The human body teems with microbes. In the first of two features, **Asher Mullard** looks at the global efforts to catalogue this vast 'microbiome'. In the second, **Apoorva Mandavilli** meets the surgeons who have a rare opportunity to watch an ecosystem being established as they transplant guts from one person to another.

THE INSIDE STORY

Any story about a human's microbes tends to invoke impressive numbers. Take the 10 trillion or so microbial cells living in the gut, which exceed the number of human cells by 10 to 1. Between them, they harbour millions of genes, compared with the paltry 20,000 estimated in the human genome. To say that you are outnumbered is a massive understatement.

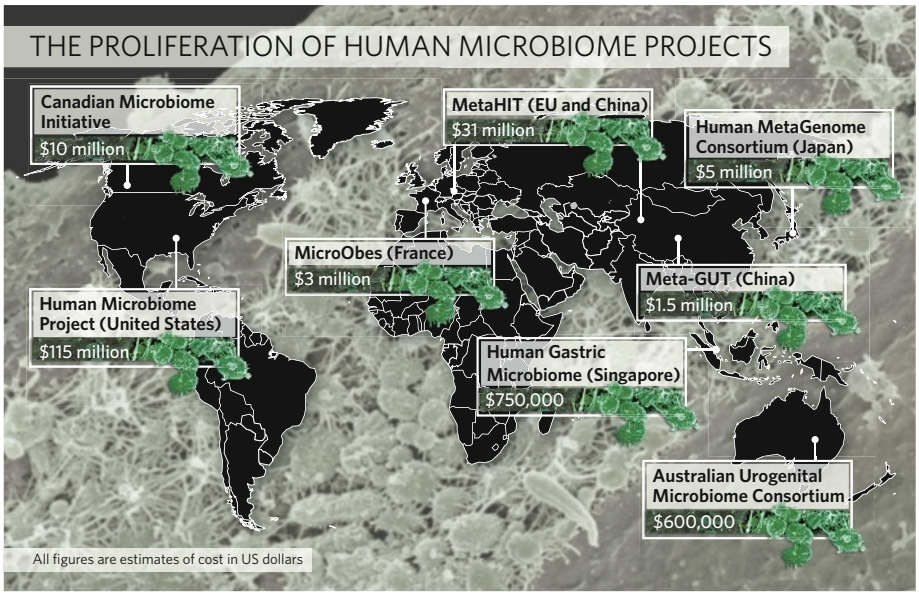
But that might not be a bad thing. There is strength in numbers; so much so, in fact, that some biologists regard a human as a 'superorganism' — a community that adds up to more than the sum of its parts. The body itself is merely one, albeit encompassing, component.

Some smaller but nonetheless striking numbers about human microbes come in cash amounts. Late last year, the US National Institutes of Health (NIH) pledged US$115 million to identify and characterize the human microbiome, the name given to the collection of microorganisms living in and on the human body. Also last year, the European Commission and various research institutes committed €20 million (US$31 million) to similar ends. And smaller sums are being thrown in by funding agencies in countries that include China, Canada, Japan, Singapore and Australia (see map).

Given the multifaceted nature of the microbiome, perhaps it is only right that it is studied by a large and varied community. The NIH's five-year Human Microbiome Project will spend much of its money identifying which bacteria are lodged where in the body and compiling a reference set of their genetic sequences. By contrast, the European Commission's four-year initiative, called Metagenomics of the Human Intestinal Tract (MetaHIT), will focus on microbial inhabitants of the gut, the main repository of the microbiota, and how they contribute to obesity and inflammatory bowel disease. Researchers involved in these and other initiatives say they will team up within a larger international consortium, but hints of competition simmer beneath the surface. "The intention is to work together," says George Weinstock, a geneticist at Washington University in St Louis, who is helping to organize the Human Microbiome Project, "but for the moment it is more about working in parallel until we can understand how to work together".

The microbes that swarm in and on the human body have always held a certain fascination for researchers. Studies over the past century have shown that mice raised in a germ-free bubble have weak immune systems, inefficient digestive systems and abnormally small internal organs. They have shown that microbes are also an essential part of human biology.

For the germ-ridden human masses who don't live in plastic bubbles, however, it has been difficult to work out exactly who these microbial passengers are and how they interact with one another. This is partly because there are so many of them, and partly because so few are easily grown in the lab.

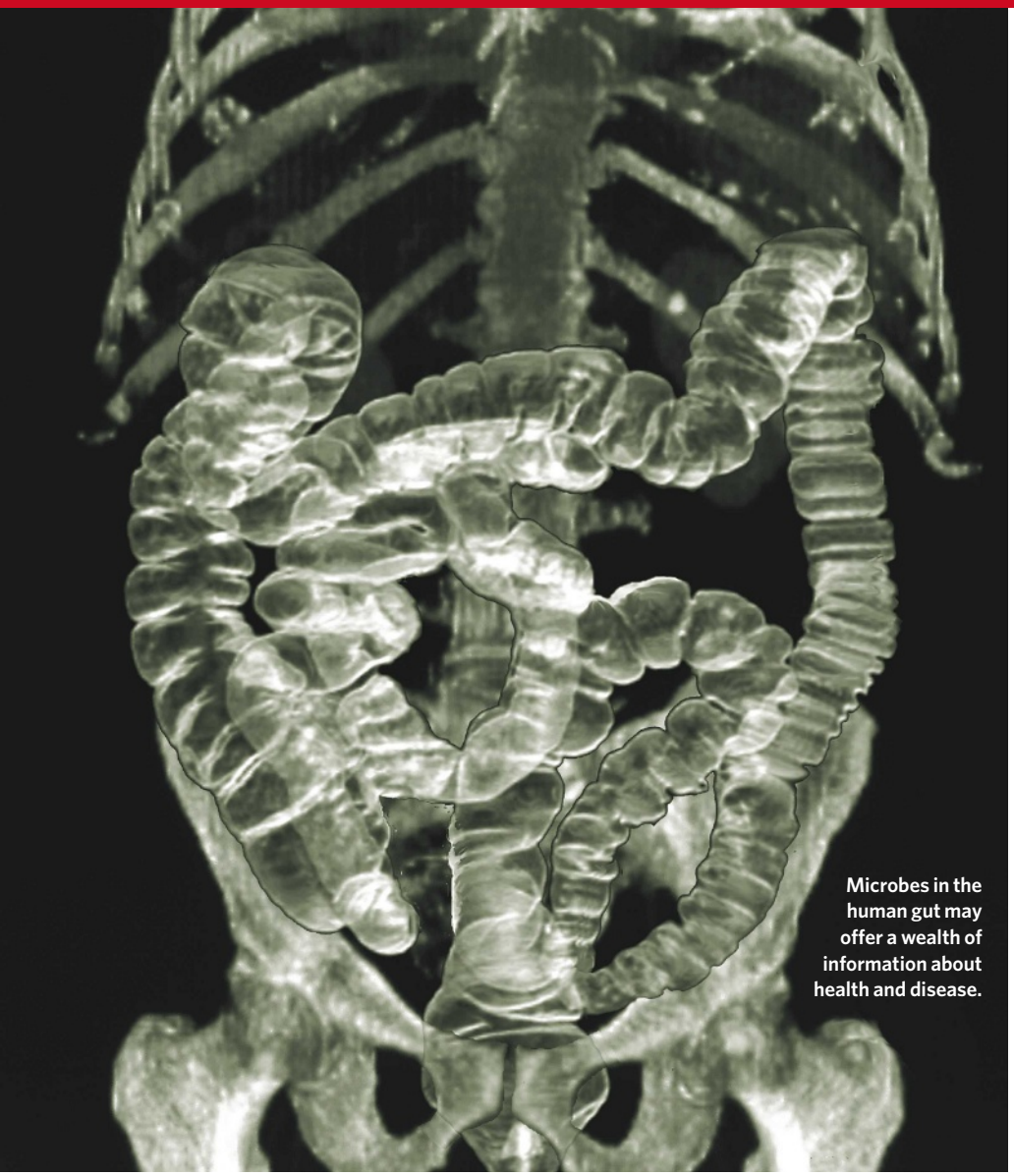


A numbers game

Things have changed in the past few years. A few million foreign genes no longer sound so daunting in the face of advanced genetic-sequencing methods that have staggering numerical endowments of their own. Using the newest technologies, researchers can process hundreds of millions of base pairs in just a few hours. So they can bypass the need to grow bacteria, exploring the human microbiome by studying genes en masse, rather than studying the organisms themselves.

In 2006, Steven Gill at the Institute for Genomic Research in Rockville, Maryland, and his colleagues, threw around some then-hefty numbers when they carried out such a metagenomic analysis of the microbes in two people's intestines. After 2,062 polymerase chain reactions and 78 million base pairs, the researchers still had only a sketch of the gut's genetics, but they had revealed an abundance of genes that are involved in producing amino acids and in other aspects of human metabolism[1]. Although interest in large-scale studies of the human microbiome has been mounting over the past few years, it was the realization that sequencing technology really was up to the task that finally prompted the money to roll in, Weinstock says.

Preliminary surveys such as Gill's have indicated that genes, age, diet, lifestyle and geography all affect which bacteria live in a person's body. But these first surveys involved too few



Microbes in the human gut may offer a wealth of information about health and disease.

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individuals and sampled too few microbes, usually from only the gut or the mouth, to provide an adequate description of the microbiome. How many bacterial species colonize the entire body remains anyone's guess. So does the question of which ones everyone shares. "One of the things that is obsessing microbiologists is: 'What is the size of the core microbiome'," says Jeremy Nicholson, a biological chemist who studies microbes and metabolism at Imperial College London.

Bringing order to chaos

The Human Microbiome Project is just the project for such obsessed microbiologists. In this, its first year, researchers will collect samples of faeces plus swabs from the vagina, mouth, nose and skin from 250 volunteers. They will sequence short, variable stretches of DNA that code for components of ribosomes in order to roughly identify which bacteria are present in each person and how many the volunteers have in common. With an estimate of diversity in hand, the researchers then plan to mine deeper. For this, they will also use shotgun sequencing to analyse many short pieces of DNA from all over the microbes' genomes and reveal which genes are present.

Like any biology project that involves large sums and grand aims, the Human Microbiome Project has brought out some critics. One challenge, they say, is that the core microbiome might be incredibly small. Even though two people may each have 1,000 types of bacteria living in their guts, they might have only 10 species in common, for instance. And the commonalities might not lie in the genes, but rather in bacteria that have the same metabolic and physiological role. The Human Microbiome Project will do little to assess the function of microbes during its first year, although it may focus on this later.

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> — Jeremy Nicholson

Sarkis Mazmanian, a microbiologist at the California Institute of Technology in Pasadena, voices another reservation. "There's very little in terms of actual application to disease as part of the initiative. The lion's share of the efforts is in sequencing." This criticism tends to be aired for all big sequencing efforts and in this case, one-quarter of the money has been earmarked to examine the role of the microbiota in health and disease. Weinstock says that the project's main goal is to build up a research community and to generate a sequence resource, akin to that developed during the Human Genome Project, so that other basic and applied questions can be tackled later on.

MetaHIT is a different sort of beast. It will concentrate almost exclusively on the role of the gut microbiota in obesity and inflammatory bowel disease. And whereas the Human Microbiome Project is initially comparing people's microbiota on a species level, MetaHIT aims to find differences in microbial genes and the proteins they express without necessarily worrying about which species they came from. "We don't care if the name of the bacteria is *Enterobacter* or *Salmonella*. We want to know if there is an enzyme producing carbohydrates, an enzyme producing gas or an enzyme degrading proteins," explains Francisco Guarner, a gastroenterologist at Vall D'Hebron University Hospital in Barcelona, Spain. We want to "examine associations between bacterial genes and human phenotypes", says Dusko Ehrlich, coordinator of MetaHIT and head of microbial genetics at INRA, the French agricultural research agency in Jouy-en-Josas.

Gut reactions

Jeffrey Gordon, a microbiologist at Washington University School of Medicine in St Louis, has already shown how illuminating these associations can be. Gordon, one of the pioneers in the human microbiome field, showed two years ago that obese and lean individuals have radically different profiles of bacteria in their guts[2]. When the obese volunteers went on a one-year diet and lost up to one-quarter of their bodyweight, their bacterial profiles changed to look more like those of the lean people. The theory, based on studies in mice[3], is that part of the propensity to gain weight might lie in 'obesity-causing' bacteria in the gut that release more calories from food than those found in lean people. Researchers hope to gain further insight into how this happens by comparing the microbial genes in thin and fat people, and the findings could help to determine whether probiotics or other interventions could be used to shape the microbiome.

Some of this work is already under way as part of MetaHIT. In Denmark, a team led by Oluf Pedersen at the Steno Diabetes Centre in Copenhagen is collecting faecal samples from

120 obese volunteers and 60 controls to tease out specific microbial genes that might contribute to obesity. A similar-sized study in Spain, led by Guarner, will compare the microbiotas of patients with inflammatory bowel disease with those of genetically matched controls and examine the effect of drugs.

Whatever their differences, those involved in the Human Microbiome Project and MetaHIT sometimes find themselves on common ground — and that comes down again to those mind-boggling numbers. How do you effectively study such a vast and unknown community? Both groups will be using shotgun sequencing, which generates scraps of sequence from the many different genes and species. To make the most of this approach, a reference sequence is needed against which researchers can compare and identify these scraps, but at present there aren't enough known bacterial gene sequences to match the fragments against. Earlier studies, such as Gill's, produced many sequences that were impossible to assign functions to.

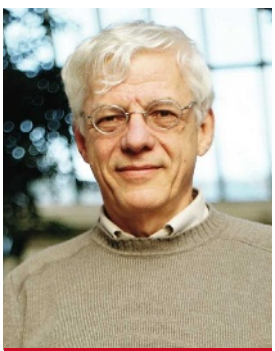
Into the unknown

Both the Human Microbiome Project and MetaHIT plan to sequence the complete genomes of hundreds of bacterial species and deposit them in a shared database. They will initially use shotgun sequencing of a few select species that can already be grown, and piece together their whole genomes from overlapping fragments. The Human Microbiome Project plans to provide 600 'reference genomes', MetaHIT will do another 100, and other sequencing efforts by the NIH and elsewhere will make additional contributions. With a broad enough reference database, researchers hope to be able to predict the genetic capabilities of some of those recalcitrant, unculturable species solely on the basis of similarities with known genes.

Even with such a reference, "it is pretty hairy from a computational biology analysis point of view", says Peer Bork, the biochemist who heads MetaHIT's computational centre at the European Molecular Biology Laboratory in Heidelberg, Germany. Even with the immense power of supercomputers to process the sequencing data, it will take some clever analysis to compare the millions of sequence reads that span thousands of species between hundreds of healthy and unhealthy people. It may be even hairier if, as many suspect, subtle genetic patterns are what is important in disease rather than the presence of a single gene or species.

In many ways, such bioinformatics brings the need for collaboration into stark relief. When all the projects are running at speed, reams of data will be generated worldwide. But because different groups are using different techniques to collect samples, extract DNA and annotate data, the data sets are difficult to compare, Bork says.

Enter the as-yet-unlaunched International Human Microbiome Consortium. Scientists from several international projects, including the Human Microbiome Project and MetaHIT, have been meeting since late 2005 to figure out how to collaborate on a range of issues such as the compatibility of data and which bacteria to sequence for the reference database. The group is already setting up infrastructure and "beginning to address the tough questions", says Weinstock. But it is too early to say how well it will grease the wheels of collaboration. Its official launch, scheduled for April, was postponed for six months to allow the NIH and the European Commission to overcome bureaucratic hurdles. Even so, optimism for the collaboration runs high, partly because its members can still pursue their own pet projects. "Talented people are doing what they think is the most important research to do, rather than being forced to do what somebody else has decided would be the best," says Ehrlich.



"Talented people are doing what they think is the most important research to do."
— Dusko Ehrlich

One of questions being addressed by the consortium is over intellectual property. As with other genomic projects, members of the consortium will be expected to release sequence data into the public domain as soon as they are generated. But this doesn't necessarily preclude disputes over intellectual property if, for instance, a particular bacterial gene proves to be a useful diagnostic marker for a disease. Another unresolved question is whether a laboratory can have one project that abides by the consortium's regulations, and another that doesn't. "There are grey areas, and I feel that until we have a test case, they will have to be watched very carefully," says Bhagirath Singh of the Canadian Institute of Health Research, who is helping to develop the Canadian Microbiome Initiative.

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"There's so much to learn, so much we don't know and so many adventures."
— Jeffrey Gordon

Participants from microbiome projects around the world say that they plan to sign up to the consortium. But the number of independent projects being launched speaks to at least some underlying competition between those involved, and some of the difficulties the group may face. In addition to differences in focus and scope, MetaHIT's operating budget is only a quarter the size of the Human Microbiome Project's. "This is giving a huge advantage to the Americans," Guarner says. "They are going to be quicker and they have more equipment." But some members of MetaHIT feel that they actually have an edge because money for their project has already been distributed and data collection is under way, whereas the Human Microbiome Project will not announce many of its funding decisions until later this year. "We have an advantage already, we have a show on the road," says Willem de Vos, a microbiologist at Wageningen University in the Netherlands and a member of MetaHIT.

Given the number of separate projects, all at such an early stage, it's almost impossible to make out where the starting line lies or who, exactly, is edging ahead. And the intention to pool data means that there may be no clear line separating them. "If it is an international consortium, it doesn't matter where the data are generated," Bork adds. "For example, we can be the pirates here, sitting at the end in Europe, and use American data to make the discoveries."

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Plundering may be unwarranted. With trillions of microbes to sift through, most researchers feel that there is more than enough of the microbiome to go around. "There's so much to learn, so much we don't know and so many adventures," Gordon says. "There's enough room for everyone." ■

Asher Mullard is an assistant editor for *Nature Reviews Microbiology* and *Nature Reviews Molecular Cell Biology*.

1. Gill, S. R. *et al. Science* **312,** 1355–1359 (2006).
2. Ley, R. E. *et al. Nature* **444,** 1022–1023 (2006).
3. Turnbaugh, P. J. *et al. Nature* **444,** 1027–1031 (2006).

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