicky requirements or can grow only in the presence of certain other microbes. A few scientists take an untargeted approach, setting up cultures with the idea that anything that grows has a good chance of being interesting; others target specific microbes that they want to understand better. Whatever the approach, cultivating something that no one has grown before requires perseverance, patience and luck.

"It's an illusion to believe that you can work on microorganisms without growing them," says Didier Raoult, director of the Mediterranean University Hospital Institute of Infection in Marseille, France. His adventures began as a relative "youngster", he says, in 1983, when, despite their reputation for being one of the more difficult bacteria to isolate and grow, he decided to study *Rickettsia*. His students possess the same spirit; some have gone so far as to defecate in the laboratory, so that they could quickly place the samples in oxygen-free conditions that support interesting microbes. Their dedication has revealed at least one new species, *Faecalibacterium timonensis*, and allowed the culture of several more, opening up a series of oxygen-sensitive microbes to laboratory scrutiny.

Gone fishing

In his more conventional hunts, using samples from patients or other volunteers, Raoult casts a wide net. His method, called culturomics[1], incorporates robotic liquid handling to create diverse culture conditions, as well as mass spectrometry and ribosomal RNA sequencing to identify what grows. Raoult estimates that it has yielded about 700 new organisms so far, mainly from the human gut.

Indeed, one of his lab's biggest challenges, Raoult says, is keeping up with naming and describing the new species. The team often chooses names that honour other investigators, reflect the disease of the person who gave the stool sample or highlight the institute's location. Recent reports, for instance, include a rod-shaped bacterium (*Gordonibacter massiliensis*) that the group named after Massilia, the ancient name for Marseilles[2]; and *Prevotella marseillensis*, from a person living in Marseilles with a *Clostridium difficile* infection[3].

Researchers such as Raoult attempt to find conditions in the lab that will accommodate new microbes, often by copying natural environments. But Slava Epstein, a microbiologist at Northeastern University in Boston, Massachusetts, goes one step further. "Why do we mimic?" he says. "Let's just cultivate organisms in nature."

Epstein's team has designed multiple devices that allow the researchers to incubate pure cultures in natural soils or sediments. One inexpensive version is the isolation chip, or ichip, which is built from a micropipette tip rack[4]. The researchers fill the holes with a microbial sample diluted in molten agar, in the hope that each chamber will contain one or a few starter microbes. Semi-permeable polycarbonate membranes on either side of the rack allow nutrients and other molecules to come into the chambers from the surrounding environment, but bar other microbes from entering.

Often, the team simply gathers a bucket of soil and keeps it in the lab, sliding in ichips so that the researchers can develop their cultures. They also occasionally leave ichips out in the natural environment, but this can lead to interference from dogs and wildlife. "The things we hate the most are crabs," Epstein says, "because they come sometimes and, with their claws, puncture our membranes."

In 2016, Epstein's then graduate student Brittany Berdy hitched a ride with a military plane to Thule Air Base, on Greenland's northwest coast, to look for microbial communities with unique adaptations to the extreme environment. "We were so far north, you had to drive south to see the Northern Lights," recalls Berdy, now at the Broad Institute of MIT and Harvard in Cambridge, Massachusetts. She waded into the chilly waters of a nearby, unnamed lake to place the ichips, and returned a couple of weeks later to retrieve them.

"It was so hard, and there were many instances where it felt like it was never going to happen."

Back in Boston, Berdy tried to mimic the conditions of the lake with different kinds of media at various dilutions. The trickiest part was matching the lake's 10 °C temperature — too chilly for a water bath, too warm for a cold room. The team finally succeeded using a refrigerator on the warmest setting, with the door slightly ajar.

Buddy system

Researchers such as Berdy, Epstein and Raoult don't know exactly what they're going to get from their cultures. But often, researchers are looking for something specific. For instance, Mircea Podar, a microbiologist at Oak Ridge National Laboratory in Tennessee, is interested in the large and diverse Saccharibacteria (formerly known as TM7), part of the community of microbes that live in the human mouth, yet not cultured in the lab until recently.

In 1996, Saccharibacteria were among the first phyla to be identified by sequencing alone, rather than from a culture, in a sample from a peat bog[5]. Although not particularly abundant in the oral microbiome, their populations rise and fall with certain diseases — including periodontis — suggesting that the bacteria have a role in health. They're also found in the human gut, as well as the mouths of dogs, cats and dolphins, and in soils, sediments and sewage. "They are kind of everywhere," says Podar.

In the early 2010s, Podar devised a plan to isolate Saccharibacteria: use the microbe's genome, which is known from single-cell sequencing, to predict which proteins are found on the surface of the cells, and then generate antibodies to artificial versions of those proteins. The researchers could use fluorescently labelled versions of those antibodies to tag the microorganisms, and isolate them from a saliva sample using flow cytometry.

The first postdoc on the project, James Campbell, used this approach to obtain several cultures containing Saccharibacteria. But it wasn't until years later, after graduate student Karissa Cross took over the project in 2014, that the team found success.

"It was so hard, and there were many instances where it felt like it was never going to happen," recalls Cross, now a postdoc at Vanderbilt University in Nashville, Tennessee. She tried liquid culture, solid culture and chocolate agar, made from lysed red blood cells, among other recipes. "It would take days to make media." Nothing worked.

Then, in 2015, other researchers reported a crucial clue: Saccharibacteria can't live alone[6]. These tiny, spherical bacteria, just 200–300 nanometres across, require a host from the phylum Actinobacteria. By trying to isolate Saccharibacteria, Podar's group had inadvertently omitted a key partner.

Finally, in the summer of 2018, Cross got DNA sequences matching Saccharibacteria from one of her co-cultures — and not just any Saccharibacteria, but probably a new family or order[7]. It was her most significant eureka moment of her graduate studies, she says. She e-mailed Podar, "I think we got it," and seconds later heard his footsteps coming down the hallway. They high-fived.

The right recipe

When it comes to feeding such fussy microbes, details matter. And an all-you-can-eat buffet of amino acids and sugars, such as those found in standard media formulations, isn't necessarily the right approach, says Jörg Overmann, a microbiologist and scientific director of the Leibniz Institute DSMZ–German Culture Collection of Microorganisms and Cell Cultures in Braunschweig. Dropping the concentration of nutrients stunts the growth of fast-growing microbes, giving the slow growers time to replicate.

Physical growth substrates matter, too. Overmann's team sometimes dangles a piece of solid surface — steel or glass, for example — in a liquid culture to provide a substrate for biofilms. "We get entirely new stuff that is entirely different from what you get on an agar plate," he says. In one study using this technique with fresh water and soil samples, the team netted more than a dozen never-before-cultured types of bacterium, including at least five new genera[8].

Kamagata's team uses bioreactors to maintain a flow of nutrients and remove waste. Keeping the overall nutrient concentration low better reflects the target organisms' marine habitat, he says. The researchers and their collaborators hung a polyurethane

sponge (like a kitchen sponge) in a reactor to culture, for the first time, a deep-sea archaeon from the eukaryote-like clade known as Asgard archaea[9].

For hints as to where to start, researchers can check the BacDive database, which lists characteristics and culture conditions for more than 80,000 cultured strains from 34 bacterial and 3 archaeal phyla. Genomic information, when available, can also provide clues, says Christian Jogler, a microbiologist at Friedrich Schiller University Jena in Germany.

But even pedestrian concerns can make a difference, Jogler warns. Rather than relying on ultrapure water-purification systems, such as Milli-Q, that many labs use, Jogler's group makes its own pure water by distilling it, twice. Milli-Q water can contain chemicals that block the growth of some cultures, he says. Plus, Jogler adds, the agar commonly used as a gelling agent might inhibit growth, so he sometimes tries alternatives such as gellan gum.

Even the way that the agar is prepared can be important, Kamagata's group has found. When agar is heat-sterilized together with phosphates, it produces hydrogen peroxide that prevents some microbes from growing. Autoclaving the components separately eliminates the problem, and has allowed the team to grow previously uncultivated microbes[10].

Patience is key. It took Kamagata and his colleagues more than 12 years to grow their archaeon, tentatively christened '*Prometheoarchaeum syntrophicum*'. But once microbiologists obtain the first culture of a new organism, that microbe usually grows faster.

Epstein calls the process domestication. He suggests that during the first, sluggish growth cycle, some microbes alter their epigenome — the molecular markers on DNA that control gene expression — to adapt to lab conditions. Then, they grow faster.

Earth and sky

Now, Epstein is developing technology to isolate and cultivate new microbes entirely *in situ*.

He calls the devices Gullivers, in honour of the adventurer in Jonathan Swift's 1726 book *Gulliver's Travels*. Gullivers are little boxes filled with sterile gel, with a semipermeable-membrane surface, like that of the ichip, to allow nutrients and signals to diffuse in. A single pore, one micrometre across, allows an individual microbe to enter from the environment. That microbe should plug the entryway, but its descendants could populate the gel inside the box, forming a colony.

Eventually, Epstein says, it might be possible to get results from a Gulliver without opening or even retrieving it. Nanosensors could collect and send back data on oxygen or carbon dioxide levels, or the production of signalling compounds or antibiotics, he imagines. After dropping the device into, say, the depths of the Arctic Ocean, researchers could simply go on holiday and wait for results to pour in, he jokes.

In the coming months, Epstein plans to test Gullivers at Mount Erebus, an active Antarctic volcano. But his ultimate goal is beyond Earth, deploying the devices on potentially life-hosting bodies such as Mars or Jupiter's moon, Europa.

Time will tell whether microbes exist in such places. In the meantime, there's plenty of microbial diversity on Earth to keep researchers busy. With the right techniques, says Raoult, it should be possible to domesticate and study any microorganism — eventually.

"Unculturable", he sniffs, "is an insult to the future."

Amber Dance is a freelance science journalist in the Los Angeles area.

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CHLOE COLLYER/BLOOMBERG/GETTY

Alexa-powered tools allow researchers to quickly access laboratory-specific information.

VOICE-ACTIVATED ASSISTANTS COME TO THE LAB

The research-optimized tools enable hands-free note-taking, reminders, instrument control and more. **By Jeffrey M. Perkel**

Funsho Fakuade has spent a lot of time in the dark. As a PhD student studying ion channels and cardiac arrhythmias at Georg August University in Göttingen, Germany, his work typically involves measuring the electrical output and fluorescence intensity of cells under a microscope. But because such measurements must be made in darkness, he needed to document his experiments in the dim glow of a computer monitor. Often, he wrote nothing at all.

"I had to prioritize the information I wrote," Fakuade says. "It's one thing to be looking at the screen to look at [cellular] changes, and another thing to be trying to strain your eyes to write something down."

Then, in early 2019, team members from Berlin-based company LabTwin visited the