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The Hunt for Antibiotics in Soil

Erik Ness

Slava Epstein feels a reverence for soil.

In January, the Northeastern University microbiologist and his colleagues at NovoBiotics unveiled teixobactin—one of the most promising antibiotics of the last decade. But for Epstein, the key is how they found it, in a soil sample from a Maine field. If he's right, teixobactin may mark a new era in antibiotic discovery.

Epstein's work ranges from identifying the microbes that live on human teeth to deciphering the microbial ecology of a lake in Greenland. He's also deeply intrigued by the mystery of why so few microbes can be grown in the lab.

Almost as soon as biologists began trying to grow bacteria on purpose, they realized that only a small percentage of microorganisms would thrive in their fermentation flasks and petri dishes. Eventually this disparity was dubbed "the great plate count anomaly." It's often estimated that less than 1% of bacteria available in an environmental sample can be cultivated using standard laboratory methods.

Epstein and his colleagues neatly bypassed this cultivation puzzle with a specially designed incubation chamber—the ichip. There is nothing electronic about the ichip—the 'i' stands for "isolation." About the dimensions of a Snickers bar, the precision-milled plastic contains 384 tiny holes. These isolation chambers are populated by massively diluting an environmental sample and then submerging the chip in the mixture. Once the chip is inoculated, fine membranes are secured on both sides, and the chip is returned to the sample's original environment for two or more weeks.

Bacteria cannot pass through the membrane, but the chemicals that they need from the environment can—and this is the key. The ichip acts as a diffusion chamber, incubating the cultures in their own environment.

Eleftheria terrae was one of the thousands of bacterial cultures from that Maine field. From this newly identified species, Epstein's group isolated teixobactin, which has shown great early potential against drug-resistant infections in mice.

Epstein believes that new cultivation efforts may allow scientists to grow closer to 50% of bacteria in a sample. "Not only are we working with a novel pool of organisms," Epstein says. "We are working with a novel pool of chemicals. The rate of the discovery is approaching what it used to be in the 1950s."

Restocking the 'Bare Cupboard'

In the heyday of antibiotic discovery following World War II, microbes cultivated from the soil were rich sources of new drugs. Rutgers University microbiologist Selman Waksman has a fascination with soil-dwelling actinomycete bacteria that began when he was an undergraduate in 1916.

In the heyday of antibiotic discovery following World War II, microbes cultivated from the soil were rich sources of new drugs (**top**). **Middle:** Selman Waksman became the first soil microbiologist to win the Nobel Prize for discovering 18 antibiotics. *Photo courtesy of Wikimedia Commons*. **Bottom:** The iChip, a miniature device Slava Epstein's team created that can isolate and help grow single cells in their natural environment, and thereby provide researchers with much improved access to uncultured bacteria. *Photo courtesy of Slava Epstein/ Northeastern University.*

In 1939, Waksman's former student, René Dubos, derived tyrothricin and gramici-

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din from bacteria, and Waksman began screening his actinomycetes. Waksman eventually discovered 18 antibiotics, including streptomycin and neomycin. In 1952, he became the first soil microbiologist to win the Nobel Prize.

Over the next 20 years, a pharmaceutical arsenal was teased out of the soil: kanamycin, gentamicin, geldanamycin, dactinomycin, and lincomycin. From France came the family of rifamycins. The rainforests of Borneo yielded vancomycin. Nothing rivals Waksman's actinomycetes, however; to this day about half of all antibiotics in clinical use are derived from this diverse group.

But the pace of discovery began to flag after a few decades. Despite tantalizing evidence of staggering microbial diversity, drug prospectors kept growing the same things in their petri dishes.

Meanwhile, microbes were rapidly evolving a dangerous resistance to once-revolutionary antibiotics. According to the Centers for Disease Control, 2 million Americans deal with drug-resistant infections every year, and 23,000 die.

"The cupboard is nearly bare," warned Margaret Chan, Director-General of the World Health Organization, in 2012. "A post-antibiotic era means, in effect, an end to modern medicine as we know it. Things as common as strep throat or a child's scratched knee could once again kill."

Antibiotic research had also moved away from cultivation. Drug designers learned how to configure molecules to hit specific targets. Synthetic chemistry—with its huge libraries of compounds and high-throughput testing—yielded many pharmaceuticals, but few antibiotics.

Meanwhile, genetic sequencing was getting cheaper and more powerful, spawning approaches like "metagenomics." Instead of cultivating and identifying individual bacteria, it was now possible to simply record every gene in the environment.

Huge ventures launched to sequence everything from the oceans to the human microbiome. Omnivorous and ambitious, these ongoing cataloguing missions promised to reveal all kinds of biological secrets, including new antibiotics.

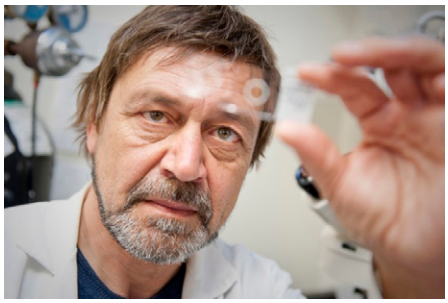
In metagenomics, computer programs sift through countless fragments of DNA—comparing, contrasting, and collating. Somewhere around half of the known genes are completely unmoored: They have no known function and are associated with no known microbe.

But just locating a gene doesn't necessarily lead to insight. Nature itself may be a better engine for discovery. Some bacteria can spawn new generations as rapidly as every 20 minutes, and many species also routinely swap out huge swathes of genetic material. Each potential rough draft of evolution creates fancier poisons, attractants, colors, and protective agents. It's in these funky molecules that novel chemical ideas emerge.

"Microbes have been interacting with each other and killing each other and communicating with each other for a long, long, long time," says Daniel Buckley, a microbial ecologist at Cornell University. "Chances are they've explored a lot of areas that we can't even imagine. The potential for discovery is tremendous."

New Cultivation Systems Yield Deeper Insights

Epstein says microbial cultivation is a neglected field, but researchers have made some progress isolating biochemically elaborate growth factors, such as cyclic AMP or homoserine lactones or sideraphores. Then, in 2002, scientists reported that simply diluting seawater had finally allowed the cultivation of a major order of oceanic bacteria. The ichip is exciting because of its broader applicability and high-throughput design.



Slava Epstein says microbial cultivation is a neglected field, but researchers have made some progress isolating biochemically elaborate growth factors. *Photo courtesy of Northeastern University.*

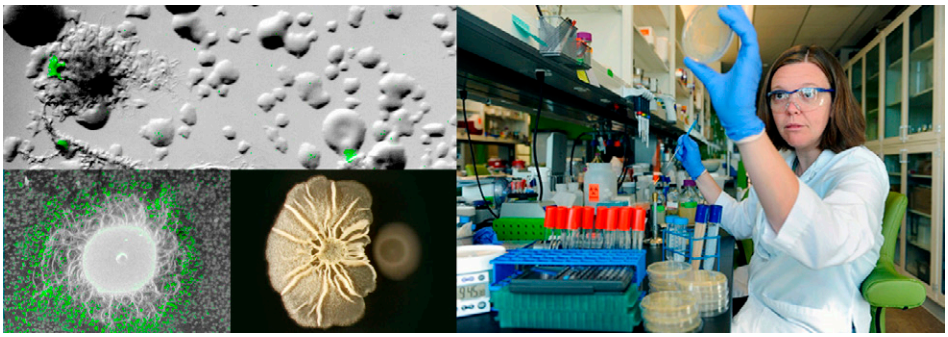
Mark Williams, a microbial ecologist in the Department of Horticulture at Virginia Tech, and his grad student Madhavi Kakumanu, spent a few years developing a similar experimental system. The inspiration: leaf litter, one of the organic building blocks of soil. "We're going through a renaissance," Williams says. "I would just be flabbergasted if in the next five years there aren't at least five new antibiotics."

In the meantime, Elizabeth Shank's lab at University of North Carolina at Chapel Hill is using a "transparent soil" first developed to study root systems. With a refractive index identical to that of water, you can see right through it.

"I call it transparent soil, but if you were a soil scientist, you would laugh me out of the room," she admits. "It is not soil in many ways. But it does capture some of the salient features that we think are going to be really important for understanding microbial interactions and their interactions with fungi and plants."

Shank's lab uses this "soil" to study carefully controlled interactions between different species of microbes. Being able to cultivate microbes is incredibly important to unraveling the impossible complexity of soil ecosystems. As her team gathers clues, it will knock out genes to figure out what's going on at the molecular level. The transparent system

also allows them to use complex imaging techniques.



Elizabeth Shank at the University of North Carolina at Chapel Hill is using a "transparent soil" to study carefully controlled interactions between different species of microbes. *Photo courtesy of UNC.*

They're already figuring out important details in biofilm formation—an important but poorly understand part of how bacteria colonize plant roots. Biofilms are also involved with drug-resistant infections on implanted medical devices, so finding a way to target biofilms could make a huge difference in defeating this problem.

For Shank, it's important to see medical potential in tandem with microbial dynamics. "Sometimes our focus on trying to find new therapeutics has narrowed our vision," she says. "We are interested in finding new molecules, but we're really much more interested in finding things ... that might be relevant to maintaining or stabilizing these complex communities. We're trying to find molecules that do things that might be important to the soil ecosystem of the microbes."

There Will Be More and More and More

While metagenomics itself hasn't yielded the expected bounty, it does suggest there is much more to discover. And that's encouraged scientists like Epstein to keep looking for more ways to culture new microbes. "You know the diversity of genes in the environment," he says. "You know the diversity of genes in the cultures you grow. You know these are totally different universes."

He believes a very useful symbiosis is developing, with cultivation feeding metagenomics and related fields while these in turn feedback to cultivation efforts. "There is no such thing as uncultivable," he declares. "The biological novelty of microbes is no longer a limiting factor. We can get a lot more than chemists can process."

The odds demand uncovering about 100 such molecules to find one that wins FDA approval. NovoBiotic, the company he helped found, has already found 25. "Teixobactin is a wonderful molecule, but at the end of the day, its fate may not matter," Epstein concludes. "Because there will be more, and more, and more. We need to scale up."