The hunt for antibiotics in soil

by Erik Ness

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Slava Epstein has 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 collected from a Maine field. If he’s right, teixobactin may mark a renaissance in antibiotic discovery.

As part of Northeastern’s Antimicrobial Discovery Center, 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 apparently available 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.

In the heyday of antibiotic discovery following World War II, microbes cultivated from the soil were rich sources of new drugs. Selman Waksman’s fascination with soil-dwelling actinomycete bacteria began as an undergraduate in 1916. He overlooked their antibiotic potential until 1939 when his former student, René Dubos, derived tyrothricin and gramicidin from bacteria. Waksman began looking for similar powers in his actinomycetes. Ultimately, his lab isolated 18 antibiotics, including streptomycin and neomycin. In 1952, he became the first—and still the only—soil microbiologist to win the Nobel Prize.

Over the next 20 years, an arsenal of pharmaceutical weapons were teased out of the soil: kanamycin, gentamicin, geldanamycin, dactinomycin, lincomycin, and more. From France came the family of rifamycins, while the rainforests of Borneo yielded vancomycin. Soil bacteria yielded both antibiotics outright and biochemical ideas to guide the invention of others. Nothing rivals Waksman’s actinomycetes; 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. The problem was well documented, if poorly understood: it was the great plate count anomaly. Despite tantalizing evidence of staggering microbial diversity, drug prospectors kept growing the same things in their petri dishes. That flash of something new was too often missing.

Something new like *Eleftheria terrae*, one of the thousands of bacterial cultures from that Maine field. From this newly identified species they 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, massively increasing the potential for discovery. “Not only are we working with a novel pool of organisms,” says Epstein. “We are working with a novel pool of chemicals. The rate of the discovery is approaching what used to be in the 1950s.” 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,” he concludes. “Because there will be more, and more, and more. We need to scale up.”

A ‘Nearly Bare’ Cupboard

Teixobactin is a timely breakthrough. Disease-causing microbes are rapidly evolving a dangerous resistance to our armamentarium, as once-revolutionary drugs are rapidly becoming useless. 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 Dr. 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.”

How did the cupboard get cleaned out? As it became more difficult to prospect for natural products in soil, drug discovery moved in other directions. Synthetic chemistry could develop huge libraries of compounds. High-throughput systems quickened the pace of testing these compounds for therapeutic potential. Scientists also learned how to configure some new drugs to hit specific molecular targets. These new techniques yielded many pharmaceuticals, but not much in the way of antibiotics.

Business conditions also changed. Antibiotics are taken by only a small percentage of patients, and usually only for about two weeks. Companies began favoring the blockbuster drugs that many patients had to take indefinitely. It’s hard to justify investing a billion dollars in development when the resulting drug might be rendered impotent by antibiotic resistance even before it can earn its keep.

Meanwhile, genetic sequencing technologies were getting cheaper and ever 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. The technique rekindled a passion for investigating microbial potential. 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.

The search for new drugs is focused on what are called secondary metabolites—though there is a move afoot to rechristen them as specialized metabolites. The vast majority of life shares basic chemistry: DNA and RNA for storing and using genetic information, digesting sugars to release energy, and building proteins for things like cell walls. As evolution progresses, fancier chemistry evolves—poisons, chemical attractants, colors, and protection. It’s in these funky molecules that novel chemical ideas emerge.

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.

“Microbes have been interacting with each other and killing each other and communicating with each other for a long, long, long time,” says SSSA member 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.”

In the search for new secondary metabolites, novel compounds can be found in new species, and soil has that, with thousands of species per gram.

But diversity runs deeper because even microbes that we call the same...
species can be vastly different. Millions of years could have elapsed—and untold generations—from a very ancient branching point. “They might have shared an ancestor in the Cretaceous. That’s when there were dinosaurs,” Buckley says. And we’re talking about organisms that sometimes spawn new generations every 20 minutes. “That’s a long time to evolve diversity.”

Compounding this genetic diversity even further is the fact that bacteria are notoriously promiscuous with their DNA—swapping out huge swathes of genetic material is just something that microbes do. For example, *Escherichia coli* is among the best studied organisms on the planet. But when scientists started sequencing different genomes of *E. coli*, among the first three they found genetic overlap between 30 and 40%. “When you think about the genomic diversity found just within *E. coli*, you realize that the genetic diversity of microbes is almost limitless,” Buckley says. Not all microbes are this multifarious, but each species has the potential for tremendous genomic diversity; and still more, secondary metabolites.

Metagenomics has encouraged scientists like Epstein to keep looking for more ways to culture new microbes, suggesting there is much more to discover. “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 the other omics fields while these in turn feed back to cultivation efforts. “There is no such thing as unculvable,” he declares. “The biological novelty of microbes is no longer a limiting factor. We can get a lot more than chemists can process.”

**Bypassing the Cultivation Puzzle with the ichip**

“All organisms that’s difficult to cultivate is difficult to cultivate for a reason,” explains Daniel Buckley. He specializes in the microbial diversity of soils and has seen all kinds of picky eaters. “Each one is like a puzzle,” he explains. They might need this vitamin, that amino acid, the proper pH, or even a secret chemical handshake from another species. Sometimes it’s as simple as highly diluted seawater; other times, it’s something more biochemically elaborate like cyclic AMP or homoserine lactones or sideraphores.

Epstein and his colleagues neatly bypassed this cultivation puzzle with a specially designed incubation chamber—the ichip. Despite its name, there is nothing electronic about Epstein’s 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 environment of the original sample 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. Rather than try to figure out what each microbe needs, the ichip acts as a diffusion chamber, incubating the cultures in their own microbial stew. More often than not, a colony forms.

If it sounds simple, it is. In fact, when other labs ask about using the ichip, Epstein discourages them. Purpose-built for high-throughput production, it’s expensive. He suggests a low-cost alternative made from a pipette tip holder and membrane. His lab is still strewn with large steel washers used the same way.

Another important element is time. Between 80 and 90% of soil microbes aren’t even active at any given time. “We are simply throwing our petri dishes away too quickly,” Epstein says.

ASA and SSSA member Mark Williams, a microbial ecologist in the Department of Horticulture at Virginia Tech, has also been thinking about the cultivation challenge. He’s not surprised that so few microbes grow in culture because historically, there hasn’t been a lot of innovation in growth media. And while Williams is impressed with Epstein’s results, he also thinks of the ichip as “kind of a microbiologist’s
system,” with its focus on individual microbes rather than the soil ecosystem. “Soil scientists might want to run their own system,” he suggests. For example, he and his grad student Madhavi Kakumanu spent a few years developing a simple experimental system for culturing microbial diversity. The inspiration: leaf litter, one of the organic building blocks of soil. First they primed the soil, mixing in sterile rice straw and letting it sit for a few months, with the general idea that this might help encourage bacteria that feed on decomposing cellulose. Their culture medium was just a piece of filter paper sitting on top of a membrane and then well-hydrated soil. Chemicals from the soil or its associated microbes could filter up through the membrane, but not microbes.

Building the system took longer than expected. Success ultimately hinged on tinkering, letting the organisms grow longer, and just looking harder. “Even on the plates that didn’t appear to have anything new, we just had to look at them closer,” he says.

“There are just so many avenues for research on novel bacteria,” Williams says. “I think it’s a wide open area. Hopefully soil scientists will continue, and in a growing way, become involved because they have expertise that is really going to make a difference in the success of many of these experiments.

“We’re going through a renaissance. I would just be flabbergasted if in the next five years there aren’t at least five new antibiotics.”

Shank’s lab uses this “soil” to study carefully controlled interactions between different species of microbes. As they gather clues, they’ll knock out genes to figure out what’s going on at the molecular level. The transparent system allows them to use complex imaging techniques.

They’re already figuring out important details in biofilm formation—an important but poorly understood part of both infections involving medical implants and how bacteria colonize plant roots. One form of the bacterium Bacillus subtilis is devoted to biofilm production. This form can be activated by chemical signals from the same species, but also by different species. Shank wants to know if this behavior is collaborative or competitive. When one species tells another to make biofilm is it because the first species wants to band together, or is it actually just tricking a competitor into staying put, so it can swim around and take all of the food?

In their search for secondary metabolites that either inhibit or stimulate biofilm formation, they have already found both. And while neither of those were new compounds, that they played a role in biofilms was unknown. Biofilms are 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.

Playing bacteria off of each other helps parse their ecological roles but could also expose another wrinkle in their chemical diversity because microbes often don’t even produce all of the compounds in their genetic cookbook. “You have these highly conserved, enormous gene clusters that require tremendous biosynthetic energy to actually make, and the bacteria never turn them on in the lab,” she says. They are only going to be produced when they are actually required for survival or fitness—or when they get the proper cues from other microbes.

“We’ve been doing a lot of co-culture to try and stimulate the production of these cryptic metabolites,” Shank says. “We’re trying pull out these pairwise interactions.”

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.”

An Astonishing Microscopic Universe

Microbial ecologists have a refreshing sense of wonder, and of just how little they know—or even can know—about the systems they study. “What is it that makes these systems so diverse across soil types?” Williams asks. “Why is it that these organisms are just so amazingly numerous?”

On the one hand, soil exists practically everywhere, knitting together life-giving processes on a continental scale. And enmeshed in it all is an astonishing microscopic universe.

Buckley believes soil scientists bring a lot to the challenge of unlocking this universe. A petri dish is a far cry from soil, which is characterized by gradients, water films, and lots of interactions at the surface of, or inside of, aggregates. “The better you understand the environment … the better you can understand how to simulate that in a way that allows the microbes to grow,” he says.

Likewise, being able to cultivate microbes is incredibly important to unraveling the impossible complexity of soil ecosystems. Experiments with whole soil simply involve too many variables.

And the more we understand the relationships between microbial diversity and soils, the more valuable that information will become, Buckley says. We’re not there yet, but knowledge of how soils vary may ultimately become very important for predicting which kinds of microbes are going to be in which environments.

Shank is amazed to consider even a cubic centimeter of soil. “The two microbes that you find on two different ends of that are never going to have anything to do with one another, right?” Geophysical processes unfold at the scale of fields or prairies. Trying to understand how these impact the microbes, at the micron scale, is a tremendous challenge. “I don’t think we’ve really bridged that gap,” she says.

“It’s a feeling of awe—nothing short of that,” Epstein says. “For the tremendous diversity and outstanding, unimaginable complexity of the bugs and the sophistication of the interactions.” Sometimes he deals with students who see bacteria as little more than tiny bags of enzymes, like sophisticated molecules.

“There is nothing further from the truth,” he says. “They are all individuals. They are all different. Not genetically, but behaviorally, phenotypically. They talk to each other. Modify their behavior as a result of this talking. You take your hat off when you see what 3 [or] 4 billion years of evolution ended up being.”

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