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Extremophile microorganisms as cell factories: support from the European Union

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Introduction

Very few research topics in the area of life sciences have shown, in just one decade, such an explosion of knowledge and offered such a vast array of possibilities for biotechnological applications as extremophiles. Only 30 years ago, there was no evidence of the existence of a new kingdom of living beings around us. The first extreme thermophile, *Thermus aquaticus*, was described in 1969 by Brock and Freeze (1969). The discovery of a large number of new extremophile microorganisms with unique properties, particularly in the last 10 years, has been compared to the discovery, exploration, and colonization of a new continent (Aguilar 1995). Extremophilic microorganisms have extended our knowledge and understanding of fundamental questions such as the origins of life. They have added a new dimension to biodiversity and have encouraged additional efforts and interest to explore what is considered the last frontier of life.

These "exotic" microorganisms find their optimal growth conditions in habitats such as hot springs, shallow submarine hydrothermal systems, or abyssal hot-vent systems where microorganisms can be found at temperatures above 100°C (hyperthermophiles). Others, the halophiles, are found in highly saline lakes or salterns. The acidophiles and alkalophiles find their natural habitats in environments with extreme pH values, either acidic (acidic sulfatara fields and acidic sulfur pyrite areas), or alkaline (freshwater alkaline hot springs, carbonate springs, alkaline soil, and soda lakes). The habitats of psychrophilic microorganisms include cold polar seas and soils and glaciers as well as deep-sea sediments, which are not only permanently cold but also at high pressure. However, extremophiles are also found in more common places. Thus, *Thermus aquaticus*, originally isolated at Yellowstone Hot Springs, was also found to be present in laundry and domestic hot-water heaters (Brock and Boylen 1973). Usually, extremophilic microorganisms are adapted to biotopes combining several stress factors of those mentioned, which indicate the richness of biological diversity and the possibilities of life to adapt to extreme habitats and to colonize unique ecological niches. A review on the ecology and habitats of extremophiles has been recently published (Kristjánsson and Hreggvidsson 1995). The unique characteristics of extremophiles provide virtually unlimited potential for biotechnological applications.

Support from the European Union to extremophiles

The European Commission has recognized extremophiles as a priority topic in the various biotechnology programs and has continuously stimulated and supported research and training in this field since the first biotechnology program of the European Union (EU), back in 1982. For a summary of the support and initiatives provided by the European Union to extremophiles in the early years of community research, technological development, and demonstration (RTD), see Magnien (1986), Aguilar (1989, 1992, 1993, 1996), and Vassarotti and Magnien (1991).

The support to extremophiles research has been significantly reinforced in the last biotechnology program (3rd Framework Programme). A large, 3-year project (1993–1996) involving 39 academic and industrial partners from all over Europe was launched, with financial support from the

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EU of more than 4.5 million of (ECU). The project was coordinated by Prof. G. Antranikian, Technical University of Hamburg-Harburg (TUHH), Germany. The project was able to develop, in a pluridisciplinary and integrated approach, the physiological knowledge and the biochemical and genetic tools needed for a better understanding and optimization of the biotechnological capabilities of extremophiles. The project was organized around three major clusters: thermophiles and psychrophiles, alkaliphiles and acidophiles, and halophiles. For each of the three different groups, multidisciplinary research was approached at the levels of environment (isolation and taxonomy), cell machinery (physiology, biochemistry, structure biology), and genetic constituents (genetic and molecular genetics, genetic engineering).

Main achievements and major breakthroughs in the extremophile project 1993–1996, 3rd Framework Programme

Only a summary of the main achievements and breakthroughs, as presented in the final report of the project, is described here. These are grouped around these areas showing a higher impact on biotechnology: isolation of new extremophiles and methods of cultivation, enzymes and low molecular weight compounds, gene regulation and control, and aspects of the bioenergetics of extremophiles. For further information, the interested reader is invited to consult the coordinator of the project or any of the 39 participating laboratories. As many as 270 publications appeared in the scientific literature at the termination of the project, of which more than 100 were joint publications involving scientists from at least two independent groups. The number of patents exceeded 10. It is likely that these figures will increase considerably for some time after the end of the project. As a spinoff, a center for Biotechnology of Extremophiles (Biotex) was created in collaboration with the Technical University of Hamburg-Harburg in May 1997. The aim of this company, which is supported by the city of Hamburg, is to transfer results and develop new products for the European industry.

Isolation and development of fermentation technology

The laser microscope was successfully used for the isolation of Archaea and Bacteria. By applying this fast technique, five different thermoacidophilic strains were isolated from smoldering slag heaps in Sweden (Kvarntrop) and were found to be related to the type species of *Sulfolobus solfataricus*. In these studies a close relationship was observed between *Acidianus infernus* and *Desulfolobus ambivalens*; the latter was therefore reclassified as *Acidianus ambivalens*. Gram-negative sulfur-oxidizing alkaliphilic bacteria and Actinomycetes were isolated from soda lake samples from Siberia and East Africa. The isolates were found to be related to the genera *Deleya*, *Halomonas*, *Methylomicrobium*, and *Mathylococcus*. Furthermore, microorganisms were isolated from Lake Bogoria that are able to grow at high temperatures (60°–80°C) and pH values (9.5–11.0). The MEE (multilocus enzyme electrophoresis) method was developed and found to be both rapid and reliable for the classification of the thermophiles *Thermus*, *Bacillus*, and *Rhodothermus*. A new halophilic species and a thermophilic species were isolated and named *Thermonema rossianum* and *Rubrobactre xylanophilus*, respectively. The phylogenetic position of extremely halophilic Archaea isolated from the Dead Sea was also determined. A range of thermophiles, halophiles, acidophiles, and alkaliphiles have been deposited in the European Extremophile Project Culture Collection in Braunschweig.

Cultivation of hyperthermophiles was performed in dialysis batch, gas-lift bioreactors as well as by complete cell recycling with cross-flow filtration techniques. This was necessary to be able to exploit extremophiles and their products such as enzymes, other proteins, lipids, and compatible solutes. By fermenting the organism in a dialysis membrane bioreactor, a dramatic increase in the maximal cell density (3.5×10^{10} cells/ml; OD, 6.0) could be observed. As a result of vessel and growth medium development, the biomass production in gas-lift bioreactors was significantly enhanced. Growth optimization was also achieved for *Thermococcus litoralis*, *Sulfolobus shibatae*, *Hydrogenobacter thermophilus*, and *Marinococcus halophilus*. The influence of various medium components such as sugars, amino acids, and vitamins on growth of the hyperthermophiles *Pyrococcus furiosus*, *Pyrococcus abyssi*, and *Aquifex pyrophilus* was investigated. Cheaper industrial-grade substrates were identified and more than 1 kg of pyrococcal cell paste was produced in gas-lift reactors. The cultivation of the hyperthermophile *Pyrococcus furiosus*, the thermoacidophile *Sulfolobus shibatae*, and the halophile *Marinococcus* M52 in dialysis membrane reactors resulted in cell yields of 2.6, 114, and 132 g/l (cell dry weight), respectively. These results demonstrate that cultivation of extremophiles to high cell densities can be achieved if modern fermentation technology is applied.

Unique enzymes from extremophiles

Amylase from the hyperthermophile *Pyrococcus woesei*, which is an interesting enzyme for the starch industry, was purified and characterized, and its gene was cloned in the mesophilic hosts *Escherichia coli* and *Bacillus subtilis*. The pullulanase from the same archaeon was crystallized and used to collect a native and two derivative intensity data sets by X-ray diffraction. The glutamate dehydrogenase (GDH; 614 DM) from this model organism was also crystallized and studied in detail. The comparative analysis of the structure of pyrococcal and clostridial GluDH revealed a major role for ion pair networks in maintaining enzyme stability. GDHs from *Thermococcus litoralis*, *Thermotoga maritima*, and *Halobacterium salinarium* were also crystallized, and dehydrogenase from *Sulfolobus solfactaricus* were prepared for further analysis. The β-glycosidase has been recently crystallized and the 3-D structure has been resolved at 2.6 Å. In addition, the DNA polymerase, an interesting enzyme for PCR (polymerase cham reaction) technology, from *Sulfolobus solfataricus* was cloned and expressed in *E. coli*.

To understand the basis of protein thermostability, the hexameric GDH from *Pyrococcus furiosus* has been investigated. The refolding pathway was analyzed, the assembly intermediates were identified, and their formation rate constants were calculated. Experiments with the monomeric pullulanase from *Pyrococcus woesei* have shown that protein refolding requires a heating step to regain structure and activity. Hybrid proteins have been made between the GDHs from the hyperthermophile *P. furiosus* and the mesophilic *Clostridium difficile*. The detailed characterization of archaeal-bacterial hybrids showed that thermal and chemical stability may be uncoupled. Chaperonin from *Sulfolobus solfataricus* has been shown to promote correct folding of several thermophilic and mesophilic enzymes from their chemical denatured state by preventing aggregation of refolded intermediates or native enzymes. Two enzymes, adenylate kinase and cytosolic pyrophosphatase, from *Sulfolobus acidocaldarius* have been investigated. Both genes were successfully expressed in *E. coli* and the proteins were crystallized.

The thermostable xylanase from *Rhodothermus marinus*, which has application potential in biopulping, has been successfully synthesized in *E. coli*. Other polymer-degrading enzymes such as protease and amylase were studied from newly isolated psychrophylic microbes. Both enzymes have been purified and characterized. Alkali-stable and -thermostable amylase and pullulanase have been identified from a newly isolated thermoalkaliphile that represents a new taxon, *Thermopallium natronophilum*. The enzyme has a pH optimum around pH 10 and a temperature optimum in the range of 65°–92°C. In addition, the most thermostable cellulase known so far has been purified from *Rhodothermus marinus*. The *P. furiosus* genes for a novel endoglucanase and several alcohol dehydrogenases have been determined and functionally expressed. Also, the serine protease (pyrolysin) has been purified and the *pls* gene coding for this pre-pro-protein has been completely sequenced. Pyrolysin, the most thermostable protease described so far, has unique features such as an extensive glycosylation. Two DNA topoisomerases were purified from *P. furiosus*. Finally, the novel ADP-dependent kinase has been purified and characterized.

Metabolism of carbohydrates and production of low molecular weight compounds

Glucose metabolism was investigated in seven strains of hyperthermophilic Archaea and Bacteria using ^{13}C-labeled substrates. *Thermococcus tenax* and *Thermotoga maritima* show considerable contributions from both the Embden-Meyerhof and the Entner-Doudoroff pathways, whereas *Thermococcus* and *Pyrococcus* strains show 100% Embden-Meyerhof and *Sulfolobus* strains use exclusively the Entner-Doudoroff pathway. The novel archaeal enzyme involved in acetate formation and ATP synthesis (acetyl-CoA synthetase) was purified from *Pyrococcus furiosus* and characterized. Several strains of *Thermotogales* were screened for the presence of solutes (low molecular weight organic compounds). A novel compound derived from di-myo-inositol-phospate was found in *Thermotoga maritima* and characterized by NMR (nuclear magnetic resonance). Interestingly, it has been shown for the first time that β-glutamate and two isomers of dimyo-inositol-phospate occur in Bacteria. Novel organic solutes (di-glycerol-phospate, galactosyl-5-hydroxylysine, and mannosylglycerate) were identified in Archaea, and their influence as an enzyme protector against heating and freeze-drying was shown. A data bank for compatible solutes was set on the basis of NMR and IR spectra. Furthermore, six novel hopanoids, bacteriohopanetetrol glycosides possessing a glycuronic acid moiety, were isolated from a thermophilic cyanobacterium, *Synechococcus* sp.

Gene regulation and control

In this project it was possible to establish a cell-free transcription system for the hyperthermophile working at 90°C. Two pyrococcal transcription factors were discovered and were found to be related in their structure and function to certain eukaryotic transcription factors. The transcriptional initiation of the *Pyrococcus furiosus pls* and celB genes have been studied in vivo as a function of the growth conditions. Various plasmids containing the complete *P. furiosus gdh* gene or carrying internal deletions were constructed that were tested for their suitability as template for in vitro transcription reactions at low and high temperatures. In addition, various mutations have been introduced in the archaeal TATA box of the pyrococcal *gdh* gene, and their effect has now been studied in the in vitro transcription system. New plasmids were isolated from *Thermococcales*, and a naturally positively supercoiled plasmid was identified for the time in vivo. Furthermore, the DNA gyrase from *Thermotoga maritima* was sequenced and led to the discovery of a new family of Topo II in hyperthermophilic Archaea. It has been also possible to isolate drug resistance marker genes for both *Sulfolobus acidocaldarius* and *Pyrococcus furiosus*. An efficient transformation procedure was developed for *Pyrococcus*, which was followed by the development of a shuttle vector between *E. coli* and *P. furiosus*. It has been also demonstrated that both the archaeal introns and their genetically engineered derivatives are mobile between hyperthermophiles. *Saccharomyces cervisiae* was proven to be a particularly adequate host for the expression of the hyperthermophilic *P. furiosus* OTCase whereas *E. coli* is not, as the result of partial proteolysis. *Thermotoga* dihydrofolate reductase can now be produced from *E. coli* recombinant cells in amounts sufficient to envisage routine purification. Furthermore, a suitable shuttle vector was constructed for moderate halophiles and conjugation was demonstrated. Native promoters from various moderate halophiles were isolated and characterized. DNA fragments influencing osmoregulation were isolated. It has been shown that the differential streptomycin transport efficiency in haloalkaliphilic bacteria was dependent on the ionic strength of the medium.

Membranes, membrane proteins, and bioenergetics of extremophiles

The investigations of the bioenergetics of extremophiles have shown that a correlation exists between the optimum growth temperature and proton permeability of the membrane. This correlation has been found for a broad range of Bacteria and Archaea. To study this phenomenon in further detail, liposomes were prepared from lipids derived from various extremophiles grown at different temperatures. Energy transduction was studied in *Picrophilus oshimae*, a thermoacidophilic archaeon that grows optimally at pH 0.7 and 60°C. This archaeon maintains its intracellular pH at 5.0 with the external pH of 0.7. Membranes of this organism were found to be equipped with a low proton permeability that allows proton-linked energy transduction. It has been also shown that energy transduction in the newly isolated anaerobic thermophile strain LBS3 was linked to sodium cycling. To elucidate the role of the sulfur-oxidizing system of acidophilic thiobacilli in the oxidation of sulfidic minerals, enzymes involved in the sulfur metabolism have been isolated and studied. Sulfite ferricyanide oxidoreductase and the organic cofactor of the tetrathionate hydrolase were isolated from *Thiobacillus acidophilus*. It has been shown that a linear megaplasmid was induced in *Thiobacillus ferrooxidans* under chemolithotrophic conditions.

The success of the project, in terms of results produced and interaction with the industrial community, resulted primarily from the commitment and scientific excellence of the groups participating in the project and from the existence and interactions with the Industrial Platform on Microbiology (IPM), as priviledge interlocutor in the dissemination and transfer of results.

Extremophiles as cell factories

A new large project, coordinated by Prof. G. Antranikian and by Prof. N. Russell (Wye College, University of London, UK), with the title "Extremophiles as Cell Factories" has been selected for support in the frame of the current biotechnology program (4th Framework Programme) with a EU contribution of almost 7 million ECU and for a duration of 3 years starting at the end of 1996. This new project involves 59 groups in all from across the EU, of which 13 are industrial companies. The primary goal of this ambitious project is to understand how extremophiles can be more productive and how European industries can exploit extremophiles and their cellular processes. This objective includes the development of innovative products and new industrial processes. The research objectives of the project will be achieved through an integrated approach involving the topics outlined in Table 1.

Project management

The main scientific coordinators of the project are Prof. Antranikian (thermophiles, acidophiles, alkaliphiles, and halophiles) and Prof. Russell (psychrophiles). The administration and financial management is performed by the TUHH-Technologie GmbH. The project has created a Committee for Exploitation of Results, which is composed of both industrial and academic representatives to increase industrial awareness of the research results and to encourage their commercial exploitation. The close cooperation with the Industrial Platform for Microbiology (IPM) ensures the dissemination of results to European industries. The Information and Public Perception Group (IPPG) is setting up both internal and external communication links within and outside the project. All research activities are organized in a management matrix structure that promotes the flow of information between the participants of the project and enhances the personal commitment of the

Table 1. Outline of the main R&D activities to be performed in the Community project "Extremophiles as Cell Factories," Framework Programme 4

Genetic engineering
Development of expression systems for selected hyperthermophiles, thermoacidophiles, haloalkaliphiles, and psychrophiles
Overexpression of novel hydrolytic enzymes in mesophilic and psychrophilic hosts
Production and application of DNA polymerases and other DNA-binding proteins
Product engineering (enzymes and proteins)
Production, structure–function relationships, engineering, and application of hydrolytic enzymes and other proteins from extremophiles
Characterization of enzymes involved in processing of nucleic acids and biotransformation
Product engineering (organic compounds)
Production, function, and application of compatible solutes and other low molecular weight organic compounds
Formation of lipids and labeled synthetic nucleotides
Cellular factories and fermentation
Development of defined media for production of enzymes and other compounds from extremophiles
Bioreactor development for the mass cultivation of extremophiles and recombinant organisms
Development of innovative processes to be exploited by the European industry

group by transferring to them some of the managerial aspects of the project (visibility, training, etc.) (Fig. 1).

The concept

The project "Extremophiles as Cell Factories" is based on complementary elements in which academia and industry concentrates on selected extremophilic organisms and industrial products. The selected microorganisms belong to the thermophiles, psychrophiles, acidophiles, alkaliphiles, and halophiles. Major attention is focused on enzymes and proteins (e.g., hydrolases, DNA-modifying enzymes, and chaperonines) and new organic compounds (e.g., compatible solutes and lipids). The project follows a task-oriented approach in an integrated way, including the necessary disciplines needed for the exploitation of extremophiles. Detailed information on the main achievements in the first year of the project can be obtained from the coordinator, Prof. Antranikian, and from Prof. Russell. This volume of *Extremophiles* also contains contributions from some participants in the community project.

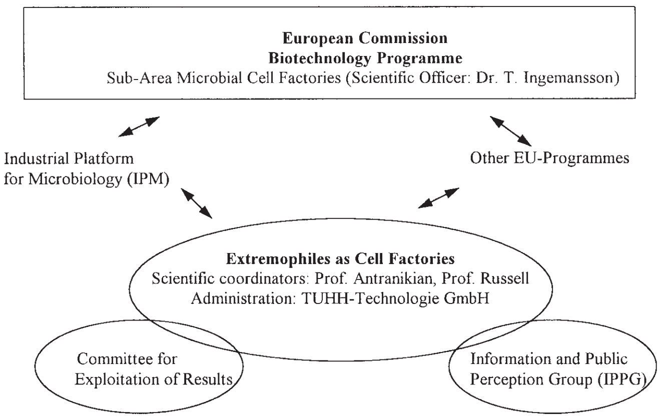

Fig. 1. Management structure of the community project "Extremophiles as Cell Factories," Framework Programme 4

Industrial opportunities from extremophiles

The application of extremophilic microorganisms in industrial processes has opened unexpected possibilities for the biotech and bio-industries. Each group of extremophiles has unique features that are not present in any other living beings. Table 2 outlines the main applications of extremophiles in the chemical, food, health, and pharmaceutical sectors.

The main reasons for selecting enzymes from extremophiles are their high stability and reduced risk of contamination of the organisms that produce them. Additional benefits during production include improved transfer rates and lower viscosity. These enzymes they are expected to fill the gap between biological and chemical processes because of their unusual properties. Cold- and heat-resistant proteins may find future applications in devices such as biosensors and biochips (Marshall 1997).

Recent developments clearly show that extremophiles are a good source of novel catalysts of great industrial interest. Polymer-degrading enzymes like amylases, pullulanases, xylanases, proteases, and cellulases would play an important role, e.g., in food, chemical, pharmaceutical, paper, pulp, or waste treatment industries. Membranes of extremophiles may contain surfactants bearing

Table 2. Biotechnological applications of extremophiles

Extremophilic organisms	Enzymes and organic compounds	Applications and products
Thermophiles, 50°–110°C	Amylases	Glucose, fructose for sweeteners
	Xylanases	Paper bleaching
	Proteases	Amino acid production from keratins, food processing, baking, brewing, detergents
	DNA polymerases	Genetic engineering, forensic science
Psychrophiles, 0°–20°C	Neutral proteases	Cheese maturation, dairy production
	Proteases	Polymer-degrading additives in detergents
	Amylases	Pharmaceuticals
	Lipases	Biosensors
	Polyunsaturated fatty acids	
	Dehydrogenases	
Acidophiles, pH < 2	Sulfur oxidation	Desulfurication of coal
Alkaliphiles, pH > 9	Cellulases	Polymer-degrading additives in detergents
	Proteases	Stabilization of volatile substances
	Amylases	Pharmaceuticals
	Lipases	
	Cyclodextrins	
	Antibiotics	
Halophiles, 3%–20% salt	Carotene	Food coloring
	Glycerol	Pharmaceuticals
	Compatible solutes	Pharmaceuticals
	Membranes	Surfactants for pharmaceuticals

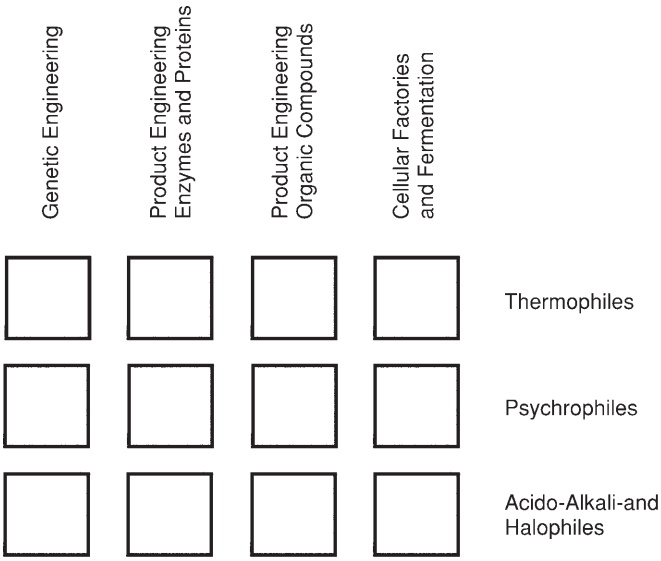

Fig. 2. Matrix organization of the community project "Extremophiles as Cell Factories," Framework Programme 4

unique stability that can be used in pharmaceutical formulations. Other important innovative products are cyclodextrins, compatible solutes, and polyunsaturated fatty acids.

Only recently has it been appreciated that cold-active enzymes and the psychrophilic (cold-loving) microorganisms from which they are derived have enormous, as yet untapped, potential for biotechnological applications in the detergent, food, and pharmaceutical industries. The applications include cold-water washing, contact-lens cleaning, environmental biosensors and on-line monitoring, bioremediations, and biotransformations, lactose hydrolysis in milk, and other food-processing applications.

The potential benefits of the extremophiles project will be the production of a new generation of biocatalysts and organic compounds that are needed for the development of more efficient industrial processes. These developments will improve existing conventional enzymatic and chemical processes and contribute to the further development and competitiveness of the European biotechnology industry.

Industrial platform for microbiology

One of the objectives of the EU's biotechnology program is to enhance, through RTD, European competitiveness. In the future this will be determined by the Member States' ability to meet a number of challenges, such as job creation and industrial competitiveness. Today there is a close link between investment in technological development and a nation's industrial strength. Biotechnology will be one of the driving forces in tomorrow's economy. In this context, the program seeks to improve the links between fundamental research and commercial applications in Europe and to promote innovation by helping to translate new ideas rapidly into new products. A novel feature in the EC RTD Programme Biotechnology has been the creation of Industrial Platforms. These are technology-driven industrial groupings established on the initiative of industry around Biotechnology RTD areas. Since the first Industrial Platform was created at the onset of the BRIDGE program (1990–1993), its value has been recognized: participation from industry in the biotechnology program has increased significantly and the number of platforms has risen to ten. Additionally, communication between academia and industry has significantly improved and could progress further through the Industrial Platforms. Additional information on the objectives, management, and interaction of biotechnology and other Life Sciences Industrial Platforms with contractors and Commission services can be found in the report by the European Commission (1997a).

The Industrial Platform for Microbiology was founded in 1995 by representatives of a number of European companies involved and interested in microbial physiology, microbial ecology, microbial taxonomy, and microbial biodiversity. It resulted from the merge of two former platforms, the Extremophiles Industrial Platform and the Microbial Identification Industrial Platform, expanding, therefore, the impact of extremophiles research on industry. With a current membership of some 15 European industries, this new entity amplifies the impact of extremophile research on industry. The objectives of the Industry Platform for Microbiology are (1) to improve the conditions for exploitation of the results of EU projects in the field of microbial biotechnology for the benefit of the EU; (2) to provide a forum for identifying suitable areas to be covered by future EU-sponsored programs; (3) to bring the industrial priorities in the field to the attention of policy makers in the Commission and in the Member States and to the academic community; and (4) to create the conditions to derive economic benefits and job creation from the exploitation of results generated in the frame of the EU biotechnology projects on microbial biotechnology.

The Industry Platform for Microbiology gives European firms the opportunity of bringing them closer to interactive and multidisciplinary scientific research being undertaken by a larger number of groups. The Commission services regard the Industry Platform for Microbiology as an excellent forum for the exchange of views in relation to extremophiles research and as suitable instrument to create opportunities for technology transfer and for industrial exploitation of results emerging from the project.[1]

Building the future

The Fifth Framework for Research, Technological Development and Demonstration (1998–2002) is currently being prepared in such a way as to ensure that European research

[1] Updated information about membership, contact persons from IPM, etc., is available at the following Internet site: http://europa.eu.int/comm/dg12/biotech/ipl.html

Table 3. Proposed structure by the European Commission on the specific programs implementing the Fifth Framework Programme

Thematic Programs	
I.	Unlocking the resources of the living world and ecosystem
	Key Actions
	Health and food
	Control of viral and other infectious diseases
	The "Cell Factory"
	Management and quality of water
	Environment and health
	Integrated development of rural and coastal areas
	Activities for generic research and development of technologies
	Life sciences for health
	Public health research
	Major natural and technological hazards
	Global environmental change
	Satellite earth observation technologies
	Biomedical ethics and bioethics
	Socioeconomic aspects of life sciences and of environmental change in the perspective of sustainable development
	Support for research infrastructures
	Biological data and resources
	Earth system observation
	Global change and natural hazards
II.	Creating a major friendly information society
III.	Promoting competitive and sustainable growth
Horizontal programs	
IV.	Confirming the international role of Community research
V.	Innovation and participation of SMEs
VI.	Improving human potential

and demonstration activities are translated more effectively than hitherto into practical and visible results. To do so, the new Framework Programme is targeting its activities on major EC policy objectives: employment, quality of life, and competitiveness of European industries. At the same time, particular effort has been made to concentrate the efforts on a reduced number of themes so as to maximize the impact.

The European Commission has proposed a Framework Programme divided in three major thematic programs and three horizontal programs (Table 3). Additional information on the current developments of the Fifth Framework Programme can be obtained from the authors of this article (see Aguilar et al. [in press]; European Commission 1997a,b) and also from web (http://europa.eu.int/comm/dg12/fp5.html).

The key action, the "Cell Factory" within Programme I, is expected to attract the interest of scientists and technologists active on the exploitation of extremophiles. The overall goal of the Cell Factory Key Action is to help community industry to exploit progress of biotechnology, particularly for applications in the fields of health, agro-food, and the environment. For these purposes it will promote development and use of key technologies for generating new bioproducts and bio-processes with high added value that can be adopted and implemented by industry.

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