Engineers embrace microbiome messiness

The gut, plant roots, ocean sediments: microbiome engineers travel to explore and model microbially complex systems.

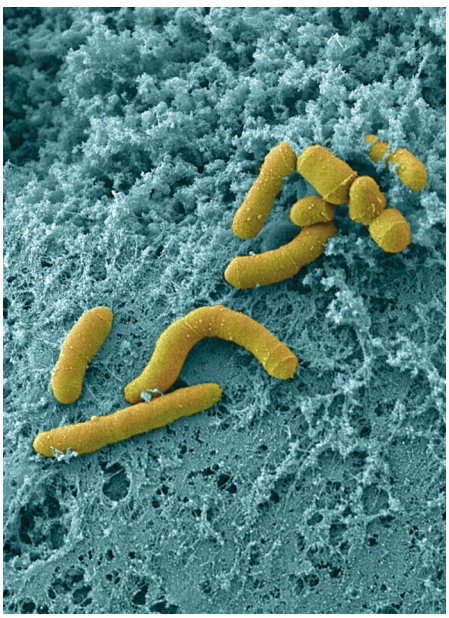
Vivien Marx

Microbiomes are bustling, evolving, complex microbial communities, as spatially and temporally variable as they are heterogeneous[1]. To take on this complexity, says Harris Wang, a researcher at Columbia University's Irving Medical Center, his lab's philosophy is: "Embrace some of the messiness of natural environments," since it will help with engineering technologies resilient to such variability. The discovery of the central role of the microbiome in health and disease has shifted medicine, says Don Ingber, director of the Wyss Institute for Biologically Inspired Engineering at Harvard University, "yet virtually all we know about its functioning is based on genomic and metagenomic correlation."

Most knowledge about microbial gene function comes from studying microbes in isolation, but microbes are rarely loners in nature, says Trent Northen, a researcher at the Lawrence Berkeley National Laboratory who directs several programs at the US Department of Energy's Joint Genome Institute. Perhaps this focus has contributed to the lack of functional understanding about so many microbial genes, which makes it urgent, he says, to study engineered microbes in community contexts to see genes, metabolites and pathways in more ecologically relevant ways. Microbiome engineering can help labs test and refine their understanding of microbiomes. Engineered microbiomes could help address conditions such as irritable bowel disease, provide environmental bioremediation or offer a better view of microbial roles in ecosystems. Labs are not yet microbiome design studios, but advancing the way they model and probe microbial communities moves them toward that goal.

From soil to gut, then back

Her lab is "ecosystem-agnostic," says Kelly Wrighton at Colorado State University. The team studies microbial ecosystems—in soil, fractured shale, the gut. "My lab tries to track metabolisms across ecosystems," she says, especially anaerobic metabolism, to explore constraints, processes and changes due to physical space or microbial interactions. These days, labs can get a good genome-resolved and strain-resolved understanding of the metabolic potential in a microbial community, says Wrighton. Next, they explore



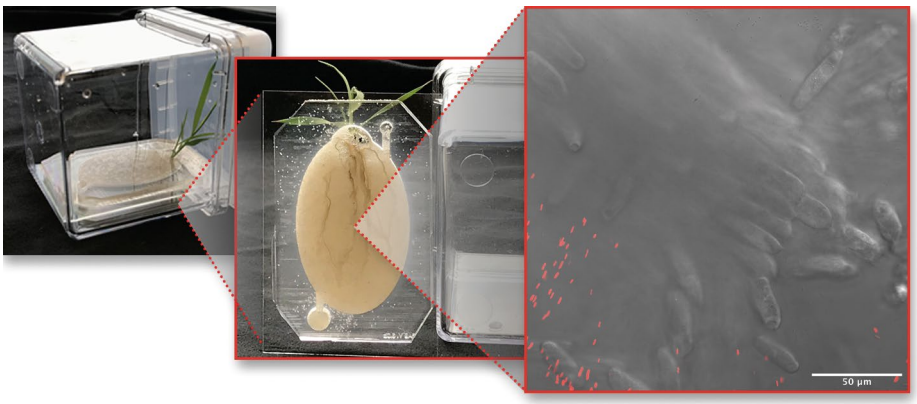
Bacteroides fragilis is a resident microbe in the human gut. Credit: Wyss Institute at Harvard University

the functional contribution of microbes across ecosystems to find conserved traits and functions "beyond just a gut but across ecosystems that are on this planet, more holistically." Using synthetic communities, her group disentangles an ecosystem's microbial complexity, tracks and monitors it, and gets time-resolved data, she says. "Ideally, we can take that information and go back to the field." For example, she studies the fractured shale wells 2,500 meters below the Earth's surface. To release natural gas, water and chemicals are pumped into the ground under high pressure. Before fracturing, rocks hold little life and water; they are "paleopasteurized," she says. Fracturing introduces microbes that can form biofilms, and there can be corrosive 'souring' in these wells, too. She explores these effects to understand microbiome metabolism also with a view to potential remedies. The lab's models and metabolic profiling indicate that a metabolic network sustains the microbiome at these depths[2]. There's cofermenting of amino acids. For example, glycine betaine, an amino acid derivative, is abundant in the fractured shale wells. Abundances of a few bacterial taxa—*Halanaerobium*, *Geotoga* and *Methanohalophilus*—help to predict the availability of carbon and nitrogen in these shales. The scientists rebuilt this shale microbiome 'microcosm' in the lab and could account for around 75% of the carbon and nitrogen cycled in the natural system. "That, to me, was the first time that I appreciated these linkages between field and lab," says Wrighton. The researchers have also been profiling methylamine cycling in shale, in soils and in the human gut. What the team has learned in shale has taught them plenty about the gut to the gut. "It's not just understanding the metabolic network in one system." Her team looks at enzymes, organisms, microbial interaction and competition across different kinds of ecosystems.

Sometimes the lab–field link fails. The team modeled soil-based microbial methane production in the lab with "beautiful" results, she says. They found the methane-production "heavy lifters" and looked for these genome-resolved strains in the field. "They weren't even there; they weren't active," she says. Lab models help to disentangle, to study cause and effect and scope out what might be manipulated how. "The field is a different beast; it gives us real contextual information," she says. Wrighton's projects involve much collaboration. Her team handles the microbiology, genomics and computational tasks. Colleagues, including the Northen lab, generate mass spectrometry, nuclear magnetic resonance spectroscopy or analytical chemical data. She uses the services of the Department of Energy's Environmental Molecular Sciences Laboratory, a facility at the Pacific Northwest National Laboratory.

Contained microbiome

Northen and his team build EcoFABs, which are see-through contained models of microbial ecosystems. One type is a seedling grown hydroponically in a 3D-printed mold into which microbes can be added. They apply methods such as mass spectrometry to capture metabolic flux and exchange within the root microbiome. The system begins as a sterile environment to assure the group is studying the intended microbiome, says Northen. Then they can make predictions about metabolite exchange and keep designing and testing synthetic microbiomes. "It's early days," he says, but EcoFABs are part of an iterative



In EcoFABs, plants are grown with a tailored root microbiome: here, *Brachypodium distachyon* with fluorescent *Pseudomonas simiae* growing next to the plant's root hairs. Credit: T. Northen, D. Chiniquy, L. Jabusch, Berkeley lab/JGI; E. Dewalt/Springer Nature

cycle of design–build–test–learn. Large-scale exometabolite profiling of microbes will give the team data for developing generalizable models. The team plans to image EcoFABs at single-cell resolution, use multicolor fluorescence imaging, build reporter constructs, and apply fluorescent environmentally sensitive probes for spatially defined analysis and to characterize microbes within their microenvironments. Among the open questions such systems can help address, says Northen, are: "Do plants modulate root exudates to select for beneficial microbes, and does this change with environmental conditions? If so, what services are the selected microbes providing for the plants? How interdependent are rhizosphere microbes?" When designing synthetic microbiomes, Northen advises carefully selecting constituent microbes. If researchers choose by phylogeny, they need to design how closely related the microbes should be. They might also choose on the basis of functional assessment of microbes. "I just don't think we know how to think about soil microbial communities yet," he says. The communities are so diverse, yet labs are limited in how they can study microbial activities and interactions, especially at scale. To amplify collective efforts, Northen hopes the research community can converge on a few complementary laboratory ecosystems so it becomes easier to compare systems across labs[3]. This is akin to agreeing on model organisms, "but we want this process to happen much faster," he says. Such an effort could be organized through the EcoFAB Steering Committee.

Hunter-gatherers

"We go into weird habitats in the world where there's cool chemistry happening and microbes are responsible," says Michelle O'Malley of the University of California, Santa Barbara. She primarily studies anaerobes, including gut microbiota, microbial communities in a landfill or microbial communities in oxygen-poor ocean sediments. One such 'weird' habitat is the rumen. Goats, for example, eat more or less anything, thanks among other things to anaerobic digesters. Lessons from such microbiomes guide how the lab builds minimal systems that can thrive on such food source diversity. They characterize the microbiome and microbial interactions,

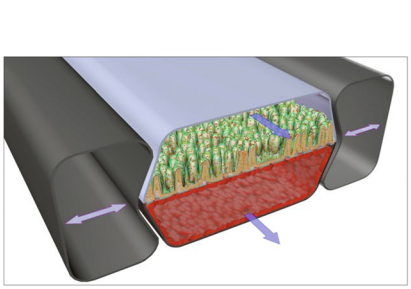
"We go into weird habitats in the world where there's cool chemistry happening and microbes are responsible," says Michelle O'Malley. Credit: UCSB

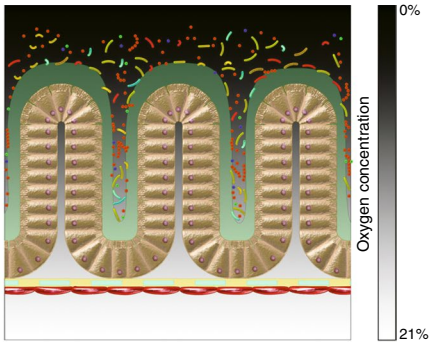
which starts with genomic reconstructions of all community members. Beyond genome assembly, the researchers assess abundances and metabolic activity. The reconstructed genomes indicate which microbes eat sugar, degrade fiber, make short-chain fatty acids or produce methane. There's a gap, says O'Malley, when it comes to follow-on questions: testing how microbes interact, how carbon is cycled and which type of information is best for a model. "In my opinion, most of what people do with the gut microbiome now is just guesswork," she says. It's tempting to see microbes as specialists rather than communities with built-in redundancy. "There's some rhyme and reason to that, even though I as an engineer struggle with that," she says. "But that's not what nature does," she says. Functional redundancy helps microbiomes recover from perturbation.

Her lab applies genome-editing tools to control different members of a microbial community. But CRISPR–Cas cannot be deployed equally well in all microbes, she finds. The team also develops ways to engineer the microbiome environment to "sculpt microbiomes," such as by limiting nutrients or promoting growth of only certain members. O'Malley's toolbox ranges from 1950s-style microbial enrichment studies in which media are used to favor growth of some microbes over others to new tools such as RNA-sequencing, genomic reconstruction, de novo assemblies, metabolic analysis and modeling. Many microbiome labs focus solely on bacteria, but not hers. She also minds the danger of bias: DNA extraction can bias captured genetic signatures because some approaches work better in bacteria; others favor fungi or archaea. "We will actually do a lot of optimization of just sample collection and prep and stability of those samples, so that we can see who's there and get a real picture," she says. It's a view that helped the team discover that, thanks to anaerobic fungi, herbivores have the largest collection of biomass-degrading enzymes of any sequenced organism[4]. "That came as a surprise to us," she says. Fungi in the rumen are often just considered pathogens, or overlooked as a result of sampling bias. Researchers usually sample the herbivore rumen liquid, which teems with bacteria. The liquid has a low abundance of fungi, which are mainly associated with food particulates and are typically discarded in sample prep, she says. Ongoing work in fiber-eating primates, including chimps and gorillas, shows that they have ample fungi in their digestive tracts, which sheds light on the human gut.

Gut-on-a-chip

Ingber and his team built and validated an intestine-on-a-chip device equipped with intestinal epithelial cells, fluid flow around the cells and an ability to mimic the gut's peristaltic movement on a chip. The researchers recently expanded[5] the model so labs can study the living complex human gut microbiome in direct contact with living human intestinal cells and its overlying mucus, says Ingber. In the human gut, oxygenated blood flows through capillaries and there is a gradient of decreasing oxygen as one crosses the tissue–tissue interface with the intestinal epithelium and moves to the lumen. The device models this with oxygenated medium in one channel and no oxygen in the other. The gut-on-a-chip can maintain a complex microbiome with over 200 different types of bacteria, anaerobes





In this gut-on-a-chip, the human gut microbiome is in direct contact with intestinal cells. There is an oxygen gradient and the device can maintain a microbiome with over 200 different types of bacteria, anaerobes and aerobes. Credit: Wyss Institute at Harvard University

and aerobes, that correspond to the human gut microbiome. In other work, Ingber and his team mimic differences in sensitivity to infection by enterohemorrhagic *Escherichia coli* in people and mice: it's mediated in large part by metabolites produced by the gut microbiome. Organoids are useful for studying cell differentiation and basic cell biology questions. They tend to be tissues, says Ingber, whereas his models are more like organs-on-chips, with access to the intestinal and capillary lumen. Organoids are closed spheres. "For this reason, you have no control of conditions at its center, and the bugs just overgrow and kill the human cells in about a day," he says. Years ago, he set out to build an intestine chip for exploring human host–microbiome interactions under controlled conditions. It's experimentally challenging that the normal human gut microbiome includes many obligate anaerobes as well as aerobes: aerobes don't like very low oxygen and vice versa, and very low oxygen kills human cells. "Therein lies the rub …" he says.

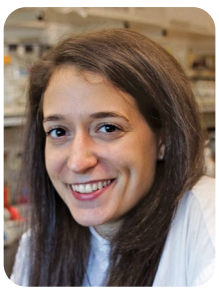
Commenting on the system, Columbia's Wang says it's an elegant way to study a microbiome. "But you are still missing some components, such as the immune system," he says. And Northen says, "I've found broad enthusiasm for these types of devices, but the proof will be in the pudding—broad acceptance will follow the impactful science." To advance such work will take a broad cross-section of expertise, such as materials scientists and people focused on microfluidics for ecosystem fabrication. One general issue with synthetic communities, says Wang, is that they do not always stay stable in complex environments. "These defined systems are really important in understanding what are the governing rules behind microbial interactions and what are the key drivers," he says. Labs can use them to focus on a small number of microbial interactions; they are experimentally and quantitatively tractable. For example, the systems help with tracking metabolites in a defined setting. "How you translate those types of knowledge bases to a really messy environment is an open challenge," he says, just as it's hard to extrapolate a well-studied predator–prey relationship to a jungle ecosystem.

Re-engineered *E. coli*

Ingber is intrigued by newly synthesized bacterial *E. coli* strains, and says, "our model could be used to advance that work." These are variants of *E. coli* called Syn61 with DNA entirely synthesized in the lab of Jason Chin at the UK Medical Research Council Laboratory for Molecular Biology[6]. The team redid the organism's four-million-base-pair genome by applying their method called REXER (replicon excision for enhanced genome engineering through programmed recombination). DNA was chemically synthesized, assembled into blocks and ferried into the bacterium. The team reduced the number of *E. coli*'s genetic codons for amino acids from 64 to 61. Two serine codons and one stop codon were swapped out at 18,000 genomic locations. It's an approach that can help synthetic biologists build organisms and is not readily accomplished with gene editing. As researchers Benjamin Blunt and Tom Ellis note in the News and Views piece accompanying Chin's paper, this synthesis and reduction of the *E. coli*'s genetic code represents "new records," with similar projects under way. "Genome minimization and codon reduction are just the first uses of this new technology, which could one day give us functionally reorganized genomes and genomes that are custom designed to direct cells to perform specialized tasks," they note. As Chin explains, beyond this way of engineering single genomes, labs could use the approach to synthetically evolve designer microbiomes. "It might be possible to assemble microbiomes composed of organisms that use different compressed genetic codes as a route to limit horizontal gene transfer between organisms in the microbiome," he says.

Thinking of evolution

For her work on microbiomes, MIT researcher Tami Lieberman applies evolutionary inference. She assesses the individualized natural history of bacteria by projecting accumulated microbial mutations into the past. Acquired adaptive mutations in genes and pathways give "an idea of what the most pressing challenges are to bacterial survival," she says, and guide how to possibly support or diminish these challenges. Given that low-frequency mutations are hard to capture with short-read sequencing and assembly, the lab cultures specific strains of aerobic and anaerobic bacteria separately. The cultures avoid microbial competition but reflect in vivo diversity. The team sequences the genomes of the many individual colonies in high throughput. The bacteria might be from a tumor, from stool samples or from a skin swab. The Lieberman lab is, for example, looking at the evolutionary course of *Staphylococcus aureus* in people with eczema. The bacterium

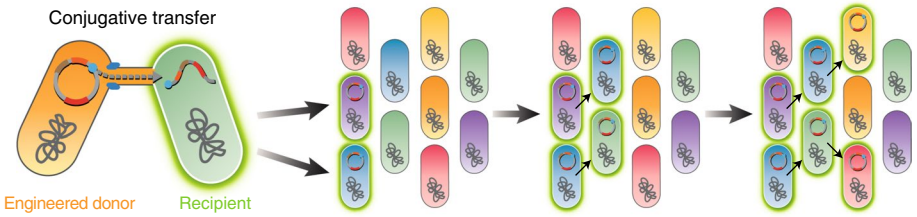


Acquired adaptive mutations in genes and pathways give "an idea of what the most pressing challenges are to bacterial survival," says Tami Lieberman.

Credit: B. Versoy for Harvard Medical School

may not be driving the condition, but lowering staph levels might help patients. Microbial populations in individuals tend to remain stable except when there's a big perturbation, such as use of antibiotics, she says. Despite plentiful exposure to external strains, the resident ones seem to beat them out. But other courses are possible. The lab tracked the lineage of *Bacteroides fragilis* in the guts of healthy people for 18 months. "What we see is that there is actually what looks like a functional diversification into two coexisting strains in that species," she says.

Commenting more generally, O'Malley says that microbiome engineers should include evolutionary considerations in their work. In microbial communities, members that were quite similar can suddenly diverge. She and her team work on the triggers of such events, which might involve horizontal gene transfer that can lend microbes a competitive, adaptive edge. "If you could figure out ways to harness that for engineering, it would be very cool," she says. Work along these lines takes place in the Wang lab.



Mobile elements help to ferry vectors from an engineered donor strain into recipients in a method called MAGIC, developed in the Wang lab at Columbia University. Credit: H. Wang, C. Ronda, Columbia Univ.; E. Dewalt/Springer Nature

Mobilome on the move

Labs can profile the microbiome in sophisticated ways but lack tools to manipulate the microbiome, says Wang. He and his team build generalizable approaches across different microbiomes and hope to make them "generalizable population-scale engineering technologies." One platform leverages mobile elements: metagenomics alteration of gut microbiome in situ conjugation (MAGIC)[7]. An *E. coli* donor strain is engineered with a designed genetic payload that is delivered via mobile elements. MAGIC is the start of a toolbox for the messy in vivo environment, he says. "We use the gut, but it could be skin, it could be any type of microbiome," says Carlotta Ronda, a postdoctoral fellow in Wang's lab; she was interviewed jointly with him. MAGIC can be used to deconvolve a system's components and gain an understanding of the complex system. The team taps into the bacterial habit of sharing materials with one another, such as exchanging plasmids or genetic elements by conjugation. It's fairly well understood, says Wang, but not its scope. Genomic analysis reveals where genes came from, which makes the analysis a kind of "genomic archaeology." Shared elements are retained only when they convey advantage, says Wang. For example, gene transfer from ocean bacteria to people's microbiota explains why some people in Japan can digest otherwise indigestible polysaccharides in seaweed. Gene transfer can be infrequent and still matter, especially when it involves antimicrobial resistance genes. By tracking networks of transfer events, the team hopes to find ways to functionally activate desirable elements in "friendly recipients," he says.

The lab continues to optimize MAGIC, says Ronda. The vectors need to lead to stable, reprogrammable function. When trying this method, labs will want to select plasmids carefully, considering how methylation and regulatory elements might affect horizontal gene transfer so they can tune constructs for their specific purpose, she says. "To some degree these mobile genetic elements are selfish elements," says Wang. They seek to propagate in as many organisms as possible. "If you can hijack that for synthetic applications, that's a way to implant new capabilities without relying on just one vehicle." And it's more likely the elements will be retained across the entire population. Wang and his lab have built synthetic ecosystems in which essential metabolites—amino acids—are 'traded'. One can quantitatively model the energy costs associated with trading, he says, by studying integration with bio-economic models and so-called microbial trade theory. But it's challenging to scale this to large, complex communities with spatial heterogeneity, temporal variability and environmental fluctuation. Engineering microbiomes means designing systems that are both robust and stable, he says. When they're not, he and his team head back to the drawing board. Labs are only just tapping into nature's metabolic capacity, says Ronda, which might lead to new approaches to fermentation and sustainable production processes.

Separately, the Wang lab has been characterizing the rate of horizontal gene transfer in natural settings[8]. They co-opted the CRISPR–Cas spacer acquisition process, which acts as a DNA recorder, says Wang. When a 'recording' strain is exposed to a microbial sample, fragments of the invading elements are captured and integrated as 'spacers' into a CRISPR array. These spacers are protective: when transcribed, they are part of the bacterial immune system. The spacers can be sequenced to identify the transferred elements. This allows one to engineer a situation, says Wang, in which a strain with this recording capability is placed in a complex environment where it will record in its array the DNA that bombards it. This record of which microbes are actively sharing in this complex environment can help the lab, for example, find new types of vectors. The recording strain might be a dedicated gene transfer system capable of keeping track of where genes go in different organisms.

Comparing, communicating, collaborating

It can be a conundrum: complex microbial systems are hard to model but, without a model, understanding is hard to achieve. In Northen's view, to take "our best guess at a community" involves iterating through the cycle of building, testing, identifying deficiencies, thinking of improvements, building a new community and then repeating the cycle. "The more groups that are studying the same systems and the greater the diversity of expertise, the faster our understanding will advance," he says. Model-builders need to be clear on their goals and on what their systems can and cannot do. "We are building 'model' ecosystems, not natural ecosystems," says Northen. The models have to provide control and reproducibility so scientists can efficiently determine causal mechanisms from which they can derive conceptual and computational models. Then the model's validity and generalizability has to be established in natural ecosystems.

The 'natural' system can be a benchmark when comparing engineered microbiomes between labs. How to best kick a model's tires? "Easy, whoever better mimics human physiology and clinical results wins," says Ingber. There is much to learn from nature, says O'Malley. "Engineers have this tendency to oversimplify any system, and that might be working against us for microbiome engineering," she says. "We need to be thinking a lot more complex than we do." To take on microbiome complexity, microbiome researchers embrace collaboration. "Labs studying different microbiomes use different methods, says Ronda, but there is "common ground." Microbiome engineering approaches need to be generalizable so they can be applied across microbiomes whether they are in soil, the plant root or gut. "There is a lot of cross-communication between different fields" she says. In her collaborations, Wrighton finds shared patterns, shared questions, shared resources. "The field," she says, "is really open to new perspectives right now." ❐

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