# The startup bugs

Burgeoning research on the human microbiome is facilitating the development of more mechanistically driven probiotics. Charles Schmidt investigates a smattering of probiotic- and microbiota-modifying therapeutic opportunities arising from commercial efforts.

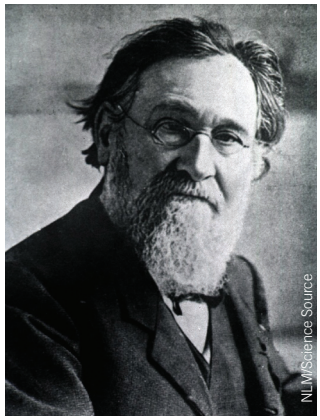
With the advent of high-throughput molecular-profiling technologies, the diversity and depth of the human microbial ecosystem—or microbiome—is coming into focus. At the same time, renewed appreciation for the effect of changes in the microbiota on human physiology is adding tantalizing wrinkles to knowledge of the action of existing treatments, such as bariatric surgery, where changes in flora after surgery have been associated with weight loss and loss of insulin resistance in obese people with type 2 diabetes[1]. What's more, traditional treatments that transfer human intestinal flora from healthy to diseased individuals have been recently validated in randomized trials[2], providing at least some clinical support for microbiome-modulating treatments.

Bolstered by these insights, companies are trying to move beyond the more general health benefits associated with probiotics. Yet these are early days for the field and scientists have a long way to go before the microbiome yields commercially approved treatments for disease. "The possibilities are exciting," says James Brown, director of computational biology at GlaxoSmithKline, in Collegeville, Pennsylvania. "We're opening up a whole new range of targets and therapeutic opportunities for treating complex, chronic diseases by modulating the gut microbiome itself."

## Setting the stage

Last June, the federally funded US Human Microbiome Project reported the first results of a massive sequencing project that details the entire human microbial makeup from 18 body sites in 242 healthy volunteers, thus opening a window into microbial diversity across populations[3,4]. Along with other large-scale sequencing programs, such as MetaHit in Europe—a European Commission–funded international consortium[5]—the project provides a reference for studies investigating how dysbiosis, an imbalance in the ratio of harmful to beneficial microbes in the body, can make people sick.

Once viewed chiefly as pathogens, microbes are now also seen as symbiotic partners that co-evolved with humans over millions of years. Developing babies grow in the relative sterility of the womb, but passage through the bacteria-laden birth canal 'seeds' a microbiome that shapes human immunity even as the growing immune system shapes the microbiome itself. By some estimates, the microbiome will eventually acquire 100 times more bacterial cells than there are human cells in the body. Beyond their roles in immunity—which include stimulating and maintaining populations of anti-inflammatory T cells—gut microbes confer many other benefits: They break fiber into digestible short-chain fatty acids, they detoxify poisons and drugs, they protect against stress-induced lesions in the gastrointestinal (GI) tract and they make vitamins.




The Nobel Prize–winning Russian scientist Elie Metchnikoff drank sour milk every day, claiming that it contained health-promoting bacteria that ward off age-related maladies.

As demonstrated by a surge in human microbiology research papers—from 500 publications in 2002 to more than 2,000 this year (See Feature by Olle, page 309)—scientists are intent on understanding how microbes and their human hosts interact. What remains elusive is a clearer picture of how changes in human microbial composition correspond with changes in health.

Evidence for a few associations exists. In 2008, a French team was able to link depleted populations of *Fecalibacterium prausnitzii*, an anti-inflammatory human bacterium, to relapsing Crohn's disease within six months of surgery[6]. The authors concluded that "counterbalancing dysbiosis using *F. prausnitzii* as a probiotic is a promising strategy in Crohn's disease treatment." And in a landmark 2006 study, Jeffrey Gordon from Washington University, in St. Louis, was able to induce excessive weight gain in germ-free, lean mice simply by gavaging them with the gut microbiota of obese mice that had been fed Western diets high in fat and sugar[7]. That result, Gordon says, helped to confirm that the gut microbiome can play causal roles in obesity. Similarly, Martin Blaser, from the New York University Langone Medical Center, found that antibiotic exposure among human babies alters the microbiome in ways that can lead to obesity and insulin resistance, which is a key risk factor for diabetes[8].

## Flora, disease and wellness

Beyond those and a few other examples, the microbiome's links with disease remain poorly characterized, according to Jens Walter, an associate professor in food technology and microbiology at the University of Nebraska in Lincoln. "We have some information about disease and dysbiotic patterns in animals, but in humans, we have very little," he says. A key need, Walter says, is to determine when microbial changes predispose, cause or exacerbate a given disease, as opposed to the disease itself causing the microbial change. That's a crucial distinction for therapeutic development, but it can only be resolved with more research, Walter says.

According to Andrew Benson, director of the Core for Applied Genomics and Ecology at the University of Nebraska, Lincoln, only a few bacterial genera, namely *Bifidobacterium*, *Clostridium*, *Bacteroides* and *Fecalibacterium* among them, predominate in the human microbiome. "These are the heavy hitters that co-evolved with the host," Benson says. And for the most part, commercial probiotics don't include these commensal human varieties. Instead, they lean toward *Lactobacillus*, a genus that barely exists at all in the human gut, according to Benson, or toward noncommensal strains of bifidobacteria, chosen largely on the basis of their ability to survive commercial packaging and a research history in microbiology showing that they can resist stomach acids, can split lactose and can trigger macrophages in the immune system (**Box 1**).

Jalil Benyacoub, head of the immunology group at Nestlé Research Center in Lausanne, Switzerland, says the probiotics industry emerged decades ago from efforts to transfer bifidobacteria—detected at high levels in the gut microbiota of breast-fed babies—into infant formula to control diarrhea. From there, he says, the industry expanded towards lactobacilli found in yogurt and fermented milk products, as an immune-boosting nutritional supplement.

Because they're not commensal, however, commercial probiotic strains don't colonize the human gut. "As soon as you stop eating them, they disappear from your body within two

## Box 1 Whence probiotics?

Food and dietary supplements companies have long claimed that probiotics in yogurt and other cultured dairy products improve digestion, boost immunity and confer other health benefits. Julian Mellentin, editor of the London-based food industry trade magazine *New Nutrition Business*, says consumers are increasingly buying into that concept. Aging populations and female consumers in particular are fueling much of the probiotic industry's growth, which is approaching $7 billion in Europe and $1.7 billion in the US, according to his analysis.

But regulatory authorities, particularly in Europe, have been thus far unimpressed with data put forth to support probiotic health claims. This year, the European Food Safety Authority (EFSA), a scientific advising body to the EU, rejected nearly every health claim put forward by the probiotics industry. In an e-mail, EFSA spokesperson Jan Op Gen Oorth wrote that the claims were rejected either because the microbes had not been sufficiently characterized, because the claimed effect was not considered beneficial (the EFSA panel opined that increasing numbers of bifidobacteria in the gut are not in themselves beneficial) or because human studies in support of the claims had not been made available. The EU stated that after December 14, 2012, food and nutritional supplements companies will no longer be allowed to communicate health benefits for their products on account of probiotic content.

Shelly Burgess, spokesperson with the US Food and Drug Administration (FDA), declined to comment on whether similar measures might be taken in the US. FDA regulates probiotics under the "generally recognized as safe" provision of the Food, Drug and Cosmetic Act. As long as a company sticks to nonspecific structure and/or function claims (that is, "improves digestive health"), it can sell the probiotics without regulatory worries. Trouble brews when companies start making more specific, drug-like claims that would otherwise require supporting evidence from human clinical trials.

In 2010, two companies—Dannon of White Plains, New York, and Nestlé, headquartered in Vevey, Switzerland—learned the hard way that drug-like claims in the absence of IND-supported data can incur regulatory wrath. After Dannon advertised that its probiotic yogurt, Activia, prevents colds in children by "strengthening the body's defenses" (achieved by speeding transit time of food in the digestive tract, they say), it was sued for false claims on December 15, 2010, by the US Federal Trade Commission (FTC), which claimed that the company had not supplied adequate human data to support such a conclusion.

Shortly thereafter, the FTC forced Nestlé Healthcare Nutrition (a division of Nestlé) to stop advertising that its probiotic beverage, BOOST Kid Essentials, prevents colds and flu by boosting immunity and helping children recover more quickly from diarrhea. In its July 14, 2010, statement, Nestlé responded that it would henceforth market the beverage only as "providing complete nutrition for kids ages 1 to 13."

Mary Ellen Sanders, a probiotics industry consultant based in Centennial, Colorado, says food and nutritional supplements companies generally balk at pursuing IND studies, especially if they involve products with a long history in the marketplace. In some cases, she says, companies have human data in support of their more specific claims. However, FDA will not consider those data if they're gathered outside the IND process, she says.

In Mellentin's view, Europe's probiotics industry will survive these regulatory setbacks. The major probiotic suppliers, he says, namely Nabisco of East Hanover, New Jersey; Chr. Hansen of Hoersholm, Denmark; and Copenhagen-based DuPont Danisco and Nestlé, are now collaborating on ways to advance human probiotic research consistent with the EFSA's regulatory demands. What's more, Mellentin points out that regardless of how they are labeled, probiotic products will remain popular simply because eating them makes people feel better. "If the products relieve symptoms, say bloating or indigestion, then consumers remember that and they become buyers," he says.

R. Balfour Sartor of the University of North Carolina School of Medicine, in Chapel Hill, feels that probiotic treatments still hold tremendous therapeutic potential. "We're beginning to see that it's possible to manipulate the microbiome—its gene expression and metabolic and transcriptional regulation—through the diet," he says. "And from limited studies, it's becoming clear that these manipulations can potentially lead to therapeutic and disease-prevention outcomes. But the food industry hasn't invested proper funds into clinical studies that could show these interventions are effective." Hard evidence of therapeutic benefits will require randomized, placebo-controlled human studies, or in other words, IND-based clinical trials.

weeks," says R. Balfour Sartor, of the University of North Carolina School of Medicine, in Chapel Hill. And Benson adds that scientists still know little about what the commercial probiotic strains actually do in the human GI tract. "There's not a lot of hard evidence," he says. "The available studies aren't as rigorous as we'd like them to be."

### Toward the clinic

This is precisely where the biotech and pharmaceutical industries are now picking up the baton. The St. Paul, Minnesota–based biotech firm Enterologics, for instance, is now preparing a specific microbe, *Escherichia coli* M17, for investigational new drug (IND) review. *E. coli* M17, which is not an endogenous human strain, has a long history: it was used by Russian clinicians for treating GI problems during the 1930s, and then later, Israeli researchers found use for it both as a human probiotic supplement and as a veterinary treatment for flock diseases in poultry and lambs. Enterologics now plans to use the microbe for treating pouchitis—an inflammatory bowel condition that afflicts some patients with ulcerative colitis after surgical removal of the colon. The precise cause of the disease remains unknown, according to Robert Hoerr, Enterologic's president and CEO. It could be an immune disorder, or it could result from gut dysbiosis after surgery. Antibiotics including ciprofloxacin provide some relief, but the drugs cause neuropathy and long-term use is inadvisable.

Probiotics could help in at least two ways, Hoerr says. They could modify microbial flora in the ileal pouch, which is created surgically from tissues in the small intestine, or they could reduce inflammation. "The observation that *E. coli* M17 reduced inflammatory markers in an animal colitis model suggests it might provide an alternative to long-term antibiotic use," Hoerr says. Enterologics has already sequenced the microbe's genome, and the company's scientists are now researching ways to ensure the microbe's stability and efficacy in a clinical setting. Hoerr points out that most people harbor "friendly, nonpathogenic *E. coli*." However, as some *E. coli* are pathogenic, Enterologics has been working to characterize M17 to avoid any potential health risks. "This also argues for careful, drug-quality production controls," Hoerr says.

Another company pursuing a live biotherapeutic product is Boston-based Vedanta

Biosciences. According to Bernat Olle, a principal at PureTech Ventures in Boston and a member of the founding team that created Vedanta, their product is indicated for a subset of inflammatory bowel disease patients with depleted gut populations of clostridia—not the toxic variety implicated in *Clostridium difficile*–associated diarrhea, but a beneficial, commensal relative. Those depleted bacteria ordinarily recruit regulatory T cells that release interleukin 10 (IL-10)—an anti-inflammatory cytokine. Comprising this 'depleted' *Clostridium* strain and other commensal microbes, Vedanta's proprietary cocktail will ideally colonize the gut and restore lost immunity to inflammatory bowel disease. "And if the treatment works in these patients, then there's a strong rationale to expand it to other autoimmune diseases where regulatory T cells aren't working properly," Olle says.

Olle says that he and other scientists working on live biotherapeutic products for the GI tract were inspired by an increasingly popular, if repugnant procedure—fecal transplant for *C. difficile*–associated diarrhea. Developed during the 1950s, fecal transplants deliver a bolus infusion of washed, suspended feces obtained from a donor (typically a relative) directly into a patient's colon; either as an enema or via endoscopy. Recent studies, including the aforementioned clinical trial, show the procedure achieves better than 90% success rates in patients who have exhausted other options. Olle says it is not clear why the process works. "It's a bit like hitting 'reset' on a computer," he says. "Only with fecal transplant, you're hitting reset on the gut ecosystem; replacing one that's diseased with another that's healthy."

David Relman, of Stanford University, cofounder of the startup Second Genomics of San Bruno, California, says fecal transplant attests to the safety and therapeutic potential of microbial treatments. "It's a poster child for the possibilities," he says.

## Beyond probiotics

But companies are also looking beyond probiotics toward other ways to modulate the microbiome for clinical benefit. Second Genome, for instance, works with prebiotics, or nondigestible starches and fibers that commensal bacteria use as food. Ideally, prebiotic treatments will favor the proliferation of targeted gut microbes that alleviate inflammatory bowel disease, *C. difficile*–associated diarrhea, diabetes and other conditions, says the company's president and CEO, Peter DiLaura.

Another company, Cambridge, Massachusetts–based Vithera Pharmaceuticals, engineers lactobacilli to release an anti-inflammatory protein called elafin. Human epithelial cells secrete elafin, but sometimes not enough to protect the gut when inflammation levels get too high, explains Vithera's founder and president Johannes Fruehauf.

Interestingly, much of the clinically oriented research in this area tends to focus on bacteria that were already identified by traditional methods in microbiology. Both Enterologics and Vedanta Biosciences work with well-known bacteria. And a British research team recently published findings in *PLOS Pathogens* showing that *C. difficile*–associated diarrhea in mice was curable with a diverse mixture of microbes cultured from the feces of healthy animals[9]. A fundamental question now is how or whether scientists can leverage sequencing data from the Human Microbiome Project, MetaHit and other programs to find entirely new biotherapeutic species and targets.

## Understanding the microbiome

University of Nebraska's Benson says he's optimistic about the microbiome's potential. By revealing its structure, including so-called keystone species that support entire assemblies of gut microbes, sequencing opens new therapeutic opportunities, he says, "When you know more about the microbiome's structure, you can introduce keystone species that build up the microbial communities that you're looking for," Benson explains. "We're a long way from doing this, but we've got the tools that we need and the field is primed for discovery." Benson's own research reveals deep connections between microbial communities and human genetics. According to his results, human genes can actually control microbial populations in the body, which leads to the conclusion that host genetics can also drive dysbiosis.

Unlocking the field's therapeutic promise is now moving along sequential research tracks. First, sequencing allows scientists to finally catalog the microbiome's full diversity. In this preliminary phase, identifying and naming new organisms has been a priority. "It's like we're going into the Amazon jungle for the first time," says Christophe Benoist, of Harvard Medical School in Boston. "We're asking simple questions, like, 'How does microbial diversity differ between males and females?'."

The next step is moving toward the field's critical frontier—to associate dysbiotic patterns with disease. Second Genome has a discovery platform that allows these types of investigation. According to DiLaura, the company uses next-generation sequencing to compare how the microbial makeup of patients sick with a given illness, such as *C. difficile*–associated diarrhea, differs from healthy baselines in the Human Microbiome Project database. They hope that these studies could reveal specific microbes that correlate with disease. And in subsequent experiments, DiLaura says, the functional effects of these organisms can be studied in germ-free mouse models.

That's the approach that Benoist is taking in research funded by the Brussels-based pharmaceutical firm UCB, which aims to mine the human microbiome for new therapies in immunological disease. Benoist and his colleagues, Dennis Kasper and Diane Mathis, also from Harvard Medical School, are testing more than 100 human gut microbes in germ-free mice. According to Benoist, the research could reveal new biotherapeutics, in addition to microbe-generated metabolites that could be useful either as drugs or as drug targets.

At GlaxoSmithKline, Brown is also trying to correlate microbial patterns with disease, but from a different angle. He uses the company's arsenal of narrow-spectrum antibiotics to knock out suspect microbes in animal models of diabetes and obesity. "If we can correlate changes in disease phenotype with antibiotic-induced shifts in the microbial community, then that goes a long way towards substantiating the causative role that microbes might [have] with a particular disease," he says.

Relman says these types of studies are a step in the right direction. But he also says that in the final analysis, prospective studies that track microbial shifts in people over time, starting before they get sick, offer the best proof of disease causation. Studies in animals have shown, he says, that giving inflammatory compounds can produce microbial patterns similar to those seen in cases of inflammatory disease. Scientists might be tempted to say that microbial shifts produce inflammation, but these studies show the reverse can also be true.

Given its extreme complexity and variation among individuals and populations, the microbiome can't be reduced to a handful of basic factors, Relman cautions. "We're talking about enormous numbers of organisms; their own interactions, their interactions with the host, and the influence of environmental factors on that whole system, he says. "The complexity is orders of magnitude greater than what we face with the human genome."

***Charles Schmidt, Portland, Maine***


1. Graessler, J. *et al. Pharmacogenomics J.* published online, doi:10.1038/tpj.2012.43 (2 October 2012).
2. van Nood, E. *et al. N. Engl. J. Med.* **368**, 407–415 (2013).
3. The Human Microbiome Project Consortium. *Nature* **486**, 207–214 (2012).
4. The Human Microbiome Project Consortium. *Nature* **486**, 215–221 (2012).
5. Qin, J. *et al. Nature* **464**, 59–65 (2010).
6. Sokol, H. *et al. Proc. Nat. Acad. Sci. USA* **105**, 16731–16736 (2008).
7. Turnbaugh, P.J. *et al. Nature* **444**, 1027–1031 (2006).
8. Cho, I. *et al. Nature* **488**, 621–626 (2012).
9. Lawley, T.D. *et al. PLoS Pathog.* **8**, e1002995 (2012).