THE BACTERIA AMONG US

A growing number of studies are linking microbes in our bodies with physiology and disease. Jeffrey Perkel speaks to researchers trying to understand this relationship and, possibly, even use it to improve human health.

Microbiome researcher Jack Gilbert. Credit: Argonne National Laboratory

When Jack Gilbert, Microbial Ecology Group Leader at Argonne National Laboratory in Argonne, Illinois, wanted to lose some weight, he didn't try traditional dieting. Instead, he tried sequencing. One of a growing number of researchers investigating the human microbiome—the collection of bacteria that live in and on us—Gilbert sequenced the bacterial flora in his own gut and used those data to design a specific diet suited to his internal microbial species diversity and its metabolic potential.

While leaving his caloric intake unaltered, he made targeted changes, replacing starchy foods such as breads and rices with whole grains, increasing his intake of animal fat, and changing when he ate. The goal, as he explains, was to “manipulate my body's microbiome and produce a slightly different microbial community structure.”

Though his data and methods are unpublished, and this “n-of-1” study lacks a control data set, Gilbert maintains his gambit worked. “I lost 40 pounds in 12 weeks,” he says. Whether this has anything to do with the microbiome remains an open question. But with each passing month it becomes increasingly clear that the microbiome exerts a powerful, and until recently largely ignored, effect on human biology. Researchers are working hard to catch up. Over the past 10 years, powered by advances in next-generation DNA sequencing technology and bioinformatics algorithms, scientists have linked humans' microbial cohabitants to a range of complex phenotypes.

In what is now a classic metagenomics study from 2006, Jeff Gordon of the Center for Genome Sciences at Washington University in St. Louis and his colleagues demonstrated that mice harboring a mutation in the leptin gene, which causes obesity, exhibited altered gut microbial communities compared to lean litter-mates, with an increased proportion of Gram-positive bacteria called Firmicutes relative to members of the Gram-negative Bacteroidetes. That, according to microbiome researcher Jonathan Eisen, Professor of Evolution and Ecology at the University of California, Davis, is perhaps unsurprising; however, what Gordon's team did next, he says, “was extraordinary.”

The researchers transferred fecal material from obese mice to lean, germ-free control animals—that is, animals that had not yet been colonized by other bacteria. Though the recipient mice were genetically normal, the treatment made them obese. “The obesity could be transmitted to another organism,” Eisen says. “It's absolutely astonishing.”

In humans, fecal microbiota transplantation is gaining rapid acceptance as a way to treat the persistent gastrointestinal distress caused by *Clostridium difficile* infection, presumably by restoring the normal microbial community that the pathogen edged out.

“The health and well-being of a plant or animal or kelp

or mushroom or any other multicellular organism is profoundly influenced by the cloud of microbes that live in and on it, even the ones that don't cause a disease," explains Eisen.

That "cloud" may contain hundreds or even thousands of distinct species, each with a unique set of features and capabilities. The trick is working out which community members drive a given biological phenotype and how they do so.

A fateful meeting

John Kirby, Professor of Microbiology and Urology at the University of Iowa, focuses on interactions and communication across a range of microbial communities. He started out studying the biology of soil microbes, but around the time the Human Microbiome Project started coalescing, he shifted focus, applying technologies developed for soil communities to the human gut. "The only difference is the fact that our digestive tract moves things from point A to point B a little bit more quickly than things move in a soil community."

Kirby, along with his graduate student Sarah Bahr, established a microbiome project in Iowa City and began delivering seminars on the topic around campus. At one event in 2011, he was approached by psychiatrist Chadi Calarge (now at Baylor College of Medicine), who wondered whether Gordon's concept of an "obesogenic" microbiome might also be able to explain the weight gains doctors were seeing in children taking the

anti-psychotic drug risperidone.

Kirby liked the idea, and the psychiatrist had already done much of the legwork, collecting a "freezer-full" of stool and other biological samples from risperidone-treated children, as well as associated clinical data. But it wasn't easy to put the idea to the test, he recalls: The team had to work out how to reproducibly extract DNA from stool, sequence it, and most importantly, crunch the numbers. In total, Kirby spent "at least \$100,000 total on the sequencing," not to mention salary and consumables costs.

But his investment paid off. By sequencing the 16S ribosomal RNA gene—which provides a genetic fingerprint of microbial identity—in both risperidone-treated and untreated individuals, Bahr and Kirby and their colleagues were able to determine that children exposed to long-term

antipsychotic treatment exhibit alterations in gut microbial communities. Specifically, the ratio of Bacteroidetes to Firmicutes in the gut drops with extended drug treatment, a change that correlates with weight gain (2).

In a follow-up study (3), the team used fecal transplants to demonstrate that, similar to obesity, risperidone-associated weight gain can be transferred from mouse to mouse. The question is, what was it about the risperidone-exposed microbiome that makes mice fat?

As it turns out, risperidone functions effectively as an antibiotic, specifically targeting the kinds of anaerobic organisms that live in the gut. The resulting microbiome causes obesity because, although the mice consume the same number of calories, their microbes are less efficient at burning them when their hosts are at rest. "The mice that gain weight in response to risperidone," Kirby explains, "are not burning calories at the same rate that another mouse is doing, even while they're asleep."

There are several possible explanations, Kirby says. The microbes could, for instance, be altering mitochondrial respiration rates in the host. Alternatively, it could be the energy diet of the bacteria themselves that is to blame. Those data aren't yet published, but Kirby says he believes the latter to be true—that by downshifting metabolic rate, the gut microbiome can induce obesity by leaving more energy on the table for the host to consume. "You're basically ... shed-

Jonathan Eisen, Professor of Ecology and Evolution at University of California, Davis, says that the health and well-being of animals are profoundly influenced by the microbes that live on it or in them. Credit: Raymond McCauley.

ding calories through bowel movement,” he explains—a conclusion he reached the old-fashioned way: by putting food, feces, microbes, and even mice in a custom-built bomb calorimeter designed by University of Iowa colleague Justin Grobe.

The data come “almost full circle to what Jeff Gordon proposed.” But whereas Gordon hypothesized that obese microbiomes were extracting more calories from the food, Kirby’s data suggest the difference is who is burning them. “It’s pretty damn exciting,” he says.

Identifying that special microbe

For all his work, Kirby has yet to answer one lingering question: Which microbial species are actually responsible for the weight gain in risperidone-treated individuals? Here lies one of the significant challenges in microbiome research at the moment. The majority of microbes, whether in the human body or outside, cannot yet be grown in the lab, making it nearly impossible to test them systematically. Researchers know which organisms are there because DNA sequencing data provide the tools for tracking the presence and shifting abundances of these organisms and their genetic capabilities. But studying species in isolation or even in defined mixtures is altogether a different problem.

That’s not to say researchers never catch a break. Diabetics, for instance, often bear unusually high *Staphylococcus aureus* concentrations on their skin, says Patrick Schlievert, Chair of Microbiology at the University of Iowa Carver College of Medicine. Earlier this year, he showed in rabbits that prolonged exposure to *S. aureus* superantigen—the molecule underlying toxic shock syndrome—can induce altered glucose metabolism and the widespread inflammation that is characteristic of diabetes mellitus type 2 (4). And in 2013, Sarkis Mazmanian, the Louis & Nelly Soux Professor of Microbiology at the California Institute of Technology, and his team

Nicole Dubilier, Director of the Symbiosis Department at the Max Planck Institute for Marine Microbiology in Bremen, Germany, has called for a large-scale international effort to examine the global microbiome. Credit: Nicole Dubilier.

demonstrated that *Bacteroides fragilis* can mitigate behavioral defects in a mouse model of autism-like behavior. That latter study even identified a single metabolite (4-ethylphenyl-sulfate) dysregulated in experimental animals, demonstrating that administration of this chemical was sufficient to alter behavior (5).

More frequently, however, it isn’t possible to narrow things down to a single organism, Mazmanian says—

perhaps because some organisms never act in isolation but instead function as part of a complex community that has evolved over millennia. “I think there’s a sharing of labor, if you will, and a coordination among organisms that then leads to definable activities.”

Researchers have come up with some strategies for simplifying the problem, however. In one recent study, Elaine Hsiao of the University

of California, Los Angeles—who was a postdoctoral fellow in Mazmanian's lab—investigated the gut microbiome's influence on serotonin production (6). Although her team wasn't able to identify a single “smoking-gun” species, according to Mazmanian, who is a coauthor on the study, they were able to narrow the field by testing a subset of gut microbes that are capable of forming spores, a group of about 50 organisms out of the 800 or so in the entire microbiome.

Importantly, Mazmanian explains, there is no “intellectual” link between spore-formers and serotonin; that phenotype is simply “an arbitrary criterion. It just lets us reduce the number of organisms to a smaller subset.”

In this case, the strategy allowed the team to determine that the spore-forming sub-microbiome was producing a critical bile salt, deoxycholic acid, which in turn induces serotonin production in the intestinal lining.

Another enrichment strategy focuses on microbes that interact with the body's defenses. The immune system has a complicated relationship with the microbiome, Mazmanian explains. Some organisms are hidden from the immune system; others, it simply ignores. Organisms in the latter class are coated with immunoglobulin-A, and researchers can exploit that difference to isolate them.

“It doesn't require any culturing, because you can just fish them out ... [and] put them into a mouse. You never have to actually grow them in a laboratory.”

Our microbes and cancer

According to Nicole Dubilier, Director of the Symbiosis Department at the Max Planck Institute for Marine Microbiology in Bremen, Germany, and coauthor of one of two recent articles calling for large-scale efforts to tackle global microbiome characterization, what is truly remarkable about the microbiome field is how rapidly it is moving, and how broad its impact is. “The last five years have revealed this incredible diversity and have also revealed how much animal and plant life is driven by and dependent on

their microbes,” she says.

And those microbes continue to prove essential in surprising ways. In early November, for instance, a pair of mouse studies established links between the microbiome and yet another vexing bit of biology: the highly variable response of tumors to anti-cancer drugs called checkpoint inhibitors. In one study, Laurence Zitvogel of the Gustave Roussy Cancer Campus in Villejuif, France and his colleagues demonstrated that the anticancer efficacy of anti-CTLA-4 antibodies for the treatment of metastatic melanoma depends upon the presence in the gut of the bacterial species *Bacteroides thetaiotaomnicon*, *B. fragilis*, and *Burkholderia cepacia* (7). The second study, led by Thomas Gajewski at the University of Chicago, established a role for *Bifidobacterium* in the anticancer properties of anti-PD-L1 antibodies (8).

According to Mathias Chamaillard, Research Director at INSERM and co-author of the Zitvogel study, the adjuvant properties of these microbes are not responsible for toxicity related to the CTLA-4 blockade but rather seem to drive the anti-tumor response by priming the immune system. *Bacteroides*, the team found, express an antigen that instructs antigen-presenting dendritic cells. These dendritic cells, in turn, secrete IL-12 and prime Th1 helper T cells, which mount the immune response to the tumor itself.

“This is maybe one reason that some patients do not respond [to the drug],” Chamaillard explains, “because we identified that there is a lot of heterogeneity among the patients in terms of abundance for this subset of commensals.”

Fecal transplantation experiments (from patients into mice) suggest that, as with obesity, drug efficacy is a transferrable trait. That raises the possibility of treating non-responding patients with probiotics to improve to treatment outcomes or, alternatively, profiling a patient's microbiome to identify those patients most likely to respond.

It'll take time to develop and test

any such therapy, of course. And nobody has yet worked out what the “ideal” human microbiome should look like in any event.

If and when they do, however, it would be wise - in this era of media microbiome hype - to keep the findings in perspective. The microbiome is only one facet of biology, says Eisen, who periodically doles out an “Overselling the Microbiome” anti-prize on his web site; so, too, are genes, environment, and frankly, luck. “We are an organism that is the sum total of all of these things,” he says. “The microbiome is just one layer.”

References

1. **Turnbaugh, P.J., et al.** 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*. 444:1027–1031.
2. **Bahr, S.M., et al.** 2015 Use of the second-generation antipsychotic, risperidone, and secondary weight gain are associated with an altered gut microbiota in children. *Transl Psych*. 5:e652.
3. **Bahr, S.M., et al.** Risperidone-induced weight gain is mediated through shifts in the gut microbiome and suppression of energy expenditure. *EBioMedicine*. Published online 27 Oct 2015. doi: 10.1016/j.ebiom.2015.10.018.
4. **Vu, B.G.** 2015. Chronic superantigen exposure induces systemic inflammation, elevated bloodstream endotoxin, and abnormal glucose tolerance in rabbits: Possible role in diabetes. *mBio*. 6:e02554-14.
5. **Hsiao, E.Y., et al.** 2013. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopment disorders. *Cell*. 155:1451–1463.
6. **Yano, J.M., et al.** 2015. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*. 161:264–276.
7. **Vétizou, M., et al.** Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science*. Published online 5 Nov 2015. doi: 10.1126/science.aad1329.
8. **Sivan, A., et al.** Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science*. Published online 5 Nov 2015. doi: 10.1126/science.aac4255.

Written by Jeffrey Perkel, Ph.D.

BioTechniques 59:324-328 (December 2015)
doi: 10.2144/000114360