Microbes in extreme environments

Auteur :
FRANZETTI Bruno, Directeur de recherche au CNRS, Institut de Biologie structurale, Université Grenoble Alpes

23-04-2019

There are few sterile places on the planet. Glacial deserts, hot springs, ocean bottoms, hypersaline environments, rocks of the earth's mantle... even these hostile environments shelter a rich biodiversity of so-called extremophilic microbes. Their study revealed a third life form different from bacteria and eukaryotes. These organisms, called Archaea, are abundant in the environment. They have evolutionary links with our cells, representing unique models for understanding the origin and functioning of complex cellular processes. Different strategies are used by extremophiles to maintain the integrity of their cellular machinery at conditions of temperature, pressure or salinity that are lethal to any other

form of life. Their study reveals the capacity of living organisms to colonize "hostile" ecosystems. The particular properties of their enzymes are currently being exploited to develop clean and sustainable industrial processes.

1. Biodiversity of extremophilic microbes

Microorganisms in the soil and oceans dominate our planet [1]. They play important roles in the regulation of major geochemical cycles and potentially constitute a reservoir of new biocatalysts for future technologies. Our knowledge of this biodiversity is still very partial, as microbiology is still too limited to human health issues. Less than 1% of environmental microorganisms can be cultured in the laboratory. In the last decade, the development of new tools (like metagenomics, that make it possible to directly analyze DNA from microbial communities) has changed our vision of microbial ecosystems{ind-text}Essembles formed by an association of living beings (or biocenosis) and its environment (biotope): biological, geological, edaphic (soil), hydrological, climate, etc. An ecosystem is characterized by interactions between living species and their surrounding environment, material and energy flows between each of the ecosystem components that allow their life and a dynamic balance over time between sustainability and evolution.{end-tooltip} [2]. Thanks to them, we have discovered environments that have long been considered incompatible with life. Despite their extreme physical and chemical conditions, they contain abundant forms of microbial life [3]
.

Figure 1. Photography of different extreme environments. Top right: hydrothermal site "Logatchev" at 3000 m depth on the Atlantic Ridge. Top left: Volcanic hydrothermal spring in the Azores. Bottom left: Antarctic ice environment. Bottom right. Hypersaline lake in the Andes. [Photos © Ifremer]

Thus, volcanic **hydrothermal springs** possess an astonishing diversity of organisms at the base of oasis of life in the abyss (Figure 1) (see focus Black smokers' ecosystems). Hypersaline environments such as large salt lakes or the Dead Sea are also populated by microorganisms that only develop when the salt concentration becomes intolerable for any other form of life. Glacial and polar environments also support rich populations of microbes. Finally, there are significant microbial communities on the ocean floor, in sediments and deep geological layers. It is estimated that 80% of terrestrial ecosystems are permanently exposed to temperatures below 5°C, often under high pressure conditions. Thus, on a global scale, extremophiles can no longer be considered as exceptions.

Little is known about the roles of extremophiles in ecosystems, including climate regulation. Recent work shows that their contribution to the production of greenhouse gases, and to the major carbon, nitrogen and nitrate cycles, is far from negligible. Often, their genome contains more than 90% of genes encoding proteins with unknown functions. For what reasons? Biological macromolecules must necessarily adapt to the physico-chemical conditions, nutritional and energy resources specific to these extreme environments. These constraints may lead to the emergence of new metabolic pathways using different substrates and co-factors than those used by "conventional" organisms.

2. The discovery of the Archaea: *a revolution*

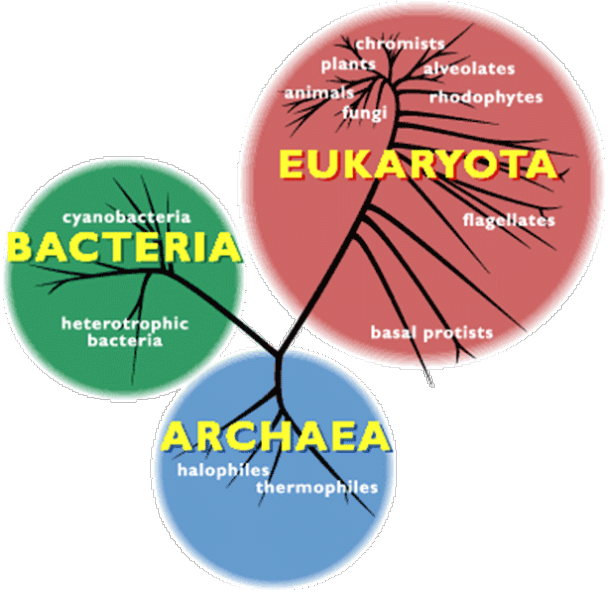

Figure 2. Phylogenetic tree of living organisms composed of three domains: bacteria, archaea and eukaryotes.

Many extremophilic organisms are **archaea,** a group of microbes that have molecular characteristics that clearly distinguish them from the other two forms of life well known to the public: bacteria and eukaryotes (Figure 2). This third life form was first proposed by the American biologist Karl Woese in 1990. Using ribosome RNA as a molecular tracer, Woese sought to reconstruct the universal tree of the evolution of life. Since then, the identification of a growing number of new archaea species and the study of their genomes has shown that this is indeed a reality [4]. As single-cell organisms without nuclei, such as bacteria (Figure 3), archaea have remarkable properties. They have their own viruses. Even more surprisingly, some archaea are **capable** of symbioses{ind-text}Intimate, lasting associations between two organisms belonging to different species that result in beneficial effects for both. The organisms involved are referred to as symbionts, or symbionts (anglicism); the largest can be named host. {end-tooltip} with complex organisms such as sponges. **Archaea are found in the gut microbiota**, which plays an important role in human health (see Human microbiotes: allies for our health). No pathogens have yet been identified in archaea, but their link to certain diseases or metabolic disorders such as obesity has been proven. The evolution and expansion of archaea and bacteria are equally complex. Archaea have an astonishing evolutionary proximity to eukaryotes. Some hypotheses suggest that archaea are at the origin of the emergence of **eukaryotes** [5] (see Symbiosis & evolution).

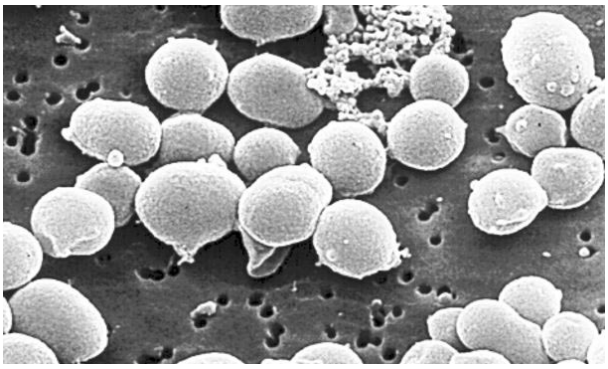

Figure 3. Electron microscopic image of an abyssal hyperthermophilic archaea: Thermococcus fumicolans [Microphotography © Ifremer].

Archaea seem particularly suitable for development under the most extreme conditions known on Earth. For this reason, they were initially considered to be poorly diversified and only associated with volcanic hot springs and hypersaline lakes. This initial vision was totally biased. Molecular ecology studies have revealed very many archaean lines in all terrestrial environments. Indeed, we are discovering more and more "non-extremophilic" archaea that would represent 25% of microbial life in oceans and soils [3]. They should therefore no longer be considered as marginal, archaic or primitive forms of life.

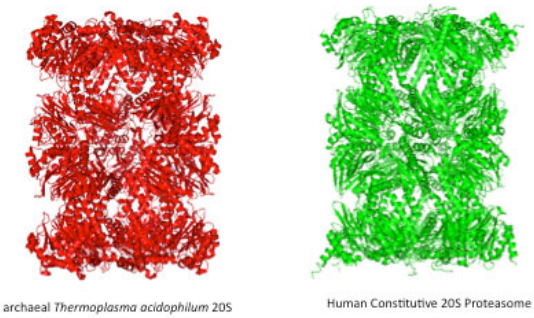

Figure 4. Molecular structure of the human proteasome (in green), a cellular machine involved in cancer. Its Archean counterpart is represented in red.

The consequences of the discovery of the archaean world are important and still poorly appreciated. This discovery changes our vision of living organisms, which was divided between prokaryotes and eukaryotes. The study of the evolution of archaea generates new scenarios to explain the appearance of cellular life [6]. Although they are single-celled organisms without nuclei, they share many processes with eukaryotic cells, including human cells [7]. Increasingly, archaea are proving to be model organisms in molecular biology. Indeed, it is always difficult to study the large cellular machines present in human cells. Their

purification and activities are difficult to control in the laboratory. The homologous systems found in archaea represent simplified versions that are much more stable and easy to produce. In addition, their extremophile character allows them to be better activated or inhibited. For these reasons, and thanks to the recent development of genetic tools, archaea represent excellent models for integrative biology combining *in vivo* studies, biochemistry, biophysics and structural biology. These studies were the first to determine the structure of many cellular machinery and are the source of many drugs such as anti-cancer drugs (Figure 4).

3. Extremophilic organisms and the limits of life

To develop, **life needs**:

carbon, the basic element of biological macromolecules;

water, the most conducive solvent for the functioning of proteins;

energy, necessary for the functioning of biological systems.

Energy is provided by light, by electron-donating elements such as metals and finally by the breaking of chemical bonds catalyzed by enzymes. The discovery of microbial communities in environments long considered sterile has shown that the physico-chemical limits within which life can develop are much more extensive than previously thought. These organisms, which are grouped under the name of extremophiles, are not in "survival" conditions but really need conditions considered hostile in order to develop [8].

In hydrothermal springs (see focus Black smokers' ecosystems), archaea and bacteria called "**thermophilic**" or "hyperthermophilic" only develop optimally at high temperatures, sometimes above 110°C. Some, isolated in the abyss such as the Marianas pit, are **piezophiles**: they need pressures exceeding 20 MPa [9]. In the seabed, lakes and glacial environments, "psychophiles" only thrive below 15°C and down to -12°C [10]. In **hypersaline** environments, open water is rare because it is largely trapped by saline ions. The result: cells are destroyed and proteins are denatured. Nevertheless, so-called "halophilic" organisms have a particular biochemistry that preserves their cellular integrity under conditions of severe dehydration [11]. Ecosystems associated with hydrothermal springs, geological effluents or hyper-saline environments often have extreme pH conditions (0 and 13). Thus, most of the organisms that live there are poly-extremophiles.

In addition to extreme and often highly fluctuating physico-chemical conditions, extremophiles are also confronted with multiple environmental stresses such as radiation or heavy metals. These agents generate free radicals that damage DNA and proteins. Most extremophilic microbes have developed DNA repair systems and protein recycling systems that enable them to resist radiation doses of up to 10,000 Gray. The exploitation of the environment's energy resources is another frontier of life that the study of extreme microbes leads us to constantly push back. Thus, extremophiles seem particularly adapted to stresses resulting from very low energy and/or nutrient flows. Thus, some live and develop very slowly in the deep sediments of the oceans or several kilometres inside the Earth's mantle [12].

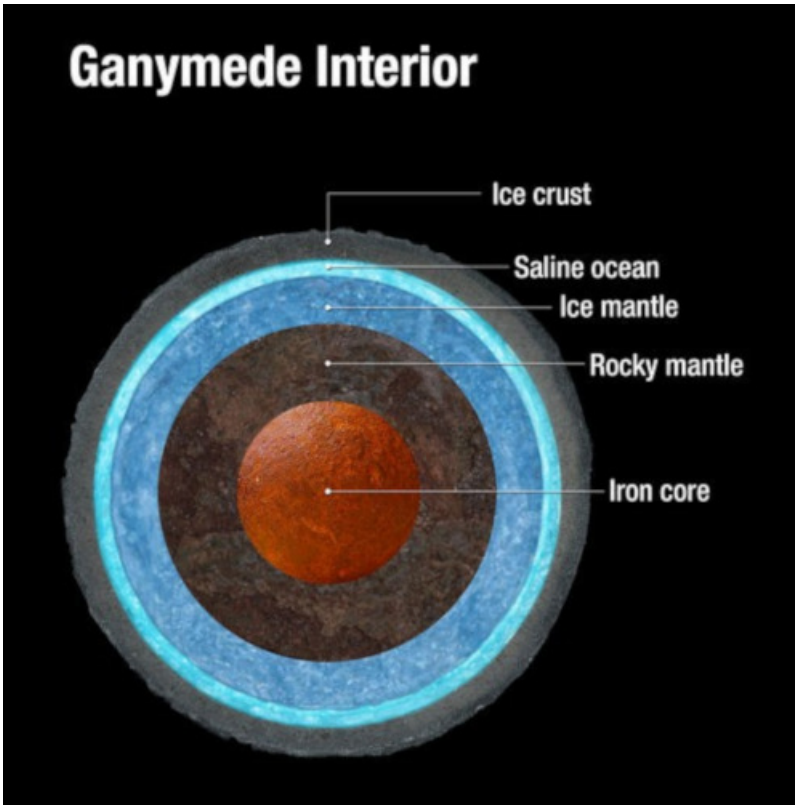

Figure 5. Representation of the interior of Ganymede. According to NASA, there is a salty ocean 100 km deep under a layer of ice [Source: Felicia Chou, NASA].

The discovery and study of archaea, more globally of extremophilic microbes, has changed our conceptions on the habitability of the Earth. They guide our ideas in the search for possible traces of life on other planets [13]. For example, microbes capable of surviving arid and cold conditions have been discovered in the Atacama Desert in Chile. Halophilic organisms are capable of developing at -15°C. Other microbes are strictly associated with high pressures, which are interesting observations to consider after the discovery of liquid brines on Mars, deep oceans and hydrothermal activities on moons of Saturn and Jupiter such as Europe and Ganymede (Figure 5).

4. How do extremophiles preserve their biological functions?

The maintenance of stable and functional biological membranes is a first condition for allowing cellular life in extreme conditions of temperature, pressure or salinity. Membranes are essential to produce energy and compartmentalize biochemical activities. Changes in the composition of membrane lipids allow them to adapt to high and low temperatures and very high pressures that affect the fluidity of membranes.

In non-extremophilic organisms, exposure to "extreme" physico-chemical conditions inactive or even denatures certain proteins. Extremophilic adaptation also consists in preserving the assembly of cellular machines and the three-dimensional folding of the polypeptide chains that constitute proteins. This folding is responsible for the biochemical activity of enzymes. Understanding the mechanisms that stabilize biological macromolecules under extreme conditions is not only useful for understanding:
(i) the origins and expansion capacities of living organisms,
(ii) the fundamental processes that govern the functioning of proteins and
(iii) the universal cellular processes designed to maintain the integrity of cellular machinery [8].

A first strategy widely used by extremophiles to preserve their cellular constituents is to synthesize and accumulate small molecules (trehalose, betaines, etc.) in the cytoplasm that stabilize molecular structures. Maintaining protein integrity and homeostasis also involves optimizing chaperone and protein modification systems to prevent aggregation, assist folding or trigger rapid destruction by intracellular proteases. These protein "quality control" systems are crucial both for the adaptation of thermophilic organisms and for psychophiles. They are not specific to extremophiles but are preserved in all living beings, including humans. Thus, systems derived from extremophilic microorganisms are simple models for understanding the fundamental mechanisms of stress response, degenerative diseases and aging processes.

The cellular mechanisms that preserve the integrity of biological macromolecules require a lot of energy from the cells. This is why the proteins of true extremophiles have highly modified properties. Acquired during evolution via mutations, these modifications stabilize proteins under conditions of high temperature, salt or pressure. However, the comparison of crystallographic structures of proteins from extremophilic organisms with their mesophilic counterparts shows little difference in

the overall architecture of the structures. On the other hand, the selected mutations generate very different biophysical properties. For example, thermophilic proteins are "frozen" at room temperature. The cause: a stiffening of the macromolecular structure due to the optimization of intramolecular interactions. This gives proteins extraordinary strength. However, while maintaining the three-dimensional structure is essential for the functioning of biological macromolecules, proteins also have overall dynamic properties. Some regions must move to recognize substrates, co-factors and perform complex biochemical functions.

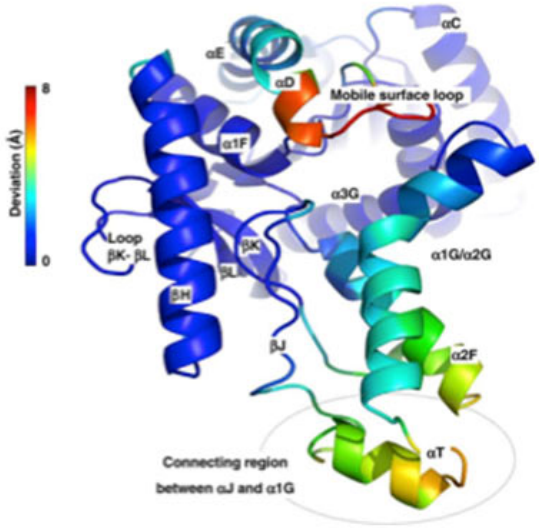

Figure 6. Crystallographic structure of an enzyme from a hyperthermophilic archaea: the yellow-orange colored regions correspond to the flexible parts necessary for the enzyme to function. The blue regions represent the rigid regions necessary for molecular edifice stability Source: Coquelle et al, 2010, ref. [14].

The comparative analysis of structures associated with molecular evolution and simulation work has identified key regions within the protein structure (Figure 6) [14]. During evolution, amino acids have been substituted in these regions. The optimal compromise between stabilization forces and local dynamics allows the protein to maintain its function in extreme conditions. There are multiple types of molecular folding, so the strategies used to adapt protein structures to environmental constraints differ. Beyond the understanding of extremophilia, this work provides a better understanding of how macromolecular structures and enzymes work. When carried out on proteins of medical interest, such studies can be used to develop drugs.

The constraints on molecular structures by different environmental parameters are not the same, resulting in different adaptive strategies. Thus, for thermophiles, the main challenge is to prevent protein folding. Adaptation consists in strengthening the forces that stabilize protein folding while maintaining significant flexibility in regions dedicated to the biochemical functioning of enzymes. The main consequence of low temperatures is to slow down the speed of chemical reactions. In psychrophilic proteins, adaptation is rather due to a modification of the active sites allowing a better catalytic efficiency, associated with a global or local relaxation of intramolecular constraints within them [10]. These modifications maintain a slow but sufficient metabolism to allow cell division. By reducing the amount of free water and interacting with polypeptide chains, salt affects protein solubility and disrupts the intramolecular interfaces that cause folding. However, halophilic proteins have accumulated mutations that allow them to counteract these effects and even interact advantageously with solvent ions [15]. These associations both contribute to stabilizing the structure while maintaining a layer of hydration necessary for the system to operate. Adaptation is so advanced here that most of the proteins from these organisms are only soluble and folded under hypersaline conditions [16]. Finally, recent research reveals molecular adaptation associated with high hydrostatic pressure conditions in the abysses and deep geological layers of the planet [9]. In this case, the cavities present within molecular structures that are mostly modified.

In all types of adaptations associated with life in "extreme" conditions, changes in protein structures profoundly alter the biochemistry and physiology of biological systems. For this reason, conditions that we consider "normal": temperatures of 37°C, salinity of 3%, atmospheric pressure, presence of oxygen, etc. are in fact hostile conditions for most extremophiles. These conditions cause stress to the cells. For example, water is a deadly solvent for halophiles [17]. These organisms accumulate almost saturated concentrations of salt in their cytoplasm, ensuring the solubility and correct folding of their proteins. On a geological scale, climatic variations of great amplitude have established extreme conditions on the surface of the planet. It is also for this reason that the notion of extremophilia must be put into perspective.

5. Usefulness of "extremozymes" for biotechnologies

Enzymes are natural products that perform chemical reactions in an ultra-efficient and non-polluting way. In a context of food

and environmental crisis requiring the development of a bio-inspired economy, the new enzymes found in the genomes of populations of extremophilic microorganisms (and called extremozymes) are of great interest [18]. Indeed, their robustness, their ability to perform chemical reactions under extreme conditions and sometimes the uniqueness of the chemical reactions they perform make them very interesting for multiple applications. For example, biotechnologies use extremozymes for the production of biofuels, bio-materials or pharmaceutical molecules. Halophilic enzymes are capable of operating in saline environments, in organic solvents and in a wide range of pH. They are used in food processing, in the paper industry as well as in the textile industry.

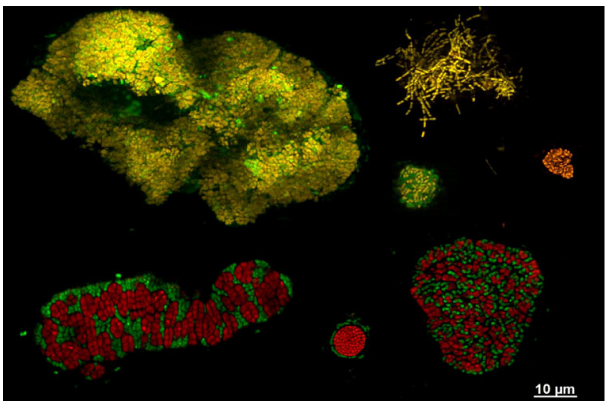

Figure 7. Images of methanotrophic archaean communities and sulfate-reducing bacteria in the sediment. [Source © Ifremer]

Thermozymes and barozymes from thermophilic and/or barophilic organisms are hyperstable enzymes that can be used for food applications under conditions that eliminate the risk of bacterial contamination. They can be used under physico-chemical conditions corresponding to multiple processes used by the textile, leather, cosmetic or pharmaceutical industries. Because of their originality, abundance and the many interactions and symbioses that govern the dynamics of bacterial and Archaean communities in extreme environments, these microbes represent a largely unexplored genetic resource (Figure 7). Thus, the search for new biocatalysts and antibiotics based on the microbial biodiversity of extreme environments is a rapidly expanding discipline requiring the development of dedicated enzymatic and structural screening and characterization platforms.

References and notes

Cover image. [Source: © Bruno Franzetti]

[1] Abdoun E. (2014) *Science & Vie 1161*, 70-77

[2] Banik J.J. & Brady S.F. (2010) *Current opinion in microbiology 13,* 603-609

[3] Cowan D.A., Ramond J.B., Makhalanyane T.P. & De Maayer P. (2015) Curr *Opin Microbiol 25,* 97-102

[4] Gribaldo S., Forterre P. & Brochier-Armanet C. (2011) *Research in microbiology* 162, 1-4

[5] Forterre P. (2015) *Frontiers in microbiology* 6, 717

[6] Gribaldo S, Forterre P, Brochier-Armanet C. (2008) Les ARCHAEA : Evolution et diversité du troisième domaine du vivant, *Bull. Soc. Fr. Microbiol.* 23(3):137-145

[7] Brochier-Armanet C., Forterre P. & Gribaldo S. (2011) *Curr Opin Microbiol* 14, 274-281

[8] Oger P. & Franzetti B. (2012) *Biofutur* 336, 36-39

[9] Jebbar M., Franzetti B., Girard E. & Oger P. (2015) *Extremophiles* 19, 721-740

[10] Cavicchioli R., Siddiqui K.S., Andrews D. & Sowers K.R. (2002) *Current Opin Biotech* 13, 253-261

[11] Oren A. (2015) *Current Opin Biotech* 33, 119-124

[12] Inagaki F. *et al* (2015) *Science* 349, 420-424

[13] McKay C.P. (2014) *Proc Natl Acad Sci USA* 111, 12628-12633

[14] Coquelle N., Fioravanti E., Weik M., Vellieux F., & Madern D. (2007) *J Mol Biol* 374, 547-562

[15] Madern D., Ebel C. & Zaccai G. (2000) *Extremophiles 4,* 91-98

[16] Vauclare P., Marty V., Fabiani E., Martinez N., Jasnin M., Gabel F., Peters J., Zaccai G. & Franzetti B. (2015) *Extremophiles* 19, 1099-1107

[17] Franzetti B. (2010) *Biofutur*, 35-38

[18] Raddadi N., Cherif A., Daffonchio D., Neifar M. & Fava F. (2015) *Applied microbiology and biotechnology* 99, 7907-7913

L'Encyclopédie de l'environnement est publiée par l'Université Grenoble Alpes - www.univ-grenoble-alpes.fr

Pour citer cet article: **Auteur :** FRANZETTI Bruno (2019), Microbes in extreme environments, Encyclopédie de l'Environnement, [en ligne ISSN 2555-0950] url : http://www.encyclopedie-environnement.org/?p=6888
