Cell

# Seeking New Antibiotics in Nature's Backyard

**As the pipeline of new antibiotics slows to a trickle, scientists are developing innovative strategies to unearth antibacterial compounds in unexpected places.**

Resistance of microbial pathogens to an increasing number of antibiotics is a serious problem. In the US alone, 90,000 people die every year from infections acquired while in the hospital. According to the Infectious Disease Society of America (IDSA), 70% of these deaths have been attributed to infection with drug-resistant bacteria, in particular methicillin resistant *Staphylococcus aureus* (MRSA). Compounding the problem, the World Health Organization warned in September of a new form of tuberculosis, XDR-TB, caused by a multidrug-resistant strain of *Mycobacterium* that leaves patients virtually untreatable with current anti-TB drugs.

Despite this threat, the pipeline of new antibiotics approved by the US Food and Drug Administration (FDA) is running dry. The number of new antibiotics is now about 60% lower than in the mid-1980s, says Brad Spellberg, an infectious disease specialist at Harbor-UCLA Medical Center in Torrance, CA. "It's a straight line down," he says. Since the 1960s, only two new classes of antibiotics have been introduced in the clinic, linezolid in 2000 and daptomycin in 2003, says Jun Wang, a senior biochemist at Merck Research Laboratories in Rahway, NJ. The IDSA estimates that about a dozen new antibiotics are in late clinical testing. But most of them, says Spellberg, are "me-too" drugs comprising modifications to existing compounds or members of known classes of antibiotics. "That doesn't help us treat drug resistant bacteria," Spellberg points out, because they are often not sufficiently different to overcome resistance.

Pharmaceutical companies are less interested in developing antibiotics than drugs that treat lifelong diseases because people only take antibiotics for a short time, notes Spellberg. "There has been at least as many drugs developed over the last 12 to 13 years for HIV as compared to all bacterial infections put together," he says. "It's all about money." It takes 250–500 patients treated with an antibiotic for every patient on a medication for a chronic disease to get the same return on investment, says Christopher Spivey, manager of business development at the nonprofit Alliance for the Prudent use of Antibiotics (APUA) in Boston, MA. "That's why companies have been walking away."

The reason that the antibiotics pipeline is running dry is not only money but also because the search has become more challenging. The first antibiotic, discovered by the British microbiologist Alexander Fleming in the 1920s, came from a mold, *Penicillium notatum*. Since then, soil-dwelling microorganisms have been the traditional source of antibiotics. But searching for antibiotics the old way—culturing soil bacteria and screening them for compounds they produce that kill bacterial pathogens—means that the same antibiotics are discovered over and over again, in part because those already identified are potent and highly concentrated, says Merck's Wang. We have run out of soil bacteria that are easy to culture, says Kim Lewis, a microbiologist at Northeastern University in Boston, MA: "As with a gold mine, you mine it out and it ends."

### Screening Goes up a Notch

Some companies are moving away from a dependence on soil bacteria, instead screening libraries of synthetic compounds for their antimicrobial properties. Pfizer researchers are using the genomic sequence information of different bacterial strains to identify bacterial survival genes. They then screen millions of synthetic chemicals to find those that interfere with the products of these essential genes. This approach has yielded three new compounds that are now in clinical trials and a few more that will be soon, says Paul Miller, head of Therapeutic Area Research for Antibacterials at Pfizer.

But this strategy is not always successful. Between 1995 and 2001, GlaxoSmithKline (GSK) did 70 high-throughput screens of synthetic chemical and other libraries for inhibitors of essential bacterial targets. The success rate was 4–5 times lower than with mammalian cell targets, says David Payne, director of microbiology at GSK in Collegeville, PA. One reason, he says, is that bacterial enzymes are harder to inhibit because they have evolved for longer and are well suited to harsh conditions. Wyeth had a similar experience. "Having had a similar degree of futility to GSK using high-throughput screening, we are certainly not going to do that ourselves in the future," says Steven Projan, vice president for biological technologies at Wyeth Research in Cambridge, MA. "Right now we are doing very little antibacterial drug discovery." Screening efforts may fail because many compounds cannot get into bacterial cells or are toxic to mammalian cells as well as bacteria or because bacteria have transporter proteins that can pump out synthetic compounds.

Given the mixed success of large screening efforts, it may be difficult to get big pharmaceutical companies interested. That's where APUA

plans to help by developing a not-for-profit screening library that would be funded by corporate sponsors and public money. This would spread the risk, and companies would have to pay less for the initial screening effort, says Michael Feldgarden, APUA's research director.

**Back to Nature**

There are plenty of antibiotic hunters who have not given up on nature. Some research groups are trying to isolate rare bacteria or antibiotics from soil and are devising methods to grow hard-to-culture soil bacteria in the lab. Others are isolating DNA directly from the soil, using it as a blueprint to produce antibiotics, or are looking for bacteria in unusual places, such as in lichens, seaweed, or deep sea mud.

Merck scientists are using a new method to screen for antibiotics at such low concentrations that they would be missed in traditional screens. First, bacteria are made more sensitive to potential antibiotics using antisense RNA to induce the microbes to make less of a certain target enzyme that is essential for survival. Inhibitors of that target enzyme then block the growth of the sensitized bacteria to a greater extent than the growth of the wild-type strain. Selecting a target that is essential for survival across a broad range of bacteria could yield an inhibitor capable of blocking the growth of many different bacterial strains. The Merck team have screened extracts of 83,000 bacterial strains from soil samples across the globe under three different growth conditions. From a South African soil sample, they isolated Platensimycin, a member of a new class of antibiotics that can kill MRSA in vitro. Platensimycin inhibits FabF, a bacterial enzyme that synthesizes membrane fatty acids.

The bacterial strain that produced Platensimycin can be cultured in the lab, but, as Julian Davies, a microbiologist at the University of British Columbia in Vancouver, points out, at least 99% of soil bacteria cannot be cultured in vitro. "We know less about soil than we do about outer space," Davies says. One gram of soil, he says, contains at least 1,000 different species of bacteria. That's why Northeastern University's Lewis is developing ways to culture "unculturable" soil bacteria, to find those strains that make new antibiotics. "We have a protocol to domesticate them," Lewis says. "They don't know that they are not in their environment." The bacteria are cultured in chambers that separate them from the environment physically, but not chemically. "We just bring big buckets of soil in the lab and then insert the chambers into the soil," he says. Lewis cofounded NovoBiotic Pharmaceuticals, based in Cambridge, MA, which uses this strategy to find new antibiotics. Already, the company has isolated thousands of bacterial strains, mainly from soil, and currently is focusing on about 200 that seem "especially interesting," Lewis says.

Meanwhile, other scientists are not even trying to culture recalcitrant soil bacteria. Instead, they isolate DNA directly from the soil, express the DNA in host bacteria in the lab, and then screen for antibiotic production—a strategy called metagenomics. One advantage is that the several dozen genes that bacteria use to synthesize antibiotics are usually arranged together in a cluster. But to get a complete cluster entails isolating a large chunk of DNA (about 100 kb), and it can be difficult for host organisms in the lab to express genes in this DNA. Not everyone is convinced that the metagenomics strategy will work. There is only proof of principle that this approach can lead to compounds with antibiotic activity, Davies says. "I don't think any of the things that have been found will become drugs."

Jo Handelsman of the University of Wisconsin, Madison has developed a way to screen for metagenomic DNA clusters that produce small molecules, some of which could be antibiotics. A reporter gene expressing green fluorescent protein detects very low concentrations of these small molecules inside the bacterial cell with the metagenomic DNA. Handelsman says this system has identified one known antibiotic, which shows that the method works. She is currently sequencing about 200 bacterial clones containing metagenomic DNA from soil collected in a remote corner of Alaska, which due to the harsh environment may yield new bacterial species. Handelsman hopes the sequences will reveal clones that look like they might synthesize new antibiotics.

The Wisconsin-based company eMetagen has identified about a dozen bacterial clones from Wisconsin agricultural soil that appear to produce compounds with in vitro activity against MRSA, says Robert Goodman, one of the company's cofounders. At least one of them might belong to a new class of antibiotics. For now, the company is focusing on two compounds to determine their structure and to do initial toxicity testing in animals. The company has been able to create libraries with an average insert size of 50 kb, he says. Next, the company hopes to get better expression of the genes in its soil bacterial library by switching from a gram-negative host (*Escherichia coli*) to a gram-positive host. "Many of the genomic DNAs we are accumulating are probably from gram-positive organisms," Goodman says.

Not everyone has had success with the metagenomics approach, however. The Lexington, MA based company Cubist Pharmaceuticals tried it for several years, but then gave up, says Jeff Alder, vice president for drug discovery and evaluation. He says too many technical steps were required, often yielding false positives after a lot of effort. "It is just so difficult to get a big piece of DNA, get it expressed and then screen and [only then] find out what's valuable," he says. Instead, Cubist researchers are trying to culture soil bacteria that are so rare that they have been overlooked. Alder says the trick is to use indicator bacteria that are resistant to most known antibiotics. Only bacterial strains that produce new compounds can kill these indicator bacteria in test assays. About 1–2 out of every 10,000 strains tested are able to kill the indicator bacteria, says Alder, and 70% of these promising bacteria have never been described before.

Soil is not the only source of new bacterial strains. Brian Austin, a microbiologist at Heriot-Watt University in Edinburgh, Scotland, has isolated bacteria from seaweed and seawater. However, they only started to make antibiotic compounds when he cultured them on a sponge from his local supermarket. So far, he has found two new compounds that show antibiotic activity against MRSA in vitro. He says both antibiotic-producing strains are now with a company for further evaluation. Sifting through marine mud, oceanographer William Fenical of the Scripps Institution in La Jolla, CA has found several new bacterial strains that produce antibacterial compounds. And Davies has isolated about 2000 bacterial strains that grow on lichens, half of which show some kind of antibiotic activity. Currently, he is focusing on two compounds that appear to be new, one of which is being further investigated by a major pharmaceutical company. Fungi, too, have not been forgotten. The Danish company Novozymes has isolated an antimicrobial peptide called plectasin from a fungus that inhabits European pine forests. Plectasin kills MRSA in mice, and Novozymes is planning an early clinical trial of this compound in 2008. The Philadelphia-based company PolyMedix has made artificial molecules with similar properties to antimicrobial peptides. Their lead compound can kill both MRSA and TB in vitro, and PolyMedix plans early clinical trials next year.

Despite these encouraging leads, one major hurdle for many small biotech companies is to pay for clinical trials once they have identified a compound. "We are going to have to do this in partnership with a drug company," says eMetagen's Goodman. Novozymes says it will pay for phase I trials but is looking for partners to take over the project after that. PolyMedix also plans to fund initial trials but is engaged in partnering discussions with major pharmaceutical companies, says Nicholas Landekic, the company's President and CEO. He says the company has raised much of the funding from selling stock and plans to apply for grants from the National Institutes of Health (NIH) to pay for part of the cost. But overall, he says, such grants could only pay for a small portion of the total costs of clinical development. Most small companies will probably have a similar mix of financing, he says, although many can't pay for clinical trials at all. "Thus many products simply do not ever get developed," Landekic says. At least for this year, NIH's National Institute of Allergy and Infectious Diseases (NIAID) is offering contracts to support several early clinical trials of antibacterial drugs, according to Michael Kurilla, Director of the Office of Biodefense Research Affairs at NIAID. "If you have a successful phase I/phase II that we have supported you are going to look very attractive to a large pharmaceutical company to take on a phase III trial," he says. Spellberg, for his part, calls for legislative incentives to get big pharmaceutical companies back into antibiotic development for the long haul. After all, he says, new antibiotics will always be needed. "Bacteria are going to become resistant to everything we come up with, it's just a matter of time."

He may well be right. According to a study in *Science* earlier this year, resistance to even the newest antibiotics, including synthetic antimicrobials, may already exist in nature. The authors screened 480 strains of soil bacteria from different environments against 21 antibiotics and found them to be resistant, on average, to 7 antibiotics, even those bacteria from remote areas. Many were resistant to the new semisynthetic antibiotic tigecycline, which was approved for use only last year. While the findings sound a note of doom, they do give us the opportunity to look for resistance mechanisms in nature as an early warning system, before they are found in hospital bacteria, says Gerard D. Wright of McMaster University in Ontario, Canada, who led the study. "Resistance is inevitable," Wright says. "There is no such thing as an irresistible antibiotic; no matter what chemistry you can think of, because the organisms have been around for so long, they figured out a way to survive. And they will."

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DOI 10.1016/j.cell.2006.11.021