ENVIRONMENTAL BIOTECHNOLOGY RESEARCH IN THE „UNIVERSITAS BIOTECHNOLOGY LABORATORY"

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The Department of Biotechnology of A. József University of Szeged, the Biological Research Center of the Hungarian Academy of Sciences, the Institute of Biotechnology of the Bay Z. Foundation for Applied Research, and the Food Technology Faculty of the Horticultural University agreed in 1996 to co-ordinate and combine forces and expertise related to the education, research, and development of biotechnology. The form of this collaboration is the „Universitas Biotechnology Laboratory", an institution without walls based on the mutual interest of the parties. Some of the joint projects are summarized as follows. Detailed presentations will follow in subsequent issues.

I. Biohydrogen

A fundamental and principal difficulty of the energy industry is that the formation of fossil fuels is much slower than the rate of their exploitation. Therefore the reserves which can be recovered in an energetically feasible manner are shrinking parallel with an increasing world-wide energy demand. Among the alternative energy carriers, hydrogen is preferred because it is easy to transport and store and it burns to environmentally friendly water vapour when utilized. Hydrogen can be produced in biological systems: solar energy captured by the photosynthetic apparatus is converted into chemical energy through water splitting, the reaction forms oxygen and can also produce hydrogen. Upon utilization, these components are combined to form water and energy is released in a cycle driven by the practically unlimited and safe energy source of the Sun (SASIKALA et al., 1993).

In addition to offering an alternative for the global energy crisis, biologically produced hydrogen may also serve as reductant for numerous microbiological activities of environmental significance. Reductants are needed to convert CO_2, atmospheric nitrogen, nitrate, or sulfate into useful and/or environmentally safe products. For example, biogas is generated from industrial, agricultural, or household waste, nitrogen is fixed by plants which decreases their need for fertilizers, and nitrate is reduced to nitrogen in drinking water thereby eliminating a public health hazard common in both developed and less developed countries.

II. Environmental biotechnology

Reductants are required in many environmental applications because most compounds, generated as waste or hazardous material, are oxidized derivatives due to the presence of an oxidizing atmosphere on Earth. These compounds can be eliminated and recycled into the global C, N, O, or S natural cycles through reduction. Biological regeneration, bioremediation, utilizes reductants generated by biological systems, i. e., hydrogen produced *in situ* (KOVÁCS et al., 1996).

II. 1. Biogas

This principle has been employed in biotechnological control and management of the complex microbiological series of events that leads to biogas formation. We have shown that anaerobic biodegradation of solid household waste, waste water sludge, or animal manure can be accelerated. Biogas formation is intensified through the microbiological manipulation of the intermediate hydrogen production steps according to our patented procedure. The method has been tested in field experiments at the landfill depository of the city of Szeged, processing about 200,000 m^3 of solid household waste yearly, and in the 2,500 m^3 fermenter of a waste water sludge treatment plant (Bácsvíz Rt., Kecskemét). The technology is also exploited in an EUREKA project of the European Union, involving Swedish, Polish, and Austrian partners.

II. 2. Denitrification

Biological hydrogen metabolism is utilized in another approach to eliminate nitrate from drinking water. It is well known that nitrate is a hazardous material endangering human health and life. Nitrate is most commonly taken up through contaminated drinking water. Nitrate accumulates in natural water reserves mainly because of the improper application of fertilizers in agriculture and because of inadequate waste water treatment (a local example of human negligence is the lack of a municipal waste water treatment facility in our home base city of Szeged). Nitrate is the most oxidized form of nitrogen and can be returned into the natural nitrogen cycle after reduction to the completely innocent nitrogen gas.

The biological denitrifying process developed at the UBL-Szeged consists of a mixed bacterial population, which is capable of reducing nitrate to nitrogen in an effective and economic way using the *in situ* produced hydrogen for reduction. Scale up work is being done in collaboration with Nitrokémia Chemical Works, Inc., a major chemical plant in Hungary producing excellent nitrate selective ion-exchange resins. The process is the subject of an other EUREKA projects at the European Union level, where the Hungarian team collaborates with Czech, Dutch, French, and German partners.

III. Hydrogenase

The understanding of molecular fundamentals of hydrogen production and utilization among microbes is a goal of supreme importance both for basic and applied research applications. The key enzyme in biological hydrogen metabolism is hydrogenase, which catalyses the formation or decomposition of the simplest molecule occurring in biology: hydrogen.

$$H_2 \Leftrightarrow 2H^+ + 2e^-$$

The simple-looking task is solved by a sophisticated molecular mechanism. The majority of hydrogenases are metalloenzymes, harbouring Ni and Fe atoms. Like most metalloenzymes, hydrogenases are extremely sensitive to inactivation by oxygen, high temperature and other environmental factors. These properties are not favourable for several potential biotechnological applications.

In metal containing biological catalysts it is the protein matrix surrounding the metal centers, which provides the unique environment for the Fe and Ni atoms which allows hydrogenases to function properly, selectively, and effectively. Therefore, the main goal of our basic research is to understand the protein-metal interaction.

The problem is not simple to address as some of the methods for scientific investigation provide information on the metal atoms themselves without directly observing the protein matrix around them. Other modern techniques at our disposal reveal details of the protein core, but do not expose the metal centres within. A combination of these two approaches, i. e., molecular biology and biophysics, is expected to uncover the fine molecular details of the catalytic action of metalloenzymes.

An important condition of successful research is the choice of the target microorganism best suited for scientific investigation. Several years ago we selected the hydrogenase enzyme(s) of photosynthetic bacteria. One of the purple photosynthetic bacteria *(Thiocapsa roseopersicina)* contains a hydrogenase, which displays outstanding stability among enzymes possessing the same catalytic function (KOVÁCS et al., 1996, RÁKHELY et al., 1998). Heat stability does not provide an advantage for the bacterium in its natural, cold water marine environment. Understanding, through molecular biological studies, the molecular factors that stabilize this hydrogenase (COLBEAU et al., 1994) is expected to help design various enzymes equipped with resistance to the inactivating effect of high temperature, oxygen, and/or proteolytic attack for future biotechnological use.

In the European Union and Associated States, research networks are formed around topics of outstanding economic and/or scientific interest. One of these networks is COST Action 8.18 „Hydrogenases and their Biotechnological Applications". COST Action 8.18 includes practically every laboratory of significant contribution to hydrogenase research, i. e., 45 laboratories from 13 European nations. The network has been co-ordinated by the head of the Szeged laboratory for the third consecutive year.

IV. Hyperthermophiles

Hyperthermophilic microorganisms grow above 80°C, and cannot multiply below 70°C. Most hyperthermophiles are archaea. Hyperthermophiles occur in various habitats, e. g., deep sea volcanic sites, hot water spring and even chimneys. By definition, enzymes operating in these unusual creatures are heat stable, therefore they offer an obvious advantage for biotechnological applications. Hyperthermophilic archaea also represent the oldest form of life on Earth. In the chemolithotroph metabolism, characteristic of hyperthermophilic archaea, hydrogen metabolism plays a crucial role. Due to the considerable technical difficulties in cultivating these microorganisms in the laboratory, only a few of the hyperthermophilic hydrogenases have been purified and characterized. We have developed a novel technique to plate hyperthermophilic archaea (RÁKHELY and KOVÁCS, 1996), this is the first major step to study their microbiology, molecular biology and genetics. This information is needed for the multiple biotechnological exploitation of hyperthermophiles.

V. Methanotrophs

Methanotrophic bacteria typically contain another metalloenzyme, methane monooxygenase (MMO). Methanotrophs utilize methane as their sole source of carbon and energy. Methane is oxidized to CO_2 through methanol, formaldehyde and formate intermediaries. The first and most important step in this sequence of reactions is the methanol conversion, catalysed by MMO.

MMO can also attack several compounds representing serious environmental and public health hazard, such as chlorinated hydrocarbons.

The solution to the molecular puzzle of MMO activity is in the intimate relationship between the protein matrix and the metal centres embedded in it, as in the case of hydrogenases.

A clear view of the methane and hydrogen metabolism in methanotrophs will be significant for advancing molecular enzymology as well as for practical utilization of methanotrophs to produce alternative energy sources and environmental protection, improving the general quality of life.

In order to fully exploit the benefits of methanotrophic biotechnology, strains that thrive at elevated temperatures are needed. Heat tolerant methanotrophs have not previously been known. We have isolated methanotrophs which grow at 55–60°C [6] and described a new species, *Methylocaldum szegediense* OR2, that grows up to 72°C (BODROSSY et al., 1997). Characterization of heat stable MMO and hydrogenase enzymes from these novel methanotrophs will lead to the development of a new generation of biocatalysts for practical use.

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