



Revista de Biologia e Ciências da Terra

ISSN: 1519-5228

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Universidade Estadual da Paraíba

Brasil

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Revista de Biologia e Ciências da Terra, vol. 9, núm. 1, 2009, pp. 1-10

Universidade Estadual da Paraíba

Paraíba, Brasil

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Life in extreme environments

Andreea Oarga¹

ABSTRACT

Extreme habitats lie outside the range of conditions in which most of organisms live. 'Extreme' includes physical extremes, e.g. temperature, radiation, pressure, and geochemical extreme, for example desiccation, salinity, pH, depletion of oxygen or extreme redox potential. Investigations of these environments are important for the study of evolution relationships, emergence of new species and various ecological relations among organisms which compensate certain environmental externalities. From such habitats new metabolites and metabolic pathways of organisms can be expected, which indicate bioremediation potential, discovery of new antibiotics, etc. Extreme habitats are populated by highly specialized organisms - extremophiles, which must - in a contrast to other species - bridge different stresses conditions. Liquid water is the *sine qua non* of life on Earth, but regarding the physical and geochemical extremes, life is present even in habitats with obvious lack of water, e.g. deserts. In such habitats environmental parameters allow existence to only specially adapted organisms live. All these facts support the idea of sustainable management with these natural features, their protection, preserving their genetic pool and popularisation in the society.

Key words: Extreme habitats, extremophiles, physical extremes, threats

1 INTRODUÇÃO

Studies from the past few decades made us to realise that on Earth where liquid water exists, there is life too. If before physical and chemical barriers were conditioning the idea of life, now another niche, 'extremophile', coupled with new data on the life outside the Earth (Rothschild & Mancinelli, 2001) and the possibility on panspermia (Richter, 1865; Thomson, 1894; Arrhenius, 1903) suggest that life may be found in more places than we think.

Our perception on environment influences our view on the definition what means extreme environment. If we think from the perspective of what means 'normal' for life, any biotope characterised by a very low or very high value of the main parameters which influence the life cycle may be characterized as an extreme environment. From ecological point of view, we ask ourselves if an extremophile 'loves' extreme environment and if an organism must be an extremophile during all their life in these conditions (Rothschild & Mancinelli, 2001).

The discovery of inhabited extreme environments and extremophilic organisms made higher possibility to find life outside of Earth, gave new pathways in biotech industry and biology field. Eukaryotes in most of the studies were often overlooked as extremophiles and the possibility of being endangered mostly from anthropogenic interventions was poorly studied.

The future perspective on the research of extremophiles is promising because of their economic potential, their role in geochemical processes, threatening issues and searching life in the Universe.

2 LIST OF EXTREME PARAMETRES AND LIFE IN THESE CONDITIONS

Extremophiles were described by Kristjansson and Hreggvidsson (1995) as those organisms which thrive beyond 'normal' environmental parametres. Normal environments would mean those with temperatures between 4 and 40°C, with pH values between 5 and 8.5, and salinity between that of

freshwater and seawater. Organisms from all three domains of life (Archaea, Eubacteria and Eukaryotes) are found at extreme conditions (Seckbach and Oren, 2007). Regarding extreme conditions, extremophiles are psychrophiles (thrive at low temperatures), thermophiles (high temperature), acidophiles (low pH), alkaliphiles (high pH), piezophiles (under extremes of pressure), xerophiles (desiccation), and halophiles (salinity) (Rothschild & Mancinelli, 2001; Magan, 2007).

'Extremes' includes physical extremes (e.g. temperature, pressure or radiation) and geochemical extremes (e.g. pH, salinity, oxygen etc.) (Rothschild & Mancinelli, 2001). Below are listed some selected environmental parameters with which extremophiles must live.

2.1 TEMPERATURE

Among the physical parameters important for life, temperature is probably the most studied (Prieur, 2007).

Temperature affects the organisms in different ways, from devastation of biomolecules till denaturing made by ice crystals. For aquatic organisms which requires O₂ and CO₂ the solubility of gases in water is correlated with temperature. High temperature creates problems and chlorophyll degrades above 70°C, what excludes photosynthesis (Rothschild & Mancinelli, 2001). Proteins and nucleic acids are normally denaturated at temperatures approaching 100°C and the fluidity of membranes to lethal level is then increased (Brock et al., 1994).

Hyperthermophilic organisms have maximum growth >80°C and psychrophilic <15°C. The first and the most hyperthermophilic organism isolated by Brock in 1977 is *Pyrolobus fumarii* (Crenarchaeota) from archaea, which has the optimum temperature for growth at 106°C and at 113°C it still continues to grow (Prieur, 2007). Some phototrophs like cyanobacteria, purple and green bacteria (Seckbach and Oren, 2007) eubacteria (*Clostridium*, *Thiobacillus*, *Bacillus* etc.) and the archaea (*Pyrococcus*, *Thermococcus*, *Sulpholobus* etc.) can have temperature optimum up to 142°C (Rothschild & Mancinelli, 2001).

The eukaryotes have the upper limit life at ~60°C and this limit is still suitable for some protozoa, algae and fungi (Mangan, 2007). Mosses have the maximum temperature of 50°C, vascular plants 48°C and fish 40°C. At high temperatures solubilization of cellular lipids can occur and cells in this case lose their integrity (Mangan, 2007).

In habitats with temperatures below 0°C we can find representatives from all major taxa. The lowest temperature for active microbes is minus 18°C. Between animals, we can mention here an insect

(*Diamesa meigen*, Chironomidae), found in 1984 in Himalaya by Kohshima, living at minus 16°C in glacier, the most cold insect habitat ever recorded.

Liquid water is not important only as a solvent but also as product or reactant in many metabolic processes (Brock et al., 1994). When temperature is low, water freezes, ice crystals destroy cell membranes. In the absence of liquid water biochemical reactions stop what results in cell death. A nematode *Panagrolaimus davidi* is the only one discovered so far which can survive when all body water freeze (Rothschild & Mancinelli, 2001).

Organisms are not only capable to live at high or low temperature, but they can modify the temperature of their habitat. Recent studies show that microbial activity can produce temperature increases in their habitat. At two crude-oil-spill sites (Bemidji, Minnesota, USA and Cass Lake, Minnesota) it was noticed a temperature increase with 3-4°C in the saturated zone. Values ranged from 6.35°C (background) to 9.19°C in the oil body in Bemidji and a similar pattern of higher temperature near the subsurface oil body (11.61°C) compared with background (7.29°C) in Cass Lake. The heating rates were determined by using theoretical methanogenic degradation energy yields and reaction rates based on observed methane and hydrocarbon fluxes. Temperature increases into the oil body shows that temperature measurements can be used to detect microbial activity within oil bodies and may help to locate hydrocarbon contaminant sources (Warren, 2008).

2.2 pH

Biological processes in normal conditions considered by humans occur in the middle of a pH spectrum (between 5 and 8.5). Organisms function best at pH values close to neutrality. The metabolic activity of cells is critical, determined by availability of inorganic ions and metabolites to the organism and all these are affected by the of hydrogen ions concentration. Very high (acidic) or very low (alkaline) concentrations of hydrogen ions will have a big effect on activity and ability of organisms to live in an environment. Acidophiles live in extremely acid waters below 2 and alkalophiles in waters with pH exceeding 10 (Le Romancer et al., 2006).

Acidophiles are defined as organisms being able to grow down to pH 1.0 and with active growth at pH <4.0 (Mangan, 2007). Due to the surface barriers of cells which are very impermeable for protons and selective mechanisms (active pumps) preventing H⁺ ions to enter in the cell, acidophiles use proton pumps as defence to maintain their intracellular pH at near neutral values (Seckbach and Oren, 2007).

There are few unicellular eukaryotes which live below pH 1 like red alga *Cyanidium caldarium* (described from natural environment at pH 0.5, however in culture it has growth optimum at pH 2-3), green alga *Dunaliella acidophila* (it survives at pH 0, and it has maximum growth rate at pH 1) (Rothschild & Mancinelli, 2001), fungi (for example *Acontium velutium* and *Cephalosporium* sp. were found at pH 0 (Mangan, 2007). Madigan (2005) concluded in his review made on anoxygenic phototrophs that there are known only two species, both purple nonsulphur bacteria (*Rhodoblastus acidophilus* and *Rhodospila globiformis*). The knowledge on diversity among acidophilic anoxygenic phototrophs is limited to two species due to unsuitability of acidic habitats for anoxygenic phototrophs and because acidic habitats have usually high level of metals which in high concentrations have negative effect on these organisms. Two species of anaerobic heterotrophs, archaea, (*Picrophilus oshimae* and *Picrophilus torridus*) were isolated from Japanese soils penetrated with solfataric gases, and show an optimal growth at pH 0.7 and 60°C (Rothschild & Mancinelli, 2001).

Alkaline environments include soda lakes, desert soils and alkaline springs where the pH reaches the value of 10. Alkalophilic organisms are defined like those which do not grow at pH<8.5 or with an optimum growth at two pH units above neutrality (Seckbach and Oren, 2007). Alkalophilic organisms are found in all domain of life tolerating pH as high as ~11 (e.g. *Chrisosporium* spp., fungi isolated from bird nests) (Mangan, 2007).

2.3 PRESSURE

Pressure causes volume changes of living organisms, decreasing fluidity of cell membranes by compressing lipids and inhibiting the biochemical reactions in cells. Sudden changes in pressure can have lethal effect.

Hydrostatic pressure is high in deep lakes and it is even more characteristic for all deep sea vent sites. Hydrostatic pressure increases at a rate of 10.5 kPa per metre depth compared with 22.6 kPa per metre for lithostatic pressure. The boiling point of water increases with pressure, and this enables that at the bottom of the sea water is liquid even at 400°C. Microorganisms inhabiting the deep sea and subsurface of Earth have the ability to survive at hydro- and litho- static pressures much greater than 1 atmosphere (atm). Studies of barotolerant (tolerance to high pressure) and barophilic (depending on high pressure) cultures of deep sea bacteria show that both types are present, and the distribution of these organisms is a function of depth (Madigan et al., 2003). Extreme piezophiles, which

can live at enormous hydrostatic pressure associated with big depths, grow well in the deep sea and even in the deep subsurface sediments deep as 1,000 m below the seafloor under anaerobic conditions (Le Romancer, 2007). Under pressurized conditions at 155°C in slurry deep sediments, prokaryotes produce H₂, CH₄, organic acids, and energy for themselves and substrates for other prokaryotes and can catalyse some reactions which are considered to be thermogenic (Parkes, 2008).

One component of pressure is gravity. Until now it was believed that on Earth organisms lived at 1 g. Space exploration included research in locations with different gravity regimes, for example launched space shuttles with variable g, Moon which have 0.17 g, Mars etc. Gravity affects human health; it has effects on microbes and directs changes in biomass (Rothschild & Mancinelli, 2001).

2.4 RADIATION

Radiation is energy which can appear like particles (e.g. neutron, protons, electrons, alpha particles or heavy ions) or electromagnetic waves (gamma rays, X-rays, UV radiation, infrared, microwaves or radiowaves). Normally extreme levels of radiation appear rarely on Earth. Man caused intense levels of irradiation are well studied due to of their role in production of goods (preserving of food, fast drying of substrates, control of pests and microorganisms), in medical applications and space travel. When the wavelength is shorter, the potential of damage of an organism is higher. Due to depletion of ozone layer in atmosphere, the exposure to UV has increased and its influence on organisms has received lately more attention. The most harmful ultraviolet radiation is UV-A: 320-400 nm, following by UV-B: 290-320 nm, and UV-C: 200-290 nm. UV affects organisms by inhibiting photosynthesis and damaging the nucleic acids.

Organisms protect themselves either actively or passively, by moving away from lethal environments, by engaging mechanisms to repair damaged cell components and by accumulation of protecting substances that protect against the harmful radiation (e.g. synthesis of carotenoids which absorb in the visible spectrum above 400 nm). One of extreme cases of resistance to irradiation is a cyanobacterium *Synechococcus* sp. isolated from an intertidal evaporitic gypsum crust from Mexico which was exposed in outer space during 2-week space flight in June 1994. In the experiment it was exposed to the high radiation (between 200 and 400 nm). Interestingly in such conditions only a small reduction in viability and activity was observed (Seckbach and Oren, 2007).

2.5 DESICCATION

Zones of water limitation represent another interesting extreme environment. Some organisms can tolerate desiccation and they enter in the state of anhydrobiosis, which is characterised by little content of intracellular water and by the absence of metabolic activity. The difference between anhydrobiosis and death is that an organism in a state of anhydrobiosis will recover, grow and reproduce when immersed in water, but a dead organism will not. When death occurs due to anhydrobiosis in the organism's body irreversible transformations of lipids, proteins, denaturation of DNA and structural breakage through Maillard reactions (chemical reactions between an amino acid and a reducing sugar, a process which requires usually heat) occur (Wharton, 2002). Although organisms theoretically cannot function in the absence of water (vital role of water in organism), there are some interesting reports on nematodes living in very dry soils in Dry Valleys of Antarctica. They probably uptake water from occasionally melted snow; however, most of their life time they are in anhydrobiosis state. Some bacteria, yeasts, fungi, plants, insects, tardigrades, rotifers, crustaceans (*Artemia salina*) can become anhydrobiotic. Bacteria have some degrees of tolerance to desiccation. Viable bacteria were isolated from sediments between 10,000 and 13,000 years old (Wharton, 2002). A cyanobacterium *Nostoc commune* showed its ability to develop a possible mechanism of desiccation tolerance. Recent studies made on a terrestrial cyanobacterium *Nostoc* sp. HK-01 showed that after application of this species to the surface of soil resulted in an increase of organic carbon and nitrogen content in the soil, and in the better growth of cultivated plants. It was also established that cultures of *Nostoc* can be applicable to improve Martian regolith up to soil characteristics by exposure of the *Nostoc* cells to high vacuum environment (10^{-5} Pa) for two weeks. After exposure *Nostoc* cells began to grow again, so they might adapt to the Martian regolith (Ohmori, 2008).

2.6 SALINITY

Water is required for all organisms to have normal cellular functioning. Water molecules enter through the semi-permeable cell membrane. To equilibrate osmotic pressure with the surrounding environment, water molecules enter in a cell. In the case of high concentration of salts, organisms must be able to adapt physiologically to such osmotic alterations. Responses to increased osmotic pressure during high-salt conditions are similar to responses of a cell during desiccation when compatible solutes such as

K⁺, glutamate, proline, glycine betaine, sucrose and trehalose accumulates and thus stabilise cells (Rothschild & Mancinelli, 2001).

Osmotic aspects of life at high salt concentrations, turgor pressure, cellular dehydration and desiccation represent osmophily. Ions requirements for life at high salt concentrations refer to halophily. These two phenomena are environmentally linked, and a halophile must cope with osmotic stress. Uptake of nutrients, protein biosynthesis and a number of enzymatic activities are affected if a shift to a high osmotic potential occurs. Microorganisms which are able to tolerate and to grow in high-salt environments are called 'osmophilic' and 'halophilic' (Madigan, 2005). Truly halophilic and/or highly halotolerant microorganisms can be found both in the bacterial, archaeal and eukaryal domains. Extreme halophiles (Archaea domain) are well known and studied (Madigan et al., 2003). Halophilic oxygenic phototrophs like halophilic cyanobacteria (e.g. *Aphanothece halophytoca*) and halophilic eukaryotic algae (like *Dunaliella salina*) notably contribute to the primary production in salt lakes (Seckbach and Oren, 2007).

2.7 OXYGEN

A big threat in nature represents reactive oxygen species. UV-A radiation has a result at 320-400 nm irradiations a photochemical production of reactive oxygen species like H₂O₂ within cells. Reactive oxygen molecules are formed in eukaryotes during mitochondrial respiration, cytochrome P450 metabolism of hydroperoxides, during production of uric acid. In aquatic systems reactive oxygen species result from photochemical reaction with H₂O₂. Hydroxyl radical are produced by ionizing radiation. The presence of oxygen can enhance DNA damages. However, oxidative damage is extremely serious when occur as a result of activity of oxygen radicals, affecting organisms from aging till cancer development and other physiological changes in living organisms (Rothschild & Mancinelli, 2001). Aerobic metabolism is thermodynamically more efficient of course than the anaerobic one. Nevertheless, organisms are found in environmental conditions where they are adapted to lethally low oxygen concentrations. A good example of this case comes from sulphur cycle in nature. Transformations of sulphur in nature are complicated due to different oxidation states. Microbial metabolism in such environments is frequently chemolithoautotrophic. Summers Engel et al. (2003) established that very diverse, yet poorly explored groups of *Epsilonproteobacteria*, which utilize reduced forms of sulphur as an energy source in strictly low oxygen

conditions, in big extent contribute to the sulphur cycle.

2.8 OTHER EXTREME CONDITIONS

Under other extreme conditions here we consider extreme environments with lethal gas composition, unfavourable redox potential, toxic or xenobiotic (synthetic) compounds and heavy metal concentration to which organisms are exposed.

Microorganisms developed a variety of mechanisms which make possible their life in the presence of toxic concentrations of metals. These mechanisms include uptake of toxic metals which enter cells via essential metal transporters, the intra- or extra-cellular precipitation of metals, and enzymatic transformations that decrease metal toxicity. Human activities influence public and environmental health by environmental pollution. The metal resistant microbes present in environment are adapted and it seems that their evolution in metal resistance depends on the contamination caused by human activity. Microbial evolution in the presence of high concentration of toxic metals is present on Earth since the evolution of life in environments where geological processes occur in the presence of metal rich deposits. In such environments microbes can be exposed to toxic levels of metals, like iron, and they can obtain energy from oxidation of such compounds. Chemolithoautotrophic acidophilic bacteria and archaea are known to tolerate metals by the presence of the metal resistance genes in their genome (Aspassia et al., 2007). Jeanthon (2000) suggested that there might exist genetic transfers in the vent environments between different phyla. He showed the possibility of genetic transfers in vents by isolating heterotrophic bacterial strains (*Acinetobacter*, *Alteromonas*, *Pseudomonas*, *Vibrio*) which showed resistance to heavy metals like cadmium, zinc, silver, arsenic and copper. 20% of the strains indicated similarities between plasmids which belong to different phyla.

Another important issue is the role of microorganisms in the transformation of chemical and radioactive components. With this topic is related a study which was made by Nazina et. al (2008) on microbial community of the deep repositories of liquid low- and intermediate-level radioactive wastes of Siberian Chemical Combine. It was shown that the microorganisms and rates of sulphate reduction, denitrification, and methanogenesis in natural formation were increased in the zone of wastes dispersion. It was found out that denitrifying bacteria and other microorganisms capable to oxidize metals due to nitrate reduction, were identified in the repository. Bacteria of the genus *Shewanella* and sulphate-reducing bacteria

were the evidence of possible biogenic precipitation and concentration of radionuclides in the deep repository for liquid radioactive wastes by reducing U(VI) (uranium) and Np(V) (neptunium) in the presence of different organic substrates.

Extremophiles are considered also those organisms which live even at biological extremes (nutritional extremes such as scarcity of nutrients, extreme of population density, parasites, and prey). Here appears an important question, if extreme conditions determine low species richness? This question can be explained, for example, that low species richness in an extreme habitat occurs due to limited size, low *in situ* productivity or low spatial heterogeneity (Tobler, 2006). One such example can be the upper part above a karst cave, named epikarst. It is a habitat with harsh conditions, e.g. darkness, and water availability or unavailability at the site. Because of its complex and heterogeneous structure (higher permeability and porosity, small fractures and solution pockets), epikarst is a hotspot of meiofauna, mostly but not exclusively aquatic, that often rivals in diversity the rest of the karst aquifer (Pipan, 2005; Culver and Sket, 2000).

Do extremophiles love or tolerate extreme environments? Extreme environments are typically constant in their physiochemical properties. Extremophilic organisms, like their physiological response to the environment constancy, show optimal growth at such conditions and thus require the extreme conditions. Some environmental extremes like radiation and vacuum include rather “tolerant” organisms than ‘loving’ organisms for such conditions.

3 EXAMPLES OF EXTREME HABITATS

3.1 DEEP-SEA ENVIRONMENTS

Deep-sea environments are dark, exposed to elevated hydrostatic pressure, and cold (average temperature 2°C). They are oligotrophic, where the trophic level depends on upper layers. Until 1977, bottoms of oceans were considered as habitat with low animal density, but now we know that there thrive bacteria, invertebrates and fish (Prieur, 2007). The discovery of deep-sea hydrothermal vents changed our perception of deep oceanic environments. In vents the temperature may reach even 400°C, and they can be found at depths ranging from 800 to 3600 m. These hydrothermal fluids are acidic, reduced and enriched with heavy metals, and inorganic molecules (hydrogen, methane, carbon dioxide, etc.). In 1977 the deep-sea “Alvin” expedition found life in the Galapagos, and in 1979, Rise, another oceanic expedition found “black smokers” which are considered the most extreme

environment discovered on Earth. Black smokers are mineral constructions from which particle-rich fluids come out with the speed up to 2 m s^{-1} , and temperatures up to 350°C . At such depths water is liquid due to high hydrostatic pressure. Black smokers are named also hydrothermal chimneys and from there thermophilic microorganisms were discovered and described (Prieur, 2007).

A new studied habitat in the deep-sea is the sub-sea-floor sediment. This habitat may contain two-thirds of Earth's total prokaryotic biomass. The deepest documented prokaryotes were till recently from 842 m. The study made by Roussel et. al (2008) provided the evidence of low concentration of living prokaryotic cells in the deepest (1626 metres below the sea floor), oldest (111 million years old) and potentially hottest ($\sim 100^{\circ}\text{C}$) marine sediments investigated.

Due to their unusual chemistry which is compatible with prebiotic chemistry leading to the origin of life, hydrothermal vents had probably critical role in evolution.

3.2 HOT SPRINGS AND GEYSERS

Geysers and hot springs are generally found in regions of young volcanic activity. Surface water percolates downward through the rocks below the Earth's surface to high-temperature regions surrounding by a magma reservoir. They have sometimes low pH and toxic elements like mercury (Aspassia et al., 2007). These environments are intensively studied by astrobiologists, evolutionary biologists and biotechnologists.

3.3 HYPERSALINE ENVIRONMENTS

Hypersaline habitats are common in hot and dry areas and can vary considerably in ionic composition. They include salt flats, evaporation ponds, natural lakes (e.g. Great Salt Lake, US) and deep-sea hypersaline basins. The largest hypersaline lake ever found on Earth on the seafloor of eastern Mediterranean Sea was recently discovered. It extends on over 100 km^2 , of seabed surface with the average depth of 100 m (Yakimov, 2008). Hypersaline environments can be considered as productive ecosystems. Halophytic organisms include a wide range of organisms like archaea, green algae, cyanobacteria, and bacteria. These organisms can cope with osmotic stress and can live even in water saturated with NaCl (Le Romancer, 2006). For example between 25 and 33% salinity bacteria (e.g. *Ectothiorhodospira halochloris*), cyanobacteria, (e.g. *Aphanothece halophytica*), green algae like *Dunaliella salina*, diatoms (*Navicula* spp., *Nitzschia* spp.) and protozoa

(*Phyllomitus and tetramites*) are found (Rothschild & Mancinelli, 2001).

3.4 EVAPORITES

Evaporite deposits usually contain halite (NaCl), gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) or anhydrite (CaSO_4). The evaporite precipitations are strongly related with water and halophilic bacteria and archaea are usually found in these habitats because they are able to survive when salts in water approach saturation point. For example, in Atacama Desert (Chile) evaporite deposits are suitable habitats for microbial life. In such habitats gypsum provides efficient protection from UV radiation and halite keeps moisture (Stivaletta and Barbieri, 2008).

3.5 DESERTS

Deserts are extremely dry, cold or hot environments, exposed to extremes of UV light irradiation and where water is always a very limiting factor for life. Prokaryotic and eukaryotic microorganisms are adapted to these extreme conditions and were found in hot desert such as Atacama Desert of Chile - the oldest, extremely dry and hot desert on Earth. The coldest and driest places on Earth are dry valleys of Antarctica. In a recent study, performed on surface sands sampled from 13 different locations in the Sahara Desert in Morocco and Tunisia, the presence of virus-like particle was reported for the first time (Le Romancer, 2006). For both hot and cold deserts, the primary inhabitants are cyanobacteria, algae and fungi living few millimetres under the sandstone rock surface. They are epilithic organisms like lichens, which favour shaded and sheltered microhabitats such as north-facing cliffs, shaded boulders and crevices (Garvie, 2008). For example in the Antarctic desert endolithic communities, which grow predominantly within the rock are based on photosynthesis (cyanobacteria, lichens, and green algae). Microbes in such environments have adapted on long periods of dry and darkness conditions combined with dustings and dry snow. Dry snow represents an important source of water (Rothschild & Mancinelli, 2001).

3.6 ICE, PERMAFROST AND SNOW

Recent discoveries in frozen or near-frozen environments have demonstrated that organisms survive even in the harshest environments. Now it is believed that the absence of life in these environments would be an exception rather than the rule. To probe the fundamental processes and controls that limit life at the extremes (frozen or near-frozen conditions) Kennicutt (2008) shows in

his excellent review recent findings which were based on the studies such as: (i) study of the transition to polar night - to test if the onset of darkness produces physiological changes that alters the functional roles of autotrophic and heterotrophic microplankton within the dry valley lakes; (ii) exploring habitats for life in permanently frozen environments - ecological, physiological and molecular adaptations are characterised for a better understanding of microbial survival in extreme frozen condition; (iii) surveying of microbial diversity in frozen environments and the biogeochemistry of ice bound microbial assemblages.

In subglacial Arctic and alpine environments active and abundant microbial communities were found which may play a significant role in biogeochemical cycling and chemical weathering process in these environments (Kennicutt, 2008).

Beneath four km thick East Antarctic ice sheet the Lake Vostok was discovered, with indirect evidence of the existence of microbial communities. This leaves open question if life really exists in the subglacial Lake (Bulat, 2008).

Permafrost is defined as ground that for at least two consecutive years the temperature remains at or below 0°C. Culture-independent analyses of the permafrost/ground ice revealed that in permafrost bacterial communities are at least as diverse as in the surface of active layer soil, but phylogenetically different (Steven et. al., 2008). The same methods were used in the study made by Steven et. al (2008) and they showed for the first time Archaea in permafrost.

Studies regarding low temperature limits for microbial life are important in our search in cryogenic systems for frozen microbial fossils or living microbial communities, and provide important clues in searching life on Mars and beyond.

3.7 ATMOSPHERE

In atmosphere the dynamic chemical and biological interactions are very complex, and organisms that survive in this environment must tolerate high UV irradiation levels, desiccation (wind drying), temperature (both extreme low and high temperatures), and atmospheric chemistry (humidity, oxygen radicals, etc.) (Griffin, 2007). Airborne bacteria exist and most of them have origin from soil, lakes, oceans, animals and humans. The 'unnatural' sources include human agricultural activities, sewage treatment, fermentation processes, etc (Mancinelli and Shulls, 1977). It is still not clear if airborne biota constitutes a functional ecosystem or it represents an aerial suspension of organisms and their spore forms (Cox and Wathes, 1991).

Airborne organisms can travel in the Earth atmosphere for thousands of kilometres and they can reach the upper atmosphere level for several kilometres above the ground (Rothschild & Mancinelli, 2001). Recent studies (Griffin, 2007) showed that the largest deserts on the planet (Sahara and Sahel regions of North Africa and the Gobi, Takla Makan and Badain Jaran deserts of Asia) are the main sources of mobilised desert top soils which travel big distances through atmosphere each year. Microorganisms in the atmosphere connected with desert soils are capable of surviving on a global scale long-range transport.

Pathogens use in general aerosolization to move from host to host, and based on this hypothesis a wide range of dust-borne pathogenic microorganisms that move great distances through atmosphere was identified. Organisms from dust clouds, with the only existing evidence till now of outbreaks of coccidiomycosis (caused by spores of the fungus *Coccidioides immitis*), do represent a risk for human health (Griffin, 2007). When humans are infected by inhalation of the spores of this fungus, in half of the disseminated cases occur meningitis. Aerosols in the atmosphere contain organic material (up to 50%) and meteorite-derived iron and nickel from stratosphere (Rothschild & Mancinelli, 2001). All these are conditions related with the origin of life (Marchant, 2000). Aerobiology is in the last period more and more popular as it represents a critical field for searching for life in the Universe and for the concept of panspermia.

3.8 SPACE

Mars

The search for life on Mars is now focused on the Martian subsurface due to the extreme life hostile conditions on the planet's surface: low atmospheric pressure, dry, high UV radiation, cold, and strongly oxidizing conditions. Because of low atmospheric pressure (0.6-0.8 kPa), liquid water may flow under the surface. Compelling with this idea, Mars may be a planet with a strong biological potential similar to the deep-subsurface or hydrothermal environments on Earth (Direito, 2008; Rothschild & Mancinelli, 2001). Due to possible biological potential of Mars, researchers are now looking for optimal protocols for the sensitive detection of biomarkers (molecules storing hereditary information) what employs molecular techniques of microbial ecology (Direito, 2008).

Europa

Europa is Jupiter's moon, where presumably exists an ocean of liquid water, which lies under ice too thick to allow photosynthesis. To prove that there life exists, carbon, organic molecules or other energy sources must be found. Lake Vostok in Antarctica with a three km thick ice layer might represents a model how a potential European biosphere might survive (Rothschild & Mancinelli, 2001).

4 THREATS FOR EXTREME LIFE

Extremophiles are organisms which thrive in extreme conditions, but can be affected like any other organisms by sudden changes in their environment caused by human activities for example, or by long term changes such as climate change.

Global warming is a threat for organisms adapted to extreme low temperatures. A good example is Antarctic environment, where increase of temperature leads to increase of atmospheric humidity, precipitations and snow melting. This leads not only to extinction of organisms that thrive in frozen or near-frozen environments, but ice melting allows even unusually assemblages of invertebrates and cryptogamic flora to develop (Kennedy, 2008).

One of the best examples to be discussed here is the deep-sea environment. The Earth's last frontier, the deepest areas of the sea, is reached now by humans due to technological development. The inhabitants of these environments feel the human impact due to their potential in the development of new products (e.g. pharmaceuticals, enzymes, cosmetics, nutritional supplement, molecular probes, and agrichemicals) and due to increasing on deep-sea fisheries. The deep-sea investigations are considered mostly to be scientific, but because of the potential of the marine environment for discovery of new drugs (antibiotics, anti-cancer, antioxidant, anti-fungal, anti-HIV, anti-tuberculosis and anti-malaria), commercial exploration increased. All these represent a considerable pressure making the deep-sea from an enormous resource a fragile ecosystem. Hydrothermal vents, cold seeps and seamounts feel the pressure of research, bioprospecting (collecting and screening of biological material for commercial purposes) and mineral exploration (Synnes, 2007). These and others unique ecosystems may be disturbed by extensive sampling. Biodiversity must be conserved for environment and for the biotechnological industry and collections should be thus limited. The deep-sea is very vulnerable for intensive fishery, even more because some of the deep-sea species have long life and reproduction cycles. *Orange roughy*, the deep-sea fish, is an endangered species which can be eradicated by

fishery, because it lives 149 years and it is becoming sexually mature only at 25 years of age. Mining industry is also interested in exploitation of hydrothermal vents due to zinc and copper mineral deposits which lay there. This altogether results in disturbance of the unique set of species, often new to science. It is important to notice that some invertebrates can be eradicated simply by collecting the bacteria with which they live in symbiosis. If sampling is performed with caution, bioprospecting of microorganisms can be done on a sustainable basis (Synnes, 2007).

CO₂ from fossil fuel burning is changing the ocean chemistry. The future impacts of higher CO₂ concentrations may cause potentially important changes such as: lowering of pH of oceans, increasing seawater temperature, changes in water stratification, lowering of O₂ concentration, changing of salinity and increasing of UV. Through all effects species biogeography, biodiversity, biogeochemical cycles, food webs, ecosystems and their function are affected (Turley, 2008).

5 THE POTENTIAL OF EXTREMOPHILES AND FUTURE PERSPECTIVES

Extremophiles are known to be useful for biotechnology by possessing a wealth of novel bioresources. They are important for economy being used in agriculture, chemical synthesis, laundry detergents and pharmaceuticals.

Enzymes from extremophiles, extremoenzymes, have a great economical potential in agricultural, chemical and pharmaceutical processes. They are used in biological processes by increasing specificity and catalytic activity, and are stable at extreme incubation conditions (Chadha and Patel, 2008). There are many enzymes from organisms living in a single extreme condition like high salinity or temperature, and very few living in dual extreme conditions. An example of this last case is an anaerobic thermohalophilic bacterium *Halothermothrix orenii*, which provides better understanding of "adaptation" of proteins to dual extremities and design of enzymes. This is especially advantageous as it gives improved stability against multiple extremities. These kinds of studies will provide information about the evolution of genes and phylogenetic evidence for missing links in evolution. Extremoenzymes can be used in the future for novel biocatalytic processes that are more accurate, faster and more environmentally friendly (van den Burg, 2003).

By studying extremophiles, evolutionary biology will not only benefit by discovering the most extreme among extremophiles and finding new taxa,

but also by understanding evolution at molecular level. For human health, extremophiles could be an indirect benefit through bioremediation. For example *Dunaliella* algae can be direct used as a nutritional supplement and antioxidant. Another use of extremophiles is their antifreeze proteins for cryoprotectants of frozen organs (Rothschild & Mancinelli, 2001).

Recently on the Mid-Atlantic Ridge peridotite-hosted hydrothermal vent named 'Lost City Hydrothermal Field' was discovered. Metabolic reactions of those microbial biofilms from this community which use energy source like hydrogen and vent autotrophs might give us information about what kind of reactions initiated chemistry of life (Baross and Brazelton, 2008).

The research of extremophiles is becoming more interesting and needed. The improvements regarding research methods in exploration combined with analytical technology give us the hope that new discoveries are forthcoming. The commercial potential represents a very promising field, although at the present moment it is just in the phase of recognition. Research of extreme environments represents the main link to study the space, formerly inaccessible environment. Of course, why to study the space, and Mars, when we have so many undiscovered things on our planet? But the facts that our resources are not permanent available, science try to find solutions for future and links in Earth evolution and origin of life. Is known that Mars contain large quantities of water in the form of ice and that clay minerals detected in oldest Martian terrains give the information that liquid water was abundant for a long period of time. All these knowledge gives the possibility that life on Mars was suitable once like on Earth in the earliest stages of life (Zegers, 2008). To study a possible colonization and terraforming on Mars, extreme environments and extremophiles are a good point to start.

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