

Extremophilies as Models for Extraterrestrial Life

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Abstract. Microbial life is globally ubiquitous. Current biological investigations have expanded our information into many habitats hitherto regarded as too harsh to harbor microbial life. Among those newly discovered microorganisms are the extremophiles consisting of the prokaryotic Archaea, Bacteria and Eukarya (i.e., Protista). Such microorganisms thrive in an endmost range of temperature levels, pH ranges, salinity, desiccation or xerophilic, pressure and other extreme conditions. Microorganisms from these severe environments may represent models for the origin of the first cells as well as a pattern for extraterrestrial life. Microorganisms from locations like the Dead Sea in Israel (where one finds hypersaline halophiles), Antarctica (psychrophilic cells) or Yellowstone National Park in USA (thermophilic microbes) may play an important role in the extraterrestrial life search. It is widely accepted that the Monera (Bacteria & Archaea) emerged as non-nucleated prokaryotes in warm habitats. Similar habitats are still located in many hot environments all over the world. This paper is a brief review the main groups of certain extremophiles, which may serve as good candidates for extraterrestrial life.

1. Introduction

Recent studies have shown that microorganisms (prokaryotes, Archaea, Bacteria, and Eukarya. i.e., Protista) have a wide range of viability and are found all over the globe. Biohabitats are spread throughout land, water and air, several of these environments are considered severe or extreme from the anthropocentric view (Seckbach 1997; Walsh & Seckbach 1999). It is reasonable to believe that life exists in many places throughout the cosmos. Among the many billions of heavenly bodies, there are galaxies with millions of stars that may harbor lower or even higher forms of living organisms (Chela-Flores, this volume). Since the laws of physics and carbon chemistry are the same everywhere, it is presumed to consider that the Sun is not the only star with planets and that Earth is not the only planet containing water and other elements essential to life. For example, Mars and Jupiter's moon Europa are currently the celestial candidates stated that may contain liquid water and perhaps microbial life (Seckbach *et al.* 1998; Shock 1997; Abyzov *et al.* 1999). Recent observations have shown terrestrial extremophilic microorganisms with long-term viability in a variety of environments such as in permafrost or deep ice and at high levels of temperature or in other parameters (Seckbach 1999). Furthermore, the Archaea and

mysterious subterrestrial bacteria living in darkness or in the steam around deep ocean volcanic vents derive their energy from sulfur, iron and other minerals and thrive without oxygen or sunlight. Such extremophiles or hyperthermophiles may serve as models for survivability of microbes in space environments (Davies 1999; Shock 1997). Fossil evidence suggests that Earth's earliest organisms were themselves "extremophiles". Extremophiles can be defined as those organisms observed in uncommon habitats (from our anthropocentric viewpoint). Kristjansson and Hreggvidsson (1995) define an extremophile as one whose optimal growth conditions are found outside of "normal" environments. With "normal" being those that have a temperature between 4 and 40 °C, pH between 5 and 8.5, and salinity between that of freshwater and that of seawater (Walsh & Seckbach 1999). Extremophilias include not only Bacteria and Archaea but also some eukaryan protists (Roberts 1999; Seckbach *et al.* 1998). Prokarya hyperextremophiles thrive at higher levels of temperature and in hypersalinity than the moderate extremophiles.

2. Prokaryotes at Extreme Conditions

Many microorganisms are adapted to ecological niches that colonize frontiers of life environments originally considered by man to be extremes. Prokaryotes show a greater flexibility in their environmental adaptations than most of the Eukarya. The latter are known to grow at "normal" or mesophilic environmental conditions. There are the extremophilias living in high or low ranges of temperature, pH, pressure and the halophiles growing in hypersalinity and in other diverse habitats. Microorganisms have the ability to adapt to many extreme environmental conditions and even to the presence of toxic compounds like petroleum (Fought & McFarlan 1999) and organic solvents such as toluene and xylenes (Ron 2000). The evidence that prokaryotes can survive for long periods without water or nutrients (Cano & Borucki 1995) makes the study of the extremophilias central to space travel and our search for extraterrestrial life. For further data on the limits of life and on extremophiles consult Nealson (1999), Seckbach (1999, 2000) as well as Horikoshi and Grant (1998).

2.1. Extreme Temperatures

2.1.1 *The Hyperthermophiles – High Temperature Lovers*

The thermophiles are microorganisms living at high temperature (Brock 1992); it was recorded that the prokaryotes grow at an optimum of 45–80 °C. Thermophiles are found in hot waters, sun-heated soils and geothermal areas (Walsh & Seckbach 1999). The prokaryotic hyperthermophiles have a temperature optima of 80 °C and even above (Madigan & Oren 1999). Extremophilias do not only tolerate these harsh conditions but also actually require them for their growth. Darwin stated that life evolved in a warm little pond, and most probably these thermophiles were the first organisms on Earth (Copland 1936; Seckbach 1994/1995, 1997). The species of thermophilic Archaea, like many of the methanogens, lie near the root in the tree of life (Valentine & Boone 2000; Pace 1997; Madigan & Marris 1997). Recently it has been well established that all life forms that cluster around the base of evolutionary and phylogenetic trees are

thermophiles (Pace 1997; Shock 1997; Stetter 1998). There is however, a certain rebuttal to this theory that has challenged the warm/hot origin of life, proposing that the first cells were cold lovers (Galtier *et al.* 1999). In the hot springs of Yellowstone National Park several species, including the bacterium *Thermus aquaticus*, grow at greater than 70 °C (Brock 1978). Some archaean cells grow near the temperature of boiling water in the Yellowstone geysers. Thermophilic sulfate-reducing bacteria have been isolated from 75 °C oil field waters in the North Sea that originated from formations 2,000 to 4,000 meters below the sea floor (Rosnes *et al.* 1991). Hyperthermophilic bacteria and Archaea grow fastest between 80 °C and 100 °C. Furthermore, they are generally unable to grow at temperature below 60 °C (Stetter 1998).

Hyperthermophilic Archaea and bacteria have been cultured from production fluids at temperature up to 110 °C from oil reservoirs 3 km below the bed of the North Sea and below Alaska's North Slope permafrost surface. The archaean *Pyrolobus fumarii* inhabits submarine hydrothermal vents and has a temperature optima of over 100 °C, it has the highest temperature tolerance (113 °C) among all forms of life (Stetter 1998; Madigan & Oren 1999). Furthermore, *P. fumarii* remains viable following an hour's treatment in the autoclave at 121 °C (Madigan & Oren 1999). An older publication suggested exceedingly high values for archaean temperature limits of cells isolated from submarine vents or "black smokers" (Baross & Deming 1983). Those archaean cells were reported to grow at 250 °C at 265 atmospheres. However, this extraordinary upper temperature limit of life has not been supported or confirmed by others.

2.1.2 Eukaryotic Thermophiles

Most Eukarya thrive at more moderate or "mesophilic" thermal environmental niches. The widespread study of thermophilic Eukarya includes the acidophilic phototroph *Cyanidium caldarium* and its cohorts (Seckbach 1994). These unicellular algae thrive in acid media (pH 1–4) and at elevated temperatures (optimal 45 °C and a maximal of 55–60 °C). Earlier reports of much higher temperature recorded for these algae (75 °C–80 °C) by Copeland (1936) and by Allen (1959) turned out to be inaccurate. For further information on the *Cyanidiaceae* see Brock (1978) and the treatise by Seckbach (1994). These algae thrive under pure CO₂ (Seckbach *et al.* 1970), grow faster and have a higher levels of photosynthesis under CO₂ treatment than those cells cultured with air bubbling (Seckbach 1994). Thermophilic (prokaryotic) cyanobacteria grow at higher temperature levels but require neutral to alkaline conditions for their growth in hot environments (Brock 1978).

2.1.3 Psychrophiles and Exobiological Application

Psychrophiles are cold-loving organisms located opposite the thermophiles on the temperature scale. These microbes live at a maximal temperature of 20 °C and a minimum of 0 °C (Morita 1975). The psychrophiles are distributed in soils, waters or even in sea ice (Vishniac 1999) and an abundance of them are in Antarctica (Abyzov *et al.* 1999; Onofri 1999) and in deep ocean waters and sediments where the environment is uniformly cold below the thermocline.

There are affluent numbers of microbes growing in very cold environments. The lowest limit for microbial growth is slightly below 0°C in cold saline solutions. Some are active at -2°C, and several microorganisms can survive freezing-thawing cycles and diurnal temperature fluctuations. In Antarctica the sea-ice is rich with phototrophic microbes, such as algae, diatoms and flagellates as well as heterotrophic flagellates, ciliates and micrometazoa (Abyzov *et al.* 1999). These photosynthetic eukaryan cells are adapted to cold media and the coloration of snow is “painted” by some algae, such as the green alga *Chlamydomonas* (red snow) and dinoflagellates (Hoham & Ling 2000).

2.2. Acidophiles – Living in Acidic Habitats

Acidophilic microorganisms are found in all three domains of life. These organisms grow at pH levels of 0 to 4. Among the acidophiles are sulfur bacteria, Archaea and phototrophic hot spring protists like the thermoacidophilic alga *Cyanidium caldarium* (Seckbach 1994, 1997), *Dunaliella acidophilum* (Pick 1999) and diatoms. This eukaryotic alga *Cyanidium caldarium* tolerates even solution of 1N H₂SO₄ (Allen 1959; Seckbach 1994). Acido-thermophilic Archaea include *Sulfolobus*, a chemoautotroph in hot springs as well as *Thermoplasma*, a heterotroph in hot coal tailings; both thrive in hot acidic habitats. The hyperthermophilic archaean *Pyrolobus fumarii* (maximum growth at 113°C) lives at pH range of 4 to 6.5 (Stetter 1998; Madigan & Oren 1999).

An abundance of protists and fungi grow in moderate acidic habitats. Some of them can be observed in rumen of animals, while others live in the digestive track of termites and other insects. The extreme acidic surrounding of the acidophiles is mainly external, the internal cellular medium has been found to be neutral. It is known that high intracellular acidity may impair the function of several organelles (chloroplast, nucleic acids, cytoplasm). *Cyanidium* has a proteinaceous cell wall, which can prevent the H⁺ ions from entering the cell. Acidophilic cells may use a proton pump or low proton membrane permeability; such a defense mechanism assists them to maintain their intracellular pH at a neutral value. Recently the accumulation of sulfuric acid has been reported in the vacuoles of Phaeophyceae algae (Sasaki *et al.* 1999).

2.3. Anaerobic Organisms

Some microbes live anoxically in the absence of free or dissolved oxygen and exploit environments unavailable to other obligated aerobes. Further, oxygen might even poison these obligated anaerobic cells. Anaerobes are distributed among all free living and symbiotic microorganisms in all three biological domains. Most endosymbionts live intracellularly in anoxic conditions within their hosts. There are protists (Protozoa) which grow anaerobically inside digestive tracks of higher organisms. Termites harbor in their hindgut amitochondrian flagellates and ciliates that are themselves hosts for endosymbiont bacteria. Similarly, bacteria grow anaerobically as internal parasites of animals (in the intestinal, rumens etc.), in sealed food cans (food poisoning) or thrive in habitats of sewage treatment plants. Methanogens produce methane from CO₂ and H₂ and are associated and harbored within symbiotic flagellated protozoa that digest cellulose. Several of these protists, e.g., the microsporidia and the diplomonads, lack mitochondria and peroxisomes and are considered to be among the early

Eukarya. Some bacteria have been found subterrestrially in sediment rocks, they have been buried there for millions of years. These deep bacteria are anaerobic and survive by consuming a diet of petroleum and other organic compounds dissolved in groundwater.

2.4. Barophiles: Deep Dark Dwelling Microbes and Bacteria in a Vacuum

The barophilic Archaea and bacteria are present in deep subterranean locations as far as 4 km below the continental crust (Fredrickson & Onstott 1996) and on the ocean floor (up to 7 km). They may remain prisoners of the deep subterrestrial zones for millions of years and serve as “living fossils” under high pressure. At greater depths it is too hot for life, measuring over 113 °C (the upper biolimits). Such deep bacteria are thermophilic with ambient temperatures of 75 °C; similar bacteria have colonized volcanically heated springs such as those in Yellowstone National Park and scalding geysers on ocean floor.

Recent space travel to the Moon and back to Earth has shown that bacteria can tolerate long periods (at least 2.5 yr) of vacuum. These observations confirm the extent of the diversity of microorganisms.

2.5. Dry (Xerophytic) Microbes and Endospore-Forming bacteria

There are numerous records of photosynthetic microbes (e.g., algae) that have survived desiccation and temperature extremes (Davis 1972). Bacteria survive periods of harsh conditions by producing endospores, which are dehydrated cells surrounded by a thick cell wall (Campbell 1990). Bacteria in bees' digestive tracts turned themselves into spores when imbedded in amber. When placed in cultures these spores were viable and have been brought back to life after being dormant for 30 million years (Cano & Borucki 1995). Similar bacteria have been revived from other tissues of higher fossilized animals.

2.6. Halophiles (Hypersaline Microbes)

Halophiles thrive in salt-rich environments. These microorganisms may grow in hypersaline water bodies such as the Great Salt Lake (Utah) or in the Dead Sea (Israel) and in saltern evaporation ponds (Oren 1998). The reddish coloration of the haloenvironment is caused by the pigmented halobacteria (containing bacteriorhodopsin and carotenes). Halophiles have been also observed in underground salt deposits (Vreeland & Rosenzweig 1999). Many of these environments harbor ancient microorganisms that may have survived millions of years in a dormant state. Square, flat, gas vacuole-containing halophilic Archaea have been observed in hypersaline environments all over (Oren 1999; Oren *et al.* 1996). The archaean *Halobacterium salinarium* and the bacterium *Actinopolyspora halophila* can grow in saturated solutions of NaCl (Madigan & Oren 1999).

3. Extremophiles and Hyperextremophiles as Models for Life Elsewhere in the Universe

3.1. Psychrophiles Life

Recent discoveries that enlarge our knowledge about the diversity of microbial life on Earth also relate to intriguing questions about the origin of life (Seckbach 1994/1995) and the possibilities that microbes survive today on other celestial bodies or even beneath the surface of planets (Davies 1999; Abyzov *et al.* 1999; Shock 1997). A few decades ago Sagan (1961) proposed an extravagant planetary engineering plan. He suggested seeding the atmosphere of a hostile planet with cyanobacteria for making these planets habitable for life. Friedmann and Ocampo-Friedmann (1995) followed this idea of Sagan's and also proposed propagating Mars with cyanobacteria for extraterrestrial terraforming. Martian life may not be on the surface but in hiding in the subsurface of the dusty red Planet (Shock 1997). The same subsurface extraterrestrial life could exist on several planets of the solar system and beyond it in the cosmos. Today the surface of Mars is inhospitable because it lacks surface liquid water (Nealson 1999), but fluids may exist in the warmer interior of the planet (Chela-Flores 1999, and this volume). The ice microbes of the terrestrial cryosphere e.g., those in Antarctica are an excellent analog for cryobacteria that might be able to survive on comets and on heavenly bodies. Drillings at Vostok Station in Antarctica have discovered microorganisms at ice depth of 3 km. Lake Vostok may provide a suitable model for the ocean that may lie underneath the ice of Europa or Mars. Archaean-like organisms are likely to be the biota existing on the subsurface of Europa in the oceans of liquid water under the heavy ice layer. Martian photos demonstrate evidence of past liquid running water, which may suggest early vestiges of life. McKay and co-authors (1996) recently published some extraordinary possible evidence of Martian fossilized nanobacteria imbedded in a number of Antarctic meteorites.

3.2. Hyperthermophiles and Anaerobic Microorganisms as Candidates for Extraterrestrial Life

Some subterrestrial life on Earth might have occurred a few kilometers below ground. These microbes remain prisoners of the deep for millions of years and serve as "living fossils". The volcanoes and their prokaryotic microbiota as well as thermoacidophilic algae may be associated with the high temperature levels of Venus (Seckbach & Libby 1970). Furthermore, photographic evidence from Mars supports ancient volcanic activity and suggests a previous presence of water that was on and below the surface of this planet. These findings may encourage life possibilities on the red planet. It is assumed that life originated in the pre-oxic world and in anoxic environments that still persist in many places on Earth such as lake sediments, guts of ruminants, and the deep waters of some marine basins. These terrestrial anaerobic microbes may point out optimal conditions for some planets that are lacking (or show a low level of) oxygen and still should be able to harbor biota that do not require aerobic conditions. The recent evidence of sulfuric acid on Europa may also be associated with microbes that are able to tolerate this corrosive solution.

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