Feature

30 December 2015

Mining the hidden treasure of the world's unknown bacteria

Almost every known antibiotic came from 1 per cent of bacteria. Now we are learning how to grow the unknown microbes, and who knows what riches we'll discover

SCOOP up a handful of dirt or wipe the inside of your cheek with a cotton swab. Not impressed? You should be. What you have there are the enigmatic rulers of the world.

You can't see them, but they make up more than half the living mass on Earth. They have the power to save lives or make us seriously ill, as well as to protect crops from sickness and droughts. They even play a large part in controlling the climate. And nearly all the antibiotics in use today have come from them.

They are, of course, microbes. But given how important they are, it is surprising how little we know of them. Imagine you were to sprinkle that random pinch of soil on to a Petri dish, that classic nursery for microbes. Not even 1 per cent of the bugs will grow. Without a thriving colony, it's next to impossible to study a bacterium. So there is no escaping the fact that most of what's out there is microbial dark matter – its identity shrouded in mystery, like the strange stuff that makes up most of the cosmos.

"We have a track record of finding useful machinery inside bacteria"

It would be handy, to say the least, if we could unmask it. Think of it like this: if we've managed to extract our life-saving antibiotics from the tiny slice of pliable microbes, what riches might await us in virgin territory? It would be criminal not to explore.

Yet before we can, we must learn to satisfy the needs of these picky microbes and tempt them to grow.

One reason this quest matters now more than ever is that we're on the verge of an antibiotic crisis. Just months ago evidence emerged that bugs in China are developing resistance to polymyxins, the antibiotics of last resort. That means we urgently need new ones.

It might sound strange that antibiotics, our weapons against bacteria, often come from bacteria. In fact different species produce these molecules to ward off competing colonies. That's traditionally how we've discovered them. We grow several species on a dish and spot the colonies that have an exclusion zone around them where nothing else can survive. That's often a telltale sign that the colony is producing a microbe-killing molecule. Then comes years of painstaking lab work to isolate that substance and check if its safe to use in humans.

Culturing the unculturables would be useful for more than just antibiotics, though. We have a track record of finding useful bits of biochemical machinery in bacteria. Take the CRISPR gene-editing technique that promises to change the face of medicine. We uncovered the tools of its trade inside microbes (*New Scientist*, 5 December, p 32).

So why do most microbes steadfastly refuse to thrive on a Petri dish? One idea is that they are simply slow growing. Another is that they might need a specific balance of chemicals that the dishes don't usually provide. So what if scientists left colonies alone for weeks rather than the standard few days and tried different mixtures of chemicals?

Microbiologist Tsutomo Hattori helped pioneer this slow, more varied approach in Japan beginning in the 1970s. One of Hattori's team, Kyung-Sook Whang, went on to work with James Tiedje at Michigan State University. There, Whang was able to culture between 5 and 10 per cent of the microbes in a given soil sample by varying the materials on the plate and giving the microbes time.

David Fredricks, who studies human microbiology at the Fred Hutchinson Cancer Research Center in Seattle, tried a similar tack. While investigating disease-causing bacteria in the vagina, he tried culturing one or two microbes at a time for weeks. For some species, that did the trick. He also tweaked the medium by adding metabolites from the human vagina and found that would coax different communities of microbes into making an appearance.

Yet none of these labs fully resolved the problem. Could it be that researchers were inadvertently harming some bacteria? One proponent of this idea is Svetlana Dedysh of Russia's Winogradsky Institute of Microbiology in Moscow. She is an expert on microbes that grow in northern wetlands, such as peat bogs, and reckons that one common lab practice – stuffing microbes with food to encourage them to grow faster – is particularly detrimental for these species, which thrive in low-nutrient environments.

World shapers

Still, a proper solution to the problem of the unculturables looked far off when Slava Epstein learned about it. It was the late 1970s and he was a student at Moscow State University, studying zoology. As he looked more closely into the interconnectedness of various species, he realised something. "You and I and *Homo sapiens* are just a small ripple," he says. "We're

inconsequential for what the Earth was like 100 million years ago, or what it is going to be a billion years after you and I die. The only force that really shapes the planet is microbes."

But Epstein was quick to appreciate the huge gulf between the microbes that popped into view under the microscope and ones he could grow. Given that we've known about this for more than a century, Epstein calls it the "oldest unresolved phenomenon in microbiology". He became obsessed with it.

Epstein wasn't entirely convinced by the attempts to tweak cultivation methods to suit a bug's particular needs. That was essentially guesswork, he reasoned. And he would go on to develop his own theory about why most microbes won't grow (see "Domesticating microbes").

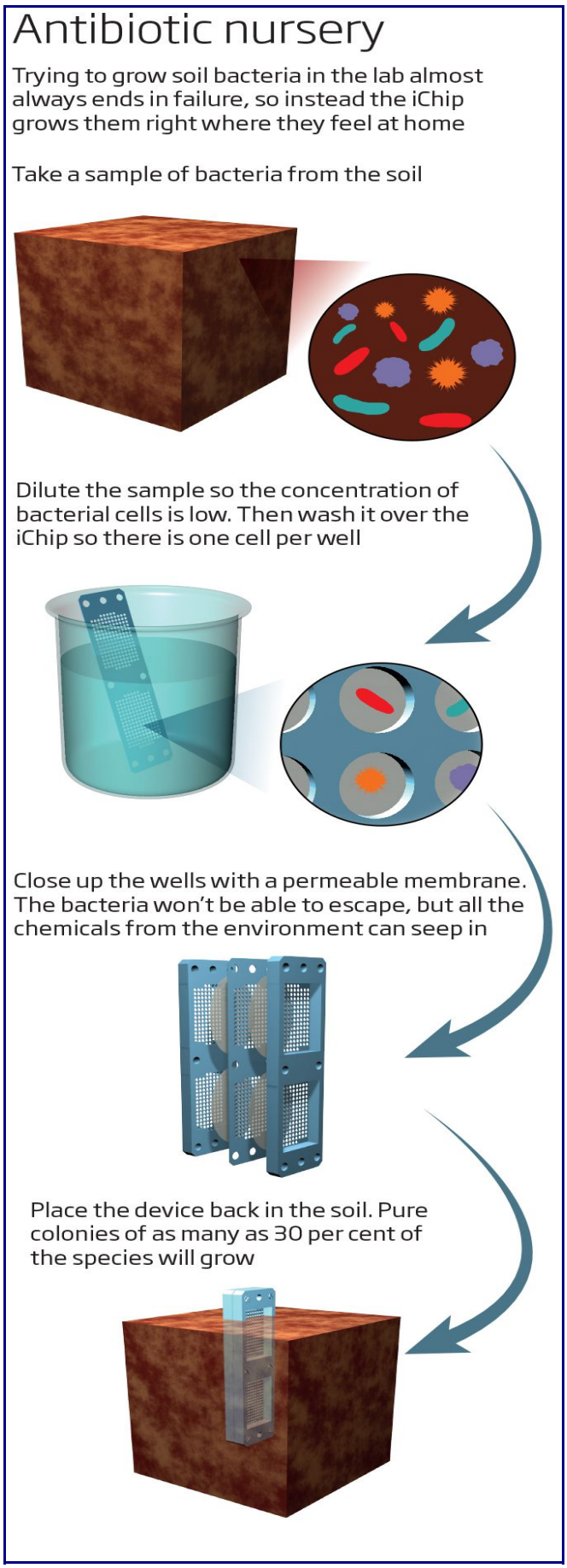
It started with a deceptively simple idea. If the problem was to do with taking microbes out of their natural environment, there was a an obvious workaround. Why not simply sample the bugs, encapsulate them in a permeable container and put them back in their favourite spot? That way microbes can grow right where they feel at home. "If we cultivate organisms in nature we don't have to guess," says Epstein. "Nature provides these microorganisms with everything they need."

The iChip keeps soil bacteria contained but happy

Translating this idea into reality wasn't easy. But eventually he managed to try it out.Epstein and his team extracted the microbes from a soil sample, parked them inside a metal disc containing a jelly-like substance called agar and sealed it on each side with a membrane. This membrane has a crucial property: it has pores that are too small to allow bacteria in or out, but all the chemicals from the soil can seep in.

The team buried the discs in the soil outside their lab at Northeastern University in Boston,

and meanwhile tried culturing microbes from the same soil in dishes. On these, just 0.1 per cent of the 10,000 soil microbe strains grew. But in the soil the picture was radically different: a whopping 20 to 30 per cent of species began to grow (*Science*, vol 296, p 1127).



This find led him to start up Novobiotic Pharmaceuticals, along with colleague Kim Lewis, to search for antibiotics.

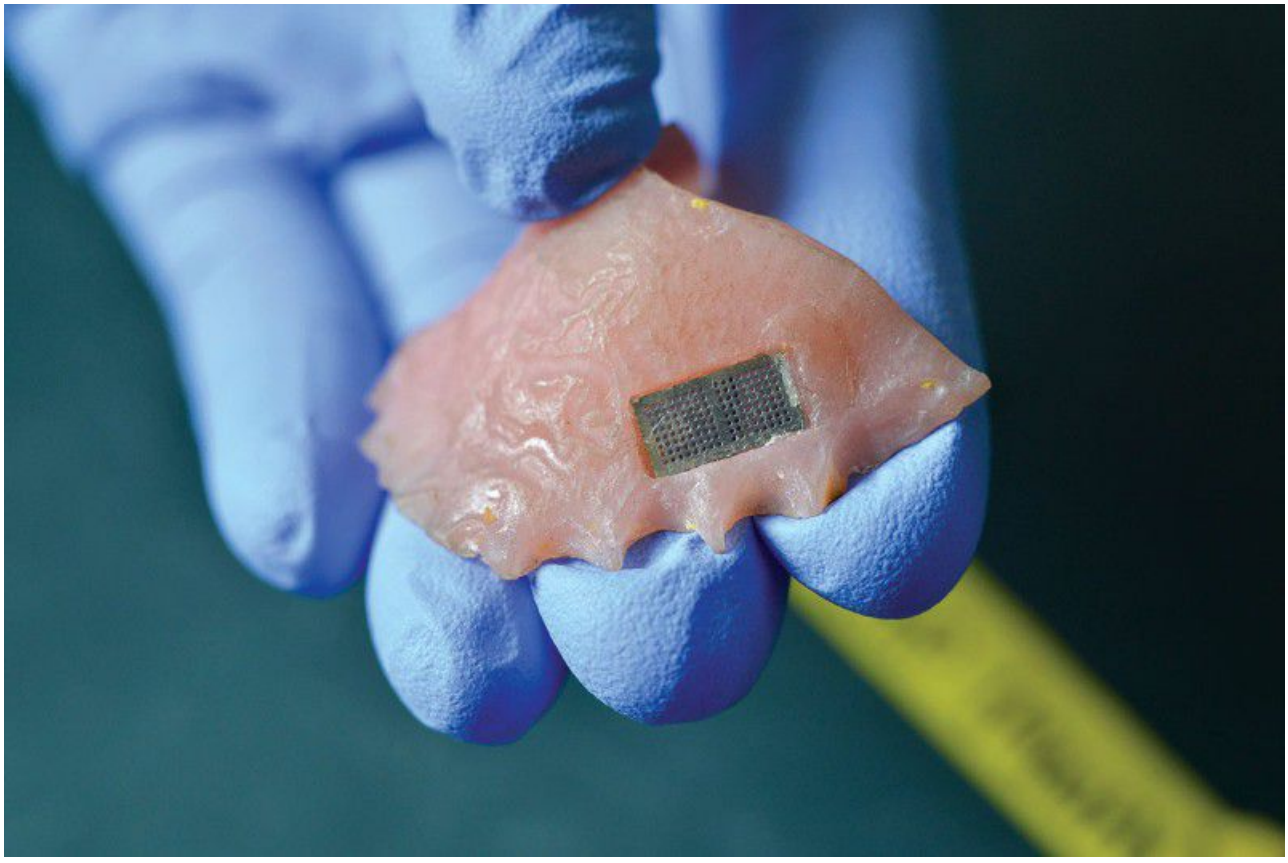
The next step was to find a way to culture species individually, which makes finding antibiotics easier. That's when the iChip came along (see diagram). Its miniature chambers hold single microbes, making it possible to grow pure colonies.

Over the past 12 years, Novobiotic has cultivated 50,000 strains of microorganism no one else could. And they've discovered 25 new antibiotics. One made headlines a year ago because it kills bugs in a new way, and one to which it is much more difficult for bacteria to develop resistance.

These 25 antibiotics will not necessarily all be useful; many look to be toxic to our cells as well as to bacteria. But the point is the speed of the approach. The firm found a new candidate antibiotic for every 2000 microbe strains it grew. That is orders of magnitude better than the pharmaceutical industry managed before it abandoned this method of discovering antibiotics, says Epstein.

Added to this, two of the 25 molecules appear to work against tuberculosis. As a result, the Bill and Melinda Gates Foundation are now funding their development into drugs. And antibiotics that are toxic to human cells often go on to be useful anticancer drugs, so they are not a lost cause.

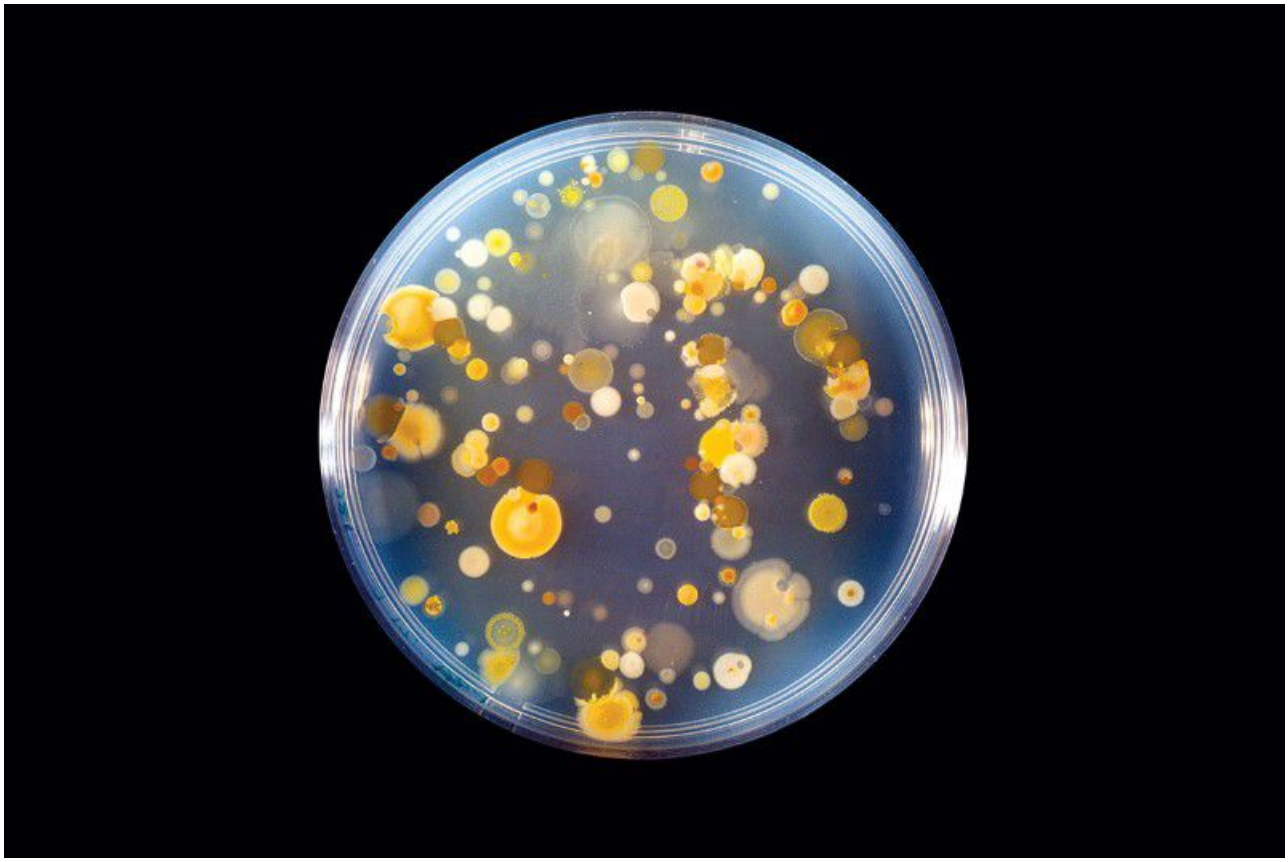
Karsten Zengler at the University of California, San Diego, is also trying to grow microbes in a way that mimics their natural environment. His technique involves coating droplets of liquid containing individual microbes in a permeable gel. He then places the resulting beads in a solution containing the same ingredients as the microbes' original environment.



Variants of the iChip could be used on the roof of the mouth

Zengler works with bacteria from our own bodies. We can already grow more than half of these bugs, but this technique can persuade the previously implacable ones to grow, Zengler says. One of the species he has tamed is *Vitreoscilla filiformis*, which lives in the skin and looks to contain a promising ingredient for anti-inflammatory skin creams.

One drawback to his approach is that it only works for microbes that live in environments from which liquid can be collected and utilised. Another is that it requires specialist equipment and techniques – though Zengler has co-founded a company to make the kit more widely available.



Then there are those who aim to study microbes without culturing them at all. To do this, they extract DNA from the environment and examine it directly. This approach, known as metagenomics, offers a way to get a snapshot of the different microbes in a given place, and it may even provide tools to develop new drugs. If you can pluck interesting-looking genes from the soil – or anywhere else for that matter – it's no big deal to engineer them into a familiar species such as *E. coli* and see what happens. Julian Davies of the University of British Columbia, Canada, is backing this approach as the path to new antibiotics. "That's the way it's going," he says. "You need enormous patience to culture bugs."

The trouble is that this technique hasn't produced any new antibiotics so far – largely because it's not obvious where the bits of DNA that govern their production would lie.

So it looks as if culturing is here to stay. That makes Epstein and Zengler's techniques all the more important. The next step is to scale them up. Novobiotic's 25 candidate antibiotics came from just a few hundred soil samples. There is scope for much more.

And why stop at Earth's top few centimetres of soil? Epstein is particularly excited about

exploring the vast number of microbial species populating the oceans, a potential treasure trove of antibiotics, he says. He hopes to create a version of the iChip that will work in marine environments. Plus, he has developed another version that sits inside a dental retainer in the roof of the mouth – and grown microbes using it already.

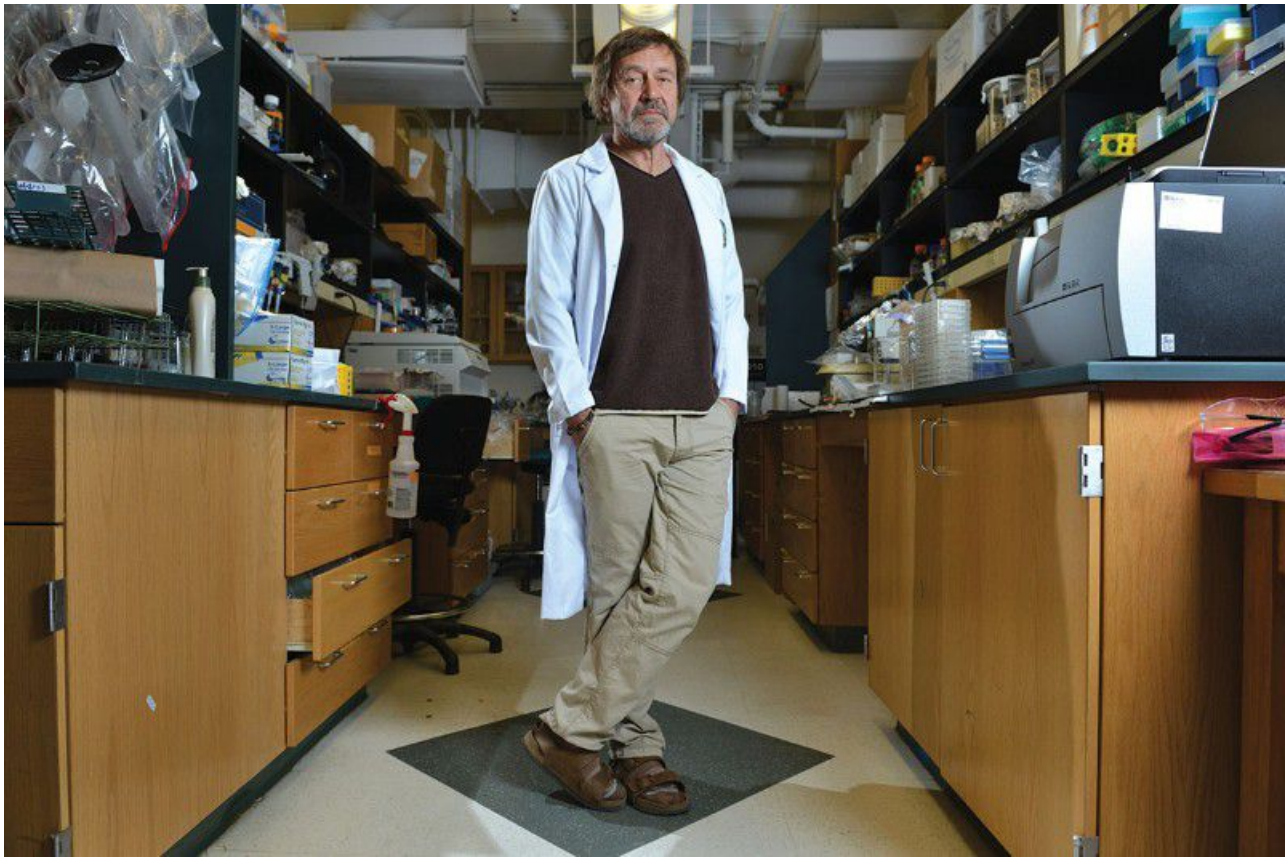
Fredricks is on the same page. He says he'd love to apply the same technique to his area of expertise, vaginal bugs, perhaps encapsulating an iChip style device in something similar to the birth control ring.

We are slowly unmasking microbial dark matter – even when it's inside our own bodies.

Leader: "Keep antibiotics useful: A new year's resolution for all of us"

(Images: KTSDesign/Science Photo Library, Crown Copyright/Health & Safety Lab/Science Photo Library, Josh Reynolds, Slava Epstein)

Domesticating microbes



Why won't most microbes grow if you tip them on to a Petri dish? It's possible that the balance of chemicals and food is not quite right, but Slava Epstein of Northeastern University in Boston has a different idea.

Many microbes in the soil naturally lie dormant for long periods of time. Epstein thinks they wake up not in response to a cue from the environment – a new food source for instance – but entirely at random. He calls these microbes "scouts". There is some evidence for their existence. Experiments in Epstein's lab and elsewhere have shown that if you leave certain samples of bacteria or fungi on a Petri dish for several months, species will begin growing at random.

This is not the whole story, however, because even if you take a colony that is actively growing in the soil and put it on a Petri dish it will often flounder.

To explain this, Epstein suggests that dormant bacterial cells could be akin to stem cells – which in animals can become any type of cell in the body. He thinks bacteria switch genes on and off to suit various environments, but once they have started to multiply they become fixed into that particular mode. That would mean if you transpose actively growing cells from one place to another they would not know what to do in the new environment.

If a microbe then enters a dormant state, that might reset its gene expression. So you would need dormant cells if you wanted to grow a colony in a new place. That can be done by growing a colony in an iChip (see diagram). The resulting mixture contains both dormant cells and actively growing ones. You have effectively increased the number of dormant cells in the colony and increased the chance that it will thrive in the lab; you have "domesticated" the microbes, Epstein says. He hopes to publish his work soon.

This article appeared in print under the headline "Make 'em happy"

By **Cynthia Graber**

Cynthia Graber is a science journalist in Somerville, Massachusetts, and presents the podcast Gastropod