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Extremophiles and the Search for Extraterrestrial Life

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ABSTRACT

Extremophiles thrive in ice, boiling water, acid, the water core of nuclear reactors, salt crystals, and toxic waste and in a range of other extreme habitats that were previously thought to be inhospitable for life. Extremophiles include representatives of all three domains (Bacteria, Archaea, and Eucarya); however, the majority are microorganisms, and a high proportion of these are Archaea. Knowledge of extremophile habitats is expanding the number and types of extraterrestrial locations that may be targeted for exploration. In addition, contemporary biological studies are being fueled by the increasing availability of genome sequences and associated functional studies of extremophiles. This is leading to the identification of new biomarkers, an accurate assessment of cellular evolution, insight into the ability of microorganisms to survive in meteorites and during periods of global extinction, and knowledge of how to process and examine environmental samples to detect viable life forms. This paper evaluates extremophiles and extreme environments in the context of astrobiology and the search for extraterrestrial life. Key Words: Extremophile—Microorganism—Genomics—Methanogen—Lake Vostok—Psychrophile—Hyperthermophile—Extraterrestrial. Astrobiology 2, 281–292.

DEFINITION AND DESCRIPTION OF THE FIELD

EXTREMOPHILES ARE ORGANISMS that not only survive, but thrive under extreme conditions. This contrasts with an organism that may tolerate and survive extreme conditions, but grows optimally under less extreme conditions. The term "extreme" is anthropocentrically derived, thereby providing a significant scope for what may be considered extreme. On Earth, there exists a truly remarkable diversity of extreme environments that are capable of supporting life. The aim of this paper is not just to overview extreme environments and their inhabitants, but to discuss specific examples that may provide clues about extraterrestrial life. It also incorporates a discussion on the use of genomics to study the biology of extremophiles and illustrates how this understanding is likely to provide important insight into the search for extraterrestrial life. Numerous comprehensive reviews of specific extreme environments can be found in the literature. A number of reviews and books with a broad coverage of extremophiles are also available (Madigan *et al.*, 1997; Atlas and Bartha, 1998; Gross, 1998; Horikoshi and Grant, 1998; Cavicchioli and Thomas, 2000; Rothschild and Mancinelli, 2001). In addition, a compilation of articles describing extremophiles will be published in the UNESCO Encyclopedia of Life Support Systems (http://www.eolss.com).

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EXTREME ENVIRONMENTS AND EXTREMOPHILES

Examples of extreme environments and resident extremophiles are provided in Table 1. High-temperature environments are most often associated with being extreme. Terrestrial surface environments include hot springs that are close to neutral pH, or acidic and sulfurous, or rich in iron. Hot subterranean areas are diverse and range from volcanically heated environments to those, such as the Great Artesian Basin in Australia, that are heated by virtue of their depth. Submarine environments include volcanic and hydrothermal vents. The latter are often described as black smokers owing to the precipitation of minerals when hot, mineral-rich volcanic fluids come into contact with cold ocean waters ($<5°C$). The present record for high-temperature growth is held by the archaeon *Pyrolobus fumarii,* which can grow in the laboratory at 113°C and is restricted to temperatures of $\geq 85°C$ (Blochl *et al.,* 1997). It is likely, however, that microorganisms capable of growth at higher temperatures will be isolated as growth has been reported on a glass slide placed in superheated water at 125–140°C inside a fumarole (Madigan *et al.,* 1997). Numerous examples of acidophilic thermophiles exist, including species of *Acidianus* [optimum pH (pH_{opt}) 2; optimum temperature (T_{opt}) 90°C] and *Sulfolobus* (pH_{opt} 2.5; T_{opt} 80°C) (Stetter, 1996). The most extreme examples of thermophilic, acid-loving extremophiles are two species of *Picrophilus* that were isolated from volcanically heated, dry soils in Japan. Not only do these Archaea tolerate these conditions, but they have optimal growth at pH 0.7 (1.2 *M* H_2SO_4) and 60°C (Schleper *et al.,* 1995). In contrast to highly acidic environments, alkaliphiles have optimal growth at pH values >10. They are often isolated from natural environments that also tend to have high concentrations of salt, and as a consequence they may be halophilic. A typical soda lake may be pH 11 and have concentrations of Na, Cl, SO_4^{2-}, and HCO_3^- of 140, 150, 20, and 70 gL^{-1}, respectively (Cavicchioli and Thomas, 2000). Members of many microbial groups have been isolated from these environments, including photosynthetic cyanobacteria and halophilic Archaea.

An interesting example of opportunism to an artificial extreme environment is the remarkably radiation-resistant bacterium *Deinococcus radiodurans,* which is able to grow in the water core of nuclear reactors. Such is the extent of growth in nuclear reactors that biocides are added to inhibit microbial growth. *Deinococcus* does not prevent radiation-induced damage to its DNA, but instead appears to have a novel DNA repair system that enables it to perform interchromosomal DNA recombination to reform a functional genome (Battista, 1997). The natural selection pressure for the evolution of this DNA-repair system is thought to be desiccation. Dehydration leads to DNA hydrolysis in a similar way to radiation damage. *Deinococcus* is a natural inhabitant of desert environments on Earth and survives by repairing DNA damage during rehydration. Artificial environments, such as radiation and toxic chemical waste dumps, also provide intense selection pressure for extremophiles. A *Rhodococcus* species isolated from a contaminated site at a chemical plant was shown not only to survive in water saturated with benzene, but to be capable of using benzene as a sole source of carbon for growth (Paje *et al.,* 1997). The abilities to tolerate and degrade toxic compounds and resist high levels of radiation have been combined in a genetically engineered strain of *D. radiodurans* (Lange *et al.,* 1998). The capacity of extremophiles to survive high levels of radiation [particularly UV (Rothschild and Cockell, 1999)] and transform toxic compounds has prompted discussion about their potential role in terraforming other planets (Slotnick, 2000).

The methanogens are a class of microorganism often associated with extreme environments. Methanogenic Archaea are the only microorganisms presently known to be able to use a variety of inorganic or organic carbon compounds for growth, and produce methane as an end product. They grow strictly anaerobically, and various members can tolerate a broad range of salt concentrations. They also span the largest thermal extremes of any class of microorganism, from up to 110°C (*Methanopyrus kandleri*) to below 0°C (*Methanogenium frigidum*). Despite their requirement for anaerobic conditions they are ubiquitous on Earth and are evidently extremely active. This is illustrated by the fact that the bulk of methane in the atmosphere is generated by methanogens and not by abiotic processes (Madigan *et al.,* 1997). Their abilities to grow without the need for an organic source of carbon or nitrogen and to colonize environments encompassing a vast

TABLE 1. EXAMPLES OF EXTREMOPHILES AND EXTREME ENVIRONMENTS

Class of extreme	*Environment*	*Organism*[1]	*Defining growth condition*	*Reference*
High-temperature growth (hyperthermophile)	Submarine vent, terrestrial hot spring	*P. fumarii* (A)	T_{max} 113°C	Blochl *et al*. (1997)
High-temperature survival	Soil, growth media contaminant	*Moorella thermoaceticum* (spore) (B)	2 h, 121°C, 15 psi	Bryer *et al*. (2000)
Cold temperature (psychrophile)	Snow, lakewater, sediment, ice	Numerous [e.g., *Vibrio, Arthrobacter, Pseudomonas* (B), and *Methanogenium* (A) spp.]	−17°C	Carpenter *et al.* (2000); Cavicchioli and Thomas (2000)
High acid (acidophile)	Dry solfataric soil	*Picrophilus oshimae/torridus* (A)	pH_{opt} 0.7 (1.2 *M* H_2SO_4)	Schleper *et al.* (1995); Johnson (1998)
High salt (halophile)	Saline lakes, evaporation ponds, salted foods	Mainly archaeal halophiles (e.g., *Halobacterium* spp. and *Halorubrum* spp.)	Saturated salt (up to 5.2 *M*)	Grant *et al*. (1998)
High alkaline (alkaliphile)	Soda lakes	*Bacillus* spp., *Clostridium paradoxum* (B), *Halorubrum* species (A)	$pH_{opt} > 10$	Jones *et al*. (1998)
Radiation (radiation-tolerant)	Soil, nuclear reactor water core, submarine vent	*D. radiodurans, Rubrobacter* spp., *Kineococcus* sp. (B), *Pyrococcus furiosus* (A)	High, γ, UV, x-ray radiation (e.g. >5,000 Gy γ radiation and >400 J m^2 UV)	Battista (1997); Di Ruggerio *et al.* (1997); Ferreira *et al*. (1999)
Toxicity (toxitolerant)	Toxic waste sites, industrial sites; organic solutions and heavy metals	Numerous [e.g., *Rhodococcus* sp. (B)]	Substance-specific (e.g., benzene-saturated water)	Isken and de Bont (1996)
High pressure (barophile or piezophile)	Deep sea	Various [e.g., *Photobacterium* sp. (B), *Pyrococcus* sp. (A)]	Deep open ocean or submarine vent (e.g., pressure in Mariana Trench is 1.1 tons cm^{-2})	Horikoshi (1998)
Low nutrients (oligotroph)	Pelagic and deep ocean, alpine and Antarctic lakes, various soils	*S. alaskensis, Caulobacter* spp. (B)	Growth with low concentrations of nutrients (e.g., <1 mg L^{-1} dissolved organic carbon) and inhibited by high concentrations	Schut *et al*. (1997)
Low water activity (xerophile)	Rock surfaces (poikilohydrous), hypersaline, organic fluids (e.g., oils)	Particularly fungi (e.g., *Xeromyces bisporus*) and Archaea (e.g., *Halobacterium* sp.)	Water activity (a_w) <0.96 (e.g., *X. bisporus* 0.6 and *Halobacterium* 0.75)	Atlas and Bartha (1998)
Rock-dwelling (endolith)	Upper subsurface to deep subterranean	Various [e.g., *Methanobacterium subterranean* (A), *Pseudomonas* sp. (B)]	Resident in rock	Atlas and Bartha (1998)

[1]A, Archaea; B, Bacteria.

range of thermal extremes and their lack of a requirement for oxygen or light provide them with the capacity to live in a wide range of organically deprived environments.

EXTREME SURVIVAL AND SPACE TRAVEL

The ability of microorganisms to live within rocks and generate a subterranean biosphere (Pedersen, 1993, 1997; Cunningham *et al.*, 1995) provides further evidence that life can exist without light or the input of external nutrients. It also provides a rationale for why microorganisms may be resident in rock samples, including meteorites. Moreover, it provides a mechanism for life to have survived during cataclysmic events brought about by large-scale planetary impacts capable of sterilizing the Earth's surface. A number of these extremophiles have been isolated, although they are not well studied. They include nitrate-reducing, sulfide-oxidizing (Gevertz *et al.*, 2000), sulfate- and iron-reducing (Nilsen *et al.*, 1996; Haveman *et al.*, 1999), iron-oxidizing (Ferris *et al.*, 1999), heterotrophic (Adkins *et al.*, 1993; Ferris *et al.*, 1999), and methanogenic (Kotelnikova and Pedersen, 1997; Kotelnikova *et al.*, 1998) microorganisms that grow at cold (Vorobyova *et al.*, 1997; Rashid *et al.*, 1999) or high (Lharidon *et al.*, 1995; Huber *et al.*, 2000) temperatures. In addition, evidence for possible fossil remains of such microorganisms has been described (Hofmann and Farmer, 2000).

The growth characteristics of the hyperthermophilic chemolithoautotrophic Archaea, such as *Methanococcus jannaschii*, have led to speculation that life may have originated on Earth by the "inoculation" and subsequent colonization of the ocean and subsurfaces by extraterrestrial microorganisms (Morell, 1996; Davies, 1998). This is referred to as panspermia and may have derived from concepts developed by Arrhenius in the early 1900s (Arrhenius, 1908). Support for this concept comes from putative fossil evidence of microorganisms and associated biological markers in meteorites from Mars such as ALH84001 (McKay *et al.*, 1996), where the interior temperature of ALH84001 has not exceeded 40°C (Weiss *et al.*, 2000). Meteorites would also provide protection from the damaging effects of UV light. Furthermore, a significant number of meteorites may be able to travel between planets in <10 years (Weiss *et al.*, 2000). Even if the travel time was extended, there is evidence to indicate the ability of microorganisms to survive for millions of years without growing. The supporting data include the resuscitation of *Bacillus* spores after preservation in amber for 25–40 million years (Cano and Borucki, 1995) and in brine inclusions within salt crystals for 250 million years (Stan-Lotter *et al.*, 1999; Vreeland *et al.*, 2000). In addition to these factors, there is evidence that microorganisms could withstand the forces generated from an impact sufficiently large to produce meteorite-sized rocks and eject them into space (Melosh, 1993; Mastrapa *et al.*, 2001). These findings imply that the panspermia hypothesis is plausible and provides impetus for the continued search for evidence of biological markers in meteorites.

COLD ENVIRONMENTS AND EXTRATERRESTRIAL LIFE

While there are arguments that the last common ancestor to life on Earth was thermophilic and that extant hyperthermophiles retain properties of the last common ancestor (Stetter, 1996), it is also argued that life may have originated in cold environments (Levy and Miller, 1998; Levy *et al.*, 2000). In addition to a potential role in the origin of life, cold-adapted microorganisms may provide insight into the search for extraterrestrial life on Mars and moons such as Europa (Blamont, 2000). The surface of Mars is cold, and life forms surviving, or multiplying in or near the surface, would need to be cold-adapted. Recently, the Labelled Release experiments performed aboard the Viking spacecraft in 1976 have been reassessed to include the possibility that they may have demonstrated biological activity in the soil samples (Paine, 2001). The potential of the soil to support life was further demonstrated in a recent preliminary report (http://www.spaceflightnow.com/news/n0105/27marsorg), where methanogens were grown in a liquid medium formed by dissolving Mars soil simulant in water. An even more provoking possibility for discovering extant extraterrestrial life is the possibility of subsurface water existing on Europa (Carr *et al.*, 1998; Hiscox, 1999; Chyba and Phillips, 2001). Subsurface lakes, even if they receive no light energy, may be able to support lithoautotrophic biological processes (McCollom, 1999). It is clear from studies of polar, alpine, and deep ocean ecosystems that microbial life proliferates in cold environments (Cavicchioli and Thomas, 2000; Cavicchioli *et al.,* 2000a), and natural microbial metabolism has been measured at temperatures of at least −17°C (Carpenter *et al.,* 2000). In the Vestfold Hills region of Antarctica, a unique ecosystem is preserved that contains numerous lakes ranging in salinity from freshwater to up to eight times that of seawater, in temperature from up to 20°C to below −10°C, and in oxygen content from aerobic to strictly anaerobic (McMeekin *et al.,* 1993). The lakes also vary in nutrient and solute level from highly ionic to extremely oligotrophic. A variety of microorganisms have been isolated and characterized (McMeekin *et al.,* 1993; Franzmann, 1996; Franzmann *et al.,* 1997b), and 16S rRNA community analyses have been performed (Bowman *et al.,* 2000a,b). The lakes are also the source of the only free-living, cold-adapted Archaea characterized (Franzmann *et al.,* 1988, 1992, 1997a). Of particular note are the cold-adapted methanogens, *Methanococcoides burtonii* (Franzmann *et al.,* 1992) and *Methanococcoides frigidum* (Franzmann *et al.,* 1997a). While *M. burtonii* utilizes organic forms of carbon (methylamines and methanol), *M. frigidum* derives its energy, carbon, and nitrogen from inorganic sources. In addition, *M. frigidum* is truly adapted to growth in the cold, displaying a theoretical growth temperature minimum of −10°C and a maximum growth temperature of 18°C.

The Antarctic subsurface lake system is an environment that is even more closely linked to what may be present on Europa. More than 70 lakes have been identified (Dowdeswell and Siegert, 1999; Siegert, 2000). The largest and most comprehensively studied is Lake Vostok. It is 230 km long and up to 50 km wide (Dowdeswell and Siegert, 1999; Jouzel *et al.,* 1999) and is 1,000 m deep in places (Gibbs, 2001), giving it a volume in excess of that of Lake Ontario in North America. There are predictions that life in Lake Vostok may have originated 30 million years ago (Duxbury *et al.,* 2001) and that microorganisms in 400,000-year-old ice may be maintained by growing in liquid veins that surround ice crystals (Price, 2000). However, the most compelling evidence that the upper ice mass (and presumably lake water) maintains viable and possibly growing microorganisms is derived from studies of ice cores drilled from the Antarctic surface through to the lake's accretion ice 3,623 m down. Based on the sampling depth, drill cores have revealed 3,000-year-old viable yeasts and actinomycetes, 38,600-year-old mycelial fungi, 110,000-year-old unicellular algae, 180,000-year-old diatom, and 200,000-year-old spore-forming Bacteria (Ellis-Evans and Wynn-Williams, 1996). From the deepest core samples (~120 m above the lake surface) four genera of Bacteria were identified by 16S rRNA sequencing of community DNA (Priscu *et al.,* 1999), and microbial respiration was indicated by the conversion of radiolabeled substrates in accretion ice samples where cells numbers were on the order of 10^2 cells mL^{-1} (Karl *et al.,* 1999). Recently, members of the bacterial genera *Sphingomonas, Methylobacterium, Brachybacteria,* and *Paenibacillus* were cultivated from accretion ice 3,593 m below the glacial ice surface (Christner *et al.,* 2001), thereby demonstrating the ability of microorganisms to survive and recover after periods in ice of ≥400,000 years. The importance of Lake Vostok (and other Antarctic subsurface lakes) as analogues for Europa and Mars is well recognized (Wynn-Williams and Edwards, 2000; Duxbury *et al.,* 2001), and there is presently a high level of consideration being directed at how to penetrate into the pristine environments, and what measurements to employ in the search for life (Newton *et al.,* 2000; Price, 2000; Wynn-Williams and Edwards, 2000; Blake *et al.,* 2001; Chyba and Phillips, 2001).

METAZOAN EXTREMOPHILES

While it is clear that most extremophiles are microorganisms, it is noteworthy that a number of metazoans (multicellular eukaryotes) can survive in extreme environments, and a few may be considered extremophiles. This implies that the search for extraterrestrial life should not be restricted to microorganisms. The Pompeii worm (*Alvinella pompejana*) is a polychaetous annelid that lives at deep-sea vents of the East Pacific Rise where it may be exposed to a highly variable mixture of vent (350°C, anoxic, acidic, and rich in CO_2 and metal sulfides) and deep-sea (2°C, mildly hypoxic) waters (Desbruyeres *et al.,* 1998). It has been reported that its posterior may be exposed to temperatures of 81°C (Cary *et al.,* 1998) with brief periods of exposure >100°C (Chevaldonne *et al.,* 1992). However, the delicate and intricate nature of the worm tubes and the technical difficulty associated with monitoring temperatures have led to a more cautious appraisal of maximum worm body temperatures of 55°C (Chevaldonne *et al.,* 2000). The recent interest in the deep-sea worms has led to the discovery of a unique composition of zinc–iron sulfide crystals within the exoskeleton of the Pompeii worm (Zbinden *et al.,* 2001). It has been proposed that these biologically produced minerals may be useful as biomarkers in fossilized paleohydrothermal vent systems.

Perhaps the best example of metazoans that can survive extreme conditions are the hydrophilous micrometazoans, also known as tardigrades. They represent a separate phylum that is related to arthropods (Nelson and Marley, 2000). More than 800 species have been described from marine, freshwater, and terrestrial habitats. One class of tardigrade is able to form a stress-resistant "tun" state. They have been recorded to survive 120 years of desiccation, 6,000 atm pressure, temperatures as low as −272°C and as high as 151°C, and x-ray bombardment 1,000 times greater than a level sufficient to kill a human, and have been shown to revive after being photographed using an electron microscope [Copley, 1999 (see also http://www.tardigrades.com); Rothschild and Mancinelli, 2001]. Their resistance relates in part to the ability to stop metabolism and replace intracellular water with trehalose, thereby preserving cellular integrity and the ability to resume growth when suitable conditions are encountered. These abilities have led to speculation that tardigrades may survive transport through outer space (Copley, 1999).

SEARCHING FOR EXTRATERRESTRIALS

What can be learned about extraterrestrial life from studying extant life on Earth? The ability of earthly microorganisms to colonize extreme environments expands the range of extraterrestrial bodies that may be candidates for extant life, or that may have harbored life in the past and therefore may retain fossil records. The discovery of Lake Vostok provided a fresh perspective on cold, oligotrophic environments with important implications for equivalent environments on Jovian moons such as Europa. The discoveries of hyperthermophiles in volcanically heated hot springs has provided a focus for candidate locations on Mars (Gulick, 2001). Recently, new types of hydrothermal vents were discovered on the seafloor in north Iceland (Marteinsson *et al.,* 2001). Unlike most marine hydrothermal systems, which are acidic and salty as a result of the discharged fluids originating from seawater seepage interacting with heated magma, these giant geothermal cones emit freshwater with a pH of 10 and temperature up to 72°C. While it is unclear from where the freshwater and indigenous microorganisms originate, they are likely to have traveled at least 1.8 km from land. These kinds of discoveries will continue to expand the possible locations that may be investigated in the search for extraterrestrial life.

When contemplating where extraterrestrial life may be found, it is clearly useful to consider which environments support life on Earth. However, the extremophiles themselves provide additional levels of information that cannot be obtained simply from physical studies of the environments. Studying the growth and survival of extremophiles will generate critical insight into developing methods for resuscitating and culturing extraterrestrial life forms when (if) they are located. Knowledge of mechanisms of survival in extremophiles will also assist the understanding of how extraterrestrial life may survive space travel (e.g., in meteorites). Biological studies will also provide important information about potential biomarkers. The importance of this latter point is highlighted by the controversy surrounding nanobes and other structures that resemble extant microorganisms and that have been found in subterranean rocks 3,400–5,100 m below the sea bed (Uwins *et al.,* 1998), in the Martian meteorite ALH84001 (McKay *et al.,* 1996), from human kidney stones (Kajander and Ciftcioglu, 1998), and in a range of other environments (Folk, 1999). The claims have prompted intense debate, which has led to a search for alternative explanations to the findings (Sears and Kral, 1998; Abbott, 1999; Kirland et al., 1999; Cisar *et al.,* 2000; Zolotov and Shock, 2000) and a discussion of the minimum size of a structure that may support life (Maniloff *et al.,* 1997; Knoll and Osborne, 1999; Cavicchioli and Ostrowski, 2002). In particular, the demonstration that mixtures of inorganic compounds may form structures that resemble the size and shape of microorganisms means that the presence of microorganisms in a sample must be substantiated by more than morphological evidence (García-Ruiz *et al.,* 2002). While this has important implications for the interpretation of fossils records such as those of the 3.5-billion-year-old cyanobacteria-like fossils (Schopf, 1993), it is also relevant to the search for viable extraterrestrial life.

Biomarkers may be biological remnants such as the presence and type of lipid remnants (Summons and Walter, 1990), the chirality of amino acids and sugars, and carbon isotope ratios (Sumner, 2001). However, it is also important to consider what responses might be expected from a microorganism when a sample is tested for the presence of life. It is evident from studies of microbial adaptation that physiological responses and the molecular mechanisms that underpin those responses are often different to what may have been expected. For example, a high mol percent G+C content of DNA would be more thermally stable than a high mol percent A+T. While hyperthermophiles may be expected to have a G+C bias, this is not generally the case. For example, *Acidianus infernus* grows optimally at 90°C but has a 31% G+C content (Stetter, 1996). If this DNA were naked it would rapidly melt at this temperature. Halophilic Archaea thrive under conditions of saturated salt (5.5 *M*). Unlike Bacteria, which adapt to high salt by excluding salt from their cytoplasm, Archaea such as *Halobacterium salinarium* accumulate potassium (5.3 *M*) and chloride (3.3 *M*) (Grant *et al.*, 1998). Rather than retain "normal" proteins, these halophilic Archaea have evolved proteins with an excess of negative surface charges, which facilitate the formation of stabilizing salt bridges or attract water and salt to form a strong hydration shell around the protein. This represents a fundamentally different mechanism of adaptation in Archaea that could not have been predicted from studying only bacterial species. The oligotrophic marine bacterium *Sphingomonas alaskensis* grows slowly irrespective of media richness, retains a constant cell size regardless of whether it is growing or starved, and does not become more stress resistant when it is starved (Eguchi *et al.*, 1996). This growth strategy contrasts with copiotrophic marine Bacteria, which undergo distinct changes in growth rate, stress resistance, and cell size. As a result of these physiotypes, it may be expected that oligotrophic Bacteria would have relatively few changes in gene expression in comparison with their copiotrophic counterparts. This, however, is not the case as it has been shown that starvation of *S. alaskensis* produces a high level of change in gene expression (Fegatella and Cavicchioli, 2000). Importantly, the capacity of this organism to display a distinct starvation response could be rationalized with the ecology of its native environment. These examples serve to highlight the importance of understanding the physiology and genetics of extremophiles in order to confidently predict their potential responses to an environmental condition, and for identifying appropriate biomarkers.

EXTREMOPHILES, GENOMICS, AND EXTRATERRESTRIALS

Identifying adaptation strategies used by extremophiles is likely to benefit from the rapid advances occurring in genomics. The number of complete genome sequences has increased from the first published for *Haemophilus influenzae* in July 1995 (Fleischmann *et al.*, 1995) to the complete genome sequences for 63 different microbial species in October 2001. The third genome sequence completed was of the hyperthermophilic archaeon, *M. jannaschii* (Bult *et al.*, 1996). A large proportion of published genomes are for extremophiles, including 11 Archaea and five Bacteria that are hyperthermophilic, thermophilic, halophilic, oligotrophic, radiation-resistant, acidophilic, or acid-tolerant. Genome sequences provide an enormous amount of information about genetic potential, and appropriate analysis of that information may reveal the basis for the specific characteristics of a cell (Pallen, 1999). This is well illustrated by analyses of genomes to elucidate the molecular determinants of protein thermostability (Thompson and Eisenberg, 1999; Cambillau and Claverie, 2000) and the analysis of the genome of a halophilic archaeon to determine mechanisms of adaptation to high salt (Kennedy *et al.*, 2001). However, depending on the cellular target of interest, it may still remain a challenge to identify the genomic basis of a particular characteristic. This is illustrated by the efforts to define the extreme radiation resistance of *D. radiodurans* (Battista, 2000), which have revealed that the radiation resistance is likely to be due to several different biological mechanisms (Makarova *et al.*, 2001). Clearly the genome provides the starting point for comprehensive *in silico* studies; however, the greatest advances will arise from global analyses of gene function. The utility of functional studies for examining extremophiles is illustrated by microarray analysis of gene expression in the acid-tolerant bacterium *Helicobacter pylori* (Ang *et al.,* 2001) and cell cycle control in *Caulobacter crescentus* (Laub *et al.,* 2000). Other functional studies include proteomic analysis of growth rate control and starvation control of gene expression in the oligotrophic bacterium *S. alaskensis* (Fegatella and Cavicchioli, 2000; Ostrowski *et al.,* 2001) and control of flagellin genes and protein modification in *M. jannaschii* (Mukhopadhyay *et al.,* 2000; Giometti *et al.,* 2001). A further example is the structural genomic analysis of proteins from *Methanobacterium thermoautotrophicum* (Christendat *et al.,* 2000).

Rapid progress with microbial genomes will continue to provide important insight into extremophile evolution and cell function. Among the >130 microbial genome projects in progress (see NCBI at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Genome or TIGR at http://www.tigr.org/tigr-scripts/CMR2/CMR HomePage.spl), at least 10 are for extremophiles. It will be particularly interesting to observe the outcomes of analyses of genomes for the methanogenic Archaea. Genome sequences are published for *M. jannaschii* (T_{opt} 85°C) and *M. thermoautotrophicum* (T_{opt} 65°C), and are in varying stages of progress for *M. kandleri* (T_{opt} 100°C), *Methanosarcina thermophila* (T_{opt} 50°C), *Methanosarcina barkerii* (T_{opt} 40°C), *Methanococcus maripaludis* (T_{opt} 37°C), *Methanosarcina mazei* (T_{opt} 35°C), *M. burtonii* (T_{opt} 23°C), and *M. frigidum* (T_{opt} 15°C). At present, there is no complete genome sequence for any psychrophilic organism. Completing the genomes for these two Antarctic Archaea (*M. frigidum* and *M. burtonii*) will provide the first blueprint for survival and adaptation at near zero temperatures. Moreover, in association with the genomes of the above-mentioned methanogens, it will be the essential link for investigating, for the first time, cellular adaptation in a set of metabolically and phylogenetically similar organisms that cover the thermal extremes of life (Cavicchioli *et al.,* 2000b).

In essence, the availability of genome sequences is providing the information necessary to make rational interpretations about the evolution of all life forms on Earth (Tekaia and Dujon, 1999; Doolittle, 2000; Singer and Hickey, 2000; Lecompte *et al.,* 2001; Podani *et al.,* 2001; Sicheritz-Ponten and Andersson, 2001). Importantly, these studies may provide insight into the types of organisms that might be found in places where complex life is unlikely to have evolved (e.g., Mars).

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ABBREVIATIONS

pH_{opt}, optimum pH; T_{opt}, optimum temperature.

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