The chemistry of microbiome–host togetherness

Labs are stacking techniques to disentangle the chemical influences of microbiomes.

Vivien Marx

Centenarians, who have 100 birthday candles to blow out, and super-centenarians, who have 110 tiny flames between them and a bite of cake, have a lowered risk of aging-related conditions such as chronic inflammation. Why this might be has long intrigued Kenya Honda from the Department of Microbiology and Immunology at the Keio University School of Medicine in Tokyo, Japan, who also is affiliated with the RIKEN Center for Integrative Medical Sciences and the JSR-Keio Medical and Chemical Innovation Center.

An important part of this type of research is digging into the chemistry of the human gut, which to a great extent is owned and operated by microbes. The chemistry of microbes shapes plant well-being, too. A renaissance is taking place at the interface between microbiology and chemistry, says Vanessa Sperandio from the University of Texas Southwestern Medical Center's Department of Microbiology and Biochemistry. "The methodological advances are really pushing the field forward," she says. As of June 2022, Sperandio will chair the Department of Medical Microbiology and Immunology at the University of Wisconsin-Madison School of Medicine and Public Health.

In pandemic times, the word 'microbes' can readily bring danger to mind. Being a 'host' to a community of microbes — a microbiome — might not sound safe. Many microbes are indeed harmful. In work in the Sperandio lab on signaling in the human gut, the scientists found that the concentration of a small molecule produced by the microbiota, indole, influences gene expression of pathogens such as enterohemorrhagic *Escherichia coli*. The pathogens can sense indole gradients and discover niches to colonize in the gut. Findings in multiple labs point to this spatial aspect, she says. "These molecules have different distributions, and pathogens navigate the sea of chemistry."

With beneficial microbes, too, the sea of chemistry matters. Establishing causality with regard to the effects of microbes on their host, be it danger or benefit, a cause of disease or an enabler of health, is a journey. On this journey, note Harvard Medical School researcher Sloan Devlin and colleagues in the Department of Biological Chemistry and Molecular Pharmacology of Harvard Medical School's Blavatnik Institute[1], researchers apply strategies to establish "chains of evidence" that show the contributions of microbiomes to disease in their hosts. Steps include establishing associations between microorganisms and disease symptoms; teasing out the molecular mechanisms, determining which facets the microbial biochemical repertoire is having an effect on; and identifying the molecules microbes produce that shape how a host responds and ways to ameliorate the situation in a targeted way, perhaps by manipulating the microbes and what they produce.

The same is true for teasing out fundamentals about the positives of the microbe–host relationship, both in the gut and in plants. A greater understanding of the microbe–host interaction in plants will shape a better understanding of basic plant biology and could enable agriculture with lower use of fertilizers and pesticides, says Yang Bai, a researcher at the Institute of Genetics and Developmental Biology of the Chinese Academy of Sciences (CAS) in Beijing. Bai is part of a virtual center focused on plant–microbe interactions that is run by CAS and the John Innes Centre in Norwich, UK, and focuses on plant science, genetics and microbiology.

Perhaps, says Devlin, the Honda team and others can show that some microbial products in hosts promote a long healthspan. To start to understand what might be helping centenarians[2], Honda and colleagues analyzed stool samples from 319 participants of different ages, among them centenarians and super-centenarians. They isolated bacterial strains and cultivated them in anaerobic conditions; performed liquid chromatography, mass spectrometry, metagenomic and whole bacterial genome sequencing and analysis; and synthesized chemicals and studied their effect in mice. Reflecting on the many techniques, Honda says that in this area, multidisciplinary approaches are a must, given how complex and dynamic microbiota are. "I would recommend others to not hesitate to challenge new methods," he says.

What the Honda team and colleagues elsewhere in Japan and in the United States learned is that that the centenarian gut and especially one super-centenarian woman's gut, which they characterized in great detail, differ in specific ways from the guts of those under 100. A chemical good news substance produced more plentifully in the guts of centenarians and super-centenarians than in younger study participants is isoallolithocholic acid (isoallo-LCA), one of the gut's many small molecules. The result has not led him to change his diet, says Honda; after all, the team hasn't assessed which foods promote or activate the microbiome of interest.

Centenarians and super-centenarians harbor in their guts a chemical of microbial origin that is unlike what is found in the guts of people under age 100. Kenya Honda and his team at Keio University School of Medicine are studying a possible connection to longevity. Credit: davidf / Getty Images

Isoallo-LCA is one of a number of secondary bile acids that emerge during the microbially enabled chemical conversions of digestion. Secondary bile acids are known to affect metabolism and the immune system and can prevent pathogens from multiplying in the gut. The team found that in centenarians, isoallo-LCA is made in a biosynthetic pathway mediated by the enzymes 5α-reductase and 3β-hydroxysteroid dehydrogenase. Further experiments showed that isoallo-LCA hinders *Clostridium difficile* from establishing itself and causing diarrhea and inflammation. Isoallo-LCA also hinders *Enterococcus faecium*, which can cause many types of infections, often life-threatening ones that are frequently antibiotic resistant. Isoallo-LCA is bacterial in origin, made by the family of Gram-negative bacteria Odoribacteraceae. The team used mice

genetically modified to mimic human liver metabolism, says Honda. In one experiment, they exposed the mice to *C. difficile* and treated some animals with Odoribacteraceae. The treated mice warded off effects of the *C. difficile* infection whereas untreated mice could not, he says. Honda and his team are now studying how isoallo-LCA might affect longevity and using mouse models to explore whether there is causality, he says.

Digging into detergents

Stanford University researcher Michael Fischbach was involved in this centenarian gut analysis. "I think a lot about the logic of bile acid metabolism by bacteria, so that was the main topic around which we were strategizing together," he says. Much about bile acids intrigues Fischbach[3] and other scientists. Humans consume and produce cholesterol. The liver converts some cholesterol to a pool of bile acids, of which there are many chemical variations that are unequally abundant. Some play major roles, others minor, but they all share a cholesterol-like chemical structure and detergent properties.

Right after a meal, bile acids stored in the bile duct are squirted into the intestine to help solubilize dietary fat. Were there an intestinal camera, he says, "you would see a bubbly, foamy mess in which a detergent is solubilizing fat." Were there "molecular eyes," one could watch as the bile acids complex the fats and tug them farther into the body. In the lab, tearing fat out of food takes organic solvents. But the gut is a "long chemical reactor" that uses bile acids to do the job and absorb what the body needs. The bile acids are squirted into the gut and disassemble fat, circulating between the intestine and liver a dozen times a day. Around 5% of bile acids are excreted in human feces.

Many techniques needed to parse the chemical roles of gut microbiomes are used in the Devlin lab at Harvard Medical School. Here, Lina Yao (front) and Megan McCurry work at the hood. Credit: J. Knight

Complex research questions emerge, given the involvement of bile acids in metabolism, immunity and other tasks. "Now it becomes a puzzle which bile acid, which receptor, what is the biological activity?" says Fischbach. It intrigues him that bile acids are a difference between centenarians and younger people. Honda's findings deliver "an interesting wrinkle" about these molecules, and he looks forward to studies that reveal how the molecules work.

Bile acids were "long relegated to the role of undistinguished detergents," as Devlin and her colleagues point out[4]. But they are increasingly being seen as the material basis of molecular messages from the microbiome — and ones that affect host physiology. What's tough about investigating this interaction is a lack of tools. They are needed, says Devlin, to regulate messages or control their complex biosynthesis. Others can probe messages, for instance, not by changing the molecules or the amount of them, but by exploring function. Tools are needed to interrogate messages by controlling or blocking them. And others might be used to turn the biosynthesis that leads to such messages on or off, says Devlin.

There is the step of processing cholesterol in the liver into primary bile acids, then further conversion to secondary bile acids. Bile salt hydrolase enzymes from gut microbes deconjugate amino acids from primary bile acids, which must take place before other bacterial enzymes — from the bile acid–inducible operon in specific species of *Clostridia* — can act on the deconjugated primary bile acids to convert them into secondary bile acids, says Devlin.

The Devlin lab team looks for chains of evidence to show the contributions of microbiomes to their hosts. From left to right, Wei Li, Ari Adhikari, Megan McCurry and Lina Yao. Credit: J. Knight

Human feces contains an estimated 50 such secondary bile acids. Some are prevalent in only the millimolar range, which means more may have escaped detection.

The Devlin lab team along with Harvard immunology colleague Jun Huh and his team have found a microbial biosynthetic pathway in the gut that produces isoallo-LCA. It's an intersecting pathway to the one found by the Honda lab, says Devlin. The two pathways start from different bile acids, but the last several enzymatic steps to isoallo-LCA are the same, she says. Together with Curtis Huttenhower and his team at the Harvard Chan School of Public Health, the Devlin lab found that isoallo-LCA levels are lower in people with inflammatory bowel diseases such as Crohn's and ulcerative colitis than in people without these conditions. Bacterial genes responsible for isoallo-LCA's biosynthesis are also less highly expressed. The Devlin and Huh labs have identified gut bacteria and enzymes that produce isoallo-LCA and 3-oxo-lithocholic acid (3-oxo-LCA) and found that both of these small molecules dampen inflammatory flames and help to keep the gut's immune system balanced as a host–microbiota network.

For this work, the team cultivated bacteria from stool, used ultra-performance liquid chromatography and mass spectrometry to identify producers of 3-oxo-LCA and isoallo-LCA, and then sequenced 16S ribosomal DNA to determine the identity of the microbial producers of these small molecules. For example, a number of families within Actinobacteria and Firmicutes produce 3-oxo-LCA. The scientists found that the small molecule inhibits differentiation of inflammatory T helper cells expressing interleukin-17a (T_H17 cells)[5]. Isoallo-LCA induces anti-inflammatory regulatory T cells and 3-oxo-LCA inhibits T_H17 differentiation. More recently, they identified the gut bacteria and enzymes involved in converting lithocholic acid into 3-oxo-LCA and another metabolite, iso-LCA, that inhibits T_H17 differentiation[6]. The scientists suggest that bacterially produced T_H17 cell-inhibitory bile acids could help to reduce the risk of disorders such as inflammatory bowel disease.

Down the road, such insight can offer clues to disorders that currently lack good treatments, such as bowel disorders, metabolic diseases and liver cancers. Outside of her work at Harvard, Devlin consults for Takeda Pharmaceutical and Axial Therapeutics. Taken together with Honda's findings, she says, the work suggests that "isoallo-LCA is a 'beneficial' — anti-inflammatory, anti-infectious — molecule produced by human gut bacteria." The Devlin lab has developed inhibitors[4] that the team hopes can serve as a chemical tool to investigate the interaction between primary and secondary bile acids and the human gut. The compounds inhibit gut bacterial bile salt hydrolase enzymes, which convert primary to secondary bile acids. In their library of inhibitors, the basic construct is a bile acid core motif and an electrophilic 'warhead,' — for example, ones used in cancer drugs, such as kinase inhibitors. One version of this inhibitor is 'clickable' to also enable experiments for studying this interaction with regard to how the molecule engages with its receptor and to detect any off-target interactions across the bacterial and mammalian proteome. Studying host–microbiome interactions takes studying the microbes as well as studying the host in targeted ways to disentangle the complex cross-species relationships.

Germ-free life

In 1885, Louis Pasteur mused about a possible experiment in which an animal is raised from birth in a germ-free environment[7]. Life under these circumstances, he noted, "would become impossible." To study the process of digestion, he continued, one could systematically add specific microbes to food. Actually and surprisingly, says Fischbach, animals reared completely germ-free stay alive. "They're not healthy, but they appear surprisingly normal in light of being completely microbe free," he says.

A major project in the Fischbach lab is to build a mouse model system of a complex gut microbiome that is close to the "native scale" and, at the same time, has a defined microbial composition. For this challenging project, physician-scientist Alice Cheng in his lab has been performing fearless wizardry, he says. To make a microbial community within range of that in a typical human gut, more than 100 bacterial strains must be grown separately and then mixed together. What this enables is a way to do experiments that leave out a strain or add a genetically modified one. Both scientifically and psychologically, it's challenging and not yet perfected, he says, but it's "on its way" to being a system that can enable the type of mechanistic experiments scientists in other fields take for granted.

Microbiome chemistry one-two

Integrating metagenomics with metabolomics, and thus essentially chemistry, "can be quite powerful," says Vanessa Sperandio. A lab will usually start with the most prevalent metabolites, and metagenomics informs on which members of the microbiota have the genetic material to synthesize, use or break down such molecules.

The techniques labs might use can involve metagenomics and analysis, mass spectrometry, analytical chemistry, and in vitro and in vivo microbiology. Depending on the scientific question, an early step might be culturing bacteria and looking for small molecule production, says Sloan Devlin. Or a scientist will have mined metagenomic data and found that certain bacteria are differentially abundant in health versus disease, "so you culture those particular bacteria and look for the molecules they produce," she says. Or a lab might take a more unbiased culturing approach, assess a variety of human-associated bacteria, find molecules the microbes make by applying mass spectrometry, and scan the bacterial genomes to hunt for genes that might encode enzymes involved in producing that produce the molecules.

When in vitro work confirms the hunches and researchers find the genes that matter for producing the molecule, "you can then zoom out," says Devlin, and look at metagenomic, metabolomic and metatranscriptomic datasets such as those from people with a certain condition and compare them with data from people who do not have this condition to check for difference in microbial abundances.

What can emerge is a correlative relationship between the bacteria, the molecules they make, the genes that produce the molecules, and human health, says Devlin. Given that testing causality in people isn't possible, researchers turn to a mammalian system, usually rodents — in particular, germ-free mice, which are colonized with bacteria that produce the molecule or molecules of interest. This is a way to see whether, when bacteria in the mouse produce the molecule, it leads to it a phenotype similar to the one of interest in people, such as increased or decreased inflammation.

Many groups are exploring what the microbiome is doing in and for the host. Bringing functional insight into metagenomics is a positive, says Michael Fischbach. It can reveal not just individual differences between people in bacterial taxa present or absent but offer a gauge of which microbial functions are up or down. These characterizations get researchers "closest to the phenotypes we care about," such as, for instance, how well checkpoint inhibitors work in cancer or how much weight an animal gains or loses.

The human gut is more or less oxygen free. With anaerobic techniques, the Fischbach lab at Stanford University is developing defined microbiota to model native conditions in the gut. Here, physician-scientist Alice Cheng uses anaerobic chambers to cultivate bacteria, making a microbial community similar to the one in a typical human gut. Credit: L. Cicero; A. Jacobson

The scale has been daunting. Our gut is more or less oxygen-free so making these communities is high-end anaerobic microbiology, for which all steps are performed in anaerobic chambers. A main concern, says Fischbach, has been: "would we be able to do an experiment the same way twice?" Getting experimental conditions to behave reproducibly is hard even in simple systems, and here is a complex system of 100 bacteria strains. "Many of those bacteria are a pain in the ass," he says. "The term of art is 'fastidious', and so they inherently are difficult to deal with."

Preliminary data indicate the system delivers reproducible results. When the team grows 104 or 119 bacterial strains separately and mixes them together, the communities are never identical. "On Wednesday, a strain of *Bifidobacterium* will refuse to grow, and on Friday, a strain of *Ruminococcus* will throw a fit," he says. The relative abundances of strains will differ from run to run. Nevertheless, the researchers observe a kind of "ecological tailwind" that levels out the system to be almost the same way every time. When the microbiota are put into the gut of a germ-free mouse, the resulting communities look remarkably similar.

Now he and his team are getting these defined microbial communities into other labs' hands, who in turn will change, adapt and improve this method. Researchers can colonize their mice and know exactly which bacterial strains are present or absent as they explore immunologic or metabolic phenotypes and hunches about how microbial composition connects to a particular condition.

As long as the tubes stay on ice and the microbes are given to the mice quickly after uncapping, those experimenters do not need to practice anaerobic culture, he says. For now, the lab's plan is to provide others with quality-controlled, defined microbiomes in frozen tubes. "This is a temporary approach until the lab has determined how to make it easy for others to do," he says. This community the lab is developing is free for other academic labs to use. In the longer term this will not be sustainable for his lab, though. The technology has been licensed to Federation Bio, which Fischbach founded and which explores "bugs as drugs," including complex communities.

Plant microbes at scale

Without microbes, plants can live a healthy life, in controlled lab conditions with sufficient nutrients, says CAS researcher Bai. Such conditions include axenic hydroponic systems, which are aseptic and involve culturing the plant in agar, not soil, which harbors many microbes. In the wild, plants need and use microbes. The plant leaf microbiome is distinct from the root microbiome. Nitrogen-fixing bacteria such as *Rhizobium* in the plant root may fix nitrogen and deliver it to plants as nitrates. The plant root has hundreds of other types of microbes, which extract organic carbon from the plant for their own needs.

As a postdoctoral fellow with Paul Schulze-Lefert and others at the Max Planck Institute for Plant Breeding Research, Bai developed a high-throughput cultivation method to capture more than 50% of root bacterial taxa in the lab[8,9]. Previous studies had indicated less than 1% of microbes can be cultivated, says Bai, which was not going to shine any kind of meaningful light on microbiome function.

The method, he says, is adapted from one developed for gut microbiome research by Jeffrey Gordon and his lab at Washington University to reconstitute a model of human gut microbiota. This method isolates bacteria in high throughput from plants, cultivates them and inoculates plants in a germ-free system to reconstitute the microbiomes.

As part of this project, Bai and colleagues, including Julia Vorholt at ETH Zurich, isolated bacteria from *Arabidopsis thaliana* leaves and roots and adopted a barcoded pyrosequencing scheme to classify nearly 8,000 'colony-forming units' on the basis of 16S ribosomal DNA sequences. The team colonized germ-free *A. thaliana* plants and used an inert soil substitute called calcined clay that was sterilized. They sterilized the seeds with ethanol as well. In their gnotobiotic reconstitution experiment, they then isolated and cultured bacterial communities from leaves and roots, inoculated plants and reconstituted these microbiota in the lab-grown plants. The microbial composition showed much overlap between microbiota found in plants and the reconstituted microbiota.

Bai and his group have thousands of pure bacterial cultures. Views will vary, he says, but a microbiome of around 20–30 bacterial strains is what his team uses to shape the key facets of a reconstituted plant microbiome. "I also usually do individual inoculations with an individual microbe to dissect the function of a microbial community," he says. To get an overview, Bai uses genomic tools. Large-scale sequencing and metagenomic analysis of strains can establish which microbial strains are in a given location, and they hint at function. Then the team focuses on the few strains that dominate the interaction and explore what the microbes are producing and how these molecules affect the plant. Not all changes inform as to a given scientific question. When measuring these small molecules, "we look for big differences," he says. "People can do a very beautiful heat map," he says, laughing about the many possible measurements and the limited insight that might provide.

To explore microbial mechanism then takes combining analytical chemistry, metagenomic analysis and gene editing. To explore function and to see which microbes help the plant survive or thrive, the Bai lab knocks out genes in the plant and in the microbial genomes. Gene editing is applied to deepen the mechanistic studies. The Bai lab mainly works with *Arabidopsis* and rice.

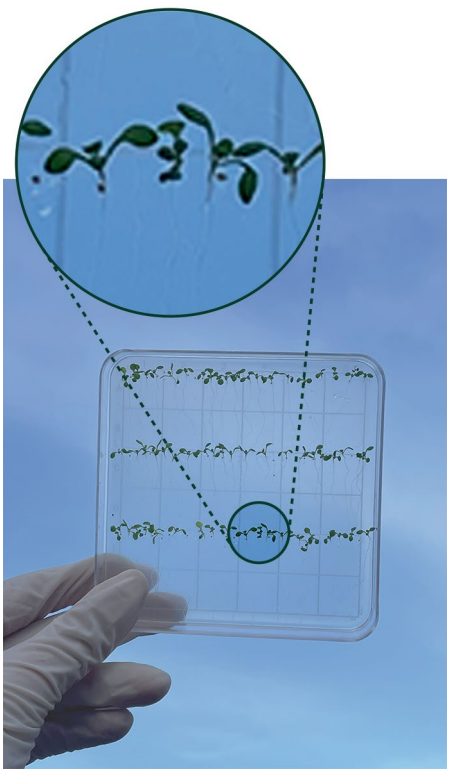
Plant and animal scientists working on microbe–host interactions have much in common, he says, in terms of methods and goals. To home in on defined interactions, they cultivate microbes and inoculate sterile or germ-free plants. A comprehensive understanding of plant root microbes and the detailed mechanisms of the microbial interactions with plants would be beneficial for basic research as well as agriculture, but the tools have been lacking to gain such an understanding. This is true even though the plant community has long considered

the interaction between plants and microbes. It was, says Bai, environmental microbiologists, studying microbes in, for example, wastewater runoff from mines and, separately, in the ocean, who kicked off the study of microbial communities, he says. Metagenomic methods emerged from such projects and were adapted for work on the human gut. The plant community benefited, too, for its work on microbe–plant interaction.

Progressing from genomic analysis to functional exploration is the way forward, he says. He also works on plant hormones and is excited about recent work from other labs on hormones such as auxin. In a recent auxin study, the scientists showed the hormone is produced by both the plant and the plant's root microbiome. "It's amazing," he says, that root bacteria can produce these plant hormones too. As it turns out, "I think this is one of the beautiful examples" of a mutually beneficial interaction. The plant root's auxin helps bacteria further colonize the plant root and bacterial auxin influences plant growth.

Plant hormone levels are generally quite low, he says but their downstream effect through signaling is quite high. For now, there are no good methods to distinguish the hormone plants make from the microbially produced one. "The chemical structures are quite similar," he says. Labs are working on this, he says, and perhaps labels or sensors will emerge.

After bacterial cultivation, germ-free plants are inoculated to reconstitute microbiomes. The team works with plants in the lab and in the field; shown here are their rice plants. Credit: Y. Bai, Institute of Genetics and Developmental Biology of the CAS.



In the lab of Yang Bai, plants such as *Arabidopsis* are inoculated in a germ-free system with cultivated microbiota to reconstitute the microbiomes. Credit: Y. Bai, Institute of Genetics and Developmental Biology of the CAS.

Combinatorial thinking

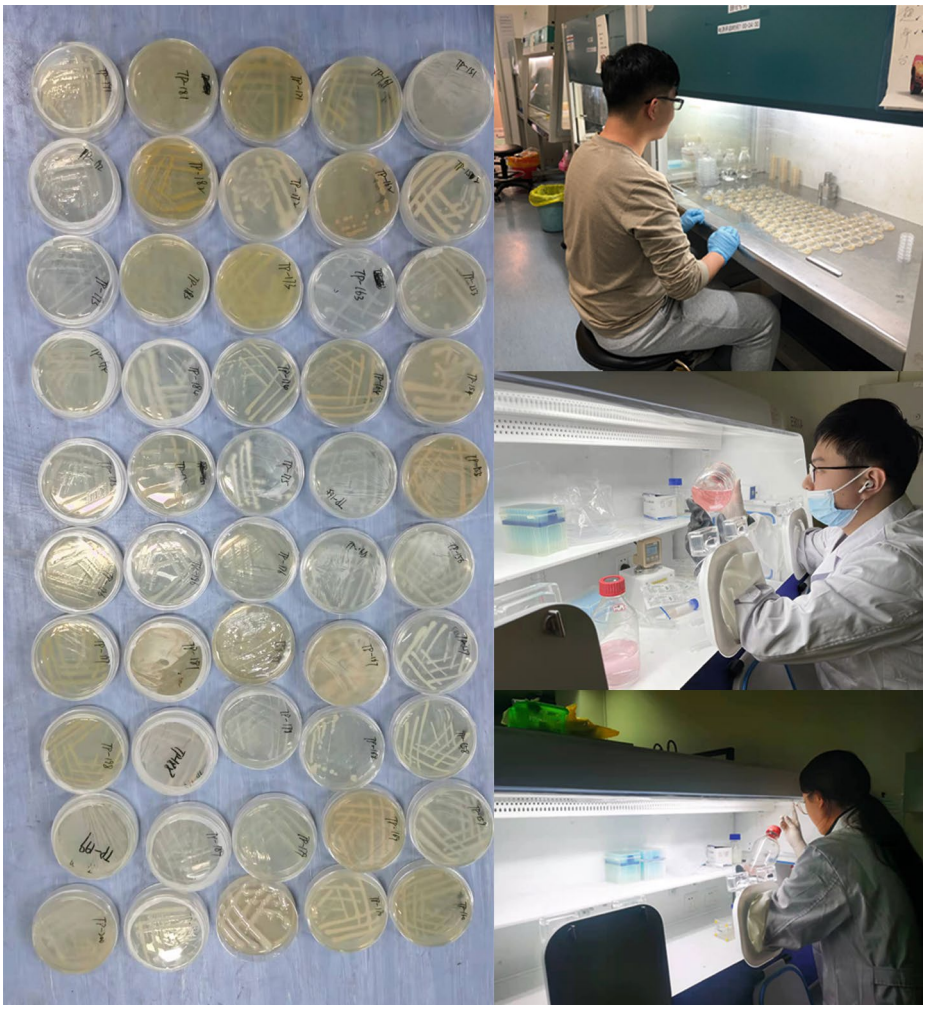
Bai's lab has members with different backgrounds, and he collaborates with others for the expertise he needs, such as in analytical chemistry. He prepares his trainees for the multifaceted exploration of microbe–host interaction and reminds them of the microbial and plant side to the scientific questions they pursue. And he emphasizes "logical training." One PhD student usually can only handle one aspect, such as tasks related to editing microbial genes. But, he says, all need a project-level understanding of the analysis. Not all students have to become programmers, but they need to appreciate a software tool's analytical logic. They "need to understand what the computer scientists are doing," he says, and gauge, for instance, whether the statistical analysis is properly done.

"Root microbiome research needs a new way of thinking," he says. To get to "a systematic description of what happens in nature," he says, scientists should combine a reductionist approach to their labwork and dissection of function with the opposite, what he calls "holistic thinking."

Some labs studying host–microbe interactions focus mainly on metagenomic and genomic sequencing and analysis; others focus mainly on mechanism. Researchers need to find evidence that this interaction leads to important changes under natural conditions. Whether inoculating rice or mice with a microbiome, "people need to show this microbiome interacts with the host in nature," says Bai. In the future, he says, researchers will want "to be sure what we study is important in nature, it happens in nature," he says, "and, when it comes to plants, that what is found in the lab can be an effective tool to affect crops in the field."

Meeting up

Indeed, says Devlin, "bioinformaticians and chemists do not hang out together usually." But the microbiome field has done well to bring together people at conferences, at least before the pandemic. It's gotten much harder to mingle at virtual conferences and meet people, she says. "I would also say that the research environment you're in can help." She's in Boston, across the 'Longwood Quad' from the lab of Curtis Huttenhower. Boston has a "a high density of microbiome research," she says, which includes Harvard, the Massachusetts Institute of Technology, the Broad Institute of MIT and Harvard, Northeastern University and the Boston area



The Bai lab uses a method he adapted from gut microbiome research. Bacteria are isolated from plants in high throughput, then cultivated. From top to bottom, Weidong Liu, Runze Wang and Na Zhang. Credit: Y. Bai, Institute of Genetics and Developmental Biology of the CAS.

hospitals. "Proximity to other researchers in the microbiome field does help," she says.

The integration between chemistry, microbiome and microbiologists is getting better, says Sperandio. "However, the issue for publishing or getting multidisciplinary projects funded is still a challenge," she says. One must assemble panels with diverse expertise, "let them go over their expertise in these projects, and try to enhance the communication on the parts that are not in their wheelhouse."

Some in the chemistry community see him as someone who has "fallen off the wagon," says Fischbach. His PhD in chemistry has given him the "chemistry mindset" to want to know where every atom is and how something works down to the atomic level. "And that spirit still drives all of my work."

As the field grows and changes, studying the microbiome's chemistry to assess microbiome function and its myriad interactions with a host is slowly emerging, he says. In the early days of his lab, to obtain a deep understanding of microbiomes, he and his team applied genomics and bioinformatics. They scoured bacterial genomes and metagenomes to find the genes responsible for making molecules. This gives "insight into what even to look at in the first place."

But it was frustrating to not have experimental systems to study what a molecule does in the host, he says. When looking at a complex molecule that soil or marine microbes make, he wondered, "how did a bug figure out to make this?" Metagenomics tells labs which strains are present, but knowing the molecules a microbiome produces tells one more about what the microbiome is doing. These thoughts led to work on model systems that allow a "clean comparison" that help deliver a mechanistic understanding of what a microbiome contributes to a host and what role a molecule might play in disease.

An exciting dynamic emerges at the nexus of these two kinds of tools: making complex microbial communities and genetically manipulating many 'bugs' in that community. It means "you could begin thinking of the microbiome in the same way that people who study mice think of the mouse," he says. Researchers can knock out a gene in a mouse and study its function as long as it's not essential to the animal. Much of what is known about human biology stems from such precise genetic manipulation of mice. "I think we would love to be able to do the same in the microbiome," he says.

The intersection between metagenomics and chemistry is only just emerging: "I think we see the beginnings of it," he says. A human being does not have a sequencer in his or her intestine to yield the exact composition of their microbiome, but the effects of what the microbiome produces are felt and measurable. "The kernel of this is the chemicals," he says. Some are diffusible; some are attached to cells; some enter the bloodstream perhaps even at concentrations as high as those of synthetic drug compounds.

"I think that the functional layer on top of metagenomics doesn't yet include chemistry to the full extent; really, to much of an extent at all," says Fischbach. "But that's in progress." Studies will hopefully emerge that stand the test of time in explaining how the microbiome works. That this is taking a while is not criticism of the field; it's "about the natural progression of studies in the area." Research in a number of labs is being framed with this multifaceted perspective, he says, and more are "joining the party." ❐

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