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A researcher tests donor stool before it is used for faecal microbiota transplantation.

Engineering the microbiome

Modified bacteria and carefully formulated microbial communities could form the basis of new living treatments. **By Claire Ainsworth**

Although it happened almost a decade ago, Willem de Vos still vividly remembers his colleagues being told to halt the clinical trial they had been running. De Vos was part of the team conducting the first randomized clinical trial of faecal microbiota transplantation (FMT) – faeces from healthy donors were used as a last-resort treatment for people with a devastating, recurrent gut infection caused by the bacterium *Clostridium difficile*. About a year in, the data and safety monitoring board overseeing the trial had seen enough: the trial needed to end. But it wasn't because the therapy didn't work – quite the opposite. The

transplants were proving so successful that it was no longer ethical to continue to give people in the control group the conventional antibiotic treatment with which the transplants were being compared. "That showed us that it worked and why it worked," says de Vos, a microbiologist at the University of Wageningen in the Netherlands and the University of Helsinki in Finland. The antibiotic-treated patients who relapsed were given the transplant, which cured them.

The *C. difficile* story is one of a growing list of examples of how the gut microbiome shapes our biology. The community of microbes that lives in the gut has been associated with many

aspects of our physiology – from conditions such as obesity to how the immune system functions and even mental health. The success of FMT in treating *C. difficile* also shows that, in principle, the ecology of the gut can be manipulated to treat disease. Now, scientists are attempting to engineer gut microbiota that will allow them to do just that.

Synthetic biologists are working at the level of individual species, engineering gut bacteria not only to deliver therapeutic payloads but also to monitor and respond to conditions inside the body. Meanwhile, synthetic ecologists are looking at the gut as an ecosystem and assembling communities of microbes that

interact to produce substances or behaviours for medical benefit. Both approaches are in their infancy, and there are challenges to getting them into the clinic. Yet the technologies are already proving to be powerful tools, allowing scientists to explore the complex microbial interactions in our internal ecosystem.

Bespoke bacteria

Engineering individual microbes has an impressive array of potential applications. Gut bacteria have been altered to produce therapeutic molecules to treat metabolic conditions, kill pathogens and trigger immune responses to cancers. A strain of *Escherichia coli* engineered to produce the proteins needed to correct rare metabolic deficiencies is now in clinical trials. And in 2018, a team in Singapore revealed gut bacteria that it had engineered to stick to colon cancer cells and secrete an enzyme that converts a substance naturally found in vegetables such as broccoli into a molecule that inhibits tumour growth. When given to mice with colon cancer, the treatment shrank tumours and reduced recurrence¹. Bacteria can even be engineered to sense signs of disease and respond by producing therapeutic molecules. For example, in 2017, researchers took a gut bacterium commonly used as a probiotic and gave it the ability to detect communication signals produced by pathogenic bacteria. The probiotic bacterium then produces an antimicrobial molecule in response. The researchers showed that it helped clear infections in worms and mice².

Studies such as this show the potential of live therapeutics, but so far the engineered bacteria are comparatively straightforward systems – they produce a therapeutic molecule either at a constant rate or in response to an environmental signal. Now, researchers are looking to broaden the scope of engineered microbes and engineer bacteria with DNA containing more complex elements designed to work like electronic circuits. This is the realm of synthetic biology, a discipline that aims to apply engineering principles – such as standardized, modular components – to biological systems.

These complex feats of engineering are allowing bacteria to do simple computational tasks, such as remembering a one-off stimulus long after it has passed. For example, a team of synthetic biologists led by Pamela Silver at the Wyss Institute for Biologically Inspired Engineering at Harvard University in Boston, Massachusetts, engineered a bacterium to detect a chemical produced by inflamed gut cells. In response, the bacteria secrete a molecular signal, and continue to secrete it even if the gut inflammation dies down. The

signal can be detected in stool samples, raising the possibility of using the bacterium as a living diagnostic test for inflammatory bowel disease – which is often transient in nature and, therefore, hard to detect in the clinic. The bacteria formed a stable colony in the guts of mice for six months and responded to experimentally induced gut inflammation³. Importantly, engineered bacteria that can remember other kinds of environmental signal would allow researchers to explore conditions in different regions of the gut – something that is hard to do with conventional stool samples. “What we really would like is the bacteria to be like detectives and tell us what’s going on as they pass through,” says Silver.

Getting a genetic circuit to work in the lab is hard enough. Translating that to the messy, competitive environment of the gut microbiome presents an even greater challenge. Any modification that imposes an extra burden – say, extra protein production – on a bacterium puts it at a disadvantage, resulting in that organism either being out-competed or ditching its engineered function to survive. Partly for this reason, researchers have struggled to get many engineered bacteria to make the leap from test tube to animal models. Scientists are now working on ways around this; Silver, for example, is using genetic elements that naturally place a minimal burden on the cell.

The final hurdle will be showing that engineered bacteria are effective and safe. What’s more, unlike conventional drugs, engineered bacteria could spread into the environment and share their DNA with other bacteria. Although the chances of them surviving in the wild are thought to be low, the possibility of unforeseen consequences (not to mention the need to secure public acceptance and regulatory approval) has led researchers to explore a number of options to contain engineered bacteria, including kill switches that force bacteria to kill themselves with a toxin if their engineered circuits turn faulty or if they leave the body.

Constructing communities

While some researchers engineer individual bacteria, others are turning their attention to groups of microbes. Just as a city functions as a result of many people doing different jobs, the gut is a hive of interactions between myriad microbes carrying out different functions. Some interactions are metabolic – one bacterium might produce something that another consumes, for instance. Others are ecological, such as when one microbe inhibits the growth of another. By working together, communities of microbes produce molecules or behaviours that would not arise

from organisms acting alone.

These emerging properties of the gut microbiome have a profound effect on our biology, such as by producing vitamins or molecules that modulate our immune responses. To understand these interactions and to devise new therapies, researchers are building combinations of different bacteria known as synthetic ecosystems. For the most part, these ecosystems are made up of naturally occurring bacterial strains, although some scientists are experimenting with ecosystems containing genetically engineered microbes.

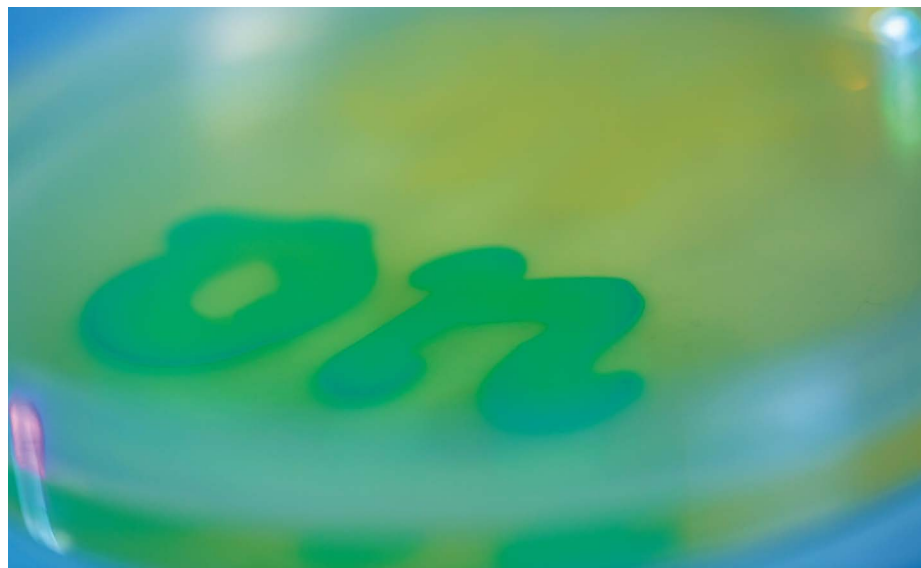
From a therapeutic point of view, synthetic ecosystems have a number of potential advantages. FMT currently relies on faecal matter provided by donors. Stool samples contain

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highly complex mixtures of microbes that vary from donor to donor, and each must be screened for pathogenic microbes. If FMTs could be stripped down to just the key species needed to treat people, simplified pathogen-free mixtures of these selected microbes could be grown in the lab. Synthetic communities would offer a standardized therapeutic with a known composition, and would lift the reliance on finding suitable donors.

Research, including a few studies in people, suggest that this approach could work. Mixtures of selected bacteria isolated from stool samples have shown promise in treating people with *C. difficile*. And it’s not just infections that could be tackled, but also conditions such as inflammatory bowel disease. In 2013, a team led by scientists in Japan identified a community of human gut microbes that could promote the activity of inflammation-damping immune cells called regulatory T cells, and showed that this could ameliorate inflammatory bowel disease in mice⁴. As well as developing therapies, stripping down conventional FMTs is allowing scientists to work out which bacteria in stool transplants are exerting a therapeutic effect – something that de Vos and his colleagues are exploring in conditions such as inflammatory bowel disease and metabolic syndrome.

One drawback of this stripping-down approach is that it limits the applications of the synthetic community to functions that already exist. There might be situations in which you would want to create a community with a new function, such as producing a vitamin



Synthetic biologists have engineered bacteria that remember the presence of a chemical and secrete a molecular signal that allows them to be identified.

or degrading a toxin. Creating new functions requires designing from the bottom up – testing different combinations of microbes, including those that don't normally co-exist in nature, until one gives the desired outcome. Doing this by trial and error in lab experiments soon becomes unwieldy, so instead researchers have turned to computer modelling.

The aim here is to predict the emergent properties of a microbial community, based on expected interactions between the microbes present. A team led by Elhanan Borenstein at Tel Aviv University in Israel created computer models of the metabolic reactions inside individual microbes, and then modelled how these would behave in the presence of another microbe's metabolism⁵. By simulating interactions between pairs of microbes, they showed how new metabolic products emerged that wouldn't be seen if the microbes acted alone. Models can simulate ecological interactions too, such as how the abundance of one microbe affects the abundances of others. This can help scientists to design microbial communities that are stable and therefore persist over time.

It's the ecology

Computer modelling and lab-grown communities allow researchers to gain a better understanding of how microbes in natural communities in the gut interact – both with each other, and with their human hosts. De Vos's team grew four different bacteria that usually live together in the mucus layer lining the gut⁶. One species, *Akkermansia muciniphila*, breaks the mucus down into compounds that other bacteria consume. The team showed that the other bacteria were not

only consuming these compounds, but were also feeding molecules they had made back to *A. muciniphila* and, in the case of butyrate, a fatty acid needed by the cells of the gut lining, to their host.

Researchers are also gaining new insights into the relationships between microbes and between microbes and their host from the creation of minimal microbiomes – constructed microbial communities containing the smallest number of species needed to create a stable ecosystem. A 2016 study showed how combining a minimal microbiome with comparative

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genomics can lead to the design of a microbial community with a desired property. Bärbel Stecher at the Ludwig-Maximilians University of Munich in Germany and her team developed the Oligo-MM¹² minimal microbiome – a collection of 12 gut microbes that helps to prevent *Salmonella enterica* from colonizing the guts of mice lacking any bacteria of their own⁷. The 12 bacterial species excluded *Salmonella* almost, but not quite, as well as a conventional microbiome. By using genomics to compare their minimal microbiome with a complex one, the researchers singled out the ecosystem functions that were missing from their community, added three more bacterial species that could fill the gap, and produced a community that was as good as the conventional one at keeping *Salmonella* out. Ultimately,

researchers hope that studies such as this will allow them to design minimal microbiomes with defined therapeutic properties, such as producing butyrate or vitamins.

Perhaps the eventual application of microbiome engineering would be to combine synthetic biology and synthetic ecology. Scientists would create communities containing genetically-engineered microbes, the collective behaviour of which would deliver a therapeutic benefit. One advantage of this approach is that it would let engineers distribute different metabolic tasks between different bacteria. This means all the physiological stress of making a drug or a vitamin would not be placed on just one bacterium. A number of teams have made progress in this area, including exploiting a system that bacteria use to detect the presence of other bacteria and to modify their gene activity in response. Researchers are using this feature, known as quorum sensing, to control the behaviour of mixed populations of bacteria, to, for example, allow bacteria that compete with each other to co-exist and form a stable population.

The potential paybacks of engineering the gut microbiome are immense, but so are the challenges to reaching this goal. Of all the human microbiomes to take on, the gut microbiome is by far the largest and most complex. Much remains to be learnt about its denizens, their genes and their interactions. And that's before you get started on what the human host brings to the party. Indeed, there is so much variation between individuals that it's still not clear what a 'healthy' gut microbiome looks like (see page S6).

Even so, the potential payoffs are motivating the scientists to aim high. Borenstein hopes one day to take information about an individual – the microbes in their gut, their physiology, their diet and their genome – and use it to build a full-scale computer model of their gut microbiome. Such an advance might make it possible to design personalized interventions to treat or prevent disease.

“This is not something we'll get to in a year, or two or five,” Borenstein admits. “But we're making progress and learning a lot of interesting biology on the way.”

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