

Using biodiversity to identify biocatalysts and drug leads

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Established in 2001, LibraGen specialises in the development of bioprocesses for the production of life science molecules and the discovery of new chemical scaffolds with cross-industry applications. By focusing on untapped biodiversity, the company has developed a platform of proven proprietary technologies to identify, validate and produce high-added-value enzymes and molecules for industrial, pharmaceutical, chemical, cosmetic and environmental applications. The overall combination of LibraGen's scientific and production know-how, technology platform and research results has already achieved a number of successful partnerships in the fields of pharmaceuticals and fine chemistry.

Functional genomic technologies

LibraGen uses functional genomic technologies combined with industrial expertise to develop new bioprocesses for the discovery of novel bioactives. The company has the ability to turn the enormous genetic and chemical diversity of bacteria that cannot be cultured under conventional conditions into a source of novel biocatalysts and molecules that can be used in the development of pharmaceuticals and other chemicals.

The company's activity in new biocatalyst discovery is based on the application of its META-DNA™ metagenomic technology, as well as on the selection of accessible enzymes and culturable microbes. LibraGen also applies technologies such as molecular evolution, substrate synthesis using classical chemistry, and target or bioassay oriented screening of biomolecules to discover new biocatalysts.

LibraGen has 500 sq m of laboratory space for microbiology, molecular biology and chemistry located in its Toulouse, France facility. The company also has a bioprocessing pilot plant available for production from discovery scale to kilo-lab scale.

Research on micro-organisms to exploit biodiversity

Micro-organisms have been widely used for industrial applications, including in drug discovery, biotransformations, and in the agrochemical and industrial chemical industries. For example, about 70 per cent of existing antibiotics were discovered as or were derived from natural products synthesised by bacteria. In addition, a large number of active compounds discovered by antibiotic screening have been found to be of interest in other therapeutic areas such as cancer, diabetes, and so on. However, the compounds discovered were identified and exploited by using less than 1 per cent of the total bacterial diversity. Therefore more than 99 per cent of bacterial diversity is still inaccessible to conventional microbiology methods, and until recently one of the principal sources of new natural compounds for use as drugs and biocatalysts has been ignored.

Technical skills identify new molecules and bioprocesses

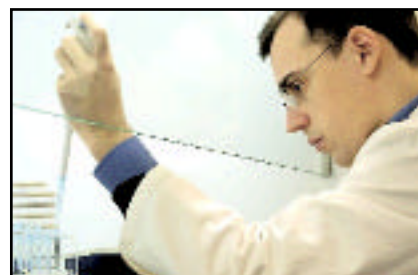
LibraGen's skills range across the whole value chain, from access to a proprietary bioresource (for example, DNA libraries) to product industrialisation for a novel molecule or for

an innovative bioprocess. The main advantage of the company's technology in comparison with current microbiological approaches for the discovery of new biomolecules is its direct access to the genes or biosynthetic pathways coding for biomolecules of interest.

Metagenomics consists of directly purifying bacterial genetic information (DNA) from the environment and is principally sourced from non-culturable bacteria. Metagenomic DNA contains a large number of different bacterial genomes, with up to 1,000 to 10,000 per gram of soil. The DNA fragments are separated out and incorporated into culturable host bacteria. As a result, the technologies provide a new approach for establishing qualified, pure, high-molecular-weight DNA libraries of unexplored bacterial origin derived from different environments. Expression of this DNA into an adapted host expression system enables the primary activity screening to identify new natural compounds and enzymatic activities.

Thus LibraGen is focused on identifying new molecules and biocatalysts of interest within its metagenomic DNA libraries. A complementary iterative screening approach has been established that includes genetic, biological activity and chemical screening. The combination of these technologies leads to identification of molecules of interest for further development at a very early stage in the process, as well as to rapid pre-purification of active extracts. This iterative screening approach is applied on the basis of the heterologous expression of cloned DNA to identify biocatalysts or natural compounds and/or search for specific chemical families encoded by known enzymes.

Genetic screening targeting specific enzyme-encoding sequences allows the identification of clones that contain these biosynthetic pathways or genes. The clones are then transferred into appropriate host bacteria expression systems to express the new biosynthetic pathways or genes and overproduce enzymes or new natural compounds.



LibraGen's highly skilled scientists apply metagenomics technology as well as molecular biology and screening of biomolecules to identify new biocatalysts.

Biological and chemical activity screening

LibraGen's technology platform and know-how have been designed to discover or improve a given biocatalytic system for the selection transformation of one or more reactants into an identified added-value product, based in particular on ensuring that the processes are economically viable. The company can supply quantities of desired product typically in the 1-100 grams range.

The company also carries out screening procedures for antibacterial, antifungal, antiproliferatives and antiviral activity identification, thus quickly identifying active recombinant clones from the metagenomic DNA libraries.

Chemical activity screening relies on analytical chemistry techniques (HPLC/UV, then MS) for detecting the presence of novel compounds and/or new metabolic pathways by comparing a culture of the strain transformed with a metagenomic DNA library fragment against the non-transformed wild type. New peaks in the chromatograms demonstrate the existence of a novel biological reaction. Using information from these preliminary analyses, LibraGen then isolates and identifies each structure/product using spectroscopic characterisation methods and then considers the possibility of chemical synthesis.

Process development and scale-up

Process development is the first operation in the industrialisation phase and consists of a range of experiments to determine parameters for fermenters (2-20 litres) and reactors (2-15 litres); the nature of media and their compositions; biological reaction kinetics and the operating parameters; and the operating time needed to attain maximum yield with the best selectivity. It is also essential to identify the technique used to isolate and recover raw product; to determine the final purification mode; and to check the reproducibility of the whole process and its robustness. It is then necessary to demonstrate the large-scale feasibility of the process. This scale-up procedure is carried out either in 100-300 litre fermenters (using metabolite or biomass production), or in a chain combining 100-300 litre fermenters and 50-100 litre chemical reactors (ie biocatalytic production of molecules). The mode of production established is then backed up with a dossier that meets international quality control and product registration principles.

Alliance with fine chemicals manufacturer

LibraGen has formed a strategic alliance with Minakem for biocatalysis and fine chemicals production. The companies say that the close integration of biocatalysis and chemical



Libragen has established a strategic alliance with fine chemicals manufacturer Minakem. Pictured is Minakem's 600-litre Hastelloy low-temperature reactor, capable of operating down to -100°C.



An 800-litre FDA-compliant pilot reactor that forms part of Minakem's fine chemical production facilities.

manufacturing know-how will deliver more cost-effective leading-edge processes. By jointly deploying their marketing and technical resources, the two companies aim to deliver significantly more cost-effective services and products to customers in many projects where there are synergies between fine chemicals production and biocatalysis.

Minakem has wide experience in process and analytical method development of complex multi-step syntheses, high-quality starting materials, intermediates and APIs for the pharmaceutical industry. The company focuses on synthesis of starting materials for the fine chemical industry in dedicated facilities with cost-effective processes; custom manufacturing of key intermediates for the pharmaceutical industry in a dedicated cGMP facility; and the manufacture of APIs, including niche API's, in FDA-inspected, cGMP-compliant facilities.

LibraGen will use its proprietary META-DNA technology platform and know-how in applied biocatalysis to generate new biosynthesis routes that will be transferred to Minakem's cGMP-compliant facilities for manufacture.

"This alliance will bring LibraGen additional openings for providing its services to the fine chemicals and pharmaceuticals industry," says Dr Renaud Nalin, chief executive officer of LibraGen. "It is a great opportunity to provide pragmatic biocatalytic processes which, when combined with Minakem's chemistry and production capabilities, offer integrated solutions for the definition and the implementation of the best industrial production processes. We will deploy all the technology available in our biocatalysis platform in these joint projects. This includes enzyme discovery, optimisation and applied biocatalysis. We are looking forward to extending our market share through this alliance." ^{SP}

FURTHER INFORMATION

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