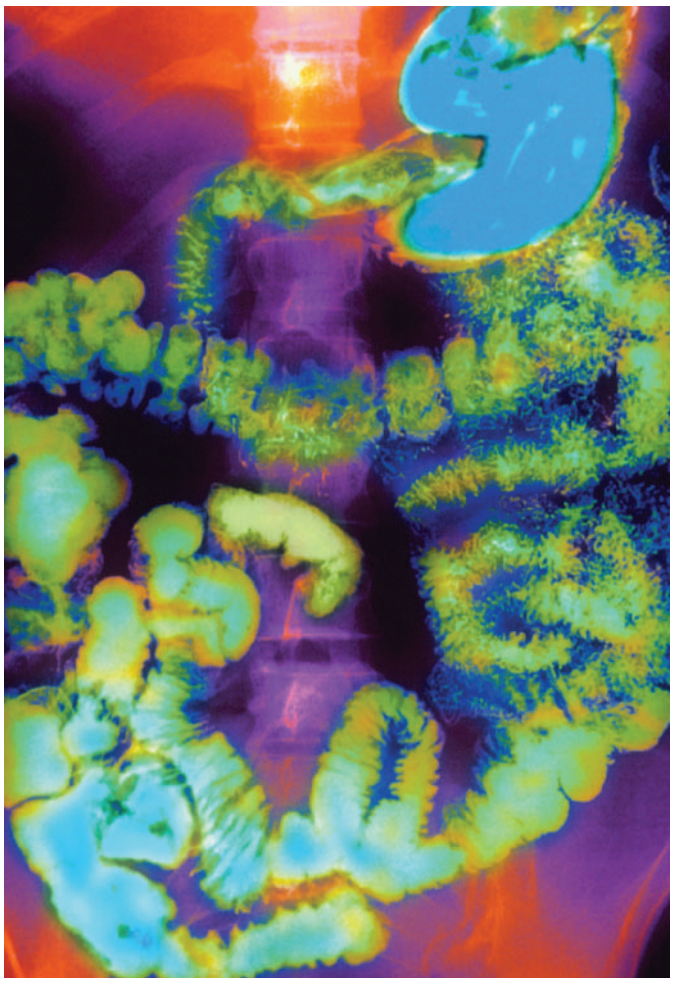
Gut reaction

Consumers are stocking up on live yoghurts and fermented drinks that claim to improve health. But is there any science behind the marketing of these 'probiotic' products? Alison Abbott investigates.

Glenn Gibson's wife prefers him to tell dinner-party guests that he works as a painter and decorator. That's understandable, because if he talks about his real job as a researcher of gut bacteria at the University of Reading, UK, the conversation all too easily turns to the source of his research material — human excrement.

For better or worse, faeces provide the best window into the microbial life of the human gut, a subject that is attracting more funding now than ever before. Partly in reaction to commercial claims that the bacteria in some yoghurts and other 'probiotic' products can boost health, the European Union (EU) has invested more than €15 million (US$19 million) since 1995 to research this poorly explored frontier.

As a result, a growing number of microbiologists are taking an interest in the ecology of the human gut. They are adapting tools previously developed for the study of microbes in oceans and soil to answer a range of questions. What lives in our gut? Do some natural gut microbes predispose us to diseases such as colon cancer? And can we change the make-up of our intestinal residents to improve our health? Gibson has even built a collection of artificial guts to study our internal microbial ecology under controlled laboratory conditions (see 'Roboguts', opposite).



BSIP DR PICHARD T/SPL

Hard to swallow? Each human gut contains about 100 species of microbe.

Hidden world

The average human intestine contains about 1.2 kilograms of bacteria plus a smattering of yeasts. So far, few of these microbes have been characterized or even identified. But this dearth of information hasn't kept companies from promoting the health value of probiotics, which contain living bacteria, and prebiotics — nutrients designed to boost populations of beneficial bacteria already living in the gut.

Probiotic dietary supplements are available in just about any form imaginable, from tubes of liquid to capsules. Some yoghurts and fermented milk drinks also promote their living contents. A typical online shop claims that its probiotic products can "strengthen the immune system, reverse the negative effects on the digestive tract of infections, antibiotics, alcohol … treat symptoms of irritable bowel disease" and more. Worldwide, the pro- and prebiotics market is now worth about US$6 billion.

But so far, the science behind these commercial boasts is rather limited. "There are a lot of bogus claims and vested interests," says Michael Blaut, head of gastrointestinal microbiology at the German Institute of Human Nutrition in Potsdam, and one of the researchers who helped to convince the EU to fund probiotics research. Although some clinical trials of probiotics have suggested a benefit, Gibson adds, few of these have been sufficiently rigorous. And even when probiotics seem to work, he says, we know too little about the normal gut ecosystem to understand why.

Soon after it was established, the EU-funded network, which includes scientists from 16 countries, discovered that the gut ecosystem is much more diverse than previously thought. Microbiologists knew that their traditional techniques of isolating and cultivating individual microorganisms were not pulling out all of the species that we live with. Many gut bacteria are notoriously difficult to grow in culture — largely because they depend on the presence of other bacterial species. But few scientists had anticipated just how diverse the ecosystem would turn out to be.

To begin to quantify the diversity, gut researchers borrowed a method from soil and ocean microbiologists that relies on comparisons of the gene for a portion of the ribosome — the cellular machine that manufactures new proteins — known as *16S*. The ribosome is so fundamental to the workings of the cell that it has changed little during evolution. That makes it easy to extract the *16S* genes from all microorganisms in a single faecal sample using the DNA-amplifying polymerase chain reaction. By looking for subtle differences between the sequences of these genes, microbiologists can gauge the number of different species present in the sample.

One surprise was that no two people have quite the same complement of bacteria. In unpublished work, molecular biologist Joël Doré of the INRA, the French agricultural research agency in Jouy-en-Josas, near Paris, has so far analysed the faeces of more than a dozen healthy adults and found the contents of each to be quite different. Although thousands of microbes can live in the gut, each person has only about 100 different species. "There is remarkably little overlap in the gut bacterial species between individuals," he says.

This is partly because of the haphazard way in which the bacteria arrive. Our guts start off in the womb completely sterile, but they are rapidly colonized with vaginal and

faecal bacteria during birth. Microorganisms from food and other environmental sources contribute to the mix during the first months of life. By the age of two at the latest, the average human gut hosts its full complement of microbial species, mixed and matched from a dozen or so dominant groups of bacteria and a longer list of rarer bacteria and yeasts.

From this point on, little changes until old age — a person's microbial complement seems to remain stable throughout adulthood, Doré says. But he has found that the faeces of people over 60 contain a much larger number of different bacteria than younger people. He suspects that the weakening barrier to new species may help explain why the elderly are more susceptible to gut infections and certain forms of cancer.

Colonic closed shop

The basis of the microbial stability that persists throughout most of our lives is still poorly understood, but is probably related to nutrient supply. By the time an infant is two years old, resident bacteria have monopolized every source of nutrients in the gut. They have also become interdependent, supplying nutrients to each other — one cell's waste is another's food. With all the nutrients accounted for, newcomers may find it hard to gain a toehold.

The stability of this ecosystem benefits not only the gut microbes, but also the human host. It prevents pathogenic bacteria, such as the various species of *Salmonella* that cause food poisoning, from taking up long-term residence. But it also means that probiotics cannot permanently change the make-up of the gut — they must be taken daily to have any effect.

What are the possible benefits? Individual species of bacteria are informally classified on a sliding scale of 'goodness' and 'badness'. Collectively, gut bacteria aid digestion by breaking down tough fibres, enzymes and other proteins. In addition, 'good' bacteria, such as species of *Lactobacillus*, *Bifidobacterium* and *Eubacterium*, are involved in fermentation reactions that produce organic acids that can be absorbed into the body and used as an energy source. 'Bad' bacteria, such as some members of the genus *Clostridium*, generate as by-products compounds including nitrosamines and cresols, which are possible carcinogens.

Commercial probiotic strains are, of course, 'good' bacteria. The probiotic milk products, yoghurts and capsules on the market generally contain *Lactobacillus* and *Bifidobacterium*. Most studies of their efficacy have been poorly controlled and have produced contradictory results, says Gibson. But a handful of well-designed clinical trials indicates that some such bacteria may help ameliorate diarrhoea[1–3] and some types of inflammatory bowel disease[4,5].

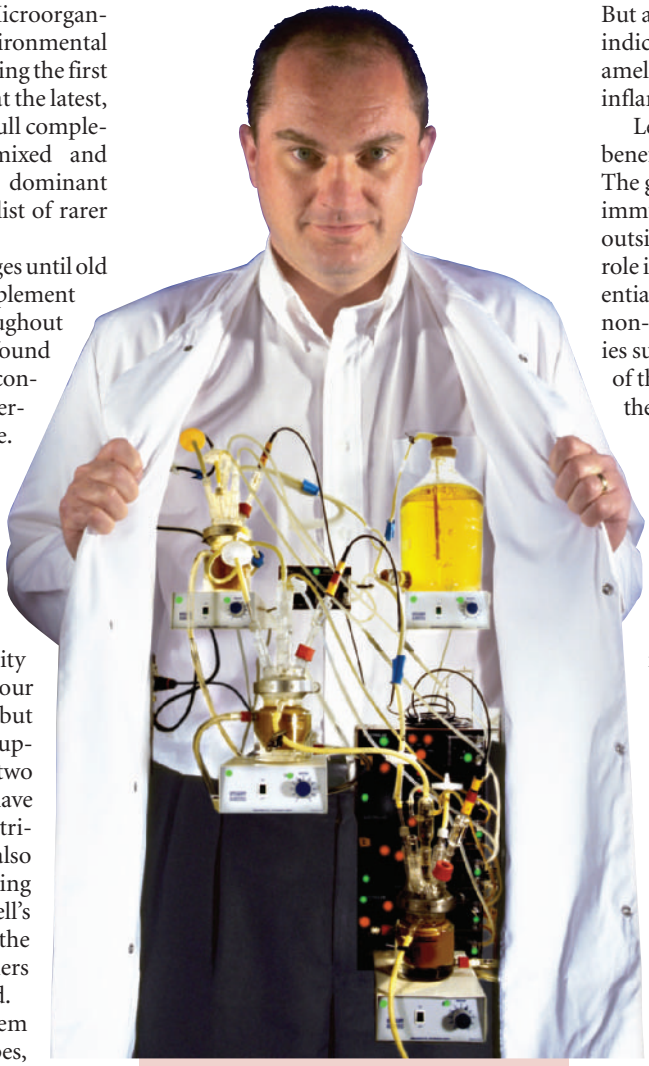
Less well documented are the claims for beneficial stimulation of the immune system. The gut, with its massive blood supply, is the immune system's primary contact with the outside world, and gut bacteria seem to play a role in teaching the immune system to differentiate between dangerous invaders and non-hostile challenges. Although some studies suggest that probiotics can affect features of the immune system, few have shown that these changes are beneficial to health.

A notable exception is a long-term study supported by the Finnish Academy of Sciences, in which pregnant women from families prone to allergies ate *Lactobacillus rhamnosus* daily. After delivery, the bacteria were given daily to the babies for the first six months of their lives. The treated infants were much less prone to allergic reactions such as eczema than controls who did not get the bacteria[6,7]. Erika Isolauri, an immunologist at the University of Turku who led the study, is now trying to determine how the treatment works. She suspects that the probiotics shift the balance between pro- and anti-inflammatory factors in the developing gut.

Friend or foe?

Other studies to assess the health benefits of probiotics are under way. With funding from the EU, for example, Doré is setting out to test a combination of *Bifidobacterium animalis* and a type of prebiotic sugar known as FOS on the gut ecosystems of young and old people in France, Germany, Sweden and Italy. "We are testing faecal samples to see whether the level of *Bifidobacterium* really does rise with this treatment, as would be expected," he says. His team will also assess how the levels of toxic and potentially carcinogenic compounds in the gut rise and fall with treatment by exposing cell cultures to extracts from the subjects' faeces. The researchers hope to determine whether suppressing 'bad' bacteria with pre- and probiotics might protect against colon cancer.

Francisco Guarner, a gastroenterologist at the Vall d'Hebron Hospital in Barcelona, Spain, is helping to organize an EU-backed clinical study involving 360 patients chronically suffering from one of two types of inflammatory bowel disease — ulcerative colitis or Crohn's disease — at centres in Ireland, Spain, Finland and France. The patients, all in remission, receive either *Lactobacillus salivarius* or *Bifidobacterium infantis*, two species that reduce gut inflammation in lab animals. The researchers then test the patients' saliva for marker molecules associated with inflammation. "Animal studies



S. ASHFIELD/SPL

Roboguts

The jumble of jars and tubes bubbling away in Glenn Gibson's laboratory smell pretty much as you would expect. They are models of the human gut. And Gibson (above), a gastroenterologist at the University of Reading, UK, uses them to investigate, among other things, the effects of new probiotics on gut microbial ecology.

He has infant guts, adult guts, ailing guts and more — around 20 in all. Each jar is a different section of colon seeded with the appropriate mix of microorganisms. Each assembly has a 'mouth' into which Gibson feeds meals, nutrients, probiotics or even harmful microbes.

The many tubes and ports give Gibson precise control over what goes on inside each of his models. For instance, by altering the speed at which food travels through them, he can give his artificial guts diarrhoea or constipation.

It may not be pretty, but Gibson says that the models are good enough to give him a fairly clear view of the events that would otherwise be hidden in the murky depths of our entrails.

CUSTOM MEDICAL STOCK PHOTO/SPL

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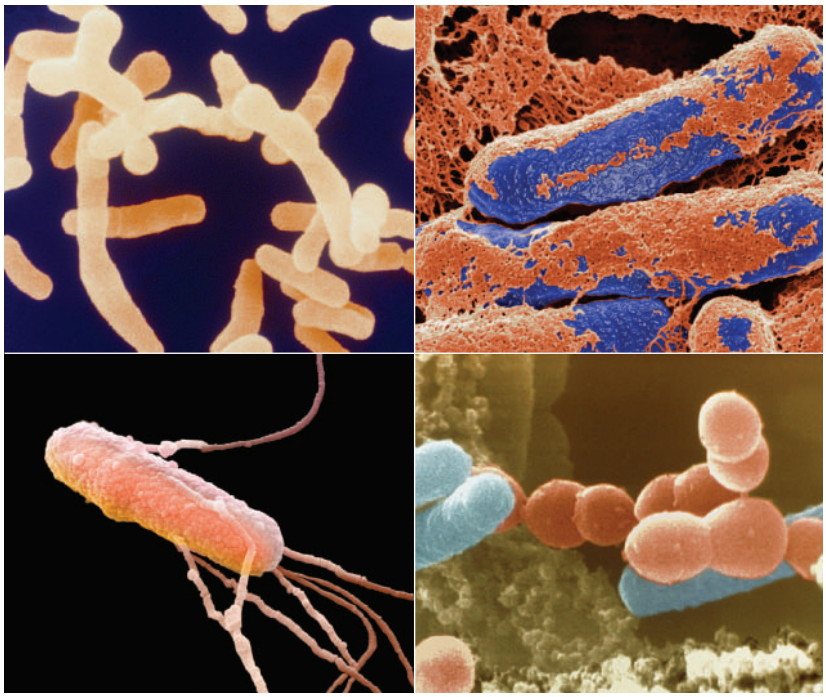

G. GAUGLER/SPL

SCIMAT/SPL

'Good' bacteria such as *Bifidobacterium* (top left) and *Lactobacillus* (blue, bottom right) may help to ward off pathogens such as *Salmonella* (bottom left) and *Clostridium botulinum*.

show that inflammatory disorders of the bowel may be helped by making the gut microbes less aggressive," says Guarner. He hopes that the probiotics will extend the patients' remission so they can reduce their reliance on immunosuppressive drugs, which have severe side effects.

Outside the EU network, Gibson is running trials at six British centres using *Lactobacillus plantarum* together with a second type of prebiotic sugar called GOS in various kinds of inflammatory bowel disease. Gibson's hypothesis is that the yeast *Candida* causes the symptoms, and he hopes that the probiotics will outcompete its growth. In a separate study, he is testing his hypothesis that certain 'bad' bacteria contribute to ulcerative colitis by generating toxic sulphur compounds such hydrogen sulphide, which smells of rotten eggs. He is giving patients FOS and GOS to stimulate the growth of competing 'good' bacteria to see whether this eases the symptoms.

Microbes on trial

In the next few years, these and other studies will help to determine how beneficial probiotics actually are. In the meantime, other salient questions are being addressed. Can we, for example, assume that probiotics are safe? One potential problem is that many probiotic strains have genes that allow them to resist antibiotics, which they might pass on to pathogenic bacteria. To address these concerns, Herman Goossens of the University of Antwerp in Belgium has acquired more than 200 commercial probiotic strains — the world's largest collection. He is systematically analysing them for their potential to transfer antibiotic resistance genes, and is also testing for any direct toxic effects that they may have.

Some experts believe that another important step will be to read the genomic sequence of every species of microbe that can colonize the human gut. They argue that a complete genomic databank would make it much easier to select species for specific probiotic effects.

To that end, the Defense Advanced Research Projects Agency, a research arm of the US military, is sponsoring a project to read all the genomes in the gut ecosystem with the same 'shotgun' method used for the privately funded effort to sequence the human genome. This approach avoids the need to separate out individual organisms. Instead, fragments of all the genomes are read off together. Computer algorithms then reassemble the fragments on the basis of overlapping sequences into complete genomes. "It's possible to conceive of doing this because the cost of sequencing has come right down," says Claire Fraser, director of The Institute for Genomic Research in Rockville, Maryland, where the work will be done.

Even if probiotics and prebiotics prove to have only modest health benefits, some scientists are considering the possibility of souping them up with genetic engineering. Many proponents of probiotics reject this idea, saying that it would be too hard to convince the public to eat live, genetically modified bacteria. But among the traits that would be useful to engineer are the ability to survive the acid environment of the stomach, a bit of 'stickiness' to help bacteria adhere to the gut lining, and so take residence for longer, and the ability to produce organic acids. Bacteria might even be engineered to deliver drugs, vitamins or vaccines[8,9].

There is already evidence that some bacteria can serve as efficient delivery vehicles. For example, Lothar Steidler of Ghent University in Belgium and his colleagues have shown that *Lactococcus lactis* genetically modified to secrete the anti-inflammatory molecule interleukin-10 can reduce colitis in mice[10]. A version for humans has also been developed that includes safety features to prevent the escape of the inserted gene into the environment[11]. A small clinical trial of this microbe is planned in Amsterdam, marking the first use of a genetically engineered bacterium as a therapeutic agent.

Unfortunately for the scientists studying probiotics, the only way forward is to delve into more human waste. Doré says he recently felt a pang of regret over that fact on a trip to visit some oceanographer friends in Marseille. "I looked out into the Mediterranean and thought: 'I'm obviously working on the wrong ecosystem'," he sighs. But the scientific challenges presented by gut bacteria are interesting enough to keep him going, he says. "It makes up for the unpleasantness." ■

Alison Abbott is *Nature*'s senior European correspondent.

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