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LECTURE TEXT

What we learn from extremophiles

Carola Schröder[1] · Christin Burkhardt[1] · Garabed Antranikian[1]

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Abstract

Extremophiles are microorganisms that love extreme conditions, such as high temperatures up to the boiling point of water or low temperatures down to below the freezing point. Moreover, some extreme microbes prefer to live in acidic or alkaline environments, under high pressure or high salinity. Three extremophilic species are presented in this article: *Lacinutrix algicola*, a psychrophilic bacterium that grows at temperatures between 0 and 25 °C, *Anaerobranca gottschalkii*, a thermophilic and alkaliphilic bacterium growing optimally at 50–55 °C under alkaline conditions, and *Pyrococcus furiosus*, a famous hyperthermophilic archaeon that prefers 100 °C for growth. These extraordinary microorganisms are examples of extremophiles that possess remarkable adaptation mechanisms and additionally produce unique enzymes called extremozymes. These robust biocatalysts can be applied in various biotechnologic processes to enable substrate conversions under extreme process conditions. Due to their unusual properties, extremophiles and extremozymes will play a pivotal role in the development of modern circular bioeconomy.

Keywords Extremophiles · Adaptation · Biotechnology · Archaea and bacteria · Extremozymes · Bioeconomy

Introduction

Extremophiles are organisms that thrive under conditions, that are considered hostile to humans. Such environments include hot springs with temperatures close to the boiling point of water or the deep sea, where low temperatures are associated with high water pressure. “Extreme” conditions not only refer to temperature, even though heat and cold are the most prominent extremes, but also to extreme pH conditions and high pressure and salinity [21]. Seasonal temperature change influences human life, but the adaptation to different temperatures includes avoidance strategies such as building houses with good insulation rather than adaptation of our coatless somatic cells to cope with exposure to cold or heat. However, extremophiles are well adapted to the extremes and prefer to grow exclusively under extreme conditions. This is why these organisms were named “extremophiles” (Latin: extremus, Greek: philiā), which means “extreme-lovers.” Depending on the particular extreme, heat-loving organisms are termed thermophiles with the corresponding superlative of hyperthermophiles, which grow best at temperatures > 80 °C up to > 110 °C [23]. Such extreme high temperatures can be found in the deep sea where water is heated up by the interior of the earth and escapes through black smokers that may have temperatures up to 400 °C (Fig. 1). Additionally, hot springs or hydrothermal vents are locations with high temperatures on the surface at volcanic islands [4]. In contrast, the name psychrophiles describes the “cold-lovers.” Except for black smokers, the deep sea presents an extremely cold and unique habitat. Moreover, the majority of the Earth’s surface is considered cold with huge areas of water and the Arctic and Antarctic representing interesting habitats with constant low temperatures.

Further extremes include high (pH 9–12) or low pH (pH 0.5–4) values (alkaliphiles or acidophiles) or high salinity such as 30% salt (halophiles). Soda lakes may combine alkaline and saline conditions. Another extreme that can be found in the deep sea is high pressure, which is required for growth of piezophiles, found at depths of up to 11 km at the Mariana Trench. All environmental conditions deviating from the “norm” are considered home by extremophiles (Fig. 2). In contrast, mesophiles love especially moderate

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✉ Garabed Antranikian
antranikian@tuhh.de

[1] Institute of Technical Microbiology, Hamburg University of Technology (TUHH), Kasernenstr. 12, 21073 Hamburg, Germany

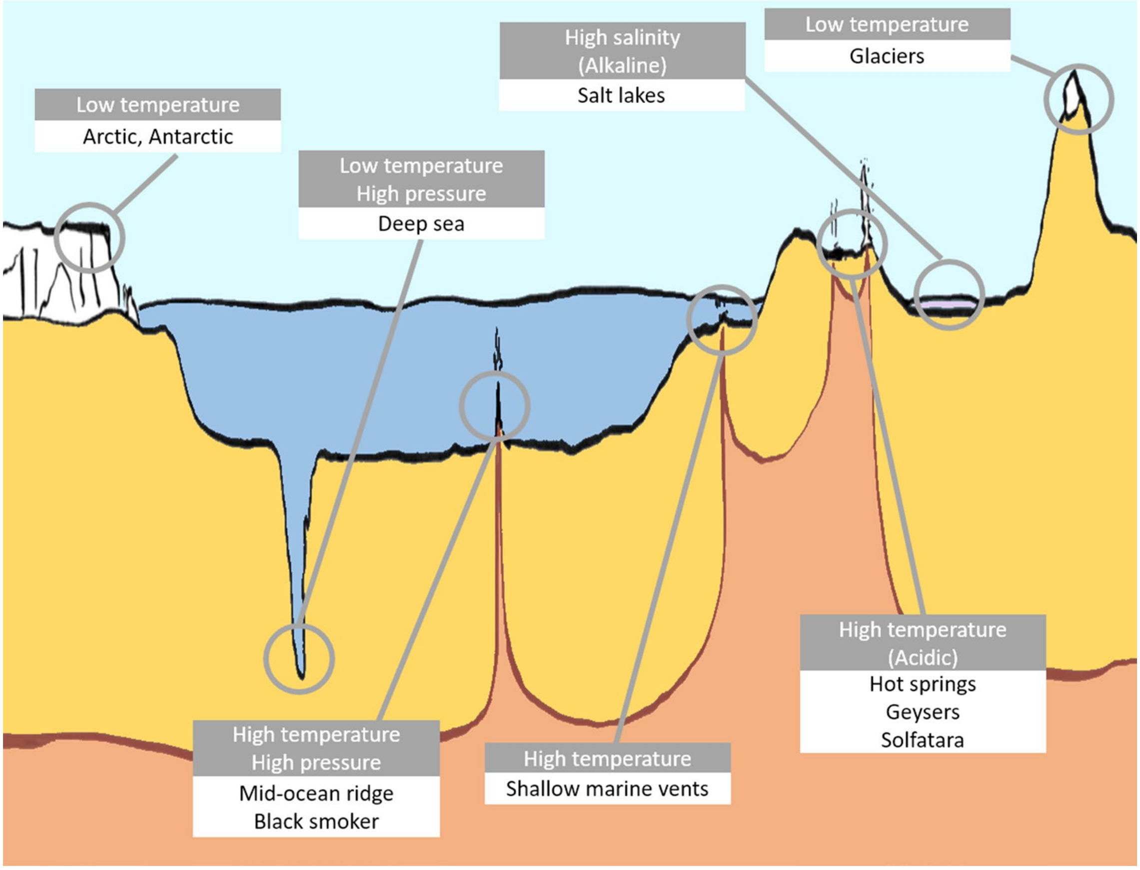


Fig. 1 Schematic representation showing examples of extreme environments found on Earth

temperatures, and neutrophils prefer a neutral pH range around 7.0.

Humans and most animals can be considered mesophilic and neutrophilic. However, some animals are considered extremophiles, such as fish that are adapted to high pressure in the deep sea [11]. They are able to thrive in depths of up to 8200 m, which equals 820 bar pressure [26]. Furthermore, tardigrades are known to survive many extremes and have become famous for their hibernation mode [22]. Nevertheless, differences between the abilities to tolerate or require extreme conditions should be distinguished. Hence, the most extreme organisms that are adapted to live optimally under these conditions are extremophilic microorganisms. These unicellular microbes not only love the extremes but literally need the extremes.

Meeting extremophiles

The tree of life contains the domain *Eukaryota*, which includes humans, animals, plants, protists and fungi, and the prokaryotic domains *Bacteria* and *Archaea*, which do not possess a nucleus within their cells. Most organisms that thrive under extreme conditions are prokaryotes. These bacteria and archaea may appear superficially similar, but when taking a closer look, the differences are immense. As representatives of numerous thermophiles, hyperthermophiles and psychrophiles, three microbial species were chosen (Fig. 3).

Lacinutrix algicola is a psychrophilic bacterium that grows at temperatures between 0 and 25 °C [16]. The name *Lacinutrix* means "lake feeder," reflecting its importance within the food chain [1]. The gram-negative, aerobic, rod-shaped and non-motile cells were initially isolated from red algae at South Shetland Islands, Antarctica, and therefore the name *algicola* (algae dweller) was given [16].

In contrast to this, *Anaerobranca gottschalkii* is a bacterium that grows only in the absence of oxygen (anaerobically) and at much higher temperatures, namely between 30 and 65°C [18]. Since optimal growth was observed at 50–55°C and additionally at pH 9.5, this bacterium is considered a thermoalkaliphile. Organisms that require more than one extreme condition for optimal growth are named polyextremophiles. *Anaerobranca gottschalkii* is gram-negative but exhibits an unusual thin cell wall and was isolated from Lake Bogoria in Kenya [18]. The name *Anaerobranca* combines

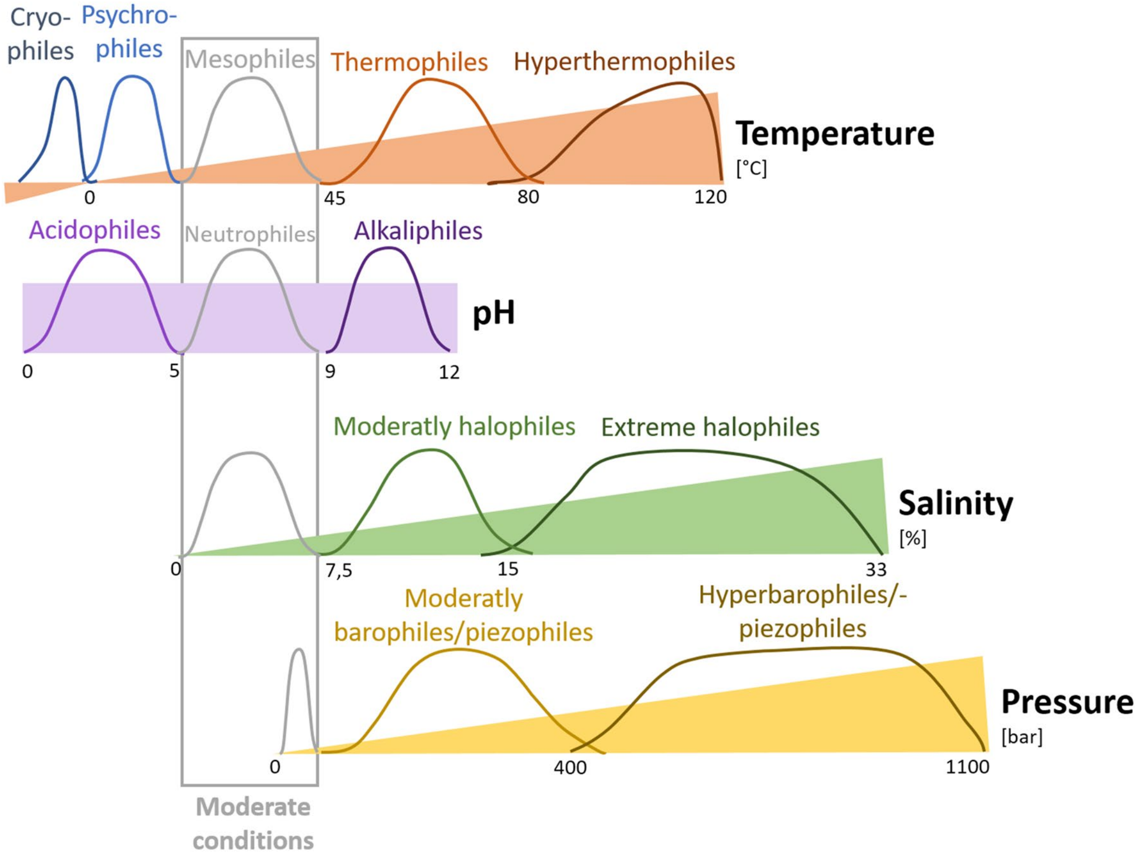
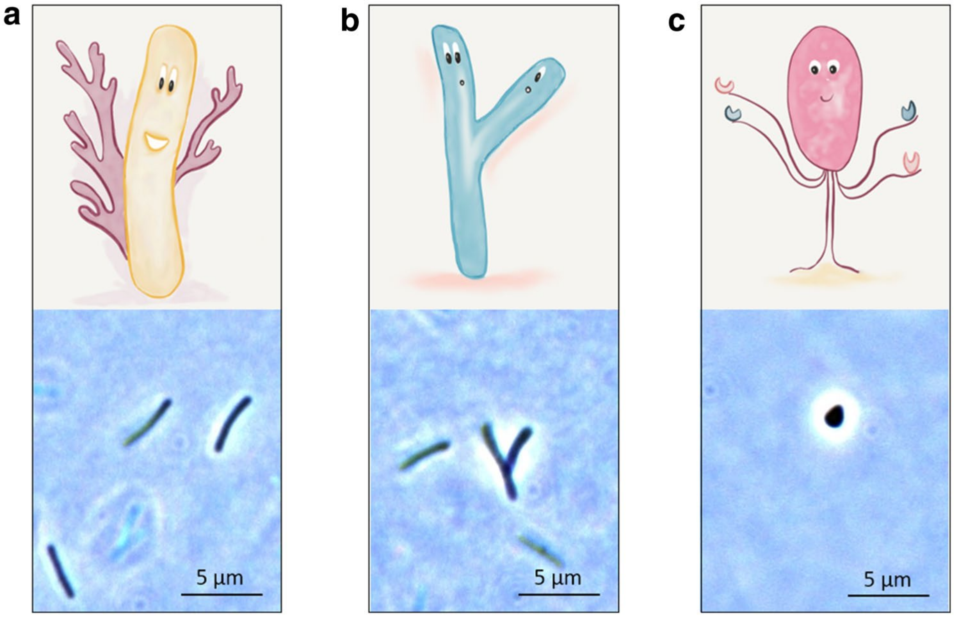


Fig. 2 Growth conditions of extremophiles encompass a comparatively broader range of temperature, pH, salt or pressure

Fig. 3 Light-microscopic images of the psychrophile *Lacinutrix algicola*, the thermophile *Anaerobranca gottschalkii* and the hyperthermophile *Pyrococcus furiosus* with additional personalized caricatures



the anaerobic lifestyle (an-aero) with the shape of the cells, which can sometimes change from elongated rod-shaped to branched (branca) forms [6]. The first *Anaerobranca* species was named *horikoshii* in honor of Koki Horikoshi, who contributed substantially to our knowledge about alkaliphiles [6]. Likewise, *A. gottschalkii* obtained its name to honor

Gerhard Gottschalk for his pioneering work on microbial metabolism [18].

Another very famous extremophile is the hyperthermophilic archaeon *Pyrococcus furiosus*, which was isolated from a shallow marine vent close to Vulcano Island (Italy). The name means "furious fireball" and refers to the extreme high optimal growth temperature of 100°C [7]. Most hyperthermophilic organisms known to date belong to the domain of *Archaea*, such as the genera *Methanopyrus*, *Pyrolobus*, *Pyrodictium* and *Pyrococcus* [24]. Cells of *P. furiosus* are spherical, motile with monopolar polytrichous flagellation and grow anaerobically [7].

The special nature of extremophiles

There are several special characteristics of extremophiles that can be briefly described. They have many unique mechanisms for adaptation that may be complex or in some cases are still not understood. Obviously, nutritional requirements must be adapted to the availability at the respective extreme environment. Adaptation to physiologic requirements may be complicated and diverse [19]. Many publications focus on the strategy of thermophiles or hyperthermophiles since heat is known to lyse cells and denature nucleic acids and proteins. To enable life at high temperatures, nucleic acids might be protected by different cellular strategies. Nucleotides are the building blocks of nucleic acid molecules, and the bases adenine and thymine connect through two hydrogen bonds, whereas cytosine and guanine are connected by three hydrogen bonds. Since a high genomic GC content, especially at the third codon position, confers greater stability, it was long assumed that this might be the main mechanism of protecting double-stranded DNA from denaturation in extremophiles [25]. Although it is beneficial, many extremophiles do not have higher GC contents compared with genomes from mesophiles. For instance, a number of *Pyrococcus* species exhibit a genomic GC content between 40.8 and 44.7% [9]. It is rather a matter of the chemical structure of nucleic acid molecules that might be modified or specialized stabilizing structures that may be formed. For example, the enzyme reverse gyrase was found in hyperthermophiles; it modifies DNA topology resulting in a stabilized macromolecule. Additionally, since temperature causes denaturation of DNA through depurination and cytosine deamination, cells of hyperthermophiles may counteract by an intracellular high salt concentration [8]. In general, heat shock proteins were described to be ubiquitous in extremophiles to assist folding proteins and refold denatured proteins [15]. Additionally, an efficient nucleic acid repair system is often characteristic for microorganisms that are exposed to extreme conditions, such as irradiation, desiccation or heat [3]. Since bacteria and archaea are distinguished in two domains of life, one difference includes the composition of membranes. Archaeal membranes exhibit ether lipids, which are in general more stable compared with the ester lipids common in bacterial membranes. Moreover, in extremophilic archaea the lipids are further strengthened, forming tetraether structures. These structures confer stability at high temperatures and alkaline milieus [13]. Besides the structural cellular components, enzymes that are produced by the cells must also be adapted to function under extreme conditions. Enzymes from psychrophiles are more flexible with fewer charged surface amino acids compared with enzymes from mesophiles. In contrast, enzymes from thermophiles exhibit a more hydrophobic core with increased electrostatic interactions conferring a more rigid character because of dense packing [20]. Especially the enzymes from extremophiles have gained great interest because of their ability to catalyze reactions under extreme conditions.

Applications of extremophiles

Biotechnology has an enormous potential especially when aiming for a sustainable biobased economy (bioeconomy). Biotechnology has already found its way into everyday life. For instance, production of lactose-free milk employs enzymes called lactose hydrolases or production of stonewashed jeans in the textile industry utilizes recombinant enzymes called cellulases. Moreover, laundry detergents contain several enzymes, and drugs are frequently produced with microorganisms [2]. Since some processes run under harsh and extreme conditions, enzymes have to function in the respective milieu. Especially for the food and beverage industry, cold temperatures are favored for preservation purposes. Therefore, cold-adapted organisms or enzymes are required. For example, low temperatures are recommended for milk treatment. Moreover, enzymes active at low temperatures and in alkaline environments are required in energy-saving cold-washing processes. For laundry detergents, enzymes such as proteases, amylases and lipases are in demand for efficient removal of textile stains. Furthermore, biocatalysts such as cellulases are required for color detergents to prevent pilling and maintain color [17]. Figure 4 gives an overview of the variety of requirements imposed on biocatalysts for application in various industrial processes.

Many processes require high temperatures because of substrate accessibility. Starch saccharification for producing sugar syrup, for example, requires high temperatures (105 °C for 5 min and 95 °C for 1 h) to facilitate liquefaction. Accordingly, heat-active and -stable starch-degrading enzymes, such as amylases and pullulanases, are required [5]. Another famous example for heat-active enzymes is the DNA polymerase from *P. furiosus*. The *Pfu* DNA polymerase is used in laboratories for amplification of DNA by

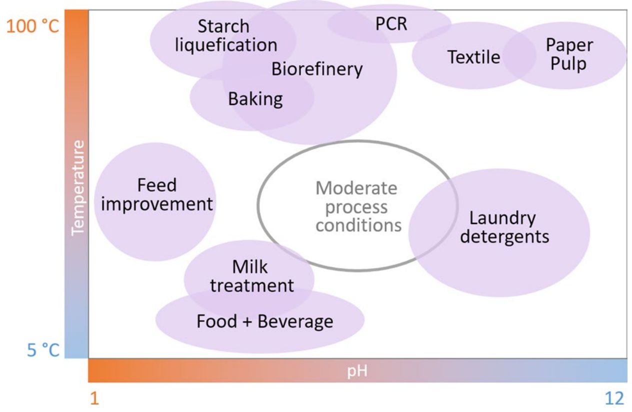


Fig. 4 Process conditions (pH and temperature) for various applications

polymerase chain reaction (PCR) and came to fame because of its extraordinary stability at the high temperatures that are required to separate the DNA double strands during the denaturation steps. Further enzymes from *P. furiosus* were characterized subsequently and showed half-lives of several days at 100 °C [12]. These polysaccharide degrading enzymes may be very interesting for biofuel production at high temperature. Biofuels of the second generation are produced in biorefineries using lignocellulosic materials, such as agricultural or forestry residues. A pretreatment of biomass is required because of the complex structure of plant cell walls that makes the polysaccharides accessible for enzymatic action. For this reason, heat pretreatment is preferable where heat-active enzymes can be applied for simultaneous degradation of available polysaccharides [14]. Accordingly, it was shown that a cellulase and a beta-glucosidase from *P. furiosus* and *P. horikoshii* could be applied for complete saccharification of cellulose [10]. Resulting sugar monomers can be used afterwards in biorefinery approaches, e.g., bioethanol production. However, many large-scale biotechnologic processes are still in development.

Conclusion

The transition of the crude oil-based industry toward a sustainable “bio-economy” relying on biomass as a renewable feedstock requires robust biotechnologic processes. Microorganisms surviving at extreme conditions represent a biotechnologic treasure chest for efficient bioprocesses by producing a large portfolio of unique biocatalysts (extremozymes) that are active under extreme temperatures, pH and high salinity. Biomass can be converted enzymatically to high-value products including chemicals, building blocks, biomaterials, pharmaceuticals, food, feed and biofuels resulting in the development of a greener bio-based industry. Furthermore, research in this field will shed light on the strategies developed by these unique microorganisms to survive in the extreme habitats of our planet.

Further reading

For German-speaking parties, the platform "MikiE – Mikroben im Einsatz" https://mikie.hoou.tuhh.de within the Hamburg Open Online University (HOOU) is recommended. Several extremophiles are introduced in more detail, and a comprehensive summary of extremophiles and their potential for industrial applications is given.

For a deeper insight and more details regarding the scientific development, the following books are recommended:

Horikoshi K, Antranikian G, Bull AT, Robb FT and Stetter KO—eds. (2011) Extremophiles handbook. Springer Japan. Print ISBN: 978-4-431-53897-4

Rampelotto PH (2016) Biotechnology of extremophiles—advances and challenges. Springer International Publishing. https://doi.org/10.1007/978-3-319-13521-2.

Krüger A, Elleuche S, Sahm K and Antranikian G. (2016) Robust biocatalysts—routes to new diversity. In: Hilterhaus L, Liese A, Kettling U, Antranikian G. (Eds.) Applied biocatalysis: from fundamental science to industrial applications. Wiley-VCH Verlag GmbH; pp. 31–51. Print ISBN: 978-3-527-33669-2.

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