BIOTECHNOLOGY

In Industry, Extremophiles Begin to Make Their Mark

This month, a Rochester, New York–based company called Genencor International introduced a new detergent additive that it promises will make cotton clothes look like new through hundreds of washings. That may sound like a routine claim in the detergent business, but it is based on a protein that is far from ordinary. The additive, an enzyme called cellulase 103, was taken from an extremophile—a microbe that thrives where few humans dare to go—and its mass-market debut represents a milestone for the fledgling industry based on these microbes. This particular bug was discovered in an alkaline lake, but other potentially useful extremophiles have recently turned up everywhere from the frigid Arctic Ocean to boiling thermal pools.

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Going to extremes. Researchers have uncovered extremophiles in Arctic ice *(above*, and brown layer, *left)*, and from saline, high-pH ponds *(below)*.

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This isn't the first extremophile product to go commercial. Many biologists already use an extremophile enzyme, or extremozyme, everyday—the Taq polymerase. Isolated in 1965 from a microbe that thrives in a bubbling thermal pool in Yellowstone National Park, Taq is the heart of the polymerase chain reaction, and companies are still fighting over its patent and annual sales of $80 million. But cellulase 103 is aimed at a much broader—and bigger—market: It is "the first large-scale industrial application of a product from an extremophile," boasts Scott Power, a protein chemist with Genencor International's labs in Palo Alto, California.

Its introduction is one sign of growing industrial interest in these enzymes, which can work at more than 100 degrees Celsius or at a pH of 10. New research initiatives, new companies, even a new journal (*Extremophiles*, launched in February) are melding basic biology with commercial development. Research agencies are surveying the natural world with renewed vigor and finding rapid new ways to identify and produce extremozymes. Such proteins could help process chemicals or food, or even lead to new classes of antibiotics. "We have yet to fully realize the benefits of these organisms," notes Mark Madden, a biochemist at Stratagene in La Jolla, California.

Even if the microbes' own proteins don't prove to be useful, chemists hope to learn from them how to redesign conventional enzymes to perform in harsh conditions. "[Extremophiles] are totally expanding our view of what enzymology is," says molecular biologist Francine Perler of New England Biolabs Inc. in Beverly, Massachusetts.

The industrial launch of extremozymes has been a long time in coming, however. Although the success of Taq polymerase seemed a hot start for extremophile biotech, few firms rushed to follow it up with other applications. And those that did often found it difficult to produce or purify enough enzyme to study—let alone to scale up for industrial use. Indeed, although enterprising microbiologists began collecting extremophiles from bizarre corners of the world more than 30 years ago, until recently they have gotten little return for their efforts.

Given that history, some experts urge caution. "There are people trying to make a whole big story about [extremophiles], but they are not the Holy Grail," says molecular biologist Glenn Nedwin of Novo Nordisk Biotech Inc. in Davis, California. Still, several companies and research agencies are now betting that risky investments in these microbes will yield big payoffs.

From soda lakes to suds. Genencor, for example, is eyeing what Power says is a potential $600 million market for detergent enzyme additives. The company is counting on cellulase 103's ability to break down the microscopic fuzz of cellulose fibers that traps dirt on surfaces of cotton textiles, without harming the cotton fibers as much as other additives do. And because it comes from bacteria in alkaline soda lakes of varying temperatures, this cellulase performs under a broader range of conditions than other additives. It will work at the pH of soapy wash water—hot or cold, says Power.

The bacteria behind the enzyme were collected from soda lakes on several continents—although Genencor won't reveal exactly where. Scientists worldwide are now surveying a host of extreme environments, from cold to acid to high salt, for organisms that might yield similarly useful enzymes. They are finding plenty of microbes to work with, says John Baross, an evolutionary microbiologist at the University of Washington, Seattle. Publications on alkaliphiles alone, for example, surged from a half dozen to more than 100 annually in the past few years.

Two of Baross's University of Washington colleagues, marine microbiologist Jody Deming and chemical engineer Barbara Krieger-Brockett, have focused on cold-adapted organisms, or psychrophiles (from the Greek for cold-loving). They isolated organisms from sediments on the Arctic Ocean floor and have begun to purify their enzymes, which could perform such jobs as removing fat effectively in cold water—a handy trait for soaps.

For some researchers, the search for extremophiles has been a career-long occupation. Take Japanese microbiologist Koki Horikoshi of the Japan Marine Science and Technology Center in Yokosuka. Horikoshi has been hunting extremophiles for decades in deep-sea sediments, and he filed several hundred patents on microbial enzymes—including one used in Japan as a detergent additive—long before the name extremophile was even coined. Only now, after his patents have run out, have some of the enzymes become widely used (*Science*, 3 January 1992, p. 28). But he's not discouraged—far from it.

His latest effort, part of DEEPSTAR, a $3-million-a-year program, has so far collected 1000 different organisms from deep-sea mud and 2500 strains from the Marianas Trench—the deepest place on Earth, almost 11,000 meters down. They have found psychrophiles as well as "barophiles"—microbes that love high pressures. The most useful of Horikoshi's new crop of organisms, however, may be those that flourish in organic solvents toxic to most other life.

He and DEEPSTAR colleagues Chiaki Kato and Akira Inoue found microbes that, for unknown reasons, manage to thrive in toluene, benzene, cyclohexane, and kerosene, sometimes at solvent concentrations of up to 50%. The microbes turned up in both soil and deep-sea muds, but were 100 times more common—and therefore much easier to isolate—in the muds. Although their precise utility is unclear, these microbes can degrade crude oil and polyaromatic hydrocarbons, and also show potential for processing substances that don't readily dissolve in water and therefore require huge volumes of water for their production, says Horikoshi.

Extreme potential. Once researchers have extremophiles in their petri dishes, the next step is to see just what the organisms can do. In Europe, a consortium of 39 teams, funded by the BIOTECH-Programme of the European Union, just finished a 3-year, $8 million survey of organisms from high-salt, high- and low-pH, and high-temperature environments. In January, 61 laboratories embarked on the next phase: a 3-year, $12 million project "to exploit these microbes for various industries," says project leader Garabed Antranikian, a microbiologist at the Technical University of Hamburg-Harburg, Germany. "We want to modify existing chemical processes and make them more biological."



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Hot stuff. Useful heat-loving microbes have turned up in smoking deep-sea vents *(left)* and Icelandic geysers (*right*).

One focus of this project is the potential use of biodegradable extremozymes to reduce toxic waste. For example, several collaborators aim to use extremozymes instead of chlorine in paper production; they are working on a quick screening method to evaluate xylanases and hemicellulases, enzymes that can remove the glue and break down pulp fibers. Other researchers are evaluating heat-resistant enzymes that could be used to label newly made DNA, thereby reducing the need for radioactive tags in diagnostic tests in molecular biology and medicine.

In the United States, too, the extremophile community is poised to put the surveying efforts of researchers like Baross to good use. An expanding cadre of biochemists and molecular biologists is sifting through enzymes extracted from these collections. In addition, the U.S. Department of Energy (DOE) is making basic information on extremophiles widely available and easy to use. Some of that information is as basic as you can get: the genetic blueprints of these strange organisms. DOE is supporting the sequencing of the complete genomes of several extremophiles. One is already completed (*Methanococcus jannaschii*), and DOE expects several more to be finished by the end of June. This makes "these genes from these weird environments" accessible to any researcher who can call up the sequence from a database, says New England Biolabs' Perler.

And one enterprising U.S. company—based in Sharon Hill, Pennsylvania, and La Jolla, California—has adopted a scattershot technique that produces extremozymes rapidly while avoiding the time-consuming process of isolating and purifying individual genes. Founded in 1994, Recombinant BioCatalysis Inc. (RBI) recovers DNA directly from samples collected from all over the world, randomly cuts it into fragments, and inserts the pieces into bacteria, usually *Escherichia coli*. The bacteria then churn out proteins that are screened for various types of enzymatic activity under different conditions.

The company has discovered 175 new extremozymes using this approach and now provides a menu of them in kits sold through biological- and enzyme-supply companies. So, while previously researchers had to invest time and money in culturing or purifying enzymes just to see whether they might be useful, "Now there are enzymes out there to try," says chemical engineer Robert Kelly of North Carolina State University in Raleigh. Each enzyme is different, explains Eric Mathur, who directs the company's La Jolla laboratory, and scientists can see which one works best for their application.

Scaling up. RBI's rough-cut approach sidesteps another difficult problem in working with extremophiles: getting enough enzyme to work with. RBI makes enzymes the traditional way, inserting the gene into *E. coli* and using these bacteria as enzymatic factories. So it's poised to scale up production if a client requests it, although no one has yet, says Mathur. But this method doesn't work with all extremozymes: The *E. coli* sometimes fail to fold the protein into an active form, or the proteins are toxic to these bacteria. And, ultimately, *E. coli* are too inefficient for commercial production, which can require tons of enzymes.

Genencor solved this potential problem easily because cellulase 103 comes from a newly discovered species of *Bacillus*, a genus of bacteria already used as an enzyme factory in commercial operations. So the company was able to put the cellulase gene into a different *Bacillus* strain and produce the 50 to 250 metric tons needed per year.

For those enzymes for which *Bacillus* might not work, some researchers are trying to use an extremophile itself for mass production. They must first overcome a big hurdle, however: These organisms seem to be infected by few viruses capable of ferrying foreign genes into them. But some early progress has been made. Patrick Forterre of the University of Paris, South, in Orsay, France, for example, is working with a thermophile microbe called *Pyrococcus abyssi* that has plasmids—small, circular pieces of DNA that readily serve as vectors for new genes. And another European laboratory has found a virus that might transfer genes into the acid-loving extremophile, *Sulfolobus*.

Even if companies do succeed in producing large quantities of extremozymes, some experts caution that they may not find a ready market. Novo Nordisk's Nedwin says that his company—which sells enzymes to companies that process everything from foods to paper—often finds that extremozymes aren't needed because enzymes from more conventional bacteria work just fine. In his view, extremozymes' chief benefit may be to help chemists learn to tailor enzymes to be stable under harsh conditions. "What extremophiles may do, and that's a big if, is maybe give you a different starting point" for altering traditional enzymes, Nedwin says.

Market economics and tradition may also prevent the use of even the most promising extremozymes. For example, Kelly found an enzyme that breaks up a viscous natural polymer called guar gum, which is used to open crevices in bedrock to enhance the flow of oil and gas. This enzyme, from heat-loving *Thermotoga neapolitana*, can make the guar-gum solution flow more readily than current enzymes do at the high temperatures—100°C—found in deep wells. Yet coaxing traditional oil- and gas-industry companies to risk the switch has been tough. "Most of the major companies have been downsizing," Kelly explains. "It's not a good time to champion a new technology," especially because the new enzyme may not cut costs initially.

Still, Kelly and others are optimistic about the future of extremophiles in industry. "What we have today is the possibility of getting enzymes that are considerably more active at the higher temperatures and more stable," Baross argues. Hamburg's Antranikian insists that his consortium is more than ready to take on these challenges. "The time has come," he says, "to show that these microbes are really interesting for industry."

–Elizabeth Pennisi