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INSIDE this issue

The Human Microbiome: Your Own Personal Ecosystem

- Animalcules
  - 1
- Germs
  - 1
- Cultural Variety
  - 2
- A Microbial Menagerie
  - 3
- A Blank Canvas
  - 4
- Stomach Bug
  - 5
- From Battlefield to Ecosystem
  - 5
- The Great Plate Count Anomaly and its Resolution
  - 7
- Unexpected Variety
  - 8
- Weighty Effects
  - 9
- The Human Microbiome Project
  - 10
- A Microbiome Success Story
  - 12
- A New Frontier
  - 12
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The Human Microbiome:
Your Own Personal Ecosystem

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COVER: In 1964 René Dubos wrote: “Our recent studies have revealed that there exists in normal animals an abundant and characteristic microflora, not only in the large intestine, but also in all the other parts of the digestive tract, including the mouth, the stomach and the small intestine. These microorganisms should not be regarded merely as contaminants. Rather, they become so intimately associated with the various digestive organs that they form with them a well-defined ecosystem of which each component is influenced by the others, and by the environmental conditions.” Dubos’s observations as well as discoveries from other scientific disciplines and advances in scientific technologies have allowed scientists to begin exploring the effects of our personal ecosystem on health and disease.
Cover illustration: © Michael Linkinhoker, Link Studio, LLC
We are not alone. For each of the ten trillion human cells in our bodies, there are ten microbial cells living in and on us. Our own cells encode about 20,000 genes, but the sum total of genes in our body is close to a billion. The vast majority of these are the genes carried by microbes.

For more than three hundred years, scientists have observed, identified, and implicated individual microorganisms in specific diseases. More recently, with a convergence of scientific disciplines, an explosion in technical capabilities and revolutionary new ways of thinking, we are exploring the organisms with which we share our bodies. The effects of these organisms—our microbiome—on our health are only just being recognized.

Animalcules

The first person to observe microorganisms associated with the human body was Antonie van Leeuwenhoek, a Dutch merchant in the seventeenth century. Although he did not invent the microscope, he was the first to construct devices with enough magnification and clarity to see life forms as tiny as bacteria. He described and drew pictures of what he saw, including organisms from the cleft in between his teeth and gum, where he observed “many very little living animalcules, very prettily a-moving.” Not content with looking at the stuff in his own mouth, he searched out family members and neighbors, especially those with less-than-perfect oral hygiene, where he found “an unbelievably great company of living animalcules, a-swimming more nimbly than any I had ever seen up to this time.” He even described what seems to have been the intestinal pathogen *Giardia lamblia* from his own feces during a bout of diarrhea.

Leeuwenhoek reported in great depth on his findings with a sense of wonder and curiosity. He made no connection between microbes and disease, since at the time infectious diseases were thought to be caused by poisonous vapors, the influence of heavenly bodies, and bad smells.

Germs

Two centuries later, in France, Louis Pasteur studied the organisms that ferment wine and beer (beneficial organisms, to be sure). He determined that the “wrong” organisms could spoil the beverage. This led him to the idea that specific microbes could cause diseases in humans. The germ theory of disease was further expanded by Robert Koch, who was responsible for several technical and conceptual innovations that would make the modern science of microbiology possible.

At the time, microbiologists grew bacteria in liquids such as meat broths. If more than one kind of organism grew in the liquid, it was impossible to separate them. Koch, however, developed a way to obtain pure cultures. At first, he used the surface of sliced potatoes. Each of the small raised bumps that grew on the potato surface was a colony of bacteria derived from a single organism. Koch began looking for a more suitable solid on which to grow bacteria. Initially, the best solution seemed to be gelatin, an animal protein, but it was not without problems. For one thing, many bacteria consume gelatin, and those that caused disease in humans grew best at human body temperature—the point at which gelatin melts. Even on very warm days, gelatin would turn from solid to liquid.
Walther Hesse, a scientist in Koch’s lab, told his wife, Angelina, about the gelatin problem. Angelina knew that her fruit jellies stayed solid throughout the summer heat because she used agar-agar. First used in Japan in the 1600s, agar-agar (or agar) is derived from seaweed and is sometimes used today as a vegetarian substitute for gelatin. Unlike the protein gelatin, agar is a polysaccharide that is not generally used by bacteria as food, and it stays solid at temperatures used to grow the cultures.

Staples in the modern microbiology lab, agar and the Petri dish (invented by Julius Petri in the Koch lab) made it easy to isolate and grow pure cultures of bacteria descended from a single cell. By diluting a liquid culture, and then spreading it on agar in Petri dishes, bacteriologists could count the colonies and calculate how many bacteria were in the original culture.

Important as these technical innovations were, Koch’s conceptual contribution was perhaps the most influential. Koch’s postulates were formulated to determine the microbial cause of infectious disease. Using these rules, scientists could be sure that the organism in question was the perpetrator of pathogenesis and not just an innocent bystander.

In 1876, Koch was the first to associate a particular disease, anthrax, with a specific organism, *Bacillus anthracis*. He also discovered the microbial causes of tuberculosis and cholera. In the next thirty years, the bacteria that cause more than a dozen diseases, from gonorrhea to dysentery to whooping cough, were identified. For a century, Koch’s postulates were the gold standard for determining the cause of infectious disease.

**Cultural Variety**

At first, scientists could only tell one kind of bacterium from another by their shape and ability to move around. In 1884, Danish bacteriologist Hans Christian Gram introduced a stain that made bacteria easier to see under the microscope. It also turned some bacteria purple and some pink. This first differential stain, based on structural differences of the cell wall, is still used to...
classify bacteria—as either Gram-positive or Gram-negative. Today, there are many different stains that can help further identify and differentiate microbes.

Soil microbiologists were the first to use functional properties of bacteria to select for specific strains. Using agar that contained no nitrogen, they were able to selectively grow bacteria that could obtain nitrogen solely from the air. Taking a clue from soil microbiologists, medical microbiologists were able to identify and study bacteria based on their metabolic functions, such as which nutrients they can utilize or which metabolic products they excrete.

Some culture media contain indicators that change color according to pH and can thereby identify organisms that produce acidic products. Others contain chemicals that specifically inhibit or enhance growth of particular organisms.

The onset of World War I brought bacteriology to the battlefield. Soldiers wounded in the trenches often developed gangrene, which killed tissue and sometimes required limbs to be amputated. Microbiologists could identify bacteria in the wounds with microscopes, but they couldn’t cultivate the organism in the lab, no matter which special nutrients they used. The organisms that cause gas gangrene, Clostridium perfringens, are anaerobes, which only grow in the absence of oxygen. Paul Fildes and James McIntosh solved the problem by inventing a device that could create an oxygen-free environment.

Figure 5 – Gram-positive and Gram-negative: Because of the thickness and composition of their cell wall, Gram-positive bacteria retain the crystal violet stain. Gram-positive bacteria include Staphylococcus, Streptococcus, and Clostridium. Gram-negative bacteria do not retain crystal violet, so the stain is rinsed away, and, after counterstaining, the organisms appear pink under the microscope. Examples of gram-negative bacteria include E. coli, Salmonella, and the organisms that cause cholera and gonorrhea.

Illustration: © Michael Linkinhoker, Link Studio, LLC.

A Microbial Menagerie

As new culture media and techniques were developed, more disease-causing microorganisms were discovered, identified, and studied. When microbiologists turned their attention to the healthy human body, they found a zoo of organisms on just about every surface, inside and out—in the nostrils and lungs, the vagina, and even on the...
surface of the eye. The skin, depending on the site, has between 100 and 10,000 organisms per square centimeter. The richest populations of microbes occur in the gastrointestinal tract, from the mouth to the anus. Saliva contains ten million organisms per milliliter. The colon contains the largest population of organisms, and about a third of the mass of feces is microbes (100 billion microbes per gram).

This diverse menagerie consists mostly of bacteria, but it also includes fungi (especially yeasts), protozoans, viruses, and organisms from the kingdom we now call archaea. Archaea look like bacteria, but genetic analyses have determined that they are as different from bacteria as we are from trees. Many of the organisms in the gut, mouth, and vagina are anaerobes.

The residents of the menagerie—often called normal microbiota, or commensals—are not constant. Populations differ vastly between individuals and over the course of an individual’s life. While the fetus grows in an essentially sterile environment, infants are colonized quickly. Studies in experimental animals show that the organisms in the gut change in number and character after birth. In humans, a similar story emerged through the study of feces. The organisms associated with babies delivered vaginally look like those of the mother’s vagina, but babies born by cesarean section are originally colonized by organisms from the mother’s and the delivery nurses’ skins. The nature of gut microbes also differ between breast-fed and formulafed babies. A child continues to acquire new microbiota and by three years of age possesses an adult-like assortment of organisms. Even in adults, normal microbiota can vary with diet, disease, medications, puberty, climate, occupation, and other factors. Scientists suspected that the microbiota in and on our bodies may have an impact on health and disease, and they developed new techniques to figure it out.

A Blank Canvas

Just as an agar plate must be sterile in order to study the organisms used to inoculate it, studying the effects of microbes on the lives of those they inhabit is best studied in an animal with no indigenous microbes. Building on discoveries in soil science, Pasteur thought that the study of microbes in animals might benefit from the use of “pure” animals. A hen’s egg, he had suggested, could be hatched and raised in a sterile environment.

Laboratory mammals such as mice, rats, and guinea pigs would require a more complicated procedure, finally perfected in the 1950s. The animal is delivered surgically to avoid contact with the mother’s microbiome. It is raised in a sterile environment and supplied with sterile food and water. Once a colony of germ-free animals is established and maintained, they can reproduce naturally to produce germ-free offspring, since there will be no microbes to contaminate the newborns. The germ-free animal is a sort of blank canvas. Further studies can be done by introducing specific, known organisms or populations of organisms to produce what is known as a “gnotobiotic” animal (from the Greek roots meaning known and life). They can even be colonized with organisms from other animals, like humans. Research with germ-free and gnotobiotic animal models has revealed the many beneficial functions of our microbiome.

Animals raised in the absence of any sort of microbe are certainly viable, but they develop quite abnormally. The heart, lungs, and livers are smaller than those of conventionally raised animals. The most noticeable changes occur in the digestive tract. The part of the intestine called the cecum is dramatically enlarged, and the structure of the intestinal lining is altered, especially in places where microbes would normally be found in large numbers.

Animals grown without microorganisms require about 30% more calories than conventionally grown animals to maintain their weight. Studies in germ-free animals
have helped us understand the importance of microbes in human nutrition. Nutrients that our own cells cannot utilize are readily gobbled up by our gut microbes. Most complex carbohydrates and plant polysaccharides, for example, cannot be broken down by human enzymes. Gut microbes ferment these large molecules into smaller molecules that can be absorbed and used for energy by human cells. In the process, they may produce gas; gut microbes are the main culprit in flatulence. Gut microbes also produce nutrients that we couldn’t get otherwise, such as vitamin B12 and vitamin K, an important blood-clotting factor.

Microbes form a barrier between us and the outside world. The microbes in our guts and on our skin and mucous membranes colonize those surfaces, preventing disease-causing organisms from gaining a foothold by competing for nutrients and blocking attachment sites. They also secrete compounds that inhibit pathogens by altering the local environment (sometimes lowering pH, for example) or directly antagonizing pathogens.

Animals raised in a germ-free environment are much more susceptible to infectious disease than their conventionally raised cohorts for another reason: the immune system is dependent on the normal microbiota for its development. It is believed that the microbes normally found in the gut educate the immune system to attack microbial invaders that may cause disease, while tolerating beneficial microbes.

**Stomach Bug**

For many years, it was thought that no microbes could survive the highly acidic environment in the stomach. As recently as the 1990s, medical textbooks stated that there were no pathogens in the stomach and that the only organisms that could be found there were transients that had been swallowed but did not stick around.

Although German scientists had observed a spiral shaped organism in the stomach in 1875, they were unable to grow the organism in the lab, so it was forgotten. However, in 1971, Australian pathologist Robin Warren began examining tissue from patients with stomach ulcers. He consistently found the distinctive S-shaped bacteria and shared his findings with internist Barry Marshall.

The medical consensus at the time was that gastritis and ulcers were caused by too much acid—from stress or spicy food, for example. Warren and Marshall used Koch’s postulates to investigate the possibility that ulcers were caused by a bacterium called *Helicobacter pylori*. (See Breakthroughs in Bioscience article, “Helicobacter pylori and Ulcers: A Paradigm Revisited”) *H. pylori* infection, in most cases, does not cause symptoms, while in some, it causes gastritis and ulcers. Marshall and Warren were awarded the Nobel Prize in 2005 for their discovery that *H. pylori* infection was a major cause of chronic gastritis, peptic ulcers, and some types of stomach cancer. This discovery increased our understanding of the connection between chronic infection, inflammation, and cancer. Treatment of *H. pylori* infection with antibiotics became the treatment of choice for ulcers, and the incidence of stomach cancer fell. We have now learned that *H. pylori* can be protective for some types of gastrointestinal disease including gastroesophageal reflux disease (GERD).

A single organism in a sparsely populated part of the body presents a complicated picture if it can both cause and prevent disease. What of the situation farther downstream, in the intestines, where the number and diversity of microbes increases dramatically? The task of determining what is protective and what is pathogenic becomes even more complicated.

**From Battlefield to Ecosystem**

As a college student in France, René Dubos disliked chemistry and microbiology. A chance meeting with Selman Waksman onboard a ship across the Atlantic in 1924, however, found himself in Waksman’s lab at Rutgers University studying the relationship between soil...
After finishing his doctoral degree, another chance meeting brought him to Oswald Avery’s lab at the Rockefeller Institute. Like so many investigators before him (and many to follow), Dubos looked to the soil. He isolated several drugs from soil bacteria, including the antibiotic gramicidin. His success encouraged others to investigate soil bacteria for antibiotics. Selman Waksman was one of those investigators. Waksman discovered streptomycin and credited his former student Dubos, noting that “to obtain the desired results required an analytical mind, an original coordination of all the facts, and especially a new philosophy.”

The microbiological paradigm. He studied the interactions of microorganisms with their environment and with each other. In contrast to the warlike model associated with the germ theory, in which specific disease-causing microorganisms were “the enemy,” Dubos promoted a more complex model, calling the human digestive tract an ecosystem. He changed the conversation from Us Against Them to We’re All in This Together.

In 1964 he wrote: “Our recent studies have revealed that there exists in normal animals an abundant and characteristic microflora, not only in the large intestine, but also in all the other parts of the digestive tract, including the mouth, the stomach and the small intestine. These microorganisms should not be regarded merely as contaminants. Rather, they become so intimately associated with the various digestive organs that they form with them a well-defined ecosystem of which each component is influenced by the others, and by the environmental conditions.”

Dubos believed that the true character of microbes could not be discovered by studying them in isolation. Microbes inhibit and enhance each others’ growth. They communicate with each other and alter their environments. It was important, but complicated work. The ecosystem model emphasized the interactions between organisms, but nobody knew what most of them were. How can you study an ecosystem if you don’t know what most of the organisms are? It would be like trying to study a forest by just looking at the squirrels.

**Figure 8 – René Dubos:** René Dubos studied the ecosystems within us and around us. In his later years, Dubos was an outspoken environmentalist, concerned with environmental influences on human health. He won a Pulitzer Prize for Nonfiction in 1969 for So Human an Animal: How We Are Shaped by Surroundings and Events. He coined the phrase “Think globally, act locally.”

*Image credit: Rockefeller Archive Center*

**MICROBIOME: AN ECOLOGICAL COMMUNITY**

The term “microbiome” was coined in 2001 by Nobel Prize-winning microbiologist Joshua Lederberg, “to signify the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and have been all but ignored as determinants of health and disease.”

Lederberg used the language of ecologists. Those investigating the microbiome often use the tools developed by microbial ecologists to identify the organisms in and on us and to understand the relationships between them. The relationships can take several forms.

- **SYMBIOSIS:** Any interaction between two or more species.
- **MUTUALISM:** Both species benefit and depend on each other for survival.
- **COMMENSALISM:** One species benefits, while the other is not affected.
- **PARASITISM:** One species benefits, while the other is harmed.
- **COMPETITION:** One species deprives another of a resource (such as a nutrient).
- **ANTIBIOSIS:** One organism damages the other through a secretion (such as an antibiotic).

Within an ecological community such as the microbiome there are niches, each with its own inhabitants. The organisms that live behind your ear, for example, are different from those between your toes.
The Great Plate Count Anomaly and its Resolution

Although the concept of studying the microbiome as an ecosystem was groundbreaking, the technology did not exist at the time to make that study possible. One major hurdle was known as “The Great Plate Count Anomaly.” The number of bacteria in a sample (from a person or a lake, for example) counted directly under the microscope did not match the number of colonies that grew on agar plates. Scientists could see more microbes—sometimes a hundred times more—than they could cultivate in the lab. It seemed most microbes were “viable but not cultivable.” Since the only way to study them was to grow them in the lab, the majority of these microbes remained uncharacterized mystery organisms. It was unknown what else was out there—and in us.

Once again, environmental microbiologists led the way. Vexed by the Great Plate Count Anomaly, they hypothesized that something about the environment from which they obtained their samples was needed for the organisms to grow in the lab (just as anaerobic bacteria from gangrenous wounds would only grow in an environment devoid of oxygen). They devised methods to cultivate microbes in conditions that mimicked the natural environment using chambers that would allow nutrients and other molecules—but not other organisms—to diffuse into the culture. They also used “helper” organisms from the same environment to provide needed growth factors. For example, bacteria need iron, but environmental iron cannot get into the cell unless it is bound to a bacterial product called a siderophore. Some microbes cannot make their own siderophores and must use siderophores produced by other organisms to obtain their iron.

**Figure 9 – Metagenomics:** A sample is taken (A) directly from the environment (such as soil, water, feces, or skin). DNA is extracted (B), broken into random fragments (C), sequenced (D), and analyzed (E) with the help of computers. Analysis of specific genes, such as 16SrDNA, will reveal which organisms are present in the sample and how they are related (phylogeny). Analysis of other genes and comparison to sequences of known genes can yield clues to their potential functions. Illustration: © Michael Linkinhoker, Link Studio, LLC.
Finally, environmental microbiologists got past the cultivation problem by skipping it altogether. They applied sensitive DNA-based technologies to look directly at the genes. It was a culture-independent way to study complex bacterial communities.

Metagenomics (also called community genomics, environmental genomics, or population genomics) examines the genes of a population of microbes as a whole, instead of individual organisms. DNA is obtained from microbial samples taken directly from the environment without culturing them. The extracted DNA contains a mixture of genes from all the organisms in the sample.

Specific genes can be analyzed using polymerase chain reaction (PCR), which makes millions of copies of specific sections of DNA between the short sections recognized by defined sequences called primers. Environmental microbiologists used PCR to analyze 16S rDNA, the gene that encodes part of the protein-assembling ribosomes found in all bacterial cells. This gene has sections that are common to all bacteria, as well as highly variable sections, that can be used to identify individual organisms and to see how closely related they are to each other. These investigators used 16S rDNA sequencing to identify 16S rDNA sequences gave microbiologists a lot of information about what types of organisms were present and how they were related, but it was only one gene. There were literally millions of genes in the uncultivable organisms, and we knew nothing about them.

Another kind of metagenomics (sometimes called “shotgun” metagenomics) is used to examine all the genes in a population. Random genes in a sample taken directly from the environment are analyzed using automated high-throughput technologies, which can quickly sequence vast numbers of genes. The new sequences can be compared to the sequences of known genes to guess their function or can be inserted into domesticated bacteria to find out for sure. Because the number of different genes that can be found in an environmental sample is so huge, advanced computational techniques, called bioinformatics, are needed to make sense of it all.

**Unexpected Variety**

David Relman, a postdoctoral fellow in Stanley Falkow’s lab at Stanford in the late 1980s early 1990s, studied a disease of the skin and lymph nodes of AIDS patients called bacillary angiomatosis. Although investigators could see what looked like bacteria in the lesions when they examined the tissue under the microscope, nobody was able to cultivate any sort of organism.

Falkow suggested to Relman that he try some molecular techniques to solve the problem, just as environmental microbiologists were doing at the time. Relman set to work finding a way to identify organisms that couldn’t be grown in the lab by looking for their genes.

Relman used the 16S rDNA technique on diseased tissue and found a sequence that was closely related to the organism that causes cat scratch disease. In the paper describing the experiment, he said, “We expect that this approach will be applicable to other infectious diseases with unclear causes and will expand our understanding of interactions between humans and microbes.”

In designing his experiment using this extremely sensitive technique, Relman was careful to choose tissues that did not have a lot of other organisms in them so that he could find the organism that was causing the disease more easily. Then, he said, “it became clear to me that what I was considering the background problem was actually an interesting topic unto itself.” He began to look at parts of the body that he knew contained numerous organisms.

He started with the mouth. His own mouth.

“I had this idea as I was getting ready to go to my dentist. I went into work first, picked up some sterile collection tubes, brought them with me to the dentist’s office, and asked him, as he was cleaning my teeth, would he mind putting this stuff into these tubes, instead of throwing it out.” At the time, about 500 different organisms were known to inhabit...
the gum pocket, some harmless, and some that can cause diseases such as dental cavities, gingivitis, and periodontal disease. Relman and his colleagues compared the results of the 16S rDNA analysis with traditional culture techniques. The molecular technique revealed a more diverse population than the culture technique, with many types never before observed in humans.

Relman then turned his attention to the stomach, where he discovered, in addition to *H. pylori*, over one hundred new types of bacteria living there. This led to a study of the lower gastrointestinal tract, home to trillions of microorganisms, the vast majority of them uncultivated and unstudied. In a study directed by Karen Nelson of the J. Craig Venter Institute, the fecal DNA from two healthy adults was sequenced by the 16S rDNA and metagenomic methods. This study revealed that the metabolic potential of the microbes in the intestines was significantly greater than that of human cells alone. The authors described humans as “superorganisms,” which use both host and microbe functions to obtain energy and nutrients from food, to synthesize vitamins and amino acids, and to break down drugs and toxins.

**Weighty Effects**

Even without knowing exactly what organisms are in the microbiome, evidence continues to mount that they have profound effects on health. Jeffrey Gordon and colleagues at Washington University in St. Louis studied the effect of gut microbes on obesity. In one set of experiments, they transplanted gut microbes from mice with genetic or dietary obesity into germ-free mice. The recipients gained more weight (and fat) than germ-free mice that received gut microbes from lean mice.

Another study by Gordon’s group transplanted gut microbes from humans into germ-free mice. Gut microbes from four sets of twins, each pair with one lean and one obese twin, were transplanted directly (without any attempt to cultivate them). The mice that received microbes from the obese twins got fat, and those that were colonized with microbes from the lean twin did not, but only when they were housed separately. When they were housed together, the mice transplanted with obese microbes acquired microbes from the lean mice and all the mice remained lean.

More studies followed, many with intriguing results, but one question remained unanswered. What is a healthy microbiome? The variations between individuals, laboratories, techniques, and protocols made it difficult to sort out. To what extent are the differences biologically relevant—associated with health or disease—or natural variation? A systemic way to answer the question would
be a huge undertaking and would require a coordinated effort from many researchers.

**The Human Microbiome Project**

In 2003, the first complete human genome was published and hailed as a milestone of scientific discovery. (See the *Breakthroughs in Bioscience* article, “Individualized Medicine: Genetically Fine-Tuning Prevention, Diagnosis, and Treatment of Disease”)

On the heels of the Human Genome Project, funded by the National Institutes of Health (NIH) and the Department of Energy, scientists recognized that the human genes were only part of the story. To fill in the other genome—the human microbial genome—would require an even larger effort.

As is often the case, technological advances allow us to ask new questions in ways that were not possible a decade ago. The Human Genome Project took thirteen years, cost about three billion dollars, and required the cooperation of many universities and other research institutions in the U.S., United Kingdom, France, Germany, Japan, and China. In 2014, the same number of genes could be sequenced in days, at a cost of about $1,000, in a single lab.

The first phase of the Human Microbiome Project (HMP), a five-year study beginning in 2007, coordinated the work of over 200 researchers in 80 institutions. The study was designed to compare the microbiomes at multiple body sites over a large number of people and to look at changes in individual microbiomes over time. Standardized procedures were created to minimize sampling differences between laboratories. The goal was to construct a reference database of sequences for the genes of all the organisms in the samples, both the cultivable and the (much more numerous) uncultivable microbes.

The study, funded by the NIH, recruited 300 subjects (149 men and 151 women), age 18 to 40. Since the purpose of the study was to find out what constitutes a healthy microbiome, people with certain medical conditions were excluded from the study. To minimize the effects of external factors, participants were instructed to refrain from using certain medications and were given a kit of personal care products to use for a period before the study. The investigators asked subjects numerous questions about their medical history, family situation, diet, lifestyle, occupation, and so on.

Samples were taken from four sites on the skin (behind each ear and inside each elbow), six sites on the soft tissue inside the mouth, the teeth above and below the gumline, the nostrils and, from women, three sites in the vagina. Subjects also provided saliva and stool samples. Some subjects gave samples two or three times over the course of the study to see if (and how) the organisms changed over time.

Once the difficult work of collecting samples and subject information was accomplished, there was even more complex work: processing and sequencing the samples. To identify and classify the organisms, investigators used the 16S rDNA method. Metagenomic analysis was used to determine the potential

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**Figure 12 – Lean/obese twin study:** On the same diet, germ-free mice transplanted with gut microbes from an obese twin gain fat, while those given microbes from a lean twin do not. When the mice are housed together, the mice with the microbes from the obese twin acquire the microbes from the mouse with the lean microbes and do not gain fat.

*Illustration: © Michael Linkinhoker, Link Studio, LLC.*
functions of the genes in the microbial community. The end result was a mountain of data that required serious computer power and new kinds of bioinformatics.

On June 13, 2012, NIH director Francis Collins announced the completion of a reference database constructed from 5,000 samples. They identified at least 10,000 distinct microorganisms and eight million microbial genes—360 times as many microbial genes as human genes.

The main finding was that there are many different microbiomes that are considered healthy. The individual genetic signatures of the microbiomes vary widely between individuals. Metabolic function is more important than microbial composition; what they do is more important than what they are. If a microbiome is disrupted (by antibiotics, for example) it could eventually move back to a healthy state, but perhaps not with the identical microbial species.

Knowing what is “normal” and being able to diagnose or treat a condition or disease based on microbiota are two very different things. Using the data generated by the Human Microbiome Project, research continues on the effects of the microbiome on human health, especially in the gut, which has strong connections to the immune system, the nervous system, and other aspects of health and disease. The potential uses of the data generated by the Human Microbiome Project have yet to be fully realized, but it is clear that it will be a valuable resource for future research.

**THE MICROBIOME IN HEALTH AND DISEASE**

Every day it seems the news is full of stories of a new association between the microbiome and some disease or condition. The microbiome likely does affect many aspects of our health, but the complexities will take years to untangle. Research continues on potential diagnostic or therapeutic aspects of the microbiome.

Asthma and allergies
Diabetes
Obesity and malnutrition
Crohn’s disease and ulcerative colitis
Gastrointestinal cancers
Psoriasis and atopic dermatitis
Rheumatoid arthritis
Muscular dystrophy
Fibromyalgia
Multiple sclerosis
Anxiety
Depression
Schizophrenia

**Figure 13 – Organisms in parts of the body:** Microbes can be found anywhere the body has contact with the environment, including the digestive tract from mouth to anus, and the vagina. The composition of microbes in different parts of the body can vary widely. Each person’s microbiome is different and can change with diet, illness, medication, geography, and age.

*Image credit: Daryl Leja/National Human Genome Research Institute; Reprinted with permission from the Annual Review of Genomics and Human Genetics, Volume 13 © 2012 by Annual Reviews, http://www.annualreviews.org*
A Microbiome Success Story

In 2008, a 64-year-old woman came to Dr. Alexander Khoruts desperate for help. She had taken antibiotics after some surgery and to treat a case of pneumonia. As a result, she developed an infection with the bacterium *Clostridium difficile*. Despite treatment with multiple courses of antibiotics, her condition worsened. Suffering from severe diarrhea, she had lost 60 pounds in eight months.

*C. difficile* (or *C. diff*) infections are a growing problem, especially in hospitals and long-term care facilities. The U.S. Centers for Disease Control and Prevention (CDC) reports that the number of cases has tripled in the last decade, to 250,000 cases (and 14,000 deaths) per year. *C. diff* infections often occur after antibiotics have disrupted the gut microbiome, as was the case with Khoruts’s patient. Treating with more antibiotics to kill the *C. diff* may work, but its spores survive and the infection recurs.

Dr. Khoruts treated the patient with more antibiotics, with no success. He then decided to try a different strategy. Instead of killing more of the gut microbes with antibiotics, he would try to restore a normal complement of gut microbiota. For that, he turned to the patient’s husband, or more specifically to his feces, which were homogenized and reintroduced into the patient. Her diarrhea stopped within a day.

There was one small drawback. As Khoruts described it, “The olfactory potency of human fecal material revealed at the touch of a button on the blender can be quite shocking—it can empty waiting rooms.”

This was not the first fecal transplant in history. In China, Ge Hong in the fourth century and Li Shizhen in the sixteenth century successfully treated patients with fecal suspensions, which they sometimes called “yellow soup.” In the seventeenth century, Fabricius Aquapedente performed a similar procedure on cows. In 1958, Dr. Ben Eiseman and his team used a fecal enema to treat a case of pseudomembranous colitis—a severe manifestation of *C. diff* infection with a 75% mortality rate. The patient walked out the door of the hospital a few days later.

It had been assumed that this type of treatment, also called fecal microbiota transplantation (FMT), restores the gut ecosystem to a normal state, but Khoruts wanted to find out. He took a sample of the patient’s gut microbiota before the procedure and found a much less diverse population with many unusual species. After using an endoscope to introduce a preparation of the husband’s gut microbiota, he tested again. This time the profile looked more like that of her healthy husband.

As distasteful as the thought of administering human feces to a patient might be, the results have been astounding. In hundreds of subsequent cases of *C. diff*, fecal transplants have achieved over 90% cure rates. The technique is being refined to be more precise, more convenient, and less pungent.

**A New Frontier**

We now think about the organisms that share our bodies not as invaders, but as integral parts of ourselves. Using new technologies and borrowing strategies from other disciplines, we are learning what constitutes a healthy microbiome and starting to see how changes in our personal ecosystems can affect our health. New discoveries, built upon knowledge from seemingly unrelated research, may lead to new treatments for a variety of disorders. Since Dubos and Waksman isolated antibiotics from soil bacteria, more than 50,000 products of environmental microorganisms have been identified. The human microbiome has enormous potential as a source of novel drugs. New technologies are allowing scientists to isolate products of organisms in the environment and in ourselves without cultivating them in the laboratory. In addition, a more thorough understanding of the organisms and their growth requirements will expand our knowledge of basic biological processes. David Relman once said, “one of the most important ecosystems on the planet might be the human body.” Our exploration of that ecosystem is just beginning.

**ADDITIONAL SUGGESTED READING**

NIH HMP site http://commonfund.nih.gov/hmp/programhighlights

*Scientific American Magazine* June 2012 Issue “Your Inner Ecosystem”

*The New Yorker* October 22, 2012 “Germs Are Us” by Michael Specter (http://www.newyorker.com/magazine/2012/10/22/germs-are-us)


http://www.actionbioscience.org/genomics/the_human_microbiome.html

http://learn.genetics.utah.edu/content/microbiome/

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