

U-M IS INVESTIGATING HOW THE MICROBIOME MAY PROTECT US FROM DISEASE AND MAINTAIN OUR HEALTH

WHAT WE HOST



The sum total of all the microorganisms that live in and on our bodies, from bacteria to viruses to fungi, is called the human microbiome. It is the film of cells on our skin, the quiet residents of our throats and lungs, the sludge in our intestines. Inspiring descriptors like “ecosystem” and “forgotten organ,” it protects us from infections, alters our immune function, and helps us digest our meals. You are, in a very real sense, a scaffold for your microbiome. Scientists are just now gaining a foothold on understanding the importance of the microbiome. Weighing as much as 5 pounds, it contains its own separate genetic information that surpasses our own genome complexity and manufactures a vast array of molecules that influence your metabolism, digestion and even emotional health. It is only dimly understood; in fact, medical treatments often damage it. Researchers are just beginning to grasp its massive importance to the future of medicine.

In July 2013, the U-M Medical School launched the Host Microbiome Initiative, becoming just one of a handful of institutes nationwide to focus on the microbiome’s mysteries. The initiative is designed to bring together a range of medical school researchers, including microbiologists, physicians, statisticians, programmers and ecologists. Its state-of-the-art technologies — including high-throughput sequencing robots and massive computing power — allow investigators to map out which organisms live where and what, exactly, those organisms are doing. Insights into the microbiome have already led to one promising cure, and some researchers believe it may help us crack longstanding medical puzzles, like obesity, autoimmune disease and autism.

BY JENNY BLAIR • ILLUSTRATION BY ANTHONY MORROW



Championed by Senior Associate Dean for Research Steven Kunkel, Ph.D., and greenlighted by Medical School Dean James O. Woolliscroft, M.D., the initiative garnered \$5 million in funding from the Fast Forward Initiative, a program of the medical school's Office of Research. Pooled funds from all 30 medical school departments provided a further \$10 million.

That's fitting, since the microbiome is probably involved in diseases all over the body, according to Vincent B. Young, M.D., Ph.D., an associate professor in the Department of Microbiology and Immunology. He and Harry Mobley, Ph.D., the Frederick G. Novy Professor and chair of the Department of Microbiology and Immunology, co-founded the initiative.

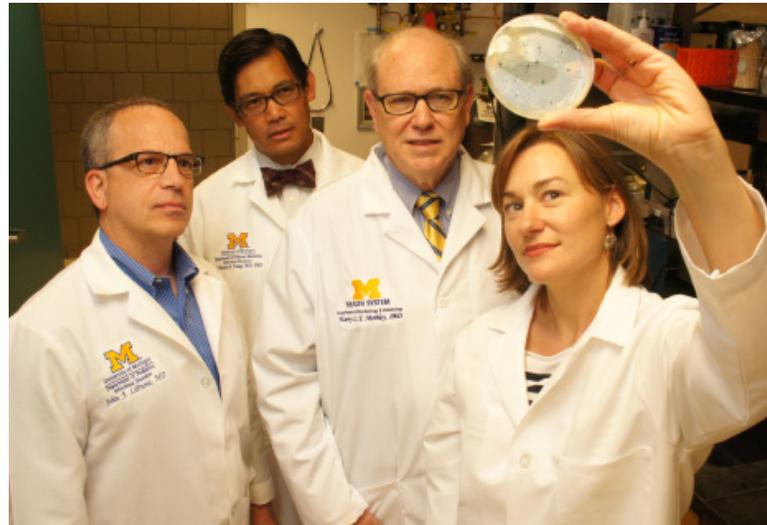
"Everything ranging from heart disease to, of course, obesity and diabetes are at least in part influenced, if not caused, by changes in microbes," Young says. "Everyone from psychiatry to endocrinology to surgery can potentially be involved."

With the Host Microbiome Initiative's tools, researchers are beginning to piece together a powerful understanding of the entire human microbiome. They have converged on the problem from radically varied backgrounds: data analysis, wastewater treatment and cystic fibrosis, among others.

John LiPuma, M.D., is a pediatrician who studies cystic fibrosis, especially the exotic lung infections that plague patients with this genetic anomaly. Once thought to be sterile, the lung turns out to host its own richly diverse portion of the human microbiome. What fascinates LiPuma, the James L. Wilson, M.D., Research Professor of Pediatrics and Communicable Diseases, is how the lung's microbial populations shift over time, especially around the time of exacerbations (when the illness takes a turn for the worse). He followed a group of CF patients over 10 years, collecting sputum samples in order to map and identify entire bacterial communities.

He uncovered an intriguing relationship: The CF lung microbiome's biodiversity is high in patients who feel well. That biodiversity diminishes during exacerbations, with the sickest CF patients' lungs dominated by a single species — a phenomenon LiPuma likens to the collapse of an ecosystem.

That work predated the initiative. But the findings were so exciting, quickly garnering scores of citations after their 2012 publication in *Proceedings of the National Academy of Sciences*, that they bolstered the case for its founding. Now, using the initiative's tools, LiPuma is conducting a new study of daily sputum samples. A better understanding of what's happening in the



Top (Left to right): Microbiome researchers John LiPuma, M.D.; Vincent B. Young, M.D., Ph.D.; Harry Mobley, Ph.D.; and Nicole Koropatkin, Ph.D. Bottom: Research associate Judy Opp demonstrates the liquid handler used for sequencing in Young's lab.

lung, he says, could help us find ways to encourage a healthier community of lung bacteria. Research like this raises questions about probiotics and, for that matter, antibiotics.

Consider a patient with CF who reports feeling worse than usual. The doctor assesses her lung ecosystem and discovers that one particular bug is dominating the scene. Perhaps instead of blitzing her with broad-spectrum antibiotics, the doctor offers a narrow-focus antibiotic that takes out that bug like a sniper, relieving pressure on the many other species hiding in the corners and ultimately boosting biodiversity. Perhaps that would help the patient return to her usual state of health.

"The more we learn about the CF microbiome," says LiPuma, who is also a professor of epidemiology at the U-M School of Public Health, "the more we realize how in the dark we've been over the years."

Through its researchers and technology, the initiative offers a formidable array of approaches to understanding the microbiome. U-M's researchers can sequence the mRNA of an individual bacterial species to understand how the bacterium is responding to their environment within a patient. They can sequence the whole bacterial genome in pieces and then use computer algorithms to reassemble them into a whole to determine "what makes bacteria tick." And then there's 16S ribosomal RNA gene sequencing, which offers a precise way to classify all the species in a mixed sample to understand how the entire community functions in health and disease.

Sequencing is only part of the picture, though. Just obtaining the organisms whose genetic information we want to know requires special techniques. To grow bacteria that thrive in environments with low or no oxygen (such as the intestine), for example, initiative researchers use custom-built laboratory hoods entirely enclosed by plastic bubbles that regulate the atmosphere with regards to oxygen and other gasses. (Normal atmospheric oxygen levels are 21 percent, but in these chambers, atmospheres with only a few percent oxygen or even no oxygen can be generated.) To work with the equipment within, researchers clean their hands, don disposable gloves, then thrust their arms into gauntlets that project into the bubble.

To investigate the "host" in the host microbiome, the initiative employs a group of extraordinary mice. These mice are raised from birth with gnotobiotic, or "germ-free," techniques,

in which they pass their otherwise ordinary lives in bubbles so sealed away from the outside world that their bodies never develop a microbiome. Technologists ensure no stray germs enter the mice, a task that requires specialized equipment and near-obsessive patience and meticulousness. Experimenters then expose these clean-slate mice to specific microbial populations to tease out how those microbes interact with a host.

And then there is the metabolome. Microorganisms don't just sit there. They pump out countless small molecules that can profoundly influence the health of the host, for better and worse. In addition, microbes can generate derivative molecules that we make, such as the bile acids that we secrete into the intestine to help digest fat. Studying these microbe-deprived substances is the purview of metabolomics, a set of experimental techniques that show not so much the identities of microbiome residents as what exactly they're doing. It's a little like learning about a country by studying its exports. None of these raw data would be of much use, though, without a way to analyze them. And genetic studies in particular generate a lot of data. A lot.

"Gobs and gobs," says Judy Opp, a research associate in the Young lab.

"Haystacks," says the Host Microbiome Initiative's program administrator, Michael Dority.

"We're swimming in data," says microbial ecologist Tom Schmidt, Ph.D., a professor of internal medicine and in the departments of Ecology and Evolutionary Biology, Civil and Environmental Engineering, and Microbiology and Immunology.

A NEW FUEL EFFICIENCY

The idea of treating the body as an ecosystem attracts scientists from very different backgrounds. Schmidt, the microbial ecologist, joined the medical school faculty last year with expertise in the microorganisms of lakes, oceans and soils. He holds a joint appointment in the Department of Ecology and Evolutionary Biology. Schmidt studies bacteria that survive on minimal amounts of oxygen--so-called microaerophiles. These organisms prefer in-between

spots in soils and sediments, places neither awash in oxygen nor devoid of it.

Schmidt had a guess about where to find microaerophiles in humans: the wall of the intestine, between the oxygen-rich outer side where the bloodstream penetrates and the zero-oxygen environment of the intestinal tract itself.

"From my experience in other environments, these transition zones between oxic and anoxic regions are just blooming with microbial activity," Schmidt says.

So it proved in the gut. When

Schmidt's team sampled just that spot along the lining of the mouse intestine, they found a thriving bacterial community, separate and distinct from the one that lives in the gut proper. Many of the species there are new to science. The team studies the germs in bubbles that keep the oxygen content at just 3%, as compared to 21% in ordinary air. They hope to learn how the bacterial community changes when inflammation of the gut lining changes its oxygen concentration.

Schmidt is also interested in

two new ways to measure bacterial growth. Some microbes are geared to pump out prodigious numbers of progeny fast. Others are better suited to efficient use of local resources. One you might think of as horsepower, the other as gas mileage.

Normal gut flora tend to be fuel-efficient. An organism like *C. diff.*, on the other hand, has a lot of horsepower. It can gain a foothold fast, much as weedy plants like dandelions or purple loosestrife easily dominate native plants in disturbed soils.

“We need help,” Schmidt adds. “We’re struggling with how to find patterns in all of the noise. How do you find the meaningful signals in there?”

At Michigan, that help has come from Patrick Schloss, Ph.D., an environmental engineer turned bioinformatician. Schloss, a professor of microbiology and immunology, formerly worked on wastewater treatment systems and found he was curious about exactly what goes on with the bacteria that digest the waste.

“As an engineer, you treat everything as a black box and say, ‘We don’t care who the microbes are, we just care that they do their job,’” Schloss recalls. “I wanted to know more. That kind of set me on a path to learning more about microbial ecology and the ways bacteria interact with each other as well as their surroundings.” He is now an associate professor in the Department of Microbiology & Immunology and studies how bacterial populations change over time during both health and disease.

A gifted programmer, Schloss released a software package in 2009 called *mothur* (pronounced “mother”), which sifts through big sequencing data sets to help researchers pick out a coherent story. It’s open-source, meaning anyone can contribute code to tweak and improve it, and it’s slender enough to work on a laptop for projects sorting through a modest amount of data. That makes it a resource for microbiome projects around the globe. But it works best when powerful computers work in parallel. The computers at U-M that Schloss employs for data analysis make use of hundreds of gigabytes of RAM.

With *mothur*, scientists can sort out relative populations of different bacterial species in a sample then look for associations between different people -- or across time in the same person. For instance, Schloss worked on a study that sampled stool from healthy people and those in various stages of colon can-

cer, looking for bacterial populations that might flag a tumor.

U-M’s bioinformatics team grew more powerful this fall with the recent hire of Evan Snitkin from the National Institutes of Health. A computer scientist, Snitkin earned global scientific and media renown as an NIH postdoctoral fellow for tracking a 2011 outbreak of the superbug carbapenem-resistant *Klebsiella pneumoniae* at the NIH Clinical Center. He used a technique called whole-genome sequencing that allows far more precision than standard epidemiological detective work, enabling his team to track the outbreak patient by patient and piece out its path ward by ward. The plummeting cost of doing whole-genome sequencing and the increasing availability of analytic programs bodes well for tracking and halting future outbreaks.

The Host Microbiome Initiative arose from conversations between Young, who is interested in the role bacteria play in gastrointestinal disease, and Mobley, who studies urinary tract infections in patients with bladder catheters. “Maybe five years ago, we began to realize that we shouldn’t just study the pathogenic bacteria, but also the ones that live on us and within us,” Mobley says. “They play a nice protective role against many (harmful) organisms.”

He’s referring in part to hospital-acquired infections that pose a serious health hazard, particularly to debilitated patients. There is vancomycin-resistant enterococcus (VRE), for example, which can infect the urinary tract, wounds, or the bloodstream. Perhaps more notorious is methicillin-resistant *Staphylococcus aureus* (MRSA), which causes life-threatening infections in the lungs, bones and elsewhere. Bacteria like these thrive when the microbiome is disrupted — often by antibiotics.

NEW EXPLORERS

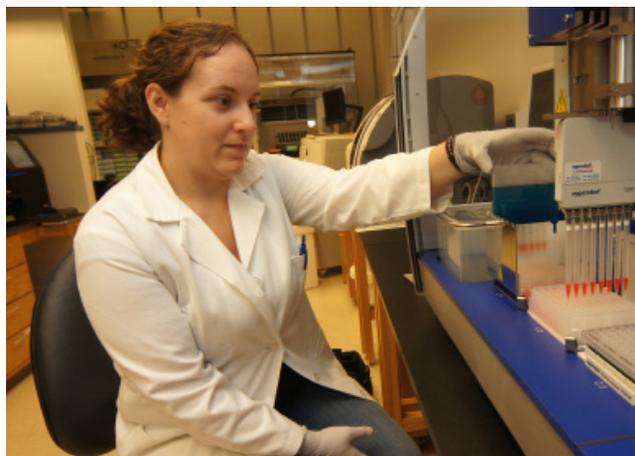
Researchers are eager to understand differences between the microbiomes of sick and well people, but it’s hard to know what to do with that information until we know what normal is. So the Host Microbiome Initiative is studying that in a massive data-collection effort, following thousands of students, community members

and hospitalized patients over time to see how their microbiomes change. What they learn will give us a sense of the wide range of healthy microbiomes, as well as potentially illustrate intriguing associations between unusual communities and disease. Some investigators at the medical school want to see if the microbiome affects their

research areas, but aren’t sure how to begin. With the Microbiome Explorers Program, the initiative swoops in to provide researchers with sufficient resources to develop and include a microbiome investigation. The program helps with experimental design, cultivation of unusual organisms, and data analysis, all free of charge.

A researcher studying obe-

city might build an associated experiment to monitor stool samples. For example, while a study of breast-implant failures can incorporate a check for bacteria on the implants. “It’s kind of like a venture capital model for the microbiome,” Young says.



Top: Harry Mobley, Ph.D., demonstrates the use of laboratory hoods to grow bacteria that thrive in environments with low or no oxygen. Bottom:

Studying drug-resistant foes like VRE and MRSA, which Mobley calls “our most feared bacteria,” is one of the initiative’s signature projects. Looking for vulnerabilities, the project monitors the genes that invasive bacteria express during infections, looking for those whose gene products seem critical for the bacteria’s survival. That information will point researchers toward agents that could target those products and treat the disease with pinpoint precision.

For yet another hospital-acquired infection, *Clostridium difficile*-induced colitis, there is now a weird but astoundingly effective treatment. Also known as *C. difficile* infection, this form of intestinal damage and severe diarrhea is a classic disease of the disturbed microbiome: It targets patients who have received one round of antibiotics too many and suffered a die-off of their normal gut bacteria. In 2013, the CDC classified it as an urgent threat, the highest threat level for antibiotic-resistant bacteria.

Standard treatment for *C. difficile* involves more antibiotics, but only a few work on the germ and the disease often recurs. (Interestingly, in people in whom the disease recurs, the gut microbiome is less biodiverse.) The finding that microbiome diversity is decreased in patients with recurrent *C. difficile* had long been suspected but was formally demonstrated in a 2008 publication from Young’s lab. This finding underlies the rationale for another approach for treating *C. difficile*: reseeded the bowel with healthy gut bacteria by putting one person’s stool into another’s intestinal tract. It’s a simple procedure called fecal microbiota transplantation, and it has revolutionized the treatment of recurrent *C. difficile* colitis. Spearheaded by John Kao, M.D., an associate professor of internal medicine and one of the initial stakeholders in the initiative, U-M’s program has helped at least 40 patients go from debilitated to well. Early indications in the scientific literature show a success rate around 90 percent.

“You could imagine we have very happy customers,” Kao says.

He recalls having trouble finding fellow gastroenterologists to join him in offering the therapy. “As a clinician, you may think of this as sort of voodoo science. ‘What are you doing? Putting stool in patients? Come on, that is almost like bloodletting!’” Kao says. “But as someone who understands the power and importance of gut microbiota, I see this as a tremendous step forward for medicine.”

Making bacteria our allies and sometimes our saviors, represents a massive shift from the conventional wisdom that treats most bacteria as enemies to be blasted with antibiotics. But scientists are eager to explore the new paradigm, to find a way to work with the microbiome — as a partner in human health.

The success for fecal transplantation for recurrent *C. difficile* has prompted initial efforts to determine if it may be useful in other conditions, including those beyond the intestine. There are reports of small numbers of patients who have had improvement following fecal transplant for conditions as varied as multiple sclerosis, diabetes and autism. Young notes, however, that none of these findings have been verified in large clinical trials as has been done with recurrent *C. difficile*.

At the U-M, the Host Microbiome Initiative is proving a crucial resource. Schloss says the research sells itself, with investigators across the medical school contacting him to discuss projects. Kao says that access to it has made him more competitive — he’s gotten favorable comments about it on federal grant applications — and calls it a classic example of team science.

“Having a team of investigators here at Michigan that share the same vision and excitement about the power of the microbiome,” Kao says, “really helps to drive science forward.” [M]

