Innovations

Digging in the dirt
TerraGen Diversity Inc. & ChromaXome Corp.

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Dirt is a microbiological metropolis, with thousands of bacterial species competing with each other for nutrients and space. The fruits of this competition, such as the antibiotics, have been some of the most important drugs ever discovered. TerraGen Diversity Inc. (Vancouver, Canada) and ChromaXome Corp. (La Jolla, California) think that the search for such drugs has just begun. They are scrutinizing the vast number of soil bacteria that have thus far been both unculturable and unexamined.

Mike Gilman, who is involved with a similar but "embryonic" project at Ariad Pharmaceuticals, Inc. (Cambridge, Massachusetts), says there is no shortage of potentially interesting compounds. "I feel like the whole world is our playground now," he says. "With this rich bounty of natural products, there are about a zillion things you would want to try them on."

Bigger is better

Natural product isolation has traditionally involved the testing of crude environmental samples, followed by painstaking and laborious purification procedures using large amounts of natural material. The idea that has all these companies excited is one aspect of the new field of combinatorial biology. It involves the isolation of large fragments of genomic DNA, fragments that can then direct the synthesis of novel natural products.

"Antibiotics require a lot of enzymes for their synthesis, but the genes are contiguous," explains Jo Handelsman, who works at the University of Wisconsin and collaborates with Gilman. An entire synthetic pathway can therefore be captured on a single bacterial artificial chromosome (BAC), and the researcher can directly test for the end product.

There are complications, however. The multiple genes on the clones retain the transcriptional signals from their diverse microbial owners, and no single host cell can guarantee the expression of all the clones. The best candidate host is one of the various species of *Streptomyces*, themselves a subset of the Actinomycetes. "The lion's share of commercially successful pharmaceuticals has come from Actinomycetes, and many of [those drugs] have come from *Streptomyces*," says David Sherman, Senior Director at ChromaXome. *Streptomyces* can express genes from most Actinomycetes, a class of bacteria that has yielded the antibiotics erythromycin and tetracycline, and the immunosuppressant FK506.

The other major problem is retrieving the DNA intact. "Your sample starts out looking like mud," says Handelsman, "and cloning from that is a challenge." Decomposing organic matter yields a complex mix of organic acids termed humic acid, and many of these compounds both degrade and copurify with DNA. "It's a major barrier to routinely generating libraries of a significant size," says Joe McDermott, research director at TerraGen. "With standard techniques you will end up with shredded DNA."

The companies have, however, isolated DNA, with insert sizes ranging from "up to 80 kb" (Ariad) to 40–100 kb (TerraGen and ChromaXome). It is difficult to determine which sizes are obtained routinely, and which only occasionally, but ChromaXome claims that the high end of their range is the "typical" size, whereas TerraGen says that inserts of around 40 kb are used in current operations.

From fish pigments to drugs

ChromaXome is a confident company for one so young. It was started just three years ago, initially to make colorful transgenic fish by manipulating pigment biosynthetic pathways. The natural product hunt began with marine bacteria, but dirt searching began in earnest when the company started a collaboration with Bristol-Myers Squibb.

"We're not just going into any old backyard and digging in the dirt," says Sherman. "We search for soil rich in Actinomycete DNA." The researchers then select for DNA fragments with homology to known drug-synthesizing genes, before expressing the DNA in *Streptomyces*. With any luck, the selected fragments will contain the genes for a whole synthesis pathway.

The next problem is one of numbers. "If you can make so many recombinants so easily," asks Sherman, "how do you screen them?" ChromaXome's solution is a technique called macrodroplets. These gel-like particles are formed when calcium ions are added to a solution of sodium alginate containing free-floating recombinant cells. On average one cell is trapped per macrodroplet. Once the cells have grown up and expressed their unique products, a reporter (such as drug-resistant bacteria) is layered on top.

According to Sherman, the partnership with Bristol-Myers Squibb has already yielded "two new natural product entities, one of them a promising anti-infective useful against many drug-resistant organisms." Other collaborations will come from ChromaXome's acquisition by Trega Biosciences Inc. Under the name Houghten

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Pharmaceuticals, Trega was an early player in the field of combinatorial chemistry, and Sherman believes the two companies are a good match. "We are taking advantage," he says, "of combinatorial biology's ability to provide novel templates for combinatorial chemistry libraries."

Shuffling and modifying

Template modification is also on TerraGen's agenda, but they are doing it *in vivo*. "If you take a host with pathways that are functioning already," says McDermott, "the libraries don't have to produce the whole pathway, they just need to modify it to produce a novel product. Most of the specificity [in drug action] is determined by the modifications, so you are really dealing with the active part of the molecule. The opportunity for picking up novel active structures is fairly high."

The modifications can be detected by screening for activity against bacteria resistant to the original drug, or by detecting the new molecule by high performance liquid chromatography and mass spectroscopy. The latter task would be nearly impossible if the bacteria to be analyzed came directly from the soil but, says McDermott, "since we are dealing with clones in the same background, if you subtract a control background you will be looking at very few differences."

Modifying existing natural products is a good idea while the size of inserts hovers around 40 kb. "With that size you can get some small pathways," says McDermott, "but others are larger than that so you're not going to get a whole pathway." Eventually, however, inserts will get bigger, and the modification program may give way to isolating entirely new pathways. "Pharmaceutical companies are really interested in truly new chemical entities," says McDermott, "because then you have the potential to modify that entity biologically or chemically, and that increases the likelihood of ending up with a real product."

Looking for lichen

For their soil samples, TerraGen has found the surrounding area of British Columbia to be sufficiently diverse. "British Columbia has quite a range of environments, from near Arctic to near desert, plus deep sea vents off Vancouver island," says McDermott. Further DNA diversity comes from the collections of lichens. Lichens are an intricate combination of a fungus and either an algal or cyanobacterial species. The fungal host provides a suitable growth environment, and the algal or cyanobacterial occupant provides fixed carbon and nitrogen. "There is a lot of very intimate signaling," says McDermott. "They seem to be truly dependent, and don't like to grow apart."

The existence of the signaling pathways suggests that the fungi may make molecules that can modify signaling pathways in both lichens and, potentially, humans. And such molecules are unlikely to have been previously described. "About one fifth of fungi are in lichen associations," says McDermott, "and these have never really been looked at because they can't be cultured. [Looking at lichens] was a natural extension of looking at [the uncultured bacteria in] soils."

Expression hosts for the lichen DNA are species of the molds *Aspergillus* or *Neurospora*. TerraGen has used the soil and lichen DNA in cell-based assays to find a number of promising natural products in three different compound classes. Some of these products target either MAP or src kinases, both possible anti-cancer targets. The major emphasis of the company will, however, be on supplying libraries to other companies for various screens.

A more focussed approach

Both TerraGen and ChromaXome select for large fragments of DNA with genes similar to known biosynthetic pathways. Although they will not disclose all of the genes that are selected for, one of the most common targets are the polyketide synthases. Polyketides are built up by stepwise combination of small carbon building blocks, to yield drugs that kill bacteria (tetracycline, erythromycin, rifamycin), fungi (amphotericin B, nystatin), or cancer cells (daunorubicin, adriamycin), suppress the immune system (FK506, rapamycin), or lower cholesterol levels (Mevacor). Manipulating polyketide pathways is the sole focus of Kosan Biosciences, Inc. (Burlingame, California). Kosan shuffles genes that are part of the same pathway but from different organisms, an approach that ChromaXome is also interested in. Kosan recently demonstrated that polyketide pathways can utilize synthetic building blocks. This should allow chemists and biologists to join forces to construct novel, 'unnatural natural products'.

Staking out territory

The natural products may be many, but the techniques used to isolate the chunks of DNA are few. TerraGen has been working in the field for longer and has a number of patent applications pending, but the first notice of allowance, the precursor to a patent, was awarded to ChromaXome in September 1997. Sherman says the patent covers a number of aspects of combinatorial biology, including both screening methods and the generation of libraries from mixed pools of genomic DNA isolated directly from the soil. But for now TerraGen's McDermott is confident that there will be room for his company to maneuver. "I don't see it as a major conflict," he says. "There is plenty of room for a number of players in this field."

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