Diversa and diversity

The words of one highly respected scholar of evolution challenge the commonly held anthropocentric and inherently progressive view of evolution:

> 'Bacteria represent the great success story of life's [evolutionary] pathway. They occupy a wider domain of environments and span a broader range of biochemistries than any other group. They are adaptable, indestructible and astoundingly diverse' – Stephen Jay Gould [*Sci. Am.* (1994) 271, 84–91].

Gould continues in his essay to propose that the emergence of humans was due to a series of quirks in natural history that would be unlikely to occur a second time, whereas the emergence of microbial life is highly predictable – almost a safe bet. Much of Gould's thesis is consistent with what drug discovery scientists have known for a long time – that microorganisms are an extremely important life form. Prospecting for new drugs from microbial metabolites has been hugely productive, both from a commercial and public health perspective. Microorganisms produce a vast range of highly diverse molecules, because they have an unrivalled diversity of biochemical reactions. Moreover, many of their metabolites have profound effects on human biochemistries, echoing the evolutionary and biochemical connections between humans and the microbial world.

Microbial domination

Chemical compounds derived from microorganisms are now in use as some of our most effective medicines, including antibiotics, immunomodulating agents and cardiovascular drugs. Moreover, recent discoveries have revealed that microbial life is much more dominant on Earth than we imagined even just a few years ago: a rich diversity of microorganisms has been discovered deep within the Earth's crust [Gold, T. *Proc. Natl. Acad. Sci. U. S. A.* (1992) 89, 6045–6049]; and an entirely new form of microbial life, the Arachea, with a highly specialized genome has been recovered from the depths of the oceans [Morell, V. *Science* (1996) 273, 1043–1045].

Extreme environments

Diversa Corporation (San Diego, CA, USA) is exploring some of the most extreme environments on Earth in the search for unique bioactive molecules that are produced by microorganisms. What makes Diversa unique among the many players in this field is the new technological approach they bring to the search; it allows them to gain access to an enormous range of microorganisms that have not been accessible by traditional approaches.

In the past, the same basic paradigm has been followed by most prospecting companies. Organisms isolated from natural samples are incubated under a variety of conditions and with several different growth media to stimulate the production of different metabolites. Extracts are then tested in a variety of screens indicative of the therapeutic areas of interest to the company. When an active compound is found, larger batches of the microorganism are cultured in sufficient quantities to extract the amounts of material needed for full biological and chemical characterization.

Figure 1. *Pompeii worms live in an extreme oceanic environment off Costa Rica. On each worm's back, bacterial hitchhickers produce enzymes that may hold the key to new protein-based catalysts for manufacturing drugs, paper, food and many other products.*

Culture problems

Although unquestionably successful in the past, this approach suffers from significant limitations. A primary problem is the need to isolate and grow each of the wide diversity of microorganisms present in the samples. 'A single sample may contain 10,000 different microbes', says Dr Jay Short, Diversa's Executive Vice President and Chief Technology Officer, but only a tiny number, maybe 0.1%, of the total microorganisms in such a sample can be isolated and grown under laboratory conditions. Short explains that it is sometimes very difficult to establish, in a laboratory setting, the conditions for which some microorganisms have become exquisitely adapted. This is especially a problem for microorganisms collected from extreme environments, which frequently harbor some of the most interesting organisms. In addition, some organisms seem to require metabolites produced by other microorganisms in the sample in order to grow. So, the very act of attempting to isolate such an organism ensures that it will not propagate itself.

Problems with scale-up

Finally, a limitation with the traditional paradigm is the problem of scale-up. Frequently, an organism that grows vigorously or produces an interesting product in small-scale culture is unresponsive to attempts to scale up production. Either the organism will not grow vigorously under the scale-up

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conditions, or (more often) it grows but no longer produces the metabolite of interest.

To overcome such problems, Diversa's microbiologists circumvent the isolation and culture step. Instead, they prepare DNA directly from the raw sample and then use it to build gene expression libraries, which are then subsequently screened for interesting enzymes or metabolites. This approach is somewhat akin to the combinatorial biosynthesis approach utilized by Kosan Biosciences (Burlingame, CA, USA), and ChromaXome (San Diego, CA, USA), who isolate DNA from various organisms and then recombine the genes to make either the natural product in a host organism or 'unnatural' natural products due to gene rearrangements in the host [Wallace, R.W. *Drug Discovery Today* (1997) 2, 505–506].

A major advantage of the Diversa approach is that DNA from all organisms in the sample is collected, providing an opportunity to detect interesting compounds produced by the more than 99% of the microorganisms in typical samples. It also allows for the production of totally novel compounds, because of the scrambling of metabolic enzymes during the production of the gene libraries.

Bacterial factory

All of the DNA is incorporated into one, well-defined bacterial factory. Although the company does not identify the bacteria used for this purpose, it claims that it is easy to grow, amenable to high-throughput screening demands, and appropriate for scale-up protocols to produce large quantities of an interesting metabolite. Moreover, it is necessary to collect only a small amount of an environmental sample to provide all of the DNA needed to make the expression libraries needed for screening. 'Even without amplifying the DNA, only a tablespoon of water is needed,' says Short. Diversa avoids amplifying the DNA it uses for libraries, because amplification has the potential to skew the genetic characteristics of the resulting DNA library.

Hydrothermal vent worm

Diversa is prospecting in extreme environments for interesting organisms. The company recently reported the discovery of the Pompeii hydrothermal vent worm (*Alvinella pompejana*), which is the most eurythermal (thriving at all temperatures) organism yet discovered [Cary, S.C., Shank, T. and Stein, J. *Nature* (1998) 391, 545–546; Figures 1 and 2]. The worm was collected from a depth of more than one and a half miles. The head of the worm experiences an average temperature of 22°C while the posterior of the worm is embedded in the ocean floor at a temperature of 81°C, a 60°C gradient in temperature throughout the body of the worm. Such organisms are a potential source of novel enzymes. In this case, an esterase was discovered that is active over a wide range of temperatures. The worm is also colonized by a unique microbial flora. 'DNA clones from the worm symbionts are currently being screened for useful compounds', says Terrance Bruggeman, Diversa's Chief Executive Officer.

Figure 2. *The deep-sea Pompeii worm (*Alvinella pompejana*), which lives in tube-like dwellings (see Figure 1),is the most heat-tolerant creature on Earth.*

Hot springs and geysers

Diversa has agreements in place that allow it to collect samples from a variety of extreme environments including the hot springs and geysers at Yellowstone National Park (USA), similar environments in Iceland and Costa Rica, and diverse habitats in Indonesia. 'We are quite literally going to the ends of the Earth to locate unique enzymes with utility in commercial processes and pharmaceutical development', says Bruggeman.

Novel enzymes

Diversa's major effort to date has been in discovering novel enzymes – a total of 12 new families of esterases and more than 300 new enzymes. Such enzymes have proved useful for such practical applications as secondary oil recovery, sanitizing and the production of chiral chemical intermediates. 'We are using enzymes as molecular tools and as effector molecules,' says Short. 'They are useful in agricultural crop improvement, insecticides and pesticides.' This aspect of the company business plan is well under way and collaborative agreements are in place with Finnfeeds International (Marlborough, UK), Boehringer Mannheim (Mannheim, Germany), Sigma-Aldrich (St Louis, MO, USA) and The Dow Chemical Company (Midland, MI, USA) to market and utilize the company's catalog of novel enzymes.

So far, the pharmaceutical aspect of the business appears to be less well developed, but Short indicates that Diversa is currently working with several partners who are screening their libraries for useful small molecules. Diversa also conducts in-house screening of its own libraries. The company is actively seeking additional partnerships.

Diversa was originally founded in 1994 as Recombinant Biocatalysis, Inc. (Sharon Hill, PA, USA). In March of 1997 the company's operations were consolidated, the new name Diversa adopted, and the entire operation was moved to San Diego.

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