THE NEW GERM THEORY

What can microbiologists who study human bowels learn from those who study the bowels of the Earth?

BY LIZZIE BUCHEN

Jillian Banfield trades in hell holes. In September, she could be found wading through the dark, hot, sulphurous innards of Richmond Mine at Iron Mountain, California, where blue stalactites ooze the most acidic water ever discovered, with a pH of −3.6. A year before that, she was pumping up a toxic soup of uranium, arsenic, molybdenum and other metals from underneath a decommissioned nuclear-processing site in Rifle, Colorado. From both sites she took samples back to her lab at the University of California, Berkeley, where she sequenced and analysed the DNA they contain in an attempt to work out which bacteria, archaea, viruses and fungi have decided to make that particular hell their home — and what it takes to survive there.

About a year ago, Banfield added a new location to her repertoire of foul study sites: the pencil-thin intestines coiled inside premature infants weighing less than 1.5 kilograms, in the neonatal intensive care unit of the University of Chicago, Illinois. Banfield had never dealt with microbes that live in humans. But her well-regarded work on the microbial communities of Richmond Mine had attracted the attention of two medical researchers.

One was Michael Morowitz, a neonatal surgeon then at the University of Chicago, and now at the University of Pittsburgh, Pennsylvania. Morowitz was studying necrotizing enterocolitis (NEC), a potentially fatal disease that destroys the bowels of premature babies. The other was David Relman of Stanford University in California, a leader in the burgeoning field exploring the human microbiota — the vast populations of microorganisms that live in and on the human body. Morowitz and Relman asked Banfield if she could help them understand the microbial mass in this unexplored landscape.

Banfield said yes — and the three struck up a collaboration. They are now bringing Banfield's techniques to bear on humans, and are sequencing and analysing microbial genes in fine detail to resolve whether hard-to-distinguish species or strains correlate with NEC and might promote it. Elsewhere, similar collaborations are linking those exploring the human microbiota in the intestine, skin, mouth and other surfaces with microbial ecologists, such as Banfield, who have already made a career out of studying microbial universes in environments such as soil, ocean water and toxic waste sites.

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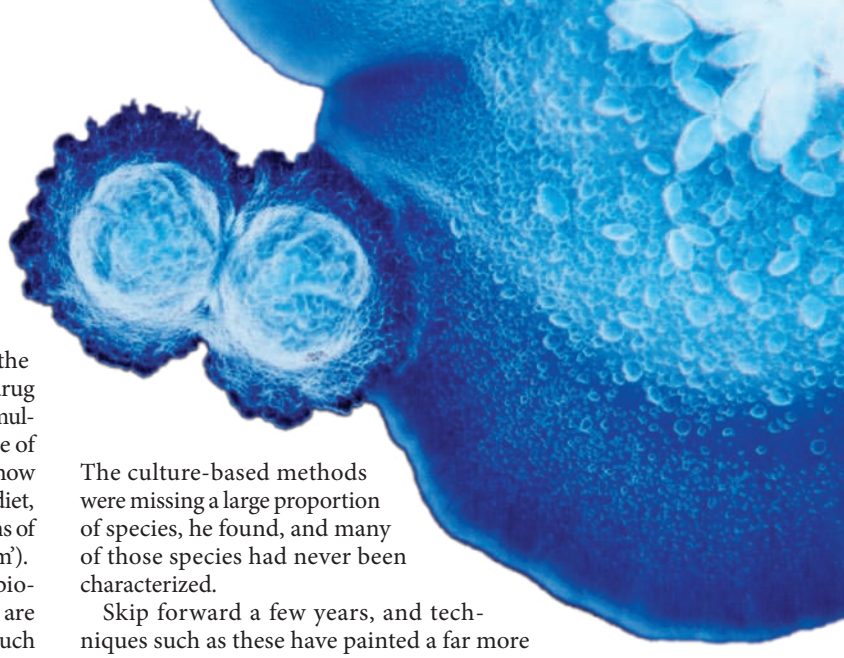
The human microbiologists need the help. Although work by Relman and many others over the past five years has gone a long way to building up a genetic catalogue of human microbiota — what types of microbes live where — it has also revealed its staggering and previously

D. READY/WELLCOME IMAGES



Composite of images of human mouth bacteria, including *Streptococcus mutans* (spherical blobs over title).

unappreciated complexity. With hundreds of interacting, coevolving species living in and on every individual, and frustratingly little species overlap between each person's microbial population, understanding the connection between microbes and health seems more daunting than ever. Researchers want to know what role the body's microbial inhabitants have in immune function, nutrition, drug metabolism and conditions as diverse as obesity, cancer, autism and multiple sclerosis. But to do so, they have to sort through an avalanche of genetic sequence to find out what microbes are in the community, how they change over the course of a day, a lifetime or after a change in diet, and which functions are served by particular microbes, combinations of microbes or microbial metabolites (see 'Exploring the superorganism').

Microbial ecologists are supplying some of the expertise and bioinformatic tools to help make sense of the data mountain. They are also bringing to the human microbial field ecological principles such as colonization, succession, resilience to change, and competition and cooperation between community members. "It's hard not to think about ecology when you enter the field," says Jeff Gordon, a leader in gut microbiology at Washington University in St Louis, Missouri. In return, specialists in human microbiology are attracting funding and attention that ecologists have sometimes struggled to find. "The arbitrary and false barriers between environmental and medical microbiology are breaking down," Gordon says.

MICROBIAL DELUGE

An infant's first exposure to microbes is at birth, as it slides out of the sterile womb, slurping up and smearing itself with its mother's vaginal fluids and faeces. From then on, life is one long microbial onslaught. New bacteria, viruses and fungi colonize every exposed organ — skin, eyes, lungs, gastrointestinal (GI) tract. But until the past decade, scientists' ability to explore these microbes en masse was hindered by historical, cultural and technological obstacles. Ever since Robert Koch advanced the germ theory of disease in the late nineteenth century, clinical microbiologists have been fixated on foreign pathogens such as *Salmonella*, Ebolavirus and *Yersinia pestis* — identifying them, growing them in isolation and determining their causal relationship with disease. Everything else living on or in the body was often dismissed as pretty inconsequential when it came to human health.

"The microbiota are bringing utensils to the dining-room table that the human host doesn't have."

Microbial ecologists have had a different perspective. Around the time Koch was growing his first pure cultures of *Bacillus anthracis* and *Mycobacterium tuberculosis*, the environmental field was beginning to recognize what became known as "the great plate count anomaly". When analysing a sample such as a drop of pond water, they saw a dramatic discrepancy between the vast number of microbial cells they could count under a microscope and the tiny number that would grow after plating the same sample. The research community, intent on studying natural ecosystems in their entirety, made efforts to work out what all the unculturables are. In 1985, a team of microbiologists published a technique that could census bacteria and archaea in a sample by sequencing the 16S ribosomal RNA gene, which is different for every species[1]. These types of genetic survey quickly became routine in the environmental microbiology field. Banfield began using them in 1995, soon after beginning her studies on the acid mine drainage at Iron Mountain.

It took longer for the clinical microbiology community to take notice. In 1999, Relman published one of the first such surveys of microbes on and in the human body, comparing a 16S survey of the plaque scraped off a healthy man's teeth with those he could grow on a culture plate[2]. The culture-based methods were missing a large proportion of species, he found, and many of those species had never been characterized.

Skip forward a few years, and techniques such as these have painted a far more sophisticated picture of human microbes as ubiquitous, abundant and indispensable, harvesting energy and nutrients from food, synthesizing essential amino acids, moisturizing the skin and playing an essential part in immune-system development. "The microbiota," as Gordon puts it, "are bringing a series of utensils to the dining-room table that the human host doesn't have." The picture bandied around nowadays is of humans and their microbial partners as a coevolved 'superorganism' in which each provides services for the other. (Gordon even employs a cultural anthropologist, who is exploring how a person's view of the self changes when they find out that, in sheer numbers of cells, they are 99% microbial.)

"There's been a real recognition that we need to include the whole human microbiota to really understand how we function as an organism," says Lita Proctor, a project director at the US National Institutes of Health in Bethesda, Maryland, which launched the 5-year, US$115-million Human Microbiome Project (HMP) in late 2007. That project, dedicated to sequencing a colossal portion of the human microbiome, is one of several with similar goals formed around the world and joined under the umbrella of the International Human Microbiome Consortium. In March, one major initiative called MetaHIT, involving the European Union and China, published a catalogue of the microbial genomes strained from the faeces of 124 people, finding more than 1,000 prevalent bacterial species across the group — and 3.3 million different microbial genes[3] (that's 150 times the number of human genes). A few months later, HMP scientists completed the sequence and analysis of 178 microbial genomes from different regions of the body and discovered genes coding for more than 30,000 different proteins[4]. "We're talking about something that's a hundred times bigger [than the human genome] that we don't have a handle on, that's intimately tied to our own health and vitality," says Proctor.

UNUSUAL SUSPECTS

Relman became familiar with Banfield and her work in the early 2000s, when she had already gained recognition for describing the dynamic structure of the acid mine drainage community and its metabolic processes. Banfield and her team developed techniques to work out, from a mass of fragmented microbial DNA sequences, which species and strains they come from. By contrast, 16S techniques and most automated bioinformatic analyses struggle to reveal differences between closely related strains, or to expose subtle genetic rearrangements.

Banfield's team first assembled fragments from the dozen or so dominant species into full genomes. Then they undertook fine-grained analyses: manually inspecting points where the DNA fragments didn't quite line up to identify closely related strains of the dominant species, and developing software to tease out the identity, metabolism and function of these and much rarer species. This helped the group work out in great detail how members of the community function and change over time[5]. And the distribution of genes also revealed the

biogeochemical processes that the community was performing.

Relman wanted to analyse human microbiomes with the same level of detail. "It was reasonably straightforward to see that this kind of approach would really be important," he says. But Banfield's approaches had been used to dissect only simple communities, such as the handful of species that can thrive in the hellish Richmond Mine, where the most abundant species make up some 40% of the cells in the community. The adult gut typically contains hundreds of microbial strains or species, and the most abundant species represent only about 4%. "It just always seemed like her system was simple enough to make it [this type of analysis] possible, whereas mine was just hopelessly not. It was worse by at least an order of magnitude," says Relman.

Then, in January 2009, Morowitz stepped in. "He just called me out of the blue one day," Banfield recalls, and spoke to her about NEC. "He was really concerned about this terrible disease. I was sort of taken by how passionate he was, and hoped I'd be able to do something that might be useful." NEC strikes about 7% of severely premature infants, but often clinicians cannot diagnose the disease to begin treatment until the symptoms, such as a ballooning belly and blood-soaked stools, are in full swing. In severe cases, surgeons must remove part or all of the intestine, and many babies die. The medical community had been searching for a causative pathogen for decades, without success. "I had a suspicion that we weren't going to find a [pathogenic] smoking gun, so I was looking around for different ways to study the problem," Morowitz says. "I became interested in people who were looking at entire communities of organisms. That's what got me reading Jill's papers."

Banfield, Morowitz and Relman realized that the preterm infant could be the perfect human testing ground for Banfield's techniques. Each tiny neonate harbours only a dozen or so species — comparable to the acid mine drainage — and the intensive care unit creates a sterile, tightly controlled environment in which to study them.

The trio's work has focused on healthy babies so far. Morowitz takes faeces samples almost daily from birth, isolates DNA, sends it to a high-throughput sequencing centre, then passes the data and clinical information to Banfield and Relman. Relman analyses the 16S sequences at every time point to get a census of the species and their abundance; Banfield then selects a few time points for more extensive sequencing and genome analysis to identify the species, strains and genes present. The team's first results, which will be published later this year, show that the techniques work: studying the first three weeks of life in a premature infant born at 28 weeks of gestation, they were able to track the rapidly changing microbial community with strain-level detail. They distinguished, for example, the changing abundance of two strains of *Citrobacter*— a species commonly found in the gut that has also been implicated in neonatal meningitis — the sequences of which are more than 99% identical. The eventual aim is to find out whether a particular strain or community structure correlates

EXPLORING THE SUPERORGANISM

Big questions about the microbial multitudes inside.

How stable is the microbial community?

After populations of intestinal microbes — the microbiota — are established, it's unclear how they change with age, shifts in diet, activity level, co-habitation or after a blast of antibiotics. One study[7] revealed that two courses of the antibiotic ciprofloxacin wreaked havoc in the gut microbes of healthy people, and that the communities never fully recovered.

Can the microbiota be used for diagnosis?

Analysis of an individual's microbes and their metabolites may reveal those directly associated with disease and those that serve as biomarkers for a wider spectrum of conditions. It may prove possible to tailor treatments to the microbiota.

Can the microbiota be changed?

Studies show a tight relationship between diet and microbiota, so it may be possible to fine-tune diet to support the most beneficial community. Prebiotics — ingesting particular foods high in fibre or vitamins that promote the growth of specific microbes — are now receiving attention from the food industry. Probiotics — microbes in food or a pill — are already common, although evidence that they have any benefits is so far equivocal.

A more radical approach is faecal transplants, and there are anecdotal accounts of such a procedure, used as a last resort, benefiting recipients whose microbial community was highly disturbed. But microbes that are innocuous in one individual could be pathogenic in another.

D. MCCARTHY/SPL

***Helicobacter pylori*: just one of the residents of the human gastrointestinal tract.**

Rather than relying on transplants of foreign faeces, "It might be wiser to bank your poop, like you do your bone marrow," says David Relman of Stanford University in California, to repopulate the intestine in the event of disease. "But you can't propagate faecal matter, so you'd be stuck with the dose that was originally frozen."

Does the microbiota affect behaviour?

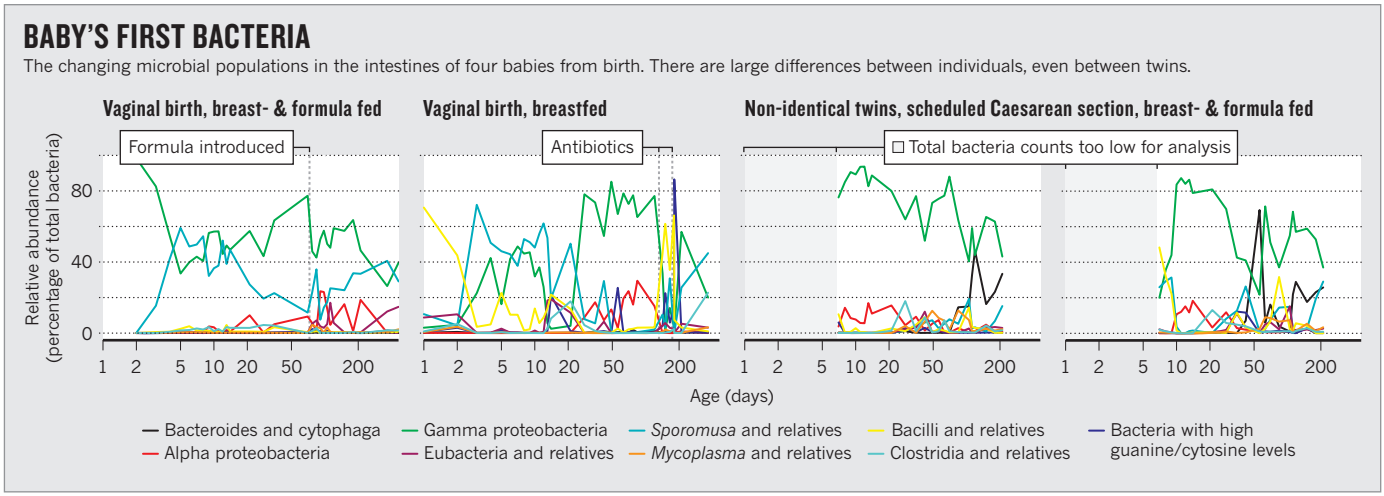
A number of conditions that affect behaviour, such as autism and schizophrenia, have been associated with digestive problems, and symptoms are often reported to be connected to diet. Microbes produce a range of compounds that can potentially affect brain activity. Some studies have suggested that the structure of microbial communities in children with autism differ from those in children without the condiiton[8]. No causal relationship has been demonstrated, but some researchers think that analysis of the microbial community may allow clinicians to diagnose these conditions and begin therapy before the symptoms even start.

How does modern life affect the microbiota?

Microbes that humans come into contact with and support might be changing dramatically, particularly in wealthier, developed countries, thanks to lavish use of antibiotics, high hygiene standards, more Caesarean sections, less breastfeeding and processed-food diets. One version of the 'hygiene hypothesis' proposes that the increasingly sterile environment in which humans are raised may alter the microbial community in the body, preventing normal development of the immune system and potentially leading to increasing prevalence of conditions such as allergies, asthma, Crohn's disease and even autism. Some now call it the 'microbiota hypothesis'. On the other hand, it may become possible to adjust microbes to help deal with modern environmental toxins.

Can the microbiota be used in forensics?

It might be possible to learn about and track criminals by analysing their microbiota — collected from body fluids or fingerprints. This might reveal what a suspect eats, where they live, how active they are and whether they have certain pets. There are numerous caveats, though, including how much the microbial community changes with time, or after someone else touches the same spot. **L.B.**



SOURCE: C. PALMER *ET AL. PLOS BIOL.* **5,** 1556–1573 (2007)

with NEC, which could help predict which babies might develop it and even give a clue to preventing it.

More broadly, the research team hopes that the simple model community provided by the neonatal gut will help them to explore the extent to which ecological principles apply to the human microbial system. "The scientific questions are really cross-cutting," says Banfield. One example, she says, is colonization — which organisms arrive first and how the community evolves (see 'Baby's first bacteria'). "It's ecological succession," Banfield says. "If you look at the surface of a pool of acid mine drainage and imagine the first organisms to arrive, it's the same as imagining a newborn baby with a sterile GI tract, and the first organisms there."

ANTIBIOTIC AFTERMATH

Other collaborations are also exploring how human microbial ecosystems adjust during illness, shifts in diet or after antibiotics. "They're probably changing all the time in response to all sorts of perturbations," says Claire Fraser-Liggett, a microbiologist at the University of Maryland School of Medicine in Baltimore, who, in collaboration with Janet Jansson, a soil microbiologist at the University of California, Berkeley, is studying microbiomes associated with the intestinal disorder Crohn's disease in identical Swedish twins. "Are these communities resilient enough to rebound to where they were before a perturbation like antibiotics? What should we be measuring in order to answer that question? What's going on in the recovery period? It leads to all these questions that ecologists have been dealing with for decades."

"Complexity's a big challenge. We're not, by any means, there yet."

Ecological concepts are also helping to account for the substantial differences that most studies have found between the microbiota of individuals — even, to a lesser extent, between identical twins. Ecology offered a likely explanation in the form of redundancy. The idea now is that every person's microbes provide a core set of genes or biological functions, regardless of the specific species encoding them[6]. "If you look at grasslands in different parts of the planet, there's a common morphology and function," says Gordon, drawing parallels. "But in different locales, the component species are quite distinct." Gordon and other researchers hope that more extensive sequencing and analysis of many individuals' microbiomes will reveal what those core functions are. Relman, meanwhile, has become interested in finding 'keystone species', rare species that nevertheless have a vital role in a community, and he is working with a colleague at Stanford, bioengineer Stephen Quake, to sequence the genomes of single microbial cells from the gut.

Yet the sheer density, diversity and complexity of the human microbiota places it in a different league from other microbial communities. "We're getting so much data," sighs Jansson. "Billions of sequences, tens of thousands of proteins and metabolites. We have data overload." The constant communication that goes on between human cells and their microbial inhabitants adds a whole extra layer of complexity. Elsewhere, the environment is more or less inert. "Complexity's a big challenge," says Relman. "We're not, by any means, there yet."

"It's a lot less clear with the Human Microbiome Project what the finish line will be," says Fraser-Liggett. "For the Human Genome Project, it was to create high-quality draft sequence for one human genome. Here, given the complexity of these communities, we're not so sanguine that we think we can yet define an endpoint." With so much diversity, "it seems like a black hole that may go on forever, which makes some of the funding agencies cringe", she says.

All this means that clinical application remains a distant prospect. Even if researchers find a convincing association between, say, a particular microbial species and a disease, they lack the tools to manipulate the microbial communities with any specificity to eliminate one species or insert another. Antibiotics tend to kill swathes of microbes, not individual species, and the inability to culture most species means that it is impossible to grow and transplant them. (There has been limited success transplanting entire globs of faeces.) Even if species-specific transplants were possible, there is no guarantee that the newcomer would survive.

So although ecology is providing a framework with which to understand the human microbiota, when it comes to applying these ideas to the clinic, old-fashioned pathogen-centric microbiology is leagues ahead. And that, says Morowitz, doesn't look like it's changing any time soon. "If you walk into any hospital and pull aside someone in a white coat and ask them about microbiology, most of what they know has to do with organisms that can be isolated when they send a blood sample down to the culture lab."

Relman, a clinician himself by training, says this focus is understandable. "They're sucked towards pathogens, and they have practical questions to deal with in the clinical workplace. They're not in need of more diversity." But that has to change, he says. "We have to get away from this monolithic, one-dimensional perspective of a one-bug-one-disease picture of health," Relman says. "The community is the unit of study." ■

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