

Life in extreme environments

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Each recent report of liquid water existing elsewhere in the Solar System has reverberated through the international press and excited the imagination of humankind. Why? Because in the past few decades we have come to realize that where there is liquid water on Earth, virtually no matter what the physical conditions, there is life. What we previously thought of as insurmountable physical and chemical barriers to life, we now see as yet another niche harbouring 'extremophiles'. This realization, coupled with new data on the survival of microbes in the space environment and modelling of the potential for transfer of life between celestial bodies, suggests that life could be more common than previously thought. Here we examine critically what it means to be an extremophile, and the implications of this for evolution, biotechnology and especially the search for life in the Universe.

Normal is passé; extreme is chic. While Aristotle cautioned "everything in moderation", the Romans, known for their excesses, coined the word 'extremus', the superlative of *exter* ('being on the outside'). By the fifteenth century 'extreme' had arrived, via Middle French, to English. At the dawning of the twenty-first century we know that the Solar System, and even Earth, contain environmental extremes unimaginable to the 'ancients' of the nineteenth century. Equally marvellous is the detection of organisms that thrive in extreme environments. Macelroy¹ named these lovers ('philos' to the Greeks) of extreme environments 'extremophiles'.

The discovery of extreme environments and the organisms that inhabit them has made more plausible the search for life outside the Earth, and even the possibility of panspermia (the transport of life from one planet to another). The discovery of extremophiles has also put vitality into the biotech industry and dreams of stock options in the minds of field biologists. The discipline has exploded during the past decade, with several reviews published on extremophiles²⁻⁴, an increasing number of meetings held⁵, genomes sequenced and patents filed, and the launch of concerted funding programmes such as the US National Science Foundation and NASA's programmes in Life in Extreme Environments, Exobiology and Astrobiology, and the European Union's Biotechnology of Extremophiles and Extremophiles as Cell Factories⁶. Here we examine what it means to be an extremophile starting from first principles. As a result, we highlight extremophiles that are often overlooked, possibly because they are eukaryotes. We then focus on the significance of extremophile research to the search for life in the Universe, and conclude with a discussion of the future of extremophile research including their economic potential.

What is an extremophile?

An organism that thrives in an extreme environment is an extremophile; in more than one extreme it is a polyextremophile. Examples of the latter would include *Sulfolobus acidocaldarius*, an archaea that flourishes at pH 3 and 80 °C (Fig. 1). 'Extremes' include physical extremes (for example, temperature, radiation or pressure) and geochemical extremes (for example, desiccation, salinity, pH, oxygen species or redox potential) (Table 1). It could be argued that extremophiles should include organisms

thriving in biological extremes (for example, nutritional extremes, and extremes of population density, parasites, prey, and so on).

'Extremophile' conjures up images of prokaryotes, yet the taxonomic range spans all three domains. Although all hyperthermophiles are members of the Archaea and Bacteria, eukaryotes are common among the psychrophiles, acidophiles, alkaliphiles, piezophiles, xerophiles and halophiles (which respectively thrive at low temperatures, low pH, high pH, and under extremes of pressure, desiccation and salinity; see <http://www.astrobiology.com/extreme.html> for an overview). Extremophiles include multicellular organisms, and psychrophiles include vertebrates.

Although these characterizations seem straightforward, three philosophical issues need further exploration. First, what is 'extreme'? Perhaps 'extreme' is in the eyes of the beholder. It is clear that to a thermophile that dies at 21 °C and a piezophile that finds atmospheric pressure 'extreme', what determines an extremophily is based on definitions that are perhaps anthropocentric. There are two possibilities that are more scientifically tenable. The first is based on an evolutionary perspective — that is, the earliest



Figure 1 Congress Pool, Norris Geyser Basin, Yellowstone National Park, USA, where Tom Brock originally isolated *Sulfolobus acidocaldarius*. The average pH is 3 and the average temperature is 80 °C. Photo taken on 20 September 2000.

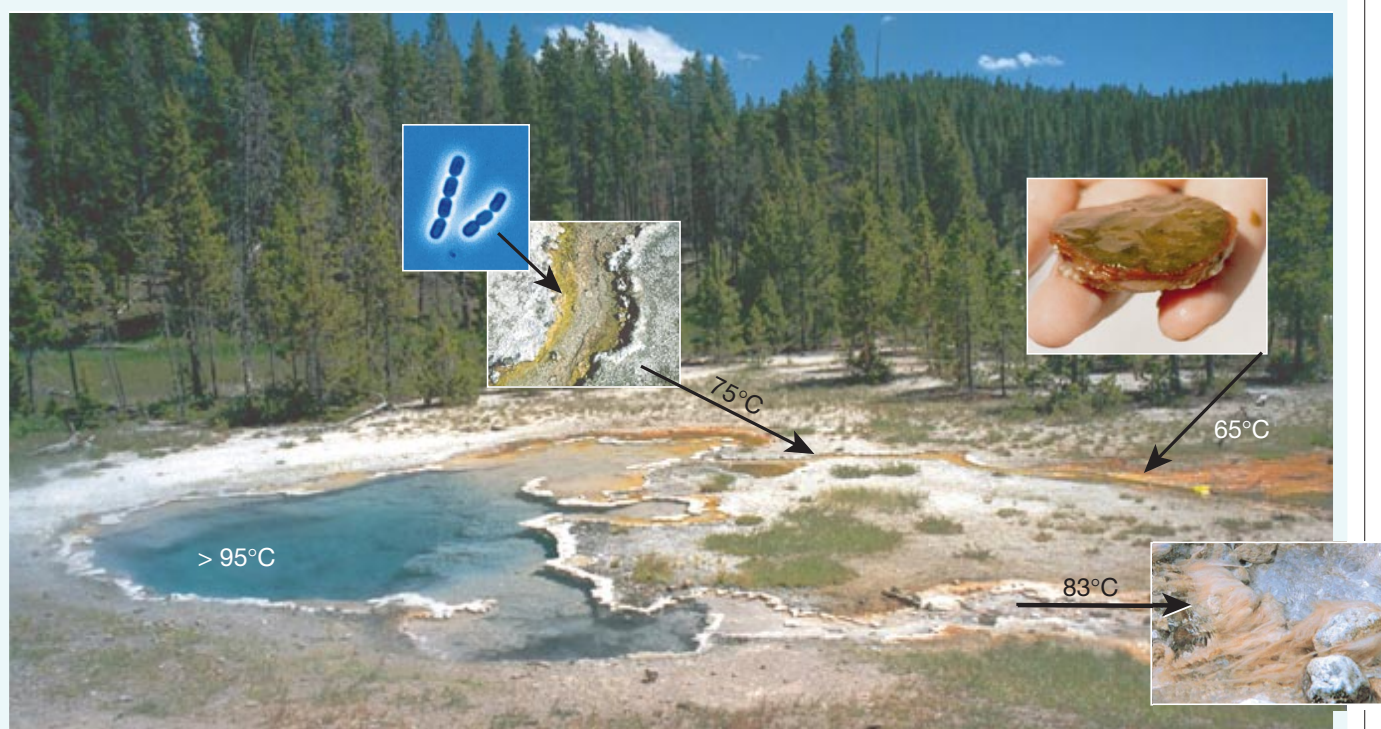


Figure 2 Octopus Spring, an alkaline (pH 8.8–8.3) hot spring in Yellowstone National Park, USA, is situated several miles north of Old Faithful geyser. The water flows from the source at 95 °C to an outflow channel, where it cools to a low of 83 °C. About every 4–5 minutes a pulse of water surges from the source raising the temperature as high as 88 °C. In this environment the pink filamentous *Thermocrinis ruber* thrives (lower right). Where the water cools to ≤ 75 °C, growth of photosynthetic organisms is permitted. The inset on left shows the growth of a thermophilic cyanobacterium, *Synechococcus*, tracking the thermal gradient across the channel. At 65 °C a more complex microbial mat forms with *Synechococcus* on the top overlaying other bacteria, including species of the photosynthetic bacterium *Chloroflexus* (upper right). The yellow object at 65 °C was part of an experimental set-up. Photo taken on 4 July 1999.

environment for life defines what is 'normal'. If life arose in a high-temperature, anoxic hydrothermal vent, any environment that deviates from that is 'extreme'. The second, which we favour, is based on a more objective, physical definition of 'extreme'. This definition is congruent with the colloquial definition, with exceptions. All physical factors are on a continuum, and extremes in the conditions that make it difficult for organisms to function are 'extreme'. For example, to maintain chemistry in an aqueous environment, cells need certain temperatures, pH and solutes, precise control over biomolecules and electric currents, and the ability to repair damage. There are certain conditions that will destroy biomolecules, such as desiccation, radiation and oxygen. Regarding the last of these conditions, oxygen forms reactive oxygen species that cause oxidative damage to nucleic acids, proteins and lipids^{7,8}. Thus, we and all other aerobes are extremophiles.

The second philosophical issue is ecological. Must an extremophile actually 'love' (remember 'philos') an extreme environment or can it merely tolerate it? In a practical sense the latter is clearly easier to determine experimentally, whereas in a biological sense the former has a certain biological and linguistic simplicity. In the last few decades of the twentieth century, numerous true extreme-loving organisms were found, thus permitting linguistic purity. But as a caveat, note that it is common for some environmental extremes (for example, radiation, vacuum or metal concentrations) to include organisms that tolerate rather than love the environment.

Third, does an organism have to be an extremophile during all life stages, and under all conditions? The bacterium *Deinococcus radiodurans*, the present gold-medallist of radiation resistance, is widely considered an extremophile par excellence. Yet, radiation resistance in *D. radiodurans* is severely diminished in stationary compared with logarithmic phase growth⁹, under increased concentrations of Mn^{2+} (ref. 10), with freezing or desiccation, and under nutrient-limited

conditions¹¹. Spores (for example, *Bacillus subtilis*), seeds and egg stages (for example, shrimp) are all far more resistant to environmental extremes than the vegetative forms. Trees, frogs, insects and fish can tolerate remarkably low temperatures during the winter as a result of seasonal shifts in physiology. Tardigrades ('water bears') in the tun state, can survive temperatures from -253 °C to 151 °C, X-rays, vacuum and, when in perfluorocarbon, pressures up to 600 MPa, almost 6,000 times atmospheric pressure at sea level¹².

Environmental extremes

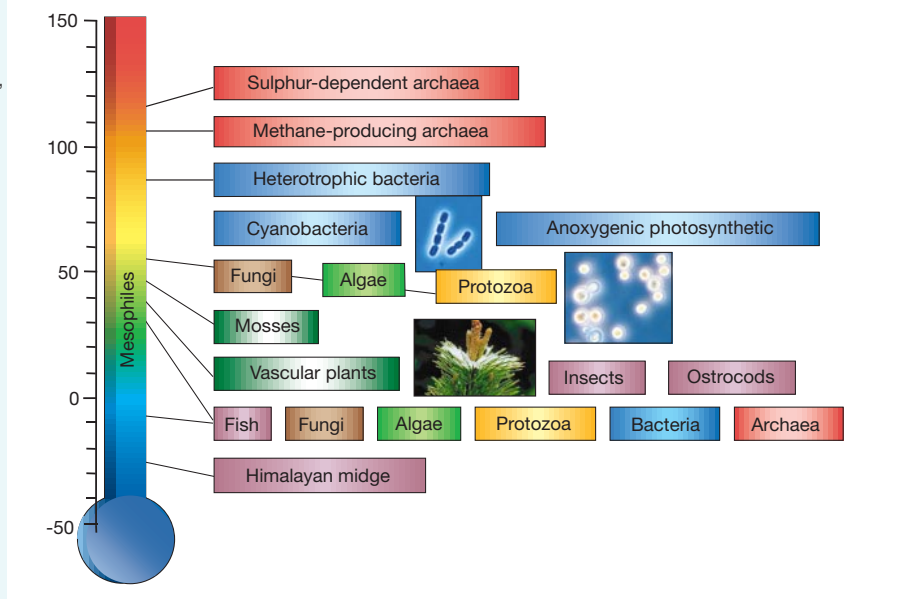
Liquid water is the *sine qua non* of life on Earth, and arguably any life in our Solar System¹³. Furthermore, life requires an input of energy, but must also be able to control energy flow. Redox chemistry is universal. As life is based on organic chemistry, such chemistry must be allowed to operate. An extremophile must either live within these parameters, or guard against the outside world in order to maintain these conditions intracellularly. With these rules in mind, we examine selected environmental parameters, summarized in Table 1.

Temperature

Temperature creates a series of challenges, from the structural devastation wrought by ice crystals at one extreme, to the denaturation of biomolecules at the other. The solubility of gases in water is correlated with temperature, creating problems at high temperature for aquatic organisms requiring O_2 or CO_2 . Temperatures approaching 100 °C normally denature proteins and nucleic acids, and increase the fluidity of membranes to lethal levels. Chlorophyll degrades above 75 °C, excluding photosynthesis (Fig. 2).

Yet, in nature thermal preferences range from hyperthermophilic¹⁴ (maximum growth >80 °C) to psychrophilic (maximum growth <15 °C). The most hyperthermophilic organisms are archaea, with *Pyrolobus fumarii* (Crenarchaeota), a nitrate-

Figure 3 Temperature limits for life. The highest and lowest temperature for each major taxon is given. Archaea are in red, bacteria in blue, algae in light green, fungi in brown, protozoa in yellow, plants in dark green and animals in purple.



reducing chemolithoautotroph, capable of growing at the highest temperatures of up to 113 °C (ref. 15). Hyperthermophile enzymes can have an even higher temperature optimum; for example, activity up to 142 °C for amylopullulanase¹⁶. There are thermophiles among the phototrophic bacteria (cyanobacteria, purple and green bacteria), eubacteria (*Bacillus*, *Clostridium*, *Thiobacillus*, *Desulfotomaculum*, *Thermus*, lactic acid bacteria, actinomycetes, spirochetes and numerous other genera) and the archaea (*Pyrococcus*, *Thermococcus*, *Thermoplasma*, *Sulfolobus* and the methanogens). In contrast, the upper limit for eukaryotes is ~60 °C, a temperature suitable for some protozoa, algae and fungi. The maximum temperature for mosses is lower by another 10 °C, for vascular plants it is about 48 °C, and for fish it is 40 °C, possibly owing to the low solubility of oxygen at high temperatures (Fig. 3).

Representatives of all major taxa inhabit temperatures just below 0 °C. Many microbes and cell lines can be preserved successfully at -196 °C (liquid nitrogen), but the lowest recorded temperature for active microbial communities is substantially higher, at -18 °C (ref. 17). Among animals, the Himalayan midge is active at -18 °C (ref. 18). Liquid water not only is a solvent for life as we know it, but also is important either as a reactant or product in most metabolic processes¹⁹. At low temperatures with nucleation, water freezes. The resulting ice crystals can rip cell membranes, and solution chemistry stops in the absence of liquid water. Freezing of intracellular water is almost invariably lethal. The only exception to this rule reported so far, outside of cryopreservation, is the nematode *Panagrolaimus davidi*, which can withstand freezing of all body water²⁰.

Radiation

Radiation is energy in transit, either as particles (for example, neutrons, electrons, protons, alpha particles or heavy ions) or electromagnetic waves (for example, gamma rays, X-rays, ultraviolet (UV) radiation, visible light, infrared, microwaves or radiowaves). Exceptional levels of radiation — sufficient to qualify for ‘extremophile’ status — rarely occur on the Earth naturally, but intense levels of UV and ionizing radiation are well-studied because of their importance to medicine, energy production, warfare and space travel. The dangers of UV and ionizing radiation range from decreased motility to inhibition of photosynthesis, but the most serious is damage to nucleic acids. Direct damage to DNA or indirect damage through the production of reactive oxygen species creates modified bases and single- and double-strand breaks.

The bacterium *D. radiodurans* is famous for its ability to withstand ionizing radiation (up to 20 kGy of gamma radiation) and UV

radiation (doses up to 1,000 J m⁻²), but this extraordinary resistance is thought to be a by-product of resistance to extreme desiccation²¹. Other organisms that can stand high levels of radiation are two *Rubrobacter* species²² and the green alga *Dunaliella bardawil*²³.

Pressure

Hominids evolved at an atmospheric pressure of 101 kPa (= 1 atmosphere = 1.013 bar), although our aquatic ancestors originated under hydrostatic pressure. Hydrostatic pressure increases at a rate of 10.5 kPa per metre depth, compared with 22.6 kPa per metre for lithostatic pressure. Pressure decreases with altitude, so that by 10 km above sea level, atmospheric pressure is almost a quarter of that at sea level. The boiling point of water increases with pressure, so water at the bottom of the ocean remains liquid at 400 °C. Because liquid water normally does not occur above ~100 °C, increased pressure can increase the optimal temperature for microbial growth, but usually by only a few degrees²⁴.

Pressure challenges life because it forces volume changes. Pressure compresses packing of lipids resulting in decreased membrane fluidity²⁵. If a chemical reaction results in an increase in volume, as most do, it will be inhibited by an increase in pressure²⁶. Although many organisms have adapted to very high pressures, a sudden change can be lethal, an effect only too well known to divers.

The Mariana trench (11° 22' N, 142° 25' E) is the world’s deepest sea floor at 10,898 m, yet it harbours organisms that can grow at standard temperature and pressure. It also has yielded obligatory piezophilic species²⁷ that can grow at 70 to 80 MPa, but not below 50 MPa.

One component of pressure is gravity. Until now, organisms on Earth have, except for brief moments, lived at 1g. Space exploration will include extended periods in locations with gravity regimes different from our own: for example, launch vehicles (variable g), the International Space Station (microgravity), the Moon (0.17g) and Mars (0.38g). Although most of the concern with the effect of gravity have focused on human health, gravitational effects also have been found for microbes and include changes in biomass production, an increase in conjugation and changes in membrane permeability in *Escherichia coli*²⁸.

Desiccation

Water possesses many properties that seem to make it the essential solvent for life. It has high melting and boiling points with a wide temperature range over which it remains liquid, and a high dielectric

constant important for its solvent action. Water expands near its freezing point, and it forms hydrogen bonds. No other compound possesses all of these traits. Thus, water limitation is an extreme environment. Organisms that can tolerate extreme desiccation enter anhydrobiosis, a state characterized by little intracellular water and no metabolic activity. A variety of organisms can become anhydrobiotic, including bacteria, yeast, fungi, plants, insects, tardigrades, mycophagous nematodes and the shrimp *Artemia salina*^{29–32}.

Mechanisms of death due to anhydrobiosis include irreversible phase changes to lipids, proteins and nucleic acids such as denaturation and structural breakage through Maillard reactions, and accumulation of reactive oxygen species during drying, especially under solar radiation^{33–35}.

Salinity

Organisms live within a range of salinities, from essentially distilled water to saturated salt solutions. Osmophily refers to the osmotic aspects of life at high salt concentrations, especially turgor pressure, cellular dehydration and desiccation. Halophily refers to the ionic requirements for life at high salt concentrations. Although these phenomena are physiologically distinct, they are environmentally linked. Thus, a halophile must cope with osmotic stress. Halophiles include a range of microbes, but some archaea, cyanobacteria and the green alga *Dunaliella salina* can withstand periods in saturated NaCl.

pH

pH is defined as $-\log_{10}[\text{H}^+]$. Biological processes tend to occur towards the middle range of the pH spectrum, and intracellular and environmental pH often fall in this range (for example, the pH of sea water is ~8.2). However, in principle, pH can be high, such as in soda lakes or drying ponds, or as low as 0 ($[\text{H}^+] = 1 \text{ M}$) and below. Proteins denature at exceptionally low pH, which is what happens during the preparation of cerviche, the Latin American seafood dish ‘cooked’ in lime juice.

Acidophiles thrive at low pH (Fig. 4). Fish and cyanobacteria have not been found below pH 4, plants and insects below pH 2–3. Several unicellular eukaryotes do live below pH 1. The best characterized is the red alga *Cyanidium caldarium*³⁶, which has been described from nature at pH as low as 0.5, although its growth optimum in culture is pH 2–3 (ref. 37; Fig. 5). The green alga *Dunaliella acidophila* can also survive pH 0, with a sharp growth maximum at pH 1 (ref. 38). Three fungi, *Acontium cylatium*, *Cephalosporium* sp. and *Trichosporon cerebriae*, grow near pH 0 (ref. 39). Archaea have also been found flourishing under extreme acidity. The aerobic heterotrophs *Picrophilus oshimae* and *Picrophilus torridus* were isolated from Japanese soils permeated with solfataric gases, and had optimal growth at pH 0.7 and 60 °C (ref. 40). *Ferroplasma acidarmanus* has been described growing at pH 0 in acid mine drainage in Iron Mountain in California⁴¹, thriving in a brew of sulphuric acid and high levels of copper, arsenic, cadmium and zinc with only a cell membrane and no cell wall.

Alkaliphiles prefer high pH, which is an equally challenging environment. As with low pH, there is often a difference of 2 or more pH units between the internal and external milieu of the cell. Protons are

scarce, creating energetic hurdles for aerobic prokaryotes with a membrane-bound ATP synthase⁴². Representatives of all domains and kingdoms of eukaryotes are able to tolerate pH as high as ~11 (Fig. 4; refs 43, 44).

Oxygen

The Earth has been anaerobic throughout most of the history of life. Today organisms inhabit environments ranging from strictly anaerobic to aerobic. Aerobic metabolism is far more efficient than anaerobic, but the exploitation of oxygen metabolism has its costs. Oxidative damage resulting from the reduced forms of molecular oxygen, especially the hydroxyl radical, is extremely serious. Oxidative damage has been implicated in an array of health problems from ageing⁴⁵ to cancer⁴⁶, and has a range of consequences in nature (L.J.R., C.L. Wilson, N. Chough and R. I. Donaldson, unpublished results).

Reactive oxygen species are a pervasive threat. There is photochemical production of such species as H_2O_2 by UVA radiation (320–400 nm) within cells⁷, and metabolic production during aerobic metabolism and photosynthesis. Other endogenous sources of reactive oxygen species in eukaryotes include mitochondrial respiration (a significant source of O_2^-), cytochrome P450 metabolism of hydroperoxides (an important source of $^1\text{O}_2$ (singlet oxygen)), production of uric acid, and oxidative bursts used in fighting pathogens in animals and plants. Exogenous sources include the photochemical production of H_2O_2 in aquatic systems⁴⁷, and the production of the hydroxyl radical by ionizing radiation. The presence of oxygen can enhance radiation-induced DNA damage⁷.

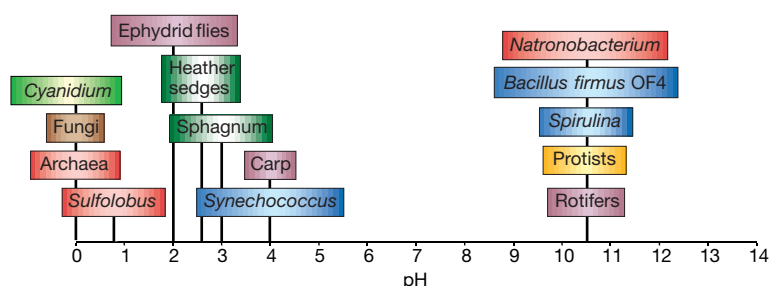
Other extreme conditions

A little creative thinking suggests other physical and chemical extremes not considered here. These include extremes in gas composition (*Cyanidium* grow in media ventilated with pure CO_2 (ref. 48)), redox potential, toxic or xenobiotic (synthetic) compounds, and heavy metal concentration⁴⁹. There are organisms that can live immersed in high levels of organic solvents⁵⁰. The electric eel (*Electrophorus electricus*) can produce, and thus must tolerate, strong electric currents.

How do they do it?

It is critical for an organism to maintain function, and the easiest approach to achieve this is to keep the external environment out. For example, *Cyanidium caldarium* and *Dunaliella acidophila* are found at pH 0.5, yet have near neutral cytoplasm^{38,51}, although this implies that extracellular proteins are acid-tolerant. The next step is to remove the problem as fast as possible. Heavy metal-resistant bacteria use an efflux pump to remove, for example, zinc, copper and cobalt, but not mercury, which is volatilized⁴⁹. If it is impossible to keep the environment out, evolutionary responses entail protective mechanisms, altering physiology or enhancing repair capabilities. Research has focused so far on three key classes of biomolecules: nucleic acids, membrane lipids and proteins. For nucleic acids, function and structure are linked inextricably. DNA is especially vulnerable to high temperature, radiation, oxidative damage and

Figure 4 pH limits for life. Examples of known pH limits for life are shown. Archaea are in red, bacteria in blue, algae in light green, assorted protists in yellow, fungi in brown, plants in dark green and animals in purple.



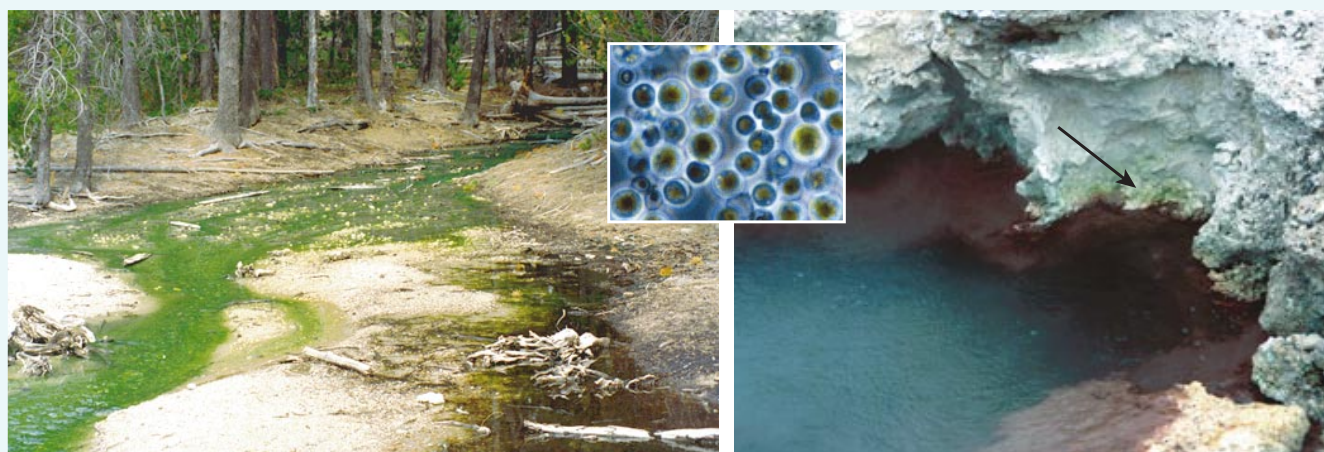


Figure 5 *Cyanidium caldarium*, Norris Geyser Basin, Yellowstone National Park, USA. The red alga *C. caldarium* can grow in the laboratory at a range of pH and temperature, but seems to be a superior competitor in nature at pH 3.3–3.5 and ~42 °C. On the left is Nymph Creek and on the right is Iron Spring. When the steam from Iron Spring cools to ~50 °C, *Cyanidium* can colonize the moist rock.

desiccation. This can lead either to convergence or to multiple ways to solve the problem of living in a particular environment. Understanding the alternatives by using extremophiles on Earth as a sample should help us understand evolutionary processes on Earth, predict them elsewhere, and be useful in commercial exploitation of extremophiles.

High-temperature adaptation

High temperature increases the fluidity of membranes. To maintain optimal membrane fluidity the cell must adjust the composition of the membrane including the amount and type (for example, saturated versus unsaturated) of lipids. Temperature also effects the structure and function of proteins⁵². Ways that proteins have evolved to cope with high temperatures include increasing ion-pair content, forming higher-order oligomers and decreasing flexibility at room temperature. Decreasing the length of surface loops is also known, in particular those loops that connect elements of secondary structure, optimize electrostatic and hydrophobic interactions, and exchange amino acids to increase internal hydrophobicity and helix propensity of residues in α -helices.

DNA at high (>70 °C) temperatures is subject normally to denaturation and chemical modification, yet the DNA of hyperthermophiles such as *Pyrococcus furiosus* is known to be more stable *in vivo* than that of a mesophile such as *Escherichia coli*⁵³. Monovalent and divalent salts enhance the stability of nucleic acids because these salts screen the negative charges of the phosphate groups, and because KCl and MgCl₂ protect the DNA from depurination and hydrolysis⁵⁴.

The G–C pair of nucleic acids is more thermostable than the A–T or A–U pairs because of the additional hydrogen bond⁵⁵. But elevated G + C ratios are not found among thermophilic prokaryotes because of the stability of the chromosomal DNA, although thermostability is correlated with G + C content of their ribosomal and transfer RNAs⁵⁶.

Low temperature

The fluidity of membranes decreases with decreasing temperature. In response, organisms increase the ratio of unsaturated to saturated fatty acids. In addition, the ability to withstand temperatures below freezing relies on two strategies: protection of the cells from ice formation by freezing avoidance, and if ice forms, protection from damage during thawing¹⁷. The proteins used in both processes are misleadingly named ‘antifreeze’ molecules — molecules that actually allow hysteresis to occur. In some terrestrial insects, hysteresis lowers the freezing point of water by 9–18 °C. Freezing of extracellular water during winter protects cells and is known from a small number of frogs, turtles and one snake⁵⁷.

Cold-temperature adaptation of protein occurs, although not always in ways that would be predicted from thermophile enzymes⁵⁸. At low temperatures there are low levels of free energy, so to decrease activation energy an enzyme must have a high degree of conformational complementarity with its substrate⁵⁹. At cold temperatures proteins become more rigid, implying that enhancing flexibility can restore function. Studies of α -amylase from the psychrophile *Alteromonas haloplanctis*, an enzyme with increased reliance of the molecular surface, a less rigid protein core and fewer interdomain interactions than mesophilic counterparts, have supported this hypothesis⁶⁰, as have studies of tubulin structure⁶¹.

Radiation and oxidative damage

Radiation and oxidative damage have always been common on Earth (L.J.R., C. L. Wilson, N. Chough and R. I. Donaldson, unpublished results). Mechanisms to avoid or repair environmentally encountered

Table 1 Classification and examples of extremophiles

Environmental parameter	Type	Definition	Examples
Temperature	Hyperthermophile	Growth >80 °C	<i>Pyrolobus fumarii</i> , 113 °C
	Thermophile	Growth 60–80 °C	<i>Synechococcus lividus</i>
	Mesophile	15–60 °C	<i>Homo sapiens</i>
	Psychrophile	<15 °C	<i>Psychrobacter</i> , some insects
Radiation			<i>Deinococcus radiodurans</i>
Pressure	Barophile	Weight-loving	Unknown
	Piezophile	Pressure-loving	For microbe, 130 MPa
Gravity	Hypergravity	>1g	None known
	Hypogravity	<1g	None known
Vacuum		Tolerates vacuum (space devoid of matter)	Tardigrades, insects, microbes, seeds
Desiccation	Xerophiles	Anhydrobiotic	<i>Artemia salina</i> ; nematodes, microbes, fungi, lichens
Salinity	Halophile	Salt-loving (2–5 M NaCl)	Halobacteriaceae, <i>Dunaliella salina</i>
pH	Alkaliphile	pH > 9	<i>Natronobacterium</i> , <i>Bacillus firmus</i> OF4, <i>Spirulina</i> spp. (all pH 10.5)
	Acidophile	low pH-loving	<i>Cyanidium caldarium</i> , <i>Ferroplasma</i> sp. (both pH 0)
Oxygen tension	Anaerobe	Cannot tolerate O ₂	<i>Methanococcus jannaschii</i>
	Microaerophile	Tolerates some O ₂	<i>Clostridium</i>
	Aerobe	Requires O ₂	<i>H. sapiens</i>
Chemical extremes	Gases		<i>C. caldarium</i> (pure CO ₂)
	Metals	Can tolerate high concentrations of metal (metalotolerant)	<i>Ferroplasma acidamanus</i> (Cu, As, Cd, Zn); <i>Ralstonia</i> sp. CH34 (Zn, Co, Cd, Hg, Pb)

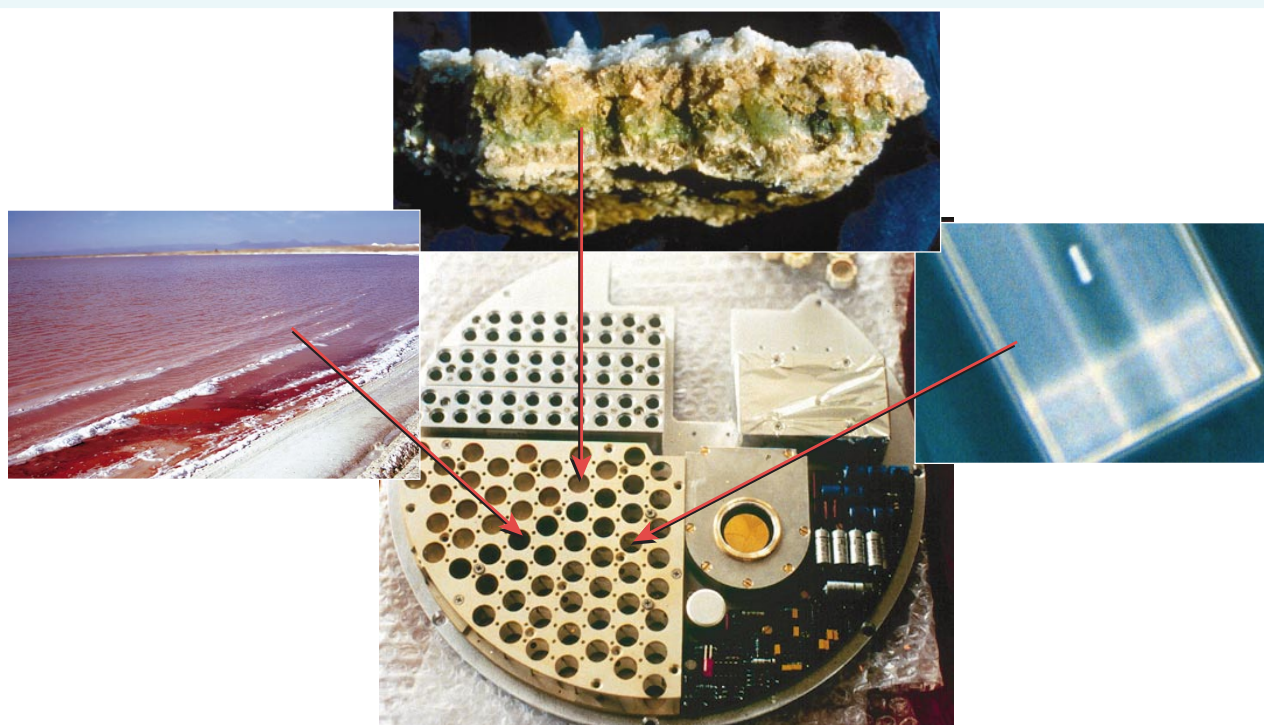


Figure 6 The BioPan halophile experiment. The BioPan facility was used to expose isolates of halophiles to the space environment in Earth's orbit during two two-week missions. Centred around the photograph of the internal sample-containing portion of the BioPan space hardware are, from left to right, a salt evaporation pond that appears red from the red-pigmented archaeal halophiles and some *Dunaliella*, an evaporite containing the cyanobacterium *Synechococcus* (Nägeli) collected from the Pacific marine intertidal zone, and a photomicrograph of a species of the extreme halophile *Haloarcula* in a NaCl crystal.

damage include production of antioxidants and detoxifying enzymes, avoidance behaviour and repair mechanisms⁶². *D. radiodurans* copes with extraordinary radiation levels by containing a unique repair mechanism that involves reassembling of fragmented DNA^{21,63}.

Pressure

Pressure is known to alter gene expression⁶⁴. When pressure increases or temperature decreases, the molecules in lipid membranes pack tighter, resulting in decreased membrane fluidity²⁴. Often organisms circumvent this problem by increasing the proportion of unsaturated fatty acids in their membranes²⁵. Pressure can also help stabilize enzymes²⁴. High pressure can damage DNA and proteins in particular⁶⁵, so survival necessitates avoidance of damage or high repair rates.

Salinity and desiccation

Many microorganisms respond to increases in osmolarity by accumulating osmotica in their cytosol, which protects them from cytoplasmic dehydration and desiccation⁶⁶. With the exception of the Halobacteriaceae, which use K^+ as their osmoticum⁶⁷, glycine betaine is the most effective osmoticum in most prokaryotes⁶⁸.

Osmotic concentration increases during desiccation, so responses are similar to those of a cell in high-salt environments. Compatible solutes such as K^+ , glutamate, glutamine, proline, glycine betaine, sucrose and trehalose accumulate away from proteins, forcing water nearby and thus stabilizing them³², and possibly stabilizing dry membranes⁶⁹. DNA damage is caused by increasing levels of desiccation from vacuum^{70,71}.

pH

Organisms that live at the extremes of pH are able to do so by maintaining their cytoplasm at the same pH as their mesophilic relatives, thus obviating the need for evolution of altered internal physiology. Active mechanisms to achieve this may involve secondary proton uptake mediated by membrane-associated antiporters. Passive mechanisms

include negatively charged cell-wall polymers in alkaliphiles⁴², and unusual bioenergetics, unusual permeability properties, positive surface charges, high internal buffer capacity, overexpression of H^+ export enzymes and unique transporters for acidophiles³⁸.

Examples of extreme environment ecosystems

Hotsprings and geysers

Hotsprings and geysers are characterized by hot water and steam, and sometimes low pH and noxious elements such as mercury. The field was reviewed by Brock⁷², and much recent work⁷³ has been inspired by evolutionary biologists, biotechnology potential and astrobiology.

Deep sea

The deep-sea environment has high pressure and cold temperatures (1–2 °C), except in the vicinity of hydrothermal vents which are underwater geysers. In vents the temperature may be as high as 400 °C (ref. 74), but water remains liquid owing to the high hydrostatic pressure. Hydrothermal vents have a pH range from about 3 to 8 (ref. 75) and unusual chemistry²⁶. In 1977 the submarine *Alvin* found life 2.6 km deep along the East Pacific Rise, a centre of sea-floor spreading. Life forms range from microbes⁷⁶ to invertebrates²⁶.

Hydrothermal vents possibly were critical to evolution. Solution chemistry of hydrothermal vent systems is compatible with prebiotic chemistry leading to the origin of life⁷⁷ (but see ref. 55). Phylogenetic evidence points to thermophiles as the last common ancestor⁷⁸. Either life arose in a vent, or only thermophiles were able to survive the last of the major impacts during the late bombardment period⁷⁹.

Hypersaline environments

Hypersaline environments include salt flats, evaporation ponds, natural lakes (for example, Great Salt Lake) and deep-sea hypersaline basins⁴³. These communities often are dominated by halophilic archaea, including square archaea⁸⁰, or *D. salina*. Other organisms are

Figure 7 Mushroom Spring, Yellowstone National Park, USA, where Tom Brock isolated *Thermus aquaticus*, the organism from which Taq polymerase was obtained.



found at 25–33% salinity, including bacteria⁸¹ (for example, *Ectothiorhodospira halochloris*), cyanobacteria (for example, *Aphanothecce halophytica*, *Phormidium* sp. and *Schizothrix arenaria*), green algae (for example, *D. salina* and *Asteromonas gracilis*), diatoms (for example, *Amphora coffeaeformis* and species of *Navicula* and *Nitzschia*) and protozoa (for example, *Blepharisma halophila* and species of *Bodo*, *Phyllomitus* and *Tetramites*). There are halophilic yeasts and other fungi, but they are not nearly as halophilic as other microbial taxa.

Evaporites

Evaporite deposits consisting primarily of halite (NaCl), gypsum (CaSO₄·2H₂O) or anhydrite (CaSO₄) and containing bacterial and algal assemblages are well known in the fossil record⁸² and are still geographically widespread⁸³. Norton and Grant⁸⁴ showed that microorganisms entrapped in fluid inclusions of growing NaCl crystals may be motile for three weeks, and may remain viable for up to six months. Rothschild and colleagues⁸⁵ demonstrated that microorganisms inhabiting gypsum halite crusts perform carbon and nitrogen fixation while inside the dry crystals of the crust for at least a year. Although highly controversial, bacteria might survive for millions of years in the fluid inclusions of salt deposits including evaporites⁸⁶.

Deserts

Deserts are extremely dry, and cold or hot. Water is always a limiting factor, so such ecosystems are often dominated by microbiotic crusts⁸⁷. The Atacama Desert is one of the oldest, driest hot deserts on Earth⁸⁸. The coldest, driest places on Earth are the dry valleys of Antarctica. The primary inhabitants for both hot and cold deserts are cyanobacteria, algae and fungi that live a few millimetres beneath the sandstone rock surface. Although the endolithic communities in the Antarctic desert are based on photosynthesis (cyanobacteria, lichens and green algae⁸⁹), these microbes have adapted to long periods of darkness and dry conditions interspersed with dustings of dry snow, that upon melting are brief sources of water⁹⁰.

Ice, permafrost and snow

From high-altitude glaciers coloured pink with ‘watermelon’ or ‘blood’ snow (often green algae with photoprotective secondary carotenoids⁹¹) to the polar permafrost, microbial life has used frozen water as a habitat. But two caveats should be noted. First, some ice contains liquid brine inclusions that provide the actual habitat for the microbes⁹². Second, some ice environments such as permafrost contain “a community of survivors”⁹³. It is unlikely that the inhabitants of such an environment actually prefer this environment, rather they have found themselves trapped in the ice and are more

resistant than others that have suffered as similar fate. Microbial communities in sea ice contain algae (mostly diatoms), protozoa, bacteria and some archaea⁹⁴.

Atmosphere

The ability of an organism to survive in the atmosphere is a function of its ability to withstand desiccation and exposure to UV radiation^{95–97}. An airborne biota exists⁹⁸, although it is unclear whether it constitutes a functional ecosystem or is merely a live, but inactive, aerial suspension of organisms and their spore forms⁹⁹. Airborne organisms may travel across the Earth for hundreds to thousands of kilometres^{98,100}, and several kilometres up into the atmosphere¹⁰⁰. We argue that this field of aerobiology is critical to the enterprise of looking for life elsewhere in the Universe and further that it could be important in panspermia. In our view, it is one of the last frontiers of biological exploration on Earth, a view supported by the recent suggestion¹⁰¹ that life could have arisen in aerosols. On the present-day Earth, aerosols contain up to 50% organic material, and can acquire a lipid coating from the water below, meteorite-derived iron and nickel from the stratosphere, and energy from solar radiation — conditions conducive to the origin of life.

Space: new categories of extreme environments

Flight technology has enabled biological studies of space. Four main environments are currently of interest: manned-flight vehicles, interplanetary space (because of the potential for panspermia), and the planet Mars and jovian moon Europa (because of the possibility of liquid water and thus life) (Table 2). Thus, it is urgent that we define the environmental envelope for life, as well as conditions conducive to the origin of life, from hydrothermal to atmospheric¹⁰¹ to hypersaline¹⁰² parameters.

Mars

Mars is, for the most part, frigid (for current temperature, see <http://emma.la.asu.edu/daily.html>). The atmosphere receives 43% as much radiation as Earth, but attenuation through the thin,

Table 2 Physical conditions prevailing in interplanetary space

Parameter	Interplanetary space
Pressure (Pa)	10 ⁻¹⁴
Solar electromagnetic radiation range	All
Cosmic ionizing radiation (Gy yr ⁻¹)	≤0.1
Gravity	<10 ⁻⁶ (varies*)
Temperature (K)	4 (varies*)

*Conditions vary depending on orientation and distance from the Sun.

Table 3 Examples of extremophiles in industry and biotechnology

Industrial process	Biomolecule	Advantages	Source organism
Hydrolysis of starch to produce soluble dextrins, maltodextrins and corn syrups	α -Amylase	High stability, aciduric, bacterial amylase	<i>Bacillus stearothermophilus</i> G-ZYME G995 (Enzyme Bio-System Ltd)
Paper bleaching	Xylanases	Decreases amount of bleach needed	Thermophiles
Prevent stalling in range of baked products	α -Amylase	Gives boost to yeast fermentation	Highest-stability bacterial amylase available, G-ZYME G-995
Food processing, baking, brewing, detergents	Proteases	Stable at high temperatures	Thermophiles
PCR reaction	DNA polymerase	No need to add additional enzyme during each cycle	Thermophiles
Cheese maturation, dairy production	Neutral proteases	Stable at low temperatures	Psychrophiles
Degradation of polymers in detergents	Proteases, amylases, lipases	Improved performance of detergent	Psychrophiles
Degradation of polymers in detergents	Cellulases, proteases, amylases, lipases	Stable at high pH	Alkaliphiles
Mariculture	Polyunsaturated fatty acids	Produced in cold temperatures	Psychrophiles
Bioremediation	Reduction of oil spills	Works efficiently in cold waters	Psychrophiles
Pharmaceuticals	Polyunsaturated fatty acids		Psychrophiles
Biosensors	Dehydrogenases		Psychrophiles
Desulphurication of coal	Sulphur oxidation		Acidophiles
Antibiotic production	Antibiotics		Alkaliphiles
Food colouring	Carotene	Inexpensive to produce	Halophiles/ <i>Dunaliella</i>
Pharmaceuticals	Glycerol, compatible solutes	Inexpensive to produce	Halophiles
Surfactants for pharmaceuticals	Membranes		Halophiles

CO₂-rich atmosphere is minimal, resulting in high surface fluxes of radiation >200 nm. Surface oxidants degrade organic carbon on the surface, which explains the negative results of the 1976 Viking missions¹⁰³. The atmospheric pressure is low (0.6–0.8 kPa), so liquid water is unstable on the surface, although hydrogeological evidence from the Mars Global Surveyor hints that liquid water may even flow today under the surface¹⁰⁴. Attention is now focused on the possibility of a subsurface biota, similar to the deep subsurface¹⁰⁵ or hydrothermal communities found on Earth.

Could life survive on the extreme harsh conditions of the martian surface? There are terrestrial organisms that hypothetically could withstand one or more of the martian extremes, but they would need protection¹⁰⁶. Mancinelli and Klovstad¹⁰⁷ demonstrated that a monolayer of *B. subtilis* spores protected by a 10- μ m-thick dust layer can survive UV exposure for weeks and probably years when exposed to a simulated martian UV-radiation flux. Thus, certain terrestrial microbes might survive on Mars.

Europa

Jupiter's moon Europa may harbour a subsurface water ocean. This putative ocean lies beneath an ice layer too thick to allow photosynthesis. However, Chyba has hypothesized¹⁰⁸ that disequilibrium chemistry in the ocean's ice cover, driven by charged particles accelerated in Jupiter's magnetosphere, could produce sufficient organic and oxidant molecules for a euroman biosphere. Lake Vostok in Antarctica possesses a perennially thick (3 km) ice-cover that precludes photosynthesis below, thus making it a good model

system for determining how a potential euroman biosphere might survive¹⁰⁹.

The space environment

The theory of panspermia, as proposed by Richter¹¹⁰, Lord Kelvin¹¹¹ and Arrhenius¹¹², holds that reproductive bodies of living organisms can exist throughout the Universe and develop wherever the environment is favourable. This implies that conditions favourable to the development of life prevailed at different locations in the Universe and at different times. Major criticisms of panspermia are that living organisms will not survive long exposure to space, and that it avoids the issue of where life began. But results of the Long Duration Exposure Facility and BioPan space experiments, which showed that microbes can survive in space, as well as the fact that organic compounds have been found in meteorites, has led to a re-examination of the feasibility of interplanetary transfer of living material, particularly microbes¹¹³.

Space is extremely cold, subject to unfiltered solar radiation, solar wind, galactic radiation, space vacuum and negligible gravity^{105,114}. At the distance of the Earth from the Sun, solar irradiance is 1,360 W m⁻². Of this, 45% is infrared light, 48% visible and only 7% UV. Space is a nutritional wasteland with respect to water and organic compounds, although comets may provide an oasis when passing a warming star.

Terrestrial organisms most likely to survive these conditions are microbes, with comets or meteorites as conveyance. Microgravity is not lethal; cold tolerance and anhydrobiosis are survivable. Until we understand transit times, we cannot address adequately the nutritional needs of organisms in transit, but we hypothesize that with the exceedingly low metabolic rates that would result from the extremes in cold and desiccation, nutritional needs would not exist. Thus, we are left with two potential 'show-stoppers': radiation and the space vacuum. Heavy ions are mutagenic or lethal to microbes¹¹⁵. Most damage to microbes exposed to space is due to UV radiation, especially during the short term, but heavy ionizing radiation has a greater probability of being lethal.

Remarkably, some terrestrial organisms can survive this highly extreme environment. This has been proven through flight experiments led by the European Space Agency with American participation (Fig. 6). Microbes tested in the space environment and then returned to Earth include *B. subtilis* spores, bacteriophage T-1, tobacco mosaic virus¹¹³, and most recently osmophilic microbes. *B. subtilis* spores will survive for years in space if either in a bilayer (or multilayer) or mixed with glucose to protect them against high solar UV-radiation flux, but if they are exposed in a monolayer they are killed within minutes¹¹³. For comparison, viruses lose viability by weeks. Although the data are controversial, *D. radiodurans* did not survive 7 months in space and the DNA had extensive breakage³⁴. Halophiles can survive for two weeks in space and probably much longer (R.L.M., M. R. Klovstad, P. Rettberg, & G. Horneck, unpublished results). The halophiles are the first example of a vegetative cell surviving exposure to the space environment.

Economic potential of extremophiles

Extremophiles have provided data that are basic to molecular biology, including information on protein folding. Evolutionary biology has benefited on two fronts. First, in the race to uncover the most extreme of extremophiles, whole new taxa have been discovered, increasing phylogenetic enlightenment. Second, the ability to survive in some extreme environments has evolved multiple times, leading to a new understanding of chance versus necessity in evolutionary pathways, especially at the molecular level. For example, the ice-binding antifreeze proteins are evolutionarily convergent, with that of the Antarctic notothenioid fish evolving from a pancreatic trypsinogen-like protease¹¹⁶.

Extremophiles have endeared themselves to multibillion-dollar industries, including agricultural, chemical synthesis, laundry

detergents¹¹⁷ and pharmaceuticals. The European Commission has supported research, training and the commercialization of technology in this area⁶ since 1982. From 1996–1999 it funded the ‘Extremophiles as Cell Factories’ project (see http://www.tutech.de/ecf/ecf1_3.htm), which is now in a phase of industry-sponsored technology transfer to European companies (G. Antranikian, personal communication). Enzymes are sought that are stable and functional in economically preferable environments, such as high or unstable temperatures¹¹⁸ (Table 3).

Enzymes from extremophiles — ‘extremozymes’¹¹⁹ — have potential in multiple areas, either by using the enzymes themselves, or by using them as sources of ideas to modify mesophile-derived enzymes. In most cases the reaction medium is aqueous, although results have indicated that aqueous/organic and nonaqueous media allow the modification of reaction equilibria and enzyme specificity, creating pathways for synthesizing novel compounds¹²⁰. The fastidious growth conditions for extremophiles means that it is often economically advantageous to express the gene in a more tractable host organism such as *E. coli*.

The canonical example of extremophile-derived enzymes in biotechnology is the source of Taq polymerase, the enzyme at the crux of the widely used polymerase chain reaction (PCR). Taq polymerase was isolated from the thermophilic bacterium *Thermus aquaticus*, an organism discovered in 1969 in Yellowstone National Park, Wyoming (ref. 121, Fig. 7). DNA polymerases from other thermophiles have been marketed by Promega Corporation as a product for high-fidelity PCR, with each having its own advantages^{122,123}.

Other extremophiles have industrial applications. For example, some Antarctic bacteria produce polyunsaturated fatty acids, an essential dietary ingredient for many aquaculture species (for example, Atlantic salmon). The bacteria are used to enrich rotifers, a food organism for larval fish¹²⁴. Antarctic bacteria have potential in bioremediation of waters following oil spills, which is a concern in cold waters¹²⁴. *D. salina* is widely used for the commercial production of β-carotenes, which it produces in response to solar radiation, and glycerol, which it produces to counterbalance external osmotic pressure¹²⁵.

Human health may benefit from extremophiles indirectly through biotechnology and bioremediation (Table 3). Direct uses include marketing of dried *Dunaliella* as a nutritional supplement, primarily as an antioxidant. Antifreeze proteins show potential as cryoprotectants of frozen organs.

What next?

Extremophile research is entering an exciting phase. The commercial potential has been recognized, but is far from being realized. Our ignorance of microbial diversity coupled with improvements in exploration and analytical technology suggest that many more discoveries will be forthcoming. The International Space Station will enhance long-term biological studies in space, improving our understanding of the scope of that formerly inaccessible environment. Colonization and terraforming of Mars will require a supporting biota, and where better to start than with extremophiles? And, when life severs its links to planet Earth it will enter new niches ripe for extremophiles, perhaps joining indigenous extraterrestrial extremophiles. □

1. Macelroy, R. D. Some comments on the evolution of extremophiles. *Biosystems* **6**, 74–75 (1974).
2. Madigan, M. T. & Marrs, B. L. Extremophiles. *Sci. Am.* **276**, 82–87 (1997).
3. Horikoshi, K. & Grant, W. D. *Extremophiles. Microbial Life in Extreme Environments* (Wiley-Liss, New York, 1998).
4. Seckbach, J. (ed.) *Journey to Diverse Microbial Worlds: Adaptation to Exotic Environments* (Kluwer, Dordrecht, 2000).
5. Cowan, D. Hot bugs, cold bugs and sushi. *Trends Biotechnol.* **16**, 241–242 (1998).
6. Aguilar, A., Ingemansson, T. & Magnien, E. Extremophile microorganisms as cell factories: support from the European Union. *Extremophiles* **2**, 367–373 (1998).
7. Tyrell, R. M. in *Oxidative Stress: Oxidants and Antioxidants* (ed. Sies, H.) 57–83 (Academic, London, 1991).
8. Newcomb, T. G. & Loeb, L. A. in *DNA Damage and Repair, Vol. 1: DNA Repair in Prokaryotes and Lower Eukaryotes* (eds Nickoloff, J. A. & Hoekstra, M. F.) 65–84 (Humana, Totowa, NJ, 1998).

9. Minton, K. W. DNA repair in the extremely radioresistant bacterium *Deinococcus radiodurans*. *Mol. Microbiol.* **13**, 9–15 (1994).
10. Chow, F. I. & Tan, S. T. Manganese(II) induces cell division and increases in superoxide dismutase and catalase activities in an aging deinococcal culture. *J. Bacteriol.* **172**, 2029–2035 (1990).
11. Venkateswaran, A. et al. Physiologic determinants of radiation resistance in *Deinococcus radiodurans*. *Appl. Environ. Microbiol.* **66**, 2620–2626 (2000).
12. Seki, K. & Toyoshima, M. Preserving tardigrades under pressure. *Nature* **395**, 853–854 (1998).
13. Ball, P. *H₂O. A Biography of Water* (Weldenfeld & Nicolson, London, 1999).
14. Morita, R. Y. Psychrophilic bacteria. *Bacteriol. Rev.* **39**, 144–167 (1975).
15. Blochl, E. et al. *Pyrolobus fumarii*, gen. and sp. nov., represents a novel group of archaea, extending the upper temperature limit for life to 113 °C. *Extremophiles* **1**, 14–21 (1997).
16. Schuliger, J. W., Brown, S. H., Baross, J. A. & Kelly, R. M. Purification and characterization of a novel amyolytic enzyme from ES4, a marine hyperthermophilic archaeum. *Mol. Mar. Biol. Biotech.* **2**, 76–87 (1993).
17. Clarke, A. in *Evolution on Planet Earth: The Impact of the Physical Environment* (eds Rothschild, L. & Lister, A.) (Academic, London, in the press).
18. Kohshima, S. A novel cold-tolerant insect found in a Himalayan glacier. *Nature* **310**, 225 (1984).
19. Franks, F. *Biophysics and Biochemistry at Low Temperatures* (Cambridge Univ. Press, Cambridge, 1985).
20. Wharton, D. A. & Ferns, D. J. Survival of intracellular freezing by the Antarctic nematode *Panagrolaimus davidi*. *J. Exp. Biol.* **198**, 1381–1387 (1995).
21. Battista, J. R. Against all odds: the survival strategies of *Deinococcus radiodurans*. *Annu. Rev. Microbiol.* **51**, 203–224 (1997).
22. Ferreira, A. C. et al. Characterization and radiation resistance of new isolates of *Rubrobacter radiotolerans* and *Rubrobacter xylanophilus*. *Extremophiles* **3**, 235–238 (1999).
23. Ben-Amotz, A. & Avron, M. *Dunaliella bardawil* can survive especially high irradiance levels by the accumulation of β-carotene. *Trends Biotechnol.* **8**, 121–126 (1990).
24. Pledger, R. J., Crump, B. C. & Baross, J. A. A barophilic response by two hyperthermophilic, hydrothermal vent *Archaea*: an upward shift in the optimal temperature and acceleration of growth rate at supra-optimal temperatures by elevated pressure. *FEMS Microbiol. Ecol.* **14**, 233–242 (1994).
25. Bartlett, D. H. & Bidle, K. A. in *Enigmatic Microorganisms and Life in Extreme Environments* (ed. Seckbach, J.) 503–512 (Kluwer, Dordrecht, 1999).
26. Van Dover, C. L. *The Ecology of Deep-Sea Hydrothermal Vents* (Princeton Univ. Press, Princeton, 2000).
27. Kato, C. et al. Extremely barophilic bacteria isolated from the Mariana Trench, Challenger Deep, at a depth of 11,000 meters. *Appl. Environ. Microbiol.* **64**, 1510–1513 (1998).
28. Cogoli, A., Iversen, T. H., Johnsson, A., Mesland, D. & Oser, H. European Space Agency Spec. Publ. No. 1105, 49–64 (1989).
29. Crowe, J. H. Anhydrobiosis: an unsolved problem. *Am. Nat.* **105**, 563–574 (1971).
30. Wright, J. C. Desiccation tolerance and water-retentive mechanisms in tardigrades. *J. Exp. Biol.* **142**, 267–292 (1989).
31. Glasheen, J. S. & Hand, S. C. Anhydrobiosis in embryos of the brine shrimp *Artemia*: characterization of metabolic arrest during reductions in cell-associated water. *J. Exp. Biol.* **135**, 363–389 (1988).
32. Potts, M. Desiccation tolerance of prokaryotes. *Microbiol. Rev.* **58**, 755–805 (1994).
33. Cox, C. S. Roles of water molecules in bacteria and viruses. *Origins Life* **23**, 29–36 (1993).
34. Dose, K. et al. ERA-experiment: space biochemistry. *Adv. Space Res.* **16**(8), 119–129 (1995).
35. Dose, K. & Gill, M. DNA stability and survival of *Bacillus subtilis* spores in extreme dryness. *Origins Life* **25**, 277–293 (1994).
36. Seckbach, J. in *Enigmatic Microorganisms and Life in Extreme Environments* (ed. Seckbach, J.) 427–435 (Kluwer, Dordrecht, 1999).
37. Doemel, W. N. & Brock, T. D. The physiological ecology of *Cyanidium caldarium*. *J. Gen. Microbiol.* **67**, 17–32 (1971).
38. Pick, U. in *Enigmatic Microorganisms and Life in Extreme Environments* (ed. Seckbach, J.) 467–478 (Kluwer, Dordrecht, 1999).
39. Schleper, C., Pühler, G., Kühlmorgen, B. & Zillig, W. Life at extremely low pH. *Nature* **375**, 741–742 (1995).
40. Schleper, C. et al. *Picrophilus* gen. nov., fam. nov.: a novel aerobic, heterotrophic, thermoacidophilic genus and family comprising archaea capable of growth around pH 0. *J. Bacteriol.* **177**, 7050–7059 (1995).
41. Edwards, K. J., Bond, P. L., Gihring, T. M. & Banfield, J. F. An archaeal iron-oxidizing extreme acidophile important in acid mine drainage. *Science* **287**, 1796–1799 (2000).
42. Krulwich, T. A., Ito, M., Hicks, D. B., Gilmour, R. & Guffanti, A. A. pH homeostasis and ATP synthesis: studies of two processes that necessitate inward proton translocation in extremely alkaliphilic *Bacillus* species. *Extremophiles* **2**, 217–222 (1998).
43. Javor, B. *Hypersaline Environments* (Springer, Berlin, 1989).
44. Jones, B. E., Grant, W. D., Duckworth, A. W. & Owenson, G. G. Microbial diversity of soda lakes. *Extremophiles* **2**, 191–200 (1998).
45. Beckman, K. B. & Ames, B. N. The free radical theory of aging matures. *Physiol. Rev.* **78**, 547–581 (1998).
46. Pourzand, C. & Tyrrell, R. M. Apoptosis, the role of oxidative stress and the example of solar UV radiation. *Photochem. Photobiol.* **70**, 380–390 (1999).
47. Cooper, W. & Lean, D. in *Encyclopedia of Earth System Science* Vol. 2 (ed. Nierenber, W. A.) 527–535 (Academic, San Diego, 1992).
48. Seckbach, J., Baker, F. A. & Shugarman, P. M. Algae survive under pure CO₂. *Nature* **227**, 744–745 (1970).
49. Nies, D. H. Heavy metal-resistant bacteria as extremophiles: molecular physiology and biotechnological use of *Ralstonia* sp. CH34. *Extremophiles* **4**, 77–82 (2000).
50. Isken, S. & de Bont, J. A. M. Bacteria tolerant to organic solvents. *Extremophiles* **2**, 229–238 (1998).
51. Beardall, J. & Entwisle, L. Internal pH of the obligate acidophile *Cyanidium caldarium* Geitler (Rhodophyta?). *Phycologia* **23**, 397–399 (1984).
52. Jaenicke, R. Stability and folding of ultrastable proteins: eye lens crystallins and enzymes from thermophiles. *FASEB J.* **10**, 84–92 (1996).
53. Peak, M. J., Robb, F. T. & Peak, J. G. Extreme resistance to thermally induced DNA backbone breaks in the hyperthermophilic archaeon *Pyrococcus furiosus*. *J. Bacteriol.* **177**, 6316–6318 (1995).
54. Marguet, E. & Forterre, P. Protection of DNA by salts against thermodegradation at temperatures typical for hyperthermophiles. *Extremophiles* **2**, 115–122 (1998).

55. Galtier, N., Tourasse, N. & Gouy, M. A nonhyperthermophilic common ancestor to extant life forms. *Science* **283**, 220–221 (1999).
56. Galtier, N. & Lobry, J. R. Relationships between genomic G + C content, secondary structures and optimal growth temperature in prokaryotes. *J. Mol. Evol.* **44**, 632 (1997).
57. Storey, K. B. & Storey, J. M. Natural freezing survival in animals. *Annu. Rev. Ecol. Syst.* **27**, 365–386 (1996).
58. Russell, N. J. Toward a molecular understanding of cold activity of enzymes from psychrophiles. *Extremophiles* **4**, 83–90 (2000).
59. Cummings, S. P. & Black, G. W. Polymer hydrolysis in a cold climate. *Extremophiles* **3**, 81–87 (1999).
60. Aghajari, N., Feller, G., Gerday, C. & Haser, R. Structures of the psychrophilic *Aeromonas haloplantis* α -amylase give insights into cold adaptation at a molecular level. *Structure* **6**, 1503–1516 (1998).
61. Willem, S. *et al.* Protein adaptation to low temperatures: a comparative study of α -tubulin sequences in mesophilic and psychrophilic algae. *Extremophiles* **3**, 221–226 (1999).
62. Rothschild, L. J. in *Enigmatic Microorganisms and Life in Extreme Environments* (ed. Seckbach, J.) 551–562 (Kluwer, Dordrecht, 1999).
63. Battista, J. R. in *DNA Damage and Repair, Vol. I: DNA Repair in Prokaryotes and Lower Eukaryotes* (eds Nickoloff, J. A. & Hoekstra, M. F.) 287–303 (Humana, Totowa, NJ, 1998).
64. Nakasone, K., Ikegami, A., Kato, C., Usami, R. & Horikoshi, K. Mechanisms of gene expression controlled by pressure in deep-sea microorganisms. *Extremophiles* **2**, 149–154 (1998).
65. Abe, F., Kato, C. & Horikoshi, K. Pressure-regulated metabolism in microorganisms. *Trends Microbiol.* **7**, 447–453 (1999).
66. Yancey, P. H., Clark, M. E., Hand, S. C., Bowler, R. D. & Somero, G. N. Living with water stress: evolution of osmolyte systems. *Science* **217**, 1214–1216 (1982).
67. Larsen, H. Biochemical aspects of extreme halophilism. *Adv. Microb. Physiol.* **1**, 97–132 (1967).
68. Le Rudulier, D. & Bouillard, L. Glycine Betaine, an osmotic effector in *Klebsiella pneumoniae* and other members of the Enterobacteriaceae. *Appl. Environ. Microbiol.* **46**, 152–159 (1983).
69. Crowe, J. H., Hoekstra, F. A. & Crowe, L. M. Anhydrobiosis. *Annu. Rev. Physiol.* **54**, 579–599 (1992).
70. Wehner, J. & Horneck, G. Effects of vacuum UV and UVC radiation on dry *E. coli* plasmid pUC19 II. Mutational specificity at the *lacZ* gene. *J. Photochem. Photobiol. B* **30**, 171–177 (1995).
71. Wehner, J. & Horneck, G. Effects of vacuum UV and UVC radiation on dry *E. coli* plasmid pUC19 I. Inactivation, *lacZ* mutation induction and strand breaks. *J. Photochem. Photobiol. B* **28**, 77–85 (1995).
72. Brock, T. D. *Thermophilic Microorganisms and Life at High Temperatures* (Springer, New York, 1978).
73. Reysenbach, A. L., Voytek, M. & Mancinelli, R. L. (eds) *Microbiology of Yellowstone* (Kluwer, New York, in the press).
74. Horikoshi, K. Barophiles: deep-sea microorganisms adapted to an extreme environment. *Curr. Opin. Microbiol.* **1**, 291–295 (1998).
75. Kennish, M. J. (ed.) *Practical Handbook of Marine Science* 2nd edn 236–237 (CRC Press, Boca Raton, 1994).
76. Karl, D. M. (ed.) *The Microbiology of Deep-sea Hydrothermal Vents* (CRC Press, Boca Raton, 1995).
77. Cody, G. D. *et al.* Primordial carbonylated iron-sulfur compounds and the synthesis of pyruvate. *Science* **289**, 1337–1340 (2000).
78. Pace, N. A molecular view of microbial diversity and the biosphere. *Science* **276**, 734–740 (1997).
79. Sleep, N. H., Zahnle, K. J., Kasting, J. F. & Morowitz, H. J. Annihilation of ecosystems by large impacts on early earth. *Nature* **342**, 139–142 (1989).
80. Oren, A. in *Enigmatic Microorganisms and Life in Extreme Environments* (ed. Seckbach, J.) 339–355 (Kluwer, Dordrecht, 1999).
81. Kamekura, M. Diversity of extremely halophilic bacteria. *Extremophiles* **2**, 289–295 (1998).
82. Bell, C. M. Saline lake carbonates within an Upper Jurassic–Lower Cretaceous continental red bed sequence in the Atacama region of northern Chile. *Sedimentology* **36**, 651–664 (1989).
83. Castanier, S., Perthuisot, J.-P., Rouchy, J.-M., Maurin, A. & Guelorget, O. Halite ooids in Lake Asal Djibouti biocrystalline build-ups. *Geobios (Lyon)* **25**, 811–821 (1992).
84. Norton, C. F. & Grant, W. D. Survival of halobacteria within fluid inclusions in salt crystals. *J. Gen. Microbiol.* **134**, 1365–1373 (1988).
85. Rothschild, L. J., Giver, L. J., White, M. R. & Mancinelli, R. L. Metabolic activity of microorganisms in gypsum-halite crusts. *J. Phycol.* **30**, 431–438 (1994).
86. Vreeland, R. H., Rosenzweig, W. D. & Powers, D. W. Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal. *Nature* **407**, 897–900 (2000).
87. Evans, R. D. & Johansen, J. R. Microbiotic crusts and ecosystem processes. *Crit. Rev. Plant Sci.* **18**, 182–225 (1999).
88. Rundel, P. W. *et al.* The phytogeography and ecology of the coastal Atacama and Peruvian deserts. *ALISO* **13**, 1–49 (1991).
89. van Thielen, N. & Garbary, D. J. in *Enigmatic Microorganisms and Life in Extreme Environments* (ed. Seckbach, J.) 245–253 (Kluwer, Dordrecht, 1999).
90. Friedmann, E. I. Endolithic microorganisms in the Antarctic cold desert. *Science* **215**, 1045–1053 (1982).
91. Bidigare, R. R. *et al.* Evidence for a photoprotective function for secondary carotenoids of snow algae. *J. Phycol.* **29**, 427–434 (1993).
92. Junge, K., Krembs, C., Deming, J., Stierle, A. & Eicken, H. A microscopic approach to investigate bacteria under in-situ conditions in sea-ice samples. *Ann. Glaciol.* (in the press).
93. Friedmann, E. I. *Viable Microorganisms in Permafrost* (ed. Gilichinsky, D. A.) 21–26 (Institute of Soil Science and Photosynthesis, Russian Academy of Science, Pushchino, 1994); cited in Vishnivetskaya, T., Kathariou, S., McGrath, J., Gilichinsky, D. & Tiedje, J. M. Low-temperature recovery strategies for the isolation of bacteria from ancient permafrost sediments. *Extremophiles* **4**, 165–173 (2000).
94. Staley, J. T. & Gosink, J. J. Poles apart: biodiversity and biogeography of sea ice bacteria. *Annu. Rev. Microbiol.* **53**, 189–215 (1999).
95. Mancinelli, R. L. & Shulls, W. A. Airborne bacteria in an urban environment. *Appl. Environ. Microbiol.* **35**, 1095–1101 (1978).
96. Cox, C. S. *Roles of Maillard Reactions in Diseases* (HMSO, London, 1991).
97. Israeli, E., Gitelman, J. & Lighthart, B. in *Atmospheric Microbial Aerosol Theory and Application* (eds Lighthart, B. & Mohr, A. J.) 166–192 (Chapman & Hall, New York, 1994).
98. Cox, C. S. *The Aerobiological Pathway of Microorganisms* (Wiley, New York, 1987).
99. Cox, C. S. & Wathes, C. M. *Bioaerosols Handbook* (Lewis, London, 1995).
100. Lighthart, B. & Mohr, A. J. (eds) *Atmospheric Microbial Aerosol Theory and Application* 68–98, 166–192 (Chapman & Hall, New York, 1994).
101. Marchant, J. Life from the skies—did droplets high in the atmosphere give birth to the first living cells? *New Sci.* **2247**, 4–5 (15 July 2000).
102. Dundas, I. Was the environment for primordial life hypersaline? *Extremophiles* **2**, 375–377 (1998).
103. Biemann, K. *et al.* The search for organic substances and inorganic volatile compounds in the surface of Mars. *J. Geophys. Res.* **82**, 4641–4658 (1977).
104. Malin, M. C. & Edgett, K. S. Evidence for recent groundwater seepage and surface runoff on Mars. *Science* **288**, 2330–2335 (2000).
105. Horneck, G. in *Evolution on Planet Earth: The Impact of the Physical Environment* (eds Rothschild, L. & Lister, A.) (Academic, London, in the press).
106. Rothschild, L. J. Earth analogs for Martian life. Microbes in evaporites, a new model system for life on Mars. *Icarus* **88**, 246–260 (1990).
107. Mancinelli, R. L. & Klovstad, M. Survival of *Bacillus subtilis* spores on space craft surfaces. *Planet. Space Sci.* **48**, 1093–1097 (2000).
108. Chyba, C. Energy for microbial life on Europa. *Nature* **403**, 381–382 (2000).
109. Stone, R. Permafrost comes alive for Siberian researchers. *Science* **286**, 36–37 (1999).
110. Richter, H. *Zur Darwinischen Lehre. Schmidts Jahrb. Ges. Med.* **126**, 243–249 (1865).
111. Thomson, W. in *Popular Lectures and Addresses* 132–205 (Macmillan, New York, 1894).
112. Arrhenius, S. Die Verbreitung des Lebens im Weltraum. *Umschau* **7**, 481–485 (1903).
113. Nicholson, W. L., Munakata, N., Horneck, G., Melosh, H. J. & Setlow, P. Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiol. Mol. Biol. Rev.* **64**, 548–572 (2000).
114. Rothschild, L. J. in *Evolution on Planet Earth: The Impact of the Physical Environment* (eds Rothschild, L. & Lister, A.) (Academic, London, in the press).
115. Horneck, G., Krasavin, E. A. & Kozubek, S. Mutagenic effects of heavy ions in bacteria. *Adv. Space Res.* **14**(10), 315–329 (1994).
116. Cheng, C.-H. C. & Chen, L. Evolution of an antifreeze glycoprotein. *Nature* **401**, 463–464 (1999).
117. Ito, S. *et al.* Alkaline detergent enzymes from alkaliphiles: enzymatic properties, genetics, and structures. *Extremophiles* **2**, 185–190 (1998).
118. Zeikus, J. G., Vielle, C. & Savchenko, A. Thermozymes: biotechnology and structure-function relationships. *Extremophiles* **2**, 179–182 (1998).
119. Hough, D. W. & Danson, M. J. Extremozymes. *Curr. Opin. Chem. Biol.* **3**, 39–46 (1999).
120. Sellek, G. A. & Chaudhuri, J. B. Biocatalysis in organic media using enzymes from extremophiles. *Enzyme Microb. Technol.* **25**, 471–482 (1999).
121. Brock, T. D. & Freeze, H. *Thermus aquaticus* gen. n., a nonsporulating extreme thermophile. *J. Bacteriol.* **98**, 289–297 (1969).
122. Mattila, P., Korpela, J., Tenkanen, T. & Pitkänen, K. Fidelity of DNA synthesis by the *Thermococcus litoralis* DNA polymerase—an extremely heat stable enzyme with proofreading activity. *Nucleic Acids Res.* **19**, 4967–4973 (1991).
123. Cariello, N. F., Swenberg, J. A. & Skopek, T. R. Fidelity of *Thermococcus litoralis* DNA polymerase (Vent) in PCR determined by denaturing gradient gel electrophoresis. *Nucleic Acids Res.* **19**, 4193–4198 (1991).
124. Nichols, D. *et al.* Developments with Antarctic microorganisms: culture collections, bioactivity screening, taxonomy, PUFA production and cold-adapted enzymes. *Curr. Opin. Biotechnol.* **10**, 240–246 (1999).
125. Ben-Amotz, A. in *Enigmatic Microorganisms and Life in Extreme Environments* (ed. Seckbach, J.) 401–410 (Kluwer, Dordrecht, 1999).

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