



MICROBIAL BIOPROSPECTING



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Brazil, due to its large diversity of biomes and ecosystems, its large territorial extension, and its location between the tropics, where the planet's largest biodiversity is, is shown to have a great potential for the generation of renewable energy.

Even with the advances regarding sustainable energy and the processing of raw materials of energetic interest, the technologies to reuse coproducts and waste still have to overcome great technical challenges. A good example of that is the reuse of agribusiness waste for production of biofuels and high value-added chemical products.

Several national and international groups are working to develop technological solutions for establishing environmentally and economically sustainable bioprocesses. Microorganisms are essential in several of those processes, and, for instance, they can be directly employed in fermentative processes, or as providers of inputs, such as enzymes, for lignocellulosic biomass degradation.

Currently, several microbial species are used in industrial processes, and the number of microbial products keeps growing, as microorganisms allow for the production of high concentrations of molecules with specific chemical characteristics and

small environmental impacts. Besides that, the ability to genetically modify microorganisms, both to increase production and to produce new molecules of interest, contributes largely to their industrial application.

Despite of all the success in the beneficial use of microorganisms for mankind, the diversity and inter-dependencies of those microorganisms still remains a field to be explored. Considering that most microorganisms cannot be easily grown in a laboratory (< 1%) and that there are approximately 10³⁰ microbial cells on Earth (a number which exceeds all stars known in the universe by nine orders of magnitude), the biotechnological potential of microorganisms is clearly underused. That has made it difficult to understand microbial ecosystems, and gone beyond our ability to discover and use new beneficial functions from microorganisms, such as new enzymes for chemical and biochemical reactions of interest, processes to increase bioremediation, biomarkers for diagnoses of diseases and therapeutic targets.

Thus, traditional and advanced bioprospecting technologies have been developed and employed in order to increase the knowledge on the microbiological diversity of functional processes in microbial ecosystems, and also for the identification and use of new biotechnological products.

Bioprospecting

Bioprospecting consists of the systematic identification, evaluation, and exploitation of the diversity of life in a certain place, and it aims to seek genetic resources for commercial purposes. Regarding microorganisms, it mainly comprises strategies to exploit culturable and nonculturable shares of the microbial biodiversity. Another method that has been used by research is the *in silico* prospecting of genome sequences of microorganisms that are already available in databases. All of that intends to identify microorganisms, genes, enzymes, and/or metabolic pathways for further biotechnological applications in industry or in research itself.

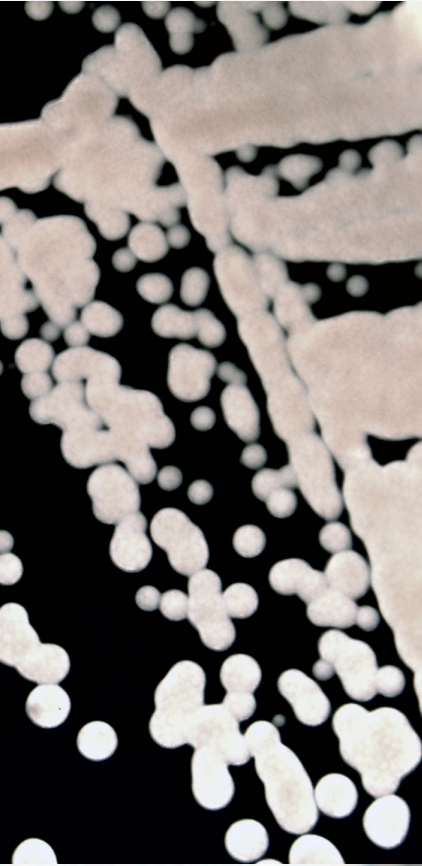


In Silico

In silico prospecting is based on discovering new genes and metabolic pathways through the analysis of genome sequences in databases. The decrease in costs for sequencing has enabled the generation of a large quantity of information on microbial genomes. Complete genomes of bacteria, fungi, and archaea have been increasingly made available by the scientific community. Prospecting those genomes through comparative genomics and evolutionary analysis methods, with the aid of bioinformatics tools, has allowed for the quick identification of new genes and enzymes with a wide range of functions and

applications. The identification and validation of new enzymes related to lignocellulosic biomass degradation or metabolism of sugars in bacteria and yeasts are good examples of where this technique can be applied.

The combination of different bioprospecting strategies for identifying new bioproducts has been employed. *In silico* bioprospecting, for example, may be used for designing molecular probes, which are then employed in strategies for prospecting culturable microorganisms or metagenomic libraries.



Nonculturable microorganisms

Bioprospecting of noncultivated microorganisms (microorganisms that cannot be cultivated with the current microbiology techniques) has been mainly performed through the use of metagenomics. In that technique, the genetic material from microorganisms in a certain environment is collected, isolated, and amplified in DNA libraries. Not only do the metagenomic libraries allow for the identification of the main microbial groups in the sampled environment, but also for its genetic characterization (DNA sequence in the microbial pool) and functional prospecting. In fact, the libraries may be prospected by specific activities, such as by hydrolytic enzymes that specifically play roles in vegetable biomass degradation. Metagenomic libraries from the most distinct environments have been built, and information on the microbiota of complex systems, such as insect intestines or oceans, have been effectively obtained and used for biotechnological applications.

Culturable microorganisms

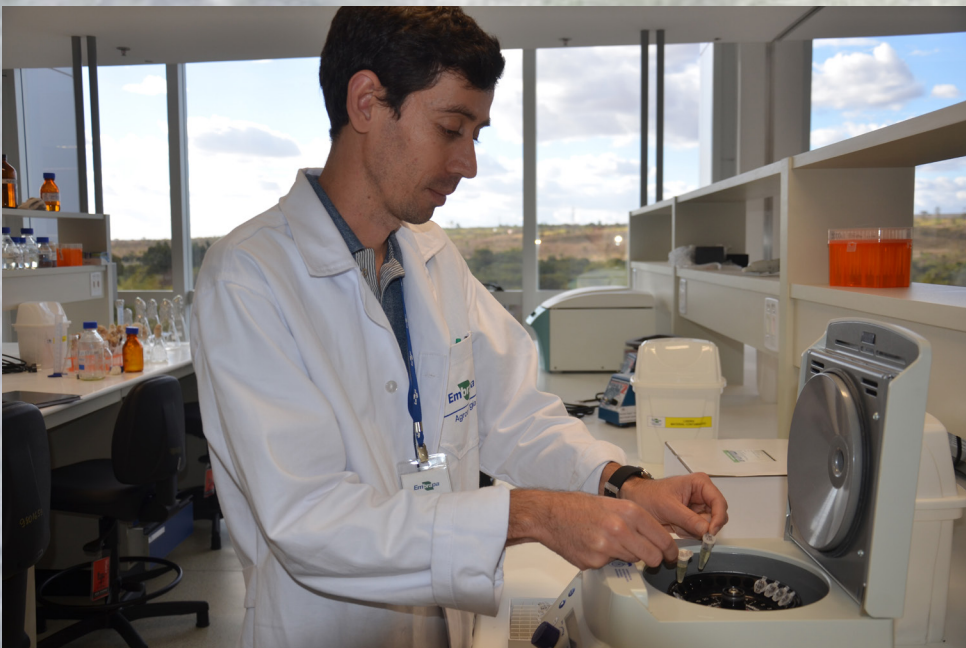
Bioprospecting of culturable microorganisms involves cultivation and selection of microorganisms for an application of interest, based on the microbial biodiversity that exists in a certain habitat or is stored in a collection. The selection of microorganisms which specifically grow in a certain source of nutrients is of great interest. Currently, less traditional, more efficient selection techniques, including the emulation of industrial conditions and the automated emulation of process conditions, has allowed for the cultivation and screening of hundreds of microorganisms for desirable traits in a single day, even if they

are complex. For example, miniaturized tests and online detection systems may be used for prospecting a large number of microorganisms that produce bioactive compounds and for identifying molecules of interest. Besides that, the cultivation of microorganisms that were otherwise said to be nonculturable has been improved by the increase in microbial physiology, biochemistry, and ecology knowledge, through the employment of large-scale phenotyping techniques, which allow for the simultaneous analysis of several traits and facilitate the optimization of culture media.

At Embrapa Agroenergia

At *Embrapa Agroenergia*, the most varied environments have been targets for prospecting microorganisms for the development of biotechnological products: soils, plants, decomposing vegetable materials, agricultural and industrial waste, and mutualistic associations among organisms such as herbivore rumen. The results obtained show that bioprospecting of microorganisms has been found to be an important tool for the development of fermentative processes. New microbial strains that are capable of metabolizing specific sugars or producing hydrolytic enzymes, both applied in the production of lignocellulosic ethanol, continue to be identified.

Besides that, aiming the development of biorefineries, microbial strains that are producers of organic compounds have been obtained, and the processes for producing such compounds from agribusiness waste (biomass and crude glycerine) are being established. In this context, bioprospecting yields a wide genetic variability of microorganisms, even those which belong to one same species. The development of new approaches for prospecting will remain to be greatly highlighted in biotechnological studies over the coming years.



||||| **Authors:** João Ricardo Moreira de Almeida (scientist), Daniela Garcia Collares e Patrícia Flávio Dias Barbosa (analysts)
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Ministry of Agriculture, Livestock and Food Supply
Parque Estação Biológica (PqEB), Av. W3 Norte (final)
70770-901, Brasília, DF, Brazil
Phone: +55 (61) 3448 1246 Fax: +55 (61) 3448 1589
www.embrapa.br/agroenergia