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8 Polyextremophiles

8.1 Introduction

Over the last decades, scientists have been intrigued by the fascinating organisms that inhabit extreme environments. Such organisms, known as extremophiles, thrive in habitats which for other terrestrial life-forms are intolerably hostile or even lethal [1–3]. They may be found thriving from the frigid environments of the Antarctic, to the superheated waters of the hydrothermal vents, from the bottom of 11-km deep ocean trenches to the high altitudes of the atmosphere, from acidic to alkaline places [4]; some may grow in toxic waste, organic solvents, heavy metals, or in several other habitats that were previously considered inhospitable for life [5]. For every extreme environmental condition investigated, a variety of organisms have shown that they not only can tolerate these conditions, but that they also often require those conditions for survival [6]. Such discoveries have fueled research aimed to understand the unique survival strategies evolved by these extremophilic forms of life [7]. Examples of extremophiles include, but are not limited to: thermophiles (high temperature), psychrophiles (low temperature), acidophiles (low pH), alkaliphiles (high pH), piezophiles (high pressure, formerly called barophiles), halophiles (high salt concentration), osmophiles (high concentration of organic solutes), oligotrophs (low concentration of solutes and/or nutrients), and xerophiles (very dry environment). The term ‘extremophile’ also includes microorganisms able to grow in the presence of high metal concentrations or high doses of radiation [8].

Whereas extremophiles are usually defined by one extreme, many natural environments pose two or more extremes [9]. To cite a few examples, many hot springs are acid or alkaline at the same time, and usually rich in metal content; several hypersaline lakes are very alkaline; the deep ocean is generally cold, oligotrophic (very low nutrient content), and exposed to high pressure. Interestingly, an increasing number of species and strains isolated from these environments have been found to tolerate multiple extremes. Such organisms are known as polyextremophiles and may be found in the three domains of life [3]. For example, hydrothermal chimney vents harbor communities of prokaryotes and multicellular organisms (e.g. tubeworms, clams, and a variety of grazers) at high pressure, temperature, and acidic pH [10].

Although the impacts of individual extremes on microorganisms have been widely researched, attempts to define their collective influences on life are scarce and we are just beginning to understand the mechanisms of evolution underlying adaptation to multiple extremes.

In this chapter, we review the knowledge gained over the last decades on the main specimens of polyextremophiles. Examples from the three domains of life are provided. These specimens give a glimpse of how intriguing and fascinating these living beings are.

8.2 Bacteria

8.2.1 *Deinococcus radiodurans*: Conan the bacterium

Deinococcus radiodurans is a polyextremophile, known for its ability to withstand a multitude of extreme environments including severe cold, dehydration, vacuum, acid, and intense radiation. For these reasons, the bacterium has been given the nickname, “Conan the Bacterium” and currently holds the Guinness World Record for the world’s toughest bacterium [11].

D. radiodurans was discovered in 1956 by Arthur Anderson at the Oregon Agricultural Experiment Station [12]. The accidental discovery of this polyextremophile occurred while trying to sterilize canned ham. After exposing the canned ham to extreme radiation it still spoiled. Anderson cultured the spoiled ham and found the only remaining bacteria to be *D. radiodurans* R1.

D. radiodurans is a red-pigmented spherical bacterium that is usually found in a tetrad grouping of four cells. Although the cell walls resemble a gram-negative bacterium, the cells stain gram positive [13]. *D. radiodurans* is an obligate aerobe and uses organic materials as its source of energy and carbon. Despite its ubiquitous presence in nature, its populations are minor compared with other bacteria occupying the same ecological niches. It is easily cultured on nutrient agar with 1% glucose at 30 °C.

The complete DNA sequence of *D. radiodurans* was published in 1999 [14], and an analysis of the genome appeared in 2001 [15]. The bacterium is a polyploid which contains four copies of the genome in its non-replicating state and ten genome equivalents during replication. This contributes to its efficient homologous recombination based repair mechanism for double-strand breaks but does not entirely explain the bacterium’s high resistance to ionizing and UV radiation.

D. radiodurans’ ability to survive in a multitude of extreme environments stems from its ability to repair intense damage to its DNA [16]. When the DNA is damaged, chromosome fragments are reconnected by a process called single-strand annealing [17]. After this, double-strand breaks are repaired through homologous recombination, utilizing the multiple copies of the genome in order to generate at least one complete copy of the genome [18]. The repair to the DNA is usually accomplished in 12 to 24 hours, and does not introduce any more errors in the genome than a normal DNA replication cycle [19]. These capabilities make *D. radiodurans* able to withstand up to 5,000 Gy of ionizing radiation without effect on viability (500 times the fatal human dose), and can survive at levels of 15,000 Gy with a somewhat lower 37% viability [20].

In recent years, novel studies have shown that high intracellular levels of manganese(II) in *D. radiodurans* protect proteins from being oxidized by radiation [21, 22]. Such studies suggest the hypothesis that protein, rather than DNA, is the principal target of the biological action of ionizing radiation in sensitive bacteria, and extreme resistance in Mn-accumulating bacteria is based on protein protection [23, 24]. According to this new paradigm, the extreme radiation resistance presented by *D. radiodurans* is due to a highly efficient protection against proteome, instead of genome, damage [25].

These unique characteristics have exciting applications. Genetically engineered strains of *D. radiodurans* are ideal candidates for cleaning up toxic waste. For example, researchers created a strain of *D. radiodurans* that can be used to detoxify ionic mercury in radioactive waste by incorporating the gene for mercuric reductase in the genome [26]. Another engineered strain can break down toluene, an organic chemical found in radioactive waste sites [27, 28]. New strains can even be used to efficiently precipitate uranium over a wide range of input U concentrations [29, 30]. *D. radiodurans* can also be used as a means of long term information storage. In 2003, scientists translated the song “It’s a Small World” into a series of base pairs that was subsequently inserted into the genome of the bacterium [31]; 100 generations later, the complete message was still intact. In the future, if we ever wish to make extremely robust storage devices, that might survive even a nuclear catastrophe, we could probably learn to store data in this bacterium.

The origin and evolution of such extreme radiation resistance has been the topic of much research and speculation, in particular, because no radiation sources on Earth are known that could produce doses comparable to those of *D. radiodurans* resistance [32]. Normal levels of background radiation on Earth are around 0.4 mGy per year, with the highest known levels being 260 mGy near Ramsar, Iran. This raises the question of why a bacterium would evolve to withstand radiation doses of 10,000 Gy when exposure to such high levels is very unlikely [33]. Some scientists pose a theory that *D. radiodurans* may have originated in outer space where cosmic radiation is very significant [34]. The bacterium could then have been brought to earth by way of a meteorite. But this is an unlikely possibility, considering the similarities of *D. radiodurans* to other prokaryotes, which suggests a terrestrial origin. Studies have indicated that the effects of dehydration on DNA are very similar to the effects of radiation [35]. Although normal strains of *D. radiodurans* are resistant to both desiccation and radiation, mutated strains have become susceptible to damage from both conditions [20, 36]. This has led some scientists to conclude that *D. radiodurans* originally evolved to withstand long periods without water, which causes very similar DNA damage to ionizing radiation [37, 38]. The radiation resistance that has come to define this bacterium may actually have been a byproduct of the mechanism for surviving dehydration.

8.2.2 *Chroococcidiopsis*

Among Bacteria, the best adapted group to various extreme conditions is the *Cyanobacteria*. They often form microbial mats with other bacteria from Antarctic ice to continental hot springs. Cyanobacteria can also develop in hypersaline and alkaline lakes, support high metal concentrations, tolerate xerophilic conditions (i.e., low availability of water), and form endolithic communities in desertic regions [2]. An interesting group is the genus *Chroococcidiopsis*, considered one of the most primitive cyanobacteria [39]. This photosynthetic, coccoidal bacterium reproduces internally by binary fission, forming small cells (baeocytes) contained within an enlarged mother cell. It occurs throughout a wide geographic range and different habitat types, encompassing different life strategies, from extreme cold (Dry Valleys in Antarctica) to extreme hot (e.g. Atacama Desert in Chile) deserts. *Chroococcidiopsis* species grow as litho-, endo-, chasmoendo-, crypto-, and hypo-liths in and on rocks; in fresh, brackish or salt waters, also as photobionts of rock and soil inhabiting lichens of the order Lichinales; and rarely as free-living cells in soil [40–45]. In its natural environment, to escape the harsh outside climate, *Chroococcidiopsis* occupies the last refuges for life inside porous rocks or at the stone-soil interfaces, where it survives in a dry, ametabolic state for prolonged periods.

Chroococcidiopsis is known for its ability to survive extreme environmental conditions, including both high and low temperatures, ionizing radiation, and high salinity. Strains of *Chroococcidiopsis* are able of withstanding years of desiccation [46], ionizing radiation up to 15 kGy [47], and UVC doses as high as 13 kJ/m² [48]. With the main prerequisites for survival in space and under Martian conditions, *Chroococcidiopsis* has been used in ground-based simulations and space exposure [49]. A monolayer survived 10 min of simulated unattenuated Martian UV-flux [50] and a multilayer overlain by grounded sandstone survived space and Martian simulations [51], while cells augmented to an epilithic community tolerated 584 days in real space [52]. Because of these abilities to survive in extreme environments, *Chroococcidiopsis* has been of interest to astrobiology [53, 54] and has been proposed as a suitable organism for the terraforming of Mars [55, 56].

Despite the great interest in this genus, many aspects of its biology remain poorly understood. Its biogeography, for example, is still debated. Fewer et al. (2002) confirmed the non-existence of biogeographical patterns suggesting close relationships between strains from very distant geographical origins [57]. However, a recent study indicates that *Chroococcidiopsis* variants from different hot and cold deserts around the world are specific to their habitat, as a result of the ancient legacy due to a very early separation of these lineages [58]. These contrasting results may be due to differences in the genetic marker used. There are studies indicating that the choice of genetic marker influences the results in the detection of biogeographic relationships.

In terms of origin and evolution, the earliest morphologically based reports of *Chroococcidiopsis* are derived from 400 million years old lichenized fossil from the

Early Devonian Rhynie Chert [59, 60]. A relaxed-clock phylogenetic analysis however indicates an age between 3.1–1.9 billion years for free-living variants of *Chroococcidiopsis* [58]. These studies indicate that the genus is one of the evolutionary oldest cyanobacteria. Nevertheless, the exact phylogenetic position of the genus *Chroococcidiopsis* is still unclear. Historically, *Chroococcidiopsis* has been classified within the order *Pleurocapsales* [61, 62], based on a unique reproduction modus by baeocytes. On the other hand, phylogenetic analyses suggest a closer relationship of this genus to the order *Nostocales* [63]. Further phylogenetic studies based on saltwater and freshwater phenotypes provide evidence that the genus is polyphyletic and should be reclassified to improve clarity in the literature [64].

So, it is clear that future research should continue with the search for mechanisms that help to understand the patterns of ecology and evolution of the genus *Chroococcidiopsis*.

8.3 Archaea

Archaea is the main group to thrive in extreme environments. Although members of this group are generally less versatile than bacteria and eukaryotes, they are quite skilled in adapting to different extreme conditions, frequently holding extremophily records [65]. Within the domain Archaea, one of the most interesting polyextremophiles is the *Halobacterium salinarum* NRC-1, as described in the following section.

8.3.1 *Halobacterium salinarum* NRC-1: a model organism

Halobacterium salinarum NRC-1 is an extreme halophilic archaeon that grows optimally at 4.3 M NaCl and is capable of growth between 2.6 and 5.1 M (29.8%) NaCl [66]. Interestingly, this microorganism is metabolically versatile. In addition to its aerobic metabolic capacity, it possesses facultative growth capabilities through anaerobic respiration, utilizing dimethyl sulfoxide (DMSO) and trimethylamine *N*-oxide (TMAO), and via arginine fermentation [67]. *H. salinarum* NRC-1 may be found in hypersaline environments all over the world, including salt production facilities, brine inclusions, salt mines, as well as natural lakes and ponds [68].

In addition to its ability to survive in hypersaline conditions (it is one of the few species known that can live in saturated salt solutions), *H. salinarum* NRC-1 is adapted to desiccation [69], ionizing radiation [70], transition metals [71], different temperatures [72], high pressure [73], oxygen limitation [74], and different regimes of UV radiation [75]. Furthermore, the long term survival of *H. salinarum* has been evidenced with the isolation of a strain from a single fluid inclusion in a 97,000-year-old halite crystal from Death Valley [76], and experiments conducted by Stan-Lotter et al. (2003) showed

that *H. salinarum* NRC-1 is able to survive a simulated Martian atmosphere (6 mbar pressure, 98% carbon dioxide, and an average temperature of -60°C) for 6 hours of exposure [77]. This incredible robustness and ability to survive and adapt to different and extreme environmental stresses makes these archaea of great interest in the search for extraterrestrial life.

H. salinarum NRC-1 is easy to culture and manipulate in the laboratory, which has made it an excellent model organism in the domain Archaea for the study of genetics, functional genomics and mechanisms of adaptive evolution [78, 79]. The genome of *H. salinarum* NRC-1 was the first halobacterial genome to be completely sequenced [80].

8.4 Eukaryota

When we think of extremophiles, Bacteria and Archaea first come to mind. However, eukaryotic life is also found actively growing in almost any extreme condition where there is a source of energy to sustain it, with the only exception of high temperature ($> 70^{\circ}\text{C}$) and possibly the deep subsurface biosphere. Recent studies based on molecular ecology have demonstrated that eukaryotic organisms are as adaptable as Bacteria and Archaea, although most habitats have not been sufficiently sampled to date. The majority of eukaryotes found living in extreme environments are microbial but many multicellular organisms are also known to thrive or tolerate such extreme conditions. In the following, we describe interesting examples of eukaryotic polyextremophiles.

8.4.1 Cyanidiophyceae

The class *Cyanidiophyceae* is comprised of asexual, unicellular red algae that are known to grow in acidic (pH 0.2–4.0) and salty (up to 10% solutions) environments and at moderately high temperatures (up to 56°C) [81, 82]. They are also known to proliferate even when exposed to a stream of 100% CO_2 or 1N H_2SO_4 solution [81] as well as tolerate heavy metals [83, 84]. For example, the microalga *Galdieria sulphuraria* tolerates desiccation [85] and high concentrations of toxic metal ions such as cadmium, mercury, aluminum, and nickel, suggesting potential application in bioremediation [83]. *G. sulphuraria* may represent up to 90% of the biomass in extreme habitats, such as hot sulfur springs with pH 0–4.

The prime habitats for *Cyanidiophyceae* are thermo-acidic environments such as warm-hot acidic springs and pools that emanate from geothermal sources [86, 87]. Among photosynthetic organisms, the environmental niche for *Cyanidiophyceae* is unique since no other phototrophs are expected to occur in this combination of low pH and elevated temperature. Furthermore, they were also observed in cryptoendolithic

layers of rocks [88, 89] and caves [90]. Heavy metals are common in the *Cyanidiophyceae* habitats, although their concentrations can vary considerably [84].

The taxonomy and phylogeny of *Cyanidiophyceae* has been quite confusing until the application of genetic approaches [91–93]. Molecular phylogenetic studies demonstrated that this group of algae is phylogenetically distinct from the main line of descent in the red algae and split quite early in geological time, i.e. ~ 1.3 Ga [94, 95]. Based on these studies, a new classification of the *Rhodophyta* (red algae) was proposed [96], with the establishment of the new subphylum *Cyanidiophytina* that included the single class *Cyanidiophyceae* (former order *Cyanidiales*).

With the total genome sequencing of *Cyanidioschyzon merolae* [97] and *G. sulphuraria* [98], studies involving *Cyanidiophyceae* species has drawn significant interest from the scientific community. Due to the simplicity of their cells and genomes, members of this ancient group may be used as biological models for providing key information on the basic and essential genes involved in the lives of photosynthetic eukaryotes.

In terms of origins and evolution, the class *Cyanidiophyceae* constitutes a very early and distinct branch of the *Rhodophyta* and may be considered pioneering eukaryotic cells on early Earth. The consensus is that the ancestor of all red algae presumably acquired a cyanobacterium as an endosymbiont [99]. According to Castenholz & McDermott (2010), the ancient eukaryotic host incorporated a thermophilic cyanobacterium as its endosymbiont, which maintained an internal pH close to neutrality, creating a comfortable climate for the cyanobacterium, which turned, in time, into chloroplasts [84].

8.4.2 Lichens

Lichens are examples of symbiotic associations between, at least, two microorganisms: a fungus (the ‘mycobiont’) and an associated photosynthetic partner (the ‘photobiont’). The photobiont can be either a green alga or a cyanobacterium, and some lichens contain both [100]. The physiological interactions that occur between the partners result in the formation of a distinctive lichen body called a thallus, with no division into root, stems, or leaves [101]. In this body, the photobionts are housed to the best advantage for photosynthesis.

The lichen lifestyle is remarkably adaptable and they often have different photobionts at different stages of the life cycle. At certain times, it may be advantageous for the lichen to contain cyanobacteria because they are able to fix atmospheric nitrogen to form amino acids and other complex organic molecules [102]. The lichen fungus is able to then break down these compounds. One of the great advantages of this ability is that lichens are extremely effective colonizers and grow in almost all natural environments and in a variety of different climatic conditions [103]. It has been estimated that they comprise the dominant components of vegetation on 8% of the Earth’s terres-

trial surface. They play a very important ecological role as carbon fixers and recyclers of the elements and nutrients nitrogen, phosphorous, and sulphur [104].

A key characteristic of lichens is that they have a remarkable ability to tolerate extreme environmental conditions and many lichen species may be considered polyextremophiles due to their high tolerance to temperature, radiation, and desiccation [105]. For example, in high mountains and polar regions, lichens are well adapted to long-term desiccation, temperatures between -40°C and 60°C , and high doses of radiation, including UV [106].

The basic physiology of lichens is what determines their efficiency at growing in extreme environments [104]. Because they lack roots, they are able to grow on rock surfaces and bark, relying on absorbing substances throughout their upper surface, which lacks a waxy protective outer cuticle. Minute pores are often present and the upper layer contains polysaccharides (carbohydrates), which attract water. The algae rest just below the surface, which also contains light-screening compounds to protect them under high levels of ultraviolet radiation. Green algae produce the sugar alcohol ribitol and cyanobacteria produce glucose (sugar), which are passed over to the fungus and rapidly converted to the sugar alcohol mannitol. This process ensures that lichens have the extraordinary ability to maintain themselves during very long dry periods. Furthermore, the huge chemical arsenal lichens possess helps ensure their survival under extreme conditions [107]. Some species contain up to 30 percent dry weight of organic compounds, which act as 'stress metabolites' [108].

Due to these interesting features, lichens have been successfully used in experiments under simulated space conditions [109–111], and they have been launched into space in the experiment Lichens [112] and Lithopanspermia [113] in order to better understand the effects of extreme space conditions on multicellular eukaryotic organisms. Such studies demonstrated that lichens are among the only organisms able to survive exposure to the complex matrix of all parameters of space including solar extraterrestrial UV radiation [114]. The thalli from this symbiotic system have certain morphologies and include adaptations, such as a thick and pigmented cortex covering the algal layer, which provide efficient shielding against the hostile parameters of outer space [115]. Many experiments performed after space exposure (as well as space and Mars simulation experiments) have shown their capacity to maintain physiological and photosynthetic activity, and their capacity to germinate and grow after being exposed to space parameters [116].

The evolution of lichens is complex and not well understood. Lichens were a component of the early terrestrial ecosystems, and the estimated age of the oldest terrestrial lichen fossil is 600 Ma [117]. In 1995, Gargas and colleagues proposed that there were at least five independent origins of lichenization [118]. However, Lutzoni et al. (2000) suggested that there may have been only a single origin of lichenization in the *Ascomycota*, but that there have been several reversals [119]. Some non-lichen-forming fungi may have secondarily lost the ability to form a lichen association. For this reason, lichenization has been viewed as a highly successful nutritional strategy [120].

The presence of a 600 million year-old fossil lichen associated with cyanobacteria [117] is consistent with an ancient origin for a lichenized state in fungi and is consistent with the “Protolichenes hypothesis” [121], which suggested that fungi existed as lichens in terrestrial paleoecosystems prior to the diversification of land plants. A conservative interpretation of recent data indicates that lichenization evolved multiple times in *Ascomycota* [122].

8.4.3 Tardigrades: nature’s toughest animal

Tardigrades (also known as waterbears or moss piglets) are microscopic invertebrates (approx. 0.1–1.2 mm) with a well-developed organization including brain and sensory organs, muscles, a complex feeding apparatus and alimentary tract, and reproductive and osmoregulatory organs [123]. They form the phylum Tardigrada with over 1,000 species identified [124]. It is an ancient group, with fossils dating from 530 million years ago, in the Cambrian period [125]. Their first observations were reported over two centuries ago by the German pastor Johann A. E. Goeze [126]. Tardigrades have representatives distributed across all continents, and in most terrestrial, freshwater and marine habitats: from the top of the Himalayas to the ocean floor [127–129].

When subjected to environmental stress, tardigrades are one of the few groups that are able to enter a state of suspended animation called cryptobiosis [130]. Depending on the environment, they may enter this state via anhydrobiosis (dehydration), cryobiosis (extremely low temperatures), osmobiosis (high salt concentration), or anoxybiosis (lack of oxygen), with desiccation induced anhydrobiosis and freezing induced cryobiosis being the most extensively studied states. While in this state (referred to as a tun), their metabolism lowers to less than 0.01% of normal and their water content can drop to 1% of normal [131].

The genetic and biochemical mechanisms behind desiccation tolerance of anhydrobiotic organisms are not fully understood yet, but several biochemical components are likely to be involved, including sugars (e.g. trehalose), stress proteins (e.g. heat shock proteins), and an efficient DNA repair system [132–136]. Such molecular mechanisms are responsible for maintaining the structural integrity of the cell components and membranes during the processes of desiccation and rehydration, as well as during the dry state.

As a likely by-product of the adaptations for desiccation and freezing, tardigrades in the dormant state show a very high tolerance to multiple extreme conditions. Interestingly, it has been reported that tardigrades in the active state also exhibit high tolerance to environmental stresses [137, 138]. Consequently, these intriguing organisms are classical examples of multicellular polyextremophiles. They are able to survive extreme temperatures from $-272\text{ }^{\circ}\text{C}$ (just 1 degree above absolute zero!) to $151\text{ }^{\circ}\text{C}$ for a few minutes [6, 139], high hydrostatic pressure of 7.5 GPa (which corresponds to the pressure at the depth of about 180 km below the surface of the Earth!) [140],

high radiation doses of 5,000 Gy (of gamma rays) and 6,200 Gy (of heavy ions) [141], and remain viable after a long period in the anhydrobiotic state (nearly 10 years) [142]. They can also tolerate exposure to CO₂, H₂S, and the biocide methyl bromide, and also immersion in ethanol, butanol, and hexanol [143–146]. In addition, they can even survive in space conditions for a limited time [147–150]. Thus, tardigrades are interesting multicellular model organisms for space and astrobiological studies [146, 151].

Tardigrades are also enigmatic in terms of evolution. The phylogenetic position of this phylum in the tree of life is still uncertain. Although it is known that Tardigrada belong to the recently recognized clade of molting animals (*Ecdysozoa*), it is still debated if they are more closely related to nematodes and nematomorphs or to arthropods and onychophorans [152–154]. MicroRNA and phylogenomic studies show that the grouping of tardigrades with nematodes found in a number of molecular studies is a long branch attraction artifact, and provide evidence that *Onychophora* is the sister group of *Arthropoda* [155]. Nevertheless, recent analyses of the organization of the nervous system in three tardigrade species speak against the proposed sister group relationship of tardigrades with nematodes [156]. They suggest instead that *Tardigrada* and *Arthropoda* are sister groups (to the exclusion of *Onychophora*).

Tardigrade genomes are generally small [157, 158], probably due to extensive gene loss, and most gene sequences show high substitution rates. This causes problems with phylogenetic reconstructions using molecular datasets due to the so-called Long Branch Attraction artifact. This means that taxa with a high substitution rate artificially cluster together. Most morphological data seem to be equally problematic, as key characteristics might have been lost due to the miniaturization of these animals and the remaining characteristics might be highly derived. Consequently, it is difficult to interpret their origin and evolution.

8.5 Conclusion

The study of recently discovered polyextremophiles have extended the known physical and chemical boundaries for life and have provided excellent models for the study and characterization of novel physiologies as well as biochemical and evolutionary pathways. The above discussion gives an overview of the potential adaptive and metabolic capacities of some specimens of these fascinating forms of life. The sequencing of their genomes is providing new insights into the evolution of interesting adaptive strategies, with *D. radiodurans* as one of the best examples; the radiation resistance that has come to define this bacterium may actually have been a byproduct of the mechanism for surviving dehydration. The authors hope that this chapter will encourage other scientists to investigate and characterize novel polyextremophiles and further expand the current limited knowledge of their ecology, biochemistry, and mechanism of adaptive evolution.

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