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Innovations

Diversa Builds a Business with Designer Bacteria

Three years ago Eric Mathur, VP of scientific affairs and founding partner of Diversa, a 330-person San Diego biotech company, went microbe hunting in the Uzon Caldera in Siberia's Kamchatka Peninsula. Accompanying him were two former Soviet bioweapons scientists under a Department of Energy proliferation-prevention program. They scraped up some sediment from an alkaline hot spring. "We isolated the genomes from the community of microorganisms residing in the hot springs," Mathur recalled. "We chopped them up, put them in laboratory hosts, and developed complex metagenomic libraries." The result was Luminase, an enzyme for bleaching paper pulp.

Luminase enzymes enable mills to reduce the amount of chlorine dioxide (ClO_2) used to bleach paper pulp by up to 28%, thus reducing both costs and pollution. Diversa is targeting a quarter of the $2 billion pulp-chemical market. Because the original microbe was collected from a hot alkaline spring, Luminase can function at high temperatures and at pH values in the 8–10 range. Under these conditions, most enzymes denature. Diversa released Luminase, as well as Cottonase, an enzyme for textiles, as a commercial product in 2004.

Diversa is one of a spate of companies that sprang up in the last ten years to use directed-evolution techniques to develop commercial products. Diversa's bioengineered enzymes are precisely tailored to various industrial-process and pharmaceutical applications, including pulp, vegetable-oil processing, animal care, aquaculture, and anti-infectives. According to the Ohio consultancy Freedonia Group, the U.S. enzyme industry in 2003 was worth $1.2 billion.

Healthcare Ventures, a venture-capital firm, incorporated the company in 1992 as a sister firm to Human Genome Sciences to develop technologies to identify and modify microbial genes with industrial potential, says Martin Sabarsky, Senior Director, Corporate Development and Investor Relations. The company went through a few unwieldy incarnations as Industrial Genome Sciences and Recombinant Biocatalysis, Inc, but was renamed Diversa as a nod to its diverse microbial sources and applications.

Diversa's first products were stable Pyrolase enzymes used in secondary oil and gas recovery. One type of Pyrolase was derived from DNA extracted from microorganisms found in a hydrothermal vent, and another version from a bug collected from a Yellowstone National Park hot spring.

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—Frances H. Arnold, professor of chemical engineering and biochemistry at Caltech

Diversa's scientists collect microorganisms in exotic locations such as deep-ocean vents, the arctic, and the digestive systems of termites on the premise that it is a lot less work to derive an enzyme that works in extreme conditions if one starts with microorganisms already adapted to living in such environments.

In order to legally collect microorganisms abroad, Diversa works within the Convention of Biological Diversity, a treaty ratified in 1992 by 188 nations. Diversa must negotiate royalties and access with the designated environmental steward from that country. Collections are usually done by local scientists and grad students, although Mathur particularly enjoys going on expeditions.

There are 20 types of amino acids that occur naturally; the specific sequence of amino acids determines the nature of the protein. At Diversa, protein molecules are comprehensively mutagenized, and the resulting enzymes are screened for desired attributes. Sometimes all the attributes already exist in the original organism.

Diversa scientists identify the gene that encodes the enzyme and extract that particular sequence of DNA from the organism. In a process of recursive ensemble mutagenesis, or "Direct Evolution," they mutate the gene extracted from the host organism and transplant it into another vector, such as a hardy *E. coli* strain that is easy to grow by standard commercial methods.

"Earlier bioengineering companies such as Novazymes and Genencor never optimized enzymes," Mathur says. Companies reinserted the engineered DNA back into the same organism to get high levels of homologous gene expression. Diversa's innovation was to metabolically engineer the host and put the engineered DNA derived from the extremophiles into hosts that can be grown in industrialized quantities. "By engineering the organism, we can engineer a high level of expression for heterologous genes," says Mathur, "then produce great quantities out of it."

The processes that the company uses to derive its enzymes and products are proprietary and expensive. "With our ultra-high throughput methods we can process a billion samples a day," Mathur says. "Gigamatrix, our premier platform, uses 400,000 wells per plate, using the same footprint but a number of wells at three orders of magnitude higher than extant drug-company robotics. Each microwell contains only 50 nl of liquid substrates." The company patented protein-engineering techniques such as Gene Site Saturation Mutagenesis (GSSM), which is a library of single-point mutations,

and GeneReassembly Technologies. The optimization problem is not simple; every enzyme usually contains between 100 and 300 amino acids and the matrix that has to be optimized is N × 20, where N is the number of amino acids in the enzyme and 20 is the number of amino acids available. The number of possible permutations is huge. Think on the order of 10^{10^6}.

Statistical methods help to reduce the number of experiments that need to be performed. This method is known as DOE, design of experiments. This algorithm was actually developed during WWII for optimizing many input/output problems and processes. DOE is commonly used in combinatorial chemistry such as drug screening. Mathur explains that they take all 19 possible changes for each amino acid and screen for phenotype improvements. Once they find the residues and amino acids that result in improvement, they combine these mutations and screen that library for the best variant. “And that is how we get around the big, giant numbers,” says Mathur.

Business Strategy

Diversa’s near-term revenue sources are industrial, chemical—such as Luminase, which was commercialized within 30 months—and animal feeds, products that have a relatively brief development cycle of six to nine months. These products only require the EPA to determine whether they are toxic, as opposed to more extensive FDA review. Medium-term (about 5 years) but higher-value-added products are pharmaceutical intermediates, such as an intermediate enzyme for Lipitor (a registered trademark of Pfizer, Inc.) synthesis. Long-term products are small-molecule drugs and protein therapeutics such as antibodies.

But how much can Diversa differentiate its products? Mathur acknowledged that understanding what the market will bear is an issue. Diversa has a group of market analysts who figure out potential market opportunities for the company’s products. “Our challenge is to come out with lead candidates really quickly,” Mathur says. “In the space of evolution, we have many competitors, but we own the space of accessing unique methods of biological diversity.” The company holds 200 patents. Some processes were patented close to 10 years ago. Because the life of a patent is 17 years, the company faces competition from potential generic enzymes as well as enzymes derived by other methods, and in other organisms. In these brutally competitive industries, innovation is only valued when it lowers costs. “It is all about costs,” Mathur says.

Applications

Diversa and its competitors are making enzymes for the production of specific compound enantiomers. Enantiomers are isomers that are mirror images of each other and differ in the way they interact with polarized light. Because all our biological functions are performed by enzymes and enzymes are enantiomer-specific, i.e., they can act on one enantiomer and not the other, specific chirality (the property of being right or left handed) is crucial for anything that relates to biological function. For example, the L isomer of Thalidomide causes birth defects, whereas the D isomer is completely safe. The FDA requires chirally pure chemicals for drugs. And enzymes are an exquisitely specific way to achieve this.

An example of an enzyme with chiral selectivity is Nitrilase. There were originally nine types of bacterial nitrilase in the public domain, and Diversa patented 237 sequences that the company had discovered with its patented approach involving functional screening of metagenomic libraries. Diversa’s nitrilases convert 3-hydroxyglutaronitrile to either (S)-3-hydroxy-4-cyanobutyric acid or (*R*)-3-hydroxy-4-cyanobutyric acid, a precursor of Lipitor. With the unmodified enzyme, only 89% of the total 3-hydroxy-4-cyanobutyric acid was the (R)-enantiomer. Diversa further bioengineered the enzyme to be 99% specific.

Enzymes that are added to animal feed to improve the digestibility of certain food elements make up another big market. Since 2003, Diversa has sold hundreds of tons of thermally stable Phyzyme XP, an enzyme that helps monogastric animals such as hogs and chickens absorb phosphorus from grain, in the U.S. alone. The product is now waiting final approval in Europe. Together with Bayer Animal Health, Diversa recently co-developed a fish vaccine for Salmonid Rickettsial Septicemia (SRS), an infection that was destroying up to 20% of the crop of Chilean salmon farmers. The vaccine eliminates the need to use antibiotics to contain the disease.

The Future

Diversa is moving into what it terms "metagenomics,” or how multiple genes act in concert to perform a task, as in a multi-step process on an assembly line. “We are a science moving beyond the biology of single organisms,” says Mathur. One of the main challenges put forth at BIO, the biotech industry meeting this year, was how to break down lignin cellulose and other biomass. Diversa is looking for the solution in termite guts. The company is sequencing everything it finds there. “We don’t know what we are looking for,” says Mathur. “All organisms have pathways to encode for small enzymes. Microbial consortia in the hindgut of termites are responsible for producing the suite of enzymes necessary for the conversion of lignocellulose to fermentable sugars.”

Professor of Chemical Engineering and Biochemistry at Caltech Frances H. Arnold was an early consultant to the precursor to Diversa and rival Maxygen. “It sounds great, but it all comes down to what it costs,” she says. She considered their main challenge to be targeting the right problems, namely those that give enzymes a chance to compete. Arnold believes that the catalyst-engineering technology is powerful but worries that process and engineering issues still limit many applications.

Another issue is that the pool of catalyst possibilities that exist in organisms that live in extreme conditions is limited. “The name of the game is to find the right catalyst,” says Arnold. “You can do this by mutation, or you can do it by finding the perfect enzyme in nature. But you have to do it quickly and efficiently.”

Although all competitors say their approaches are unique, says Arnold, “In my opinion they are pretty

much the same. Diversa stands out in having a broad platform of approaches, solving the problem from many different angles. This is good, because it is not clear a priori which approach is going to be successful."

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