BUGS ON PATROL

Smart bacteria wired up to police our gut will revolutionise the way we treat disease, says **Ed Yong**

BRETT RYDER

MATTHEW WOOK CHANG has opened an academy for assassins. His trainees are deadly. By rewiring the genes of the common gut bacterium *Escherichia coli*, Chang has created a killer that can detect, chase down and destroy microbes that make us sick.

His primary target is *Pseudomonas aeruginosa*, a bacterium that seizes any chance to infect people with weak immune systems. It can wreak havoc in hospital wards, in the lungs of those with cystic fibrosis, and in the guts of premature babies. In 2013, Chang put his assassins through their paces in a lab flask. They have since been hanging out in the guts of mice, keeping them safe. "We're about to wrap up animal studies," he says. "The survival rate of the mice was significantly increased." He is now training up assassins for other targets.

Chang, a biochemist at the National University of Singapore, is just one of a growing club of scientists who are tweaking our microbiome – the vast community of microbes that live in or on our bodies – in pursuit of better health. Armed with the tools of synthetic biology, they are stuffing bacteria with circuitry composed of new combinations of genes, turning them into precision-targeted micro-drones designed to detect and fix specific problems.

Some lie in wait for pathogens like *P. aeruginosa* or the cholera bacterium *Vibrio cholerae*, releasing lethal payloads when they have the enemy in sight. Some use the same tactics to attack cancer cells. Others can sense signs of inflammation and release chemicals that could help to treat chronic conditions like Crohn's and inflammatory bowel disease. And these tricks aren't just confined to the lab.

Synlogic is one start-up already looking to bring smart bugs to market. It has secured almost $35 million in initial funding from backers including the Bill and Melinda Gates Foundation. The company is on track to start human clinical trials by the end of 2016. "Our goal is to treat the trillions of cells that live with us but aren't part of us, the cells that are like another organism in our body," says Jose-Carlos Gutiérrez-Ramos, Synlogic's CEO.

Welcome to the age of smart probiotics, where specially designed bacterial rangers patrol the gut, reporting on the state of the environment and putting out fires. Will this tech lead to better all-round health for us all or are we jumping in where we can't yet swim?

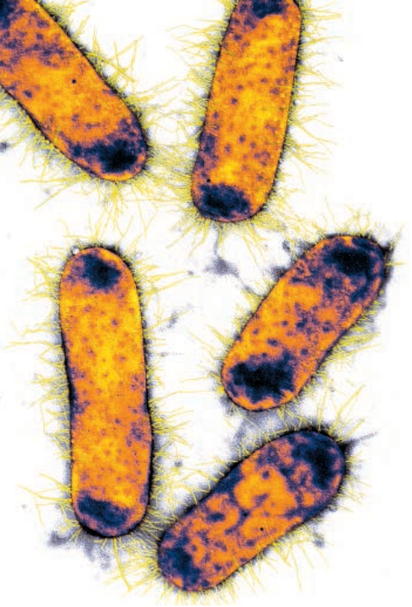
The study of our microbiome is one of the hottest fields in biology. These resident microbes train our immune system, guide the development of our growing bodies and protect us from disease – not to mention digesting our food. The microbiome is a critical part of our lives, and a mutable one: unlike our genome, which is largely static, it can readily change. Many researchers think that manipulating it could treat infectious diseases or conditions like obesity, diabetes and more.

Tinkering with our microbiome isn't

"Welcome to the age of smart probiotics, where bacterial rangers put out fires"

new. Probiotic yogurts purport to introduce beneficial microbes into our bodies, but since these species are not common gut residents, they rarely stick around for long. So probiotics have largely failed to live up to their own marketing hype. Up next are faecal transplants – the wholesale relocation of microbe-rich stool from a healthy donor to a sick one. The procedure is actually thousands of years old and has proven extremely effective at treating *Clostridium difficile* – a tenacious bacterium that causes severe, recurring diarrhoea. Donor microbes easily oust this pest, succeeding even when antibiotics fail. But faecal transplants are not problem-free. Donors must be laboriously screened to ensure they won't pass on pathogens. Plus, every stool is different, causing headaches for regulators. And, of course, there's the yuck factor.

Time for new approaches. Companies like Seres Therapeutics in Cambridge, Massachusetts, and Vedanta Biosciences in Boston are trying to identify cocktails of beneficial microbes that can both thrive in the gut and also be standardised and controlled. Other groups are forsaking naturally ›



E. coli is the microbe of choice for designers of programmable bacteria

occurring bacteria in favour of microbes engineered to perform specific jobs. Back in 2000, for example, Lothar Steidler, now at ActoGeniX in Zwijnaarde, Belgium, engineered *Lactococcus lactis* to secrete an anti-inflammatory molecule called IL-10. The bug successfully treated intestinal inflammation in mice and even made it to human trials – but, so far, without success.

These approaches have merit. Getting microbes to churn out beneficial molecules on site circumvents many of the problems that beset drug development, including the need to purify a chemical, get it into the gut, and package it to run the gauntlet of stomach acids and bile. But these engineered bugs are crude tools, designed for a single job. They are now being surpassed by a generation of smarter bacteria like Chang's that can be programmed to carry out missions tailored to the needs of an individual.

To fine-tune a microbe, you first need it to sense specific conditions. Last year, Pamela Silver at Harvard Medical School and her colleagues loaded *E. coli* with a genetic switch that allows it to record the presence of an antibiotic called tetracycline. When it encounters the drug, the switch flips, activating a gene that turns the microbe blue. By analysing the bugs that emerged in the faeces of mice fed the modified microbe, Silver could tell if the rodents had also been given tetracycline. Her microbes could sense and remember. To a large extent, the gut is a black box; food goes in, waste comes out. What happens in the intervening 8.5 metres of our intestine? Turning bacteria into surveillance drones lets us find out.

EYE OF SCIENCE/SCIENCE PHOTO LIBRARY

Silver's team were also the first to show smart bugs at work in a live animal. "It's easy to have the circuits work on the whiteboard in your office," says Synlogic co-founder Jim Collins at the Massachusetts Institute of Technology, who was involved in the study. "Biology is messy and noisy. Engineering it isn't as easy as it's sometimes presented."

Jeff Tabor at Rice University in Houston, Texas, now plans to exploit bacteria's ready-made sensors – enzymes called histidine kinases that sit on their surface and bind to different molecules. "They're the primary means through which bacteria sense their world," he says. "We've identified around 12,000 of them in the human microbiome but we only know about what 50 are sensing."

He has shown that some of these sensors are especially common in the guts of people who are obese, and he thinks they might be sensing chemicals which correlate with that state. By combining several of these sensors in a single microbe, he hopes to engineer a bug that can accurately detect when a person is at risk of obesity and other metabolic problems, ideally before symptoms show up.

The next step is wiring up detection circuits in the bacteria to those that act. Chang's strain of *E. coli*, for example, homes in on a molecule that *P. aeruginosa* cells use to communicate with each other. When it detects these signals, it swims towards them and releases two molecules – an enzyme that breaks large *P. aeruginosa* communities into more vulnerable fragments and an antibiotic that takes out the remaining enclaves. It listens, it hunts, it kills (see diagram, below).

Collins is also building a strike force. Like Chang, he is engineering strains of *L. lactis* to detect pathogens like *V. cholerae*, or *Salmonella*, and produce antibiotics that selectively kill them. So the drugs will only be released when and where they are needed. "They could serve as a prophylactic sentinel," he says. "Only individuals who are ever infected would be exposed to the molecule."

That's better for us, since we won't get swamped by needless antibiotics or anti-inflammatory compounds. It's also better for the microbes, burdened as they are by energetically expensive synthetic circuits. Until they need to swing into action, they can conserve energy and so compete more readily with their natural counterparts in the gut.

Crisis prevention

For this approach to work, however, people would have to ingest the microbes before they are sick. Collins imagines that his microbes would roll into action in a crisis like the cholera outbreak that hit Haiti in 2010 and eventually infected more than 6 per cent of the population. If communities at risk were given a cholera-killing bacterium beforehand, such epidemics could be contained. Military personnel might also swallow sentinel microbes before being deployed to foreign countries. "Traveller's diarrhoea costs the military an incredible amount of money," says Silver, who has just secured a grant from the US Defense Advanced Research Projects Agency for her work. "Every time a soldier goes overseas to serve, they get laid out for days."

The applications for smart probiotics are vast. Chang wants to engineer bacteria to convert substances in the gut into anticancer compounds. Michael Fischbach at the University of California, San Francisco, is supercharging the microbiome's ability to make small molecules, like antimicrobials that control other microbes, or neurotransmitters that affect our mood and behaviour. "We should be controlling these processes," he says. "We're interested in going orders of magnitude beyond the natural capabilities of the microbiota."

Synthetic biologists aim to combine different abilities in single organisms, like hip-hop artists remixing samples into fresh tracks. "The hope is to have a parts list," says Justin Sonnenburg from Stanford University in California. "At a certain point, this will become a plug-and-play system."

But the parts are just half the battle. You need to decide which bacterium to plug them into. Scientists like Chang and Collins are working with *E. coli* because of its reputation as a workhorse of molecular biology, plus the abundance of tools and techniques for manipulating it. But Fischbach argues that *E. coli* is a poor choice, since it is a bit-part player in the gut and found at very low levels. "I don't think they've picked the right horse," he says. Sonnenburg agrees. But switching workhorses is tricky: circuits that operate in *E. coli* won't necessarily work elsewhere, so you often have to start from scratch.

Sonnenburg's team have spent the last few years doing exactly that, developing genetic circuits for *Bacteroides thetaiotamicron* – one of the best studied common gut bacteria. The *Bacteroides* genus collectively accounts for between 30 and 50 per cent of the microbes in a Western person's gut. They are well attuned to that environment and are excellent colonisers, so won't easily dislodge unlike existing probiotics. What better candidates for donning a ranger's badge?

Yet not everyone is thrilled at the prospect of a synthetic organism taking up residence in our gut, especially when our understanding of the microbiome is still in its infancy. How do you distinguish between healthy and unhealthy communities of gut bugs? How do the multitudes of microbes influence and interact with each other? What makes them resilient or vulnerable to disturbances? Such unanswered questions make it hard to predict how the presence of an engineered microbe might rock the status quo. Would it slip seamlessly into the existing ecosystem, or turn out to be the microbial equivalent of a cane toad – a species introduced to control a pest and that then runs amok?

Without full knowledge of the risks, is it wise to work with species that are naturally great colonisers? "We'd rather not modify the microbiome long term," says Collins. Even though he is working on probiotics to treat chronic conditions like Crohn's disease, he would rather that the microbes were a temporary presence that had to be replenished with daily doses. "You don't want to aggravate these conditions," he says.

DAMON WINTER/NYT/REDUX/EYEVINE

Smart probiotics could help combat cholera outbreaks like the one in Haiti in 2010

Fischbach takes the opposite tack. With Crohn's you want to use something that will fight for a niche in the gut, he says. "It's a flip of a coin about whether the community you'll have is better or worse than the one you started out with. I'd take that flip if I knew I'd end up with a specific benefit."

To allay concerns, the engineers will need to build in safety features that allow you to pull the plug on any introduced microbes if problems emerge. At the very least, they should start with strains that don't have any obvious genes for virulence or antibiotic resistance. They can then add kill-switches that make bacteria self-destruct if they sense the right triggers. Alternatively, they can rig microbes to depend on certain nutrients, so that they simply die without a constant supply. Silver has a more ambitious strategy: she plans to engineer a group of around five gut bacteria that can only work as a team. "This gives you exquisite control, more than just building in a kill-switch," she says. "If they're all co-dependent, they'll be cleared from your system rapidly if one of them mutates or dies."

> **"Instead of modifying our own cells, we will modify the cells that live with us"**

These measures will be crucial if regulatory agencies are to approve smart probiotics. "If it's not natural, it's bucketed as a gene therapy," says Bernat Olle from Vedanta Biosciences. "Then, you have higher hurdles as you go through the regulatory process." Scientists will have to show that they can contain the microbes once they leave a patient's body, and potentially enter the environment or the water supply. "Any environmental niche that the organism can go when you poop it is regulated by some agency," says Olle. "You'll have to prove to them that the organism can be killed."

Synlogic is confident it can clear these hurdles. Last month it announced that it was developing synthetic bacteria to treat two rare but nasty metabolic conditions: urea cycle disorders, where the body cannot break down ammonia, and phenylketonuria, where the body cannot break down an amino acid called phenylalanine. In both cases, impaired gut metabolism results in a dangerous build-up of toxic substances in the bloodstream. Synlogic's smart bugs contain genetic circuits that switch on when a toxin is detected and render it harmless. The company is aiming to begin human clinical trials at the end of next year. The next step will be testing smart bugs that cure more common conditions like inflammatory bowel disease or Crohn's, says Gutiérrez-Ramos.

"Usually, when we talk about gene therapy curing a disease we are talking about correcting a defect in our own cells," he says. "But instead of modifying our own cells, we will modify the cells that live with us." ■

Ed Yong is a writer based in London. His first book, *I Contain Multitudes* – about how microbes influence the lives of every animal – will be published in 2016

Delivering the goods

Bacteria can be genetically programmed to shoot a laser beam at pathogens