Innovations

Seeking extremophiles

Recombinant Biocatalysis, Inc.

Deep-sea diving, dead-whale spotting, and the isolation of heat-loving worms are new alternative careers for chemical biologists, at least for those who join Recombinant Biocatalysis, Inc. (RBI) in San Diego, California, USA.

RBI specialises in the isolation of enzymes for industry, and the weirder the source, the better. "We identify unique habitats where there might be microbes with unique physiologies," explains Jeff Stein, a Principal Scientist in charge of microbial diversity at RBI. Once they have collected biological samples from deep-sea vents, geothermal pools, whale bones, and glaciers, RBI scientists isolate DNA and search for useful enzymes, skipping the intermediate and tricky step of culturing organisms.

This direct approach seems to be yielding results. "They are reaching out beyond organisms that are well studied," says Norman Pace, of the University of California at Berkeley, USA. "I think that is wonderful."

Boundless diversity

Even in the soil of a temperate garden, there is an enormous flowering of life. One gram of this soil contains ~10^9 genomes, representing ~10^4 different species. And that is only in the front yard — the shady back yard is a whole new universe. "The vastness makes the question of 'How many?' irrelevant," says Pace.

Perhaps, then, RBI need go no further than a San Diego garden. But RBI scientists have faith in their search for extremophiles. Although 10^4 is a lot, 10^4 of pretty much the same thing may not be so useful. Isolated populations of bacteria in extreme environments probably have enzymes with novel specificities. And enzymes from thermophilic (heat-loving) bacteria have several potential advantages.

The first is obvious: a number of chemical processes require enzymes to act, or retain their activity, at high temperatures (see the first two entries in Table 1).

But the main stumbling block for those wishing to use enzymic catalysts in industry has been lack of stability, not lack of heat-resistance. Enzymes are specific, nontoxic and biodegradable, but they go off. RBI recognised that thermophilic enzymes are stable enzymes. Even if they are to be used at mild temperatures, they last far longer than regular, mesophilic enzymes. They also resist the denaturing effects of organic solvents, and can usually operate at aqueous–organic interfaces that would inactivate normal enzymes.

Whale searching and other activities

The two best sources of thermophilic bacteria are deep-sea vents, and geothermal pools. RBI scientists have used the *Alvin* submersible to access vents at sites like the East Pacific rise, ~800 miles off Costa Rica and ~1.5 miles down. The hottest vents have core temperatures of 350–400ºC. These black smokers get their name from the plumes of precipitated metals that drift into the surrounding sea water, which has an ambient temperature of 2ºC.

The centers of the vents are sterile, but there is a temperature gradient in the surrounding chimney, and core samples turn up a wealth of thermophiles. Other samples are collected using either a slurp gun, which filters water from around the vent, or collection plates seeded with substrates likely to attract microbes and foster their growth.

Deep-sea marine animals, or rather the bacterial flora living on and in their bodies, are also a good source of biodiversity. One intriguing recent find is a worm that keeps its tail in the hot vent and its head in the cool surrounding water. RBI researchers, interested in the worm as a source of broadly adapted enzymes, don't yet know if the worm can turn around.

With their similar chemistries and temperatures, geothermal pools are the terrestrial equivalents of deep-sea vents, at least for harvesting bacteria (Figure 1). Deep-sea vents and geothermal pools also share an island-like quality, isolated biologically by their unique chemistry. For example, one pool that RBI collects from in Costa Rica, at ~95ºC and pH 0, stands in stark contrast to the surrounding rainforest.

Whale skeletons, another source of microbes, are also like isolated islands, so isolated that they are tricky to find. The US Navy helped out RBI by locating one when it was looking for a wayward Triton missile, but in other cases researchers have

Table 1

Selected applications of enzymes in industry.

Enzyme(s)	Application
Mannase	Dispersal of the galactomannans used to increase the flow of oil in a drilling operation (the other alternative is bleach). Thermophilic mannase can withstand the high temperatures at deep strata.
Endoglucanases and phosphatases	Increase the absorption of animal feed. Thermophilic enzymes survive the high-temperature extrusion process that creates the feed pellets.
Cellulases and xylanases	Bleaching of wood products. The enzymes loosen the structure of wood pulp. Paper manufactures can then use far less bleach, as the lignin can more easily escape from the plant matter.
Endoglucanases, lipases and proteases	Washing detergents. Thermophilic enzymes are useful for hospitals that wash at high temperatures for sterilization purposes; psychrophilic enzymes can be used in room-temperature washing detergents.

Figure 1

Dr. Ron Swanson, Associate Director of Genomics at RBI, collecting a sample from a geothermal vent in Yellowstone National Park, Wyoming, USA.

resorted to towing dead, beached whales out to sea and dumping them.

Isolation is not the only attraction of whale bones. "They ooze complex lipids over period of up to 20 years," says Stein. Bacterial mats that grow on the bones are a rich source of lipases and esterases, two classes of enzymes that are especially important to industry. The organisms and their enzymes are adapted to the deep-sea cold; such psychrophilic enzymes can be useful for commercial laundry detergents. More psychrophilic enzymes are turning up in earth and lichen samples from Icelandic and Antarctic glaciers.

From DNA to enzyme

Once the DNA is harvested, the job of the RBI scientists becomes more routine. The genomic DNA is cloned into libraries, which are normalised to even out the contributions of common and rare bacteria. Nanograms of DNA are enough to make a representative library, and RBI has worked hard at improving their sensitivity using various proprietary technologies. In the rare situations in which there are too few bacteria to make a library, the RBI scientists first amplify the bacteria in a mixed culture, with a liberal supply of the substrate of the enzyme they are most interested in.

Expression screening is most commonly used, because "it gives us the opportunity to select without any prior knowledge of what the genes should look like," says Jay Short, Chief Technology Officer at RBI. The RBI scientists also use sequence-based methods. In biopanning, they pass large amounts of genomic DNA over a DNA probe (such as a known esterase) to enrich for similar sequences. And random sequencing, particularly of the entire genome of the thermophilic (non-archaea) bacterium *Aquifex aeolicus*, is yielding other candidate enzymes.

Applications

The most obvious use for enzymes is the degradation of natural products (Table 1). The specificity of enzymes also makes them a powerful tool in the synthesis of pharmaceuticals and fine chemicals. Synthesis of assymetric molecules or single isomers can be difficult to achieve by any other means. The Food and Drug Administration (FDA) is particularly concerned about stereoisomers of approved drugs, which can either be inactive or, worse, toxic.

RBI has big plans for this market. "We're trying to replace enzymes already in use, and also open the door to using enzymes in other processes where they haven't been used before," says Dan Robertson, Director of Enzymology at RBI. That approach is needed, says Pace, because the biotechnology industry has, in the past, relied on "stuff that has fallen into our pockets. There has been relatively little seeking out."

RBI is developing large numbers of a given type of enzyme to fit the many possible applications. "In industrial processes," says Stein, "you have many layers of specificity that have to be optimized in any one process," including substrate specificity, and temperature and pH optima.

Once a suitable enzyme is found, process chemists add it at an ~1% molar ratio to the substrate; this is similar to the ratios used for chemical catalysts. The heat-resistant enzymes then typically last from months to years.

Reality check – the product line

As with many young biotechnology companies, not all of the enthusiastic claims are reflected in the product line. RBI prides itself on isolating enzymes using only samples of DNA, but ~50% of the enzymes that they currently market were isolated from a collection of >1500 cultured, isolated thermophiles built up by Karl Stetter of the University of Regensburg, Germany. The current products include five aminotransferases (for the synthesis of chiral amines from ketones), seven esterases and lipases, ten glycosidases (for both the hydrolysis of sugars and the synthesis of glycoconjugates), seven phosphatases, and seven cellulases.

Meanwhile, other companies have also been producing enzymes. Thermogen (Chicago, IL, USA) has also searched culturable thermophiles, and come up with a bank of esterases and lipases. They have now added an organism-discovery program.

Altus Biologics, Inc. (Cambridge, MA, USA) has taken a very different approach to the stability problem. They have made enzyme pellets by purifying, crystallizing and cross-linking known enzymes. The cross-linked crystals are stable to heat and organic solvents, and have a long shelf life.

The strength of RBI lies in its active discovery program, which promises to deliver more diverse products in the future. In the shorter term, RBI is picking up its marketing efforts, in the hopes that it can match the vastness of microbial diversity with a similarly diverse clientele.

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