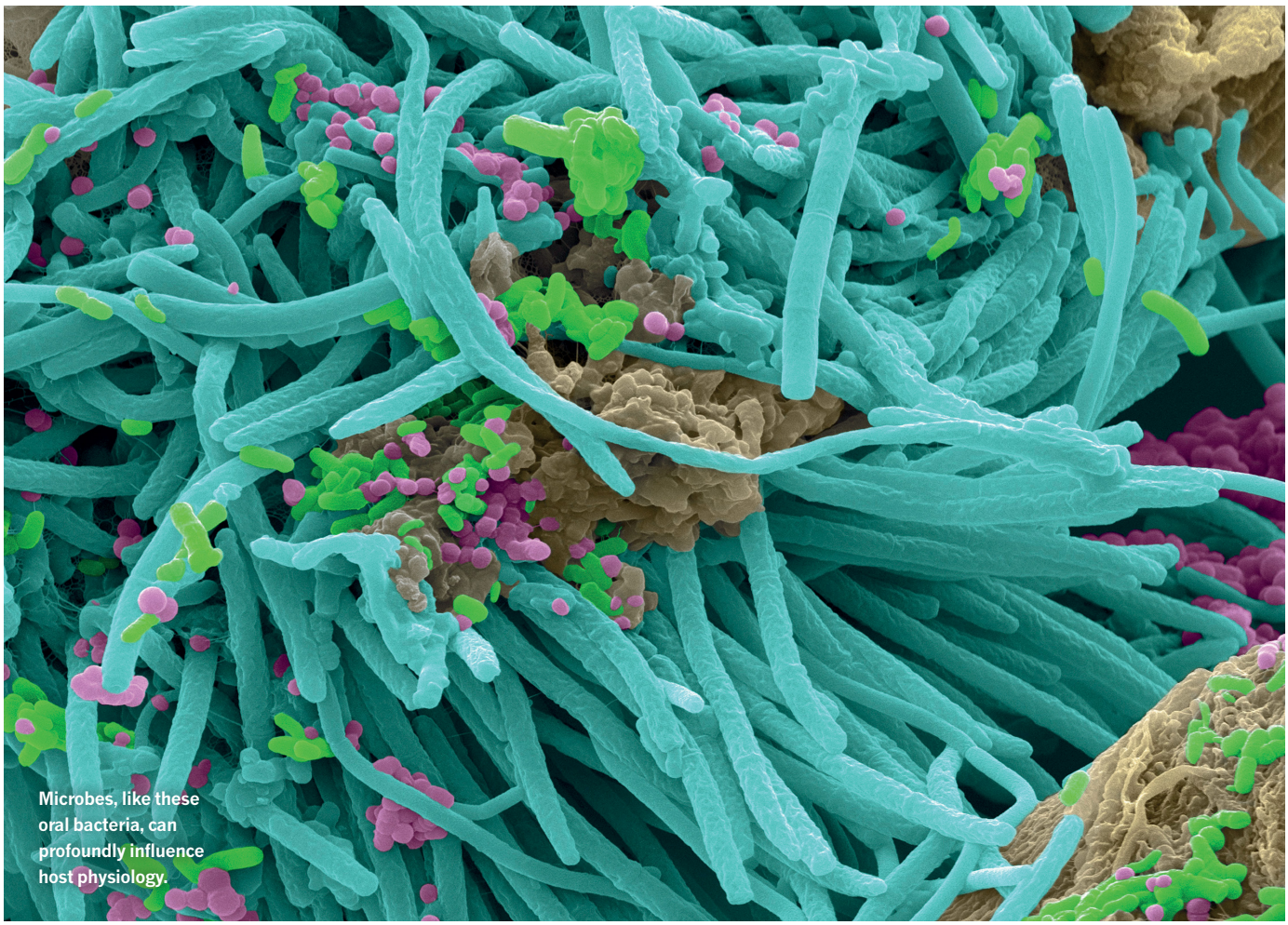
TECHNOLOGY FEATURE

MICROBIAL CHEMISTRY GAINS FRESH FOCUS

The tools of chemical biology, genomics and data mining can yield insights into the metabolites of the microbiome.

STEVE GSCHMEISSNER/SPL



Microbes, like these oral bacteria, can profoundly influence host physiology.

BY ESTHER LANDHUIS

Studies of the microorganisms that live on and inside animals' bodies have long relied on DNA sequencing, which can reveal which species abound and how these microbial communities respond to their environment. Now, the analytical methods of chemical biology, combined with genomics and computing techniques, are giving researchers insights into what these microbes are actually doing, biochemically speaking. Using mass spectroscopy and a growing suite of databases and bioinformatics tools to analyse the data, some labs are focusing on substances produced as the microbes metabolize food. These 'metabolites' serve not only as markers for charting health and disease, but also as engines of physiological change[1].

The metabolites can influence the biology of the host, and not just where the microbial communities are resident. Some such compounds reach high levels in the blood, with concentrations that can vary by more than an order of magnitude between individuals, says Michael Fischbach, a microbiologist at Stanford University in California. "These are chemicals we should know more about, because they could underlie biological differences among people."

Metabolomics — as the study of metabolites is known — is easier said than done, however. "In any given metabolomics run, we'll detect thousands of metabolites," says Erica Majumder, a biochemist at the State University of New York College of Environmental

Science and Forestry in Syracuse, New York, who studies sulfur metabolism in gut microbes.

When researchers were just starting to analyse metabolites, using a technique called liquid chromatography–mass spectrometry (LC–MS), identifying these biomolecules could take months of work. "It was really an incredibly frustrating process," says biochemist Gary Siuzdak, whose team at Scripps Research in La Jolla, California, published one of the earliest LC–MS metabolomics papers[2], in 1995.

Since then, improved instrumentation and analytical tools have shaved that time considerably. Siuzdak's lab created METLIN, a database of tandem mass spectra — which reveal structural details of molecular fragments — on more than half a million metabolites and other molecules. The lab also developed XCMS, an online platform for processing LC–MS data.

Another tool, Global Natural Product Social Molecular Networking, was created by chemist Pieter Dorrestein and his colleagues at the University of California, San Diego. It provides crowdsourced mass-spectrometry data that researchers can use to identify metabolites when official reference standards are not available. Although much work remains to be done, Siuzdak says that such tools make it possible to identify some metabolites in seconds. In 2016, fewer than 2% of mass-spectrometry signals could be matched to known metabolites, Dorrestein says. That number has now increased two- to threefold.

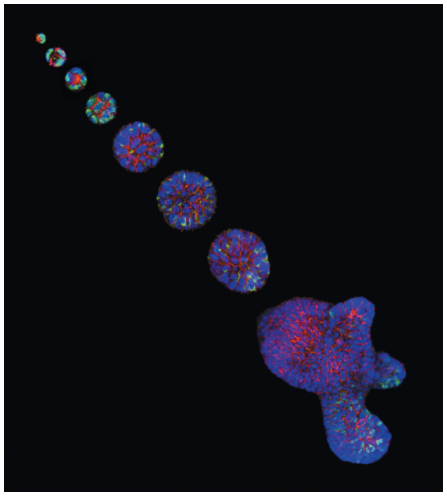
MORE WAYS TO EXPLORE

Genomics techniques are also opening up lines of exploration. One key question, addressed in two studies by Maria Zimmermann-Kogadeeva, a computational biologist at the European Molecular Biology Laboratory in Heidelberg, Germany, is how the microbiome influences drug metabolism in its host.

In the first study[3], conducted when she was a postdoc at Yale University in New Haven, Connecticut, Zimmermann-Kogadeeva and her colleagues looked at the antiviral drug brivudine, from which gut microbes produce a toxic metabolite. Zimmermann's team gave brivudine to wild-type mice or mice that lack microbiota, then measured the concentration of the drug and its metabolite over time. After identifying the microbial strains that metabolized the drug most rapidly, they systematically deactivated 2,350 bacterial genes to determine the enzyme responsible.

Next, the researchers recolonized 'germ-free' mice with bacteria lacking that enzyme. That enabled them to build a pharmacokinetic model of host–microbiome drug metabolism, an approach that could be used to estimate the microbial contribution to the digestion of foods, other drugs or endogenous metabolites.

Zimmermann and her team have also tried to quantify the microbiome's impact on oral pharmaceuticals more broadly. In a screen of 76 gut microbes and 271 oral drugs, they found



Organoids can tease apart microbial influences.

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that all microbes metabolized some of the drugs, and that 65% of the drugs studied were metabolized by at least one microbial strain[4]. The team then created libraries of bacteria, each expressing small pieces of the genomes of interest, to identify bacterial genes responsible for this metabolic activity, which they quantified using mass spectrometry.

Another question concerns the impact of microbial metabolites on the host. Indolepropionic acid (IPA), for instance, a substance that can alter the permeability of the intestinal wall, is made exclusively by gut bacteria such as *Clostridium sporogenes* from dietary tryptophan. The metabolic pathway involved, however, was unclear, until the steps were pinned down using bioinformatics, gene knockouts and mass spectrometry by a team led by Fischbach and Stanford colleague Justin Sonnenburg[5]. In a subsequent preprint[6], the team has described a CRISPR–Cas9-based system for toggling the production of bacterial metabolites, and used this to uncover a role for certain metabolites in host immunity.

"Tools make it possible to idenfity some metabolites in seconds."

Researchers are also addressing metabolite impact using organoids — lab-grown tissues that are akin to simplified organs. Stem-cell biologist Scott Magness and bioengineer Nancy Allbritton, both at the University of North Carolina, Chapel Hill, have developed a system for analysing 15,000 organoids grown in individual wells — all fitting within a square the size of a postage stamp[7]. The team built the platform using off-the-shelf and 3D-printed components, and set up an automated monitoring system using microscopy and computational image analysis. "You're never going to get a grad student or postdoc to count 15,000 wells," says Magness.

The researchers used another automated system to inject bacteria from healthy donor stool samples into the organoids, at a rate of some 90 organoids per hour (manual injection would have treated only a dozen organoids per hour). By injecting a fluorescent dye alongside the bacteria, the researchers could tell whether microbial metabolites were disrupting gut-barrier function[8].

They also demonstrated that the system could support the growth of anaerobic microbes, which predominate in the human gut. "We showed you could inject complex communities of bacteria and they would maintain a stable community over a couple days," Magness says.

A MINE OF INFORMATION

Such tools can help tease apart the microbial chemical activity of the microbiome. But to exploit and understand the metabolome, researchers also need to make use of tools such as data mining. A web tool called Metabolite Annotation and Gene Integration (MAGI), for instance, uses known biochemical pathways to generate a metabolite–gene association score, helping to correlate genetic sequences with metabolomics data[9]. "Identifying metabolites is very challenging. Likewise, identifying the function of a gene in a genome is often ambivalent," says MAGI developer Trent Northen at Lawrence Berkeley National Laboratory in California. "MAGI recognizes that metabolomic and genomic data are orthogonal, and puts those pieces of information together to help identify metabolites and identify genes."

Such tools can also help researchers home in on what's important in the research literature, Siuzdak says. "It's a new technology that's allowing us to decipher the metabolomics data more quickly." In a paper under review, Majumder describes a strategy to mine the scientific literature for clues that predict metabolite functions in specific biological contexts. She has used this to identify metabolites that might eventually help to reverse the neurodegeneration seen in multiple sclerosis. Some papers that the tool pulled up "were ones we never would have found from traditional searching, and gave us direct evidence from the literature to interpret what we saw happening in our system", she says. ■

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