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Extremophilic eukaryotes for searching life beyond the Earth

S. Onofri and A. Cassaro

Department of Ecological and Biological Sciences, University of Tuscia, Largo dell'Università snc, Viterbo, Italy

Abstract. Astrobiological studies include the study of the limits of terrestrial life and extremophilic microorganisms. Nowadays, a broad range of organisms thrive the extreme environments are known. Basing on the condition in which these organism's optimally grow, they are called as thermophilic, alkaliphilic, halophilic, psychrophilic, etc. Although the most representative group of extremophiles is in the Archaea domain, the Eukaryotic organisms demonstrate to adapt to harsh conditions, such as those experienced in Low Earth Orbit (LEO). Here, we report a brief description of the main eukaryotic extremophilic organisms and the conditions under which they survived. The study of these organisms is helpful to deepen our knowledge about the limit of life on Earth, and therefore on other celestial bodies and to hypothesize in which extraterrestrial conditions life could arise.

Key words. limits of life – Stars: atmospheres – extreme environments– extremophiles – eukaryotes

1. The limits of life on Earth

Nowadays, Earth is the only inhabited planet in our Solar System we know; its distance from the Sun and the presence of essential elements required by life, as carbon, hydrogen, nitrogen, oxygen, phosphorous and sulfur (CHNOPS) are important prerequisite for life. From the point of view of human beings, the conditions under which we are adapted seem as "normal" and "typical" for our planet. However, this anthropocentric perspective is not accurate, because if we consider the Earth as a whole, it is also characterized by environments that present rather hostile and prohibitive conditions for humans, but which allow other forms of life to proliferate (Coker 2019). In fact, what is prohibitive for humans is not necessarily prohibitive for other forms of life. The space within which life can arise and persist is determined by physico-chemical, geological and biological limits, which include temperature, water availability, pH, salinity, and pressure that interact with biological systems to define the 'habitable space' for life (Board 2007). Within these physico-chemical parameters, essential biological processes (e.g. cell growth and metabolism processes) are maintained and beyond these limits, life cannot adapt (Fig. 1). When considering the limits of life, a distinction between the survival conditions and growth conditions has to be made. The survivability of an organism could be achieved though the association of different individuals within the same species or thanks to a mutualistic relation among different species (Schulze-Makuch et al. 2015); while

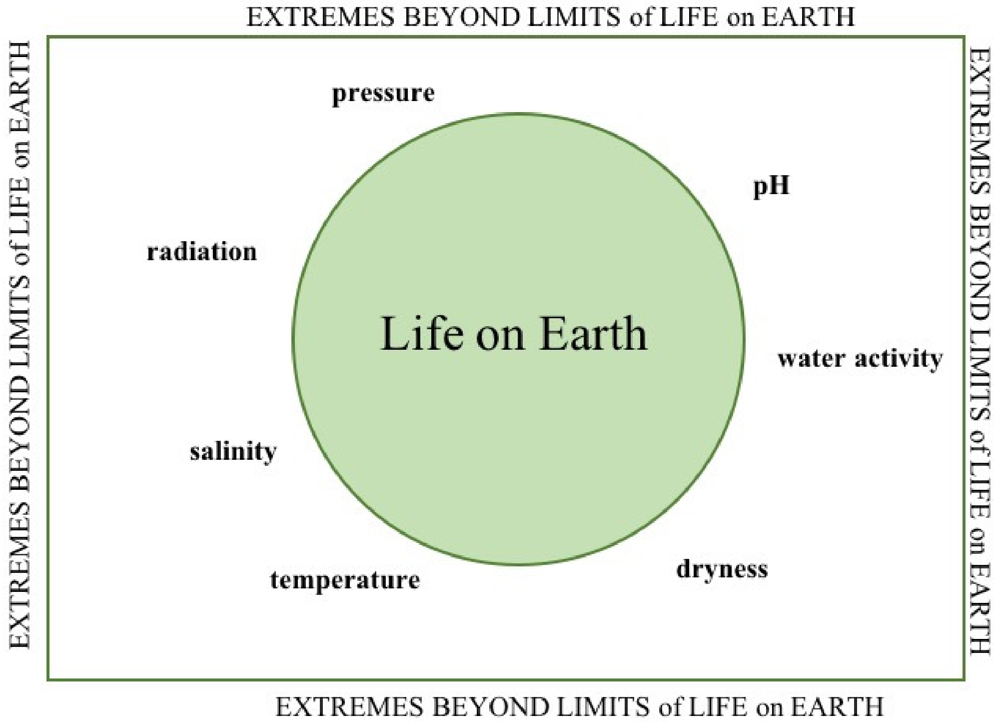

Fig. 1. Examples of physical and chemical extremes critical for complex life.

the growth limits are related to a single individual and to its ability to complete a lifecycle (Clarke 2014). On Earth, a multitude of extreme environments were discovered, where extreme includes temperature variations, radiation, depletion of oxygen, pH values, salinity, desiccation and pressure. In these habitats, conditions lie outside the "normal" range in which organisms live (Oarga 2009) and are completely inhospitable to complex organisms. The discovery of terrestrial extreme environments and the inhabiting organisms have increased the possibility to search for life beyond Earth.

2. Life under extreme conditions

Within the above-mentioned extreme parameters, life may assume a wide variety of forms, mainly dominated by microorganisms. Microorganisms are the most popular individuals found at the edge of the limit of life, because, when the conditions become extreme, a complex multicellular organism is hardly able to modify its complex biochemistry. As you get closer to the limits of life, as there is a decrease in diversity, due to the difficulty to have sufficient energy to cope with different extreme parameters. Organisms that inhabit extreme environments are distinguished into two broad categories: organisms which are easily adapted to extremes (extremotolerant) and organisms that not merely resist to extreme, but require one or more extreme conditions to survive (extremophiles) (Cavicchioli 2002). Extremophiles were described for the first time by Kristjansson and Hreggvidsson (1995) as organisms that thrive beyond 'normal' environmental parameters (temperatures between 4 and 40°C, pH values between 5 and 8.5, etc.), in conditions that are harmful for the majority of life on Earth (Oarga 2009; Gupta et al.

2014). These organisms could be categorized according to the different physico-chemical extreme in which they live (Rampelotto 2013). Many organisms live in high or very high-temperatures, such as hot springs (e.g., in the Yellowstone National Park, USA) or deep-sea hydrothermal vents. We may define thermophiles, organisms which need to growth in a temperature range between 45 and 80 °C; and hyperthermophiles, organisms that need temperature above 80 °C to growth. Otherwise, there are organisms that are adapted to low temperatures since about 20 percent of Earth hosts ice and about 70 percent of Earth is covered by oceans at temperature of 4 °C. These cold-adapted organisms are named psychrophiles, with optimal growth below 15 °C (Gupta et al. 2014). In this category, we could also distinguish cold-tolerant (psycrotolerant) that are able to growth below 15 °C. Lastly, mesophiles are organisms that can grow between about 15 and 45 °C. Organisms that exhibit an affinity with different pH values are divided into acidophiles, requiring pH value below 3 and alkalophiles, requiring pH value of 9. In addition, barophiles are organisms which grow in high-pressure environments. On Earth a variety of habitats are characterized by ions concentrations and the organisms that inhabit them are define halophiles (Rampelotto 2013). The organisms which have adapted grow in dry conditions or in the presence scarce water availability, are considered to be xerophiles; while organisms highly resistant to high levels of ionizing and ultraviolet radiation are called radiotolerant or radiophiles (Oarga 2009). Finally, endolithic organisms show their ability to growth within rock or within mineral grains (Gupta et al. 2014). Although extremophilic organisms can be divided into different categories, sometimes they cannot tolerate only one of these parameters, but several at the same time, and can therefore be defined as "polyextremophiles".

3. Organisms inhabited the extreme environments

Extremophilic organisms include individual from all life domains (i.e., Bacteria, Archaea and Eukarya, (Rothschild & Mancinelli 2001)). Archaea is the dominant group to inhabiting extreme environments (Cavicchioli 2002); have been found hyperthermophilic, acidophilic, alkaliphilic, and halophilic Archaea. For example, *Methanopyrus kandleri* is able to tolerate and grow at 122 °C (Rampelotto 2013), while *Geogemma barossii* strain 121 is able to grow and reproduce to 121 °C and also to maintain its viability for 2 hours at 130 °C (Ventura et al. 2019). The archeon *Pyrolobus fumarii* is able to grow up to 113 °C (Bloch et al. 1997); otherwise, *Haloquadratum walsbyi* has been found in halophilic environments. Some Bacteria, as *Desulforidis audaxviator*, use radiation from uranite for its metabolic pathways. The bacterium *Deinococcus radiodurans* has shown its ability to survive to ionizing radiation (up to 20 kGy of gamma radiation) and UV radiation (doses up to 1,000 Jm2) (Battista 1997). Among Bacteria, cyanobacteria are the most adapted group (Rampelotto 2013). These organisms may develop in hypersaline and alkaline lakes, high metal concentrations and tolerate xerophilic conditions. In addition, cyanobacteria are part of endolithic communities in desert regions, such as McMurdo Dry Valleys (Antarctica). For example, cyanobacteria belonging to the genus *Chroococcidiopsis* thrive in extremely arid environments such as the Dry Valleys or the driest areas of the Atacama Desert in Chile (Friedmann 1970; Warren-Rhodes et al. 2006). To date, it is known that this organism may survive for few minutes to Mars-like UV flux in de-hydrated conditions (Cockell et al. 2005), up to 15 kGy of ionizing radiation (Billi et al. 2000), and dried biofilms of *Chroococcidiopsis* cells survived in Low Earth Orbit conditions (vacuum, temperatures extreme, radiation, etc., (Billi et al. 2019)). In addition, among eukaryotes, fungi (alone or in symbiosis with cyanobacteria or algae) are the most versatile. In this work, we focus on Eukaryotic extremophiles.

4. Eukaryotic extremophiles

Many microbial eukaryotes are known as very resistant extremophilic or extremotolerant organisms. Eukaryotic microbial life may be found actively growing in almost any extreme condition, where there is a sufficient energy source to sustain it, with the exception of very high temperatures. Among extremophilic and extremotolerant microbial eukaryotic organisms, we can identify different groups, such as Algae, Plants, Metazoa and Fungi (Fig. 2). The thermo-acidophilic photosynthetic unicellular red algae of the *Cyanidiales* (Doemel & Brock 1970; Rothschild & Mancinelli 2001) and some individual of the green algae, are photosynthetic organisms mainly found in acidic waters with pH values below 4. In addition, *Cyanidiales* shown a higher temperature limit of 56 °C, while green algae below 42 °C. *Cyanidiaceae* may resist in pure CO_2 (Seckbach et al., 1970) and in high concentrations of heavy metals (Albertano& Pinto 1986) and extreme salinity (Gross et al. 2002). *Chlorosarcinopsis*, *Palmellopsis*, *Bracterococcus* green algae are representative of psycrophilic organisms (Vincent et al. 2004); for examples, the green alga *Chlamydomonas* nivalis is able to grow at 0 °C. In some cases, to resist against UV-radiation damages, chlorophytes may associate with cyanobacteria inside microbial mats in order to exploit cyanobacterial UV-screening compounds (Vincent et al. 2004). *Dunaliella parva* and *Dunaliella viridis* are halopilic green algae, found in the Dead Sea (Oren 1999). In addition, acidophilic green algae are found among the genus *Dunaliella* (i.e., *Dunaliella acidophila*) (Pick 1998). Similarly, *Chlamydomonas acidophila* populates extremely acidic lakes, but also the highly acidic Rio Tinto river (Messerli et al. 2005). The green alga *Dunaliella bardawil* shown a high amount of beta-carotene, which may be useful against excessive radiation. Regarding plant species, *Thellungiella halophila* could be classified as a salt tolerant brassicacean, used as model system to investigate salt and desiccation stressors (Bressan et al. 2001; Inan et al. 2004). In addition, one of the best models for

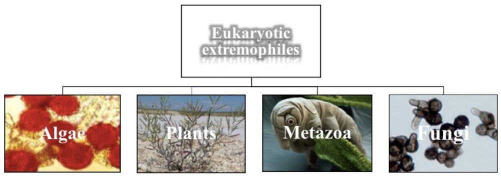

Fig. 2. Graphic representation of the main eukaryotic extremophile's groups.

desiccation tolerance is *Craterostigma plantagineum* (Linderniaceae) (Bartels & Sunkar 2005; Bartels & Salamini 2001). A large variety of metazoa have been found in extreme environments, characterized by high temperature and pressure, high salinity, and desiccation (Weber et al. 2007). Among metazoa, Tardigrades are the most utilized as test organisms in astrobiological studies. These organisms may tolerate extreme desiccation conditions, assuming a stress-resistant "tun"-state, in which they may survive temperature below 0 °C and up to 150 °C. Another important group is represented by fungi. Among fungi, the so-called "black yeasts", are considered as one of the most resistant categories. The term black fungi indicate the presence of a thick cell wall mainly composed of melanin pigments. These dark-brown pigments could be used by these organisms as protection against radiation, high temperatures and high concentration of heavy metals, desiccation and cytotoxic radicals (Butler & Day 1998; Wheeler & Bell 1988). Black fungi are able to thrive different extreme conditions. The black fungi *Hortaea werneckii* and *Hortaea acidophila* have been isolated from a suspension at pH 0.6 containing humic and fulvic acids (Holker et al. 2004). *H. werneckii* is able to survive up to 30 percent of salt concentration and is often isolated from hypersaline waters (i.e., solar salterns, (Gunde-Cimerman et al. 2000)). Acidomyces acidophilus is a polyextremophilic black fungus able to thrive in high salinity (Selbamann et al. 2008) and in low pH environments. The filamentous black fungus *Alternaria alternata* is known to grow in highly radioactive environments (Mironenko et al. 2000). Likewise, *Aspergillus versicolor* and *Cladosporium cladosporoides* species shown their ability to growth in direction of ionizing radiation sources (Zhdanova et al. 2004). *Aspergillus sydowii* isolated from 5000 meters-depth in the Central Indian Basin, possess spores able to germinate at 500 bar pressure (Raghukumar et al. 2004). Recently, *Cryomyces antarcticus* and *C. minteri* species have been isolated from McMurdo Dry Valleys (Antarctica) sandstones. These organisms could be defined as polyextremophiles, because of their extraordinary resistance against a wide range of stressors, such as high salt concentration (up to 24 percent NaCl) (Onofri et al. 2007), repeated freezing and thawing cycles (-20/+20 °C) and to high temperatures (90 °C for 1 hour) (Onofri et al. 2008) and resisted 18 months in space and Martian-like conditions on the International Space Station (Onofri et al. 2012, 2015). In particular, the black fungus *C. antarcticus* survived up to 56 kGy of gamma rays (60Co, (Pacelli et al. 2017)), accelerated iron and helium ions up to 1000 Gy (Pacelli et al. 2020; Aureli et al. 2020) and to X-ray conditions up to a dose of 0.3 Gy (Pacelli et al. 2017). However, the black fungi are not the only extremophilic fungi. *Acremonium* and *Chrysosporium spp.* have been isolated from alkaline soils of two limestone caves in Japan. *Aspergillus ustus* is considered a xerophilic fungus, which can grow at low water availability.

5. The importance to study the limits of life and the extreme environments: an astrobiological point of view

Investigating the limits of life and studying the extreme environments has become an important area of astrobiological research. First of all, the study of the limits of life on Earth allows to compare conditions experienced elsewhere in our Solar System with terrestrial conditions, trying to answer the question if there are terrestrial-like conditions suitable for life on other celestial bodies. Furthermore, the study of extreme environments and the inhabiting microorganisms is strictly related to the limits of life. Over the last few years, the study of extremophiles provided new discoveries which allow to answer in a different way to the question: "What is life and what are the limits of life"? These discoveries allow to understand the conditions required for the origin and evolution of life on early Earth and elsewhere in our Solar System. Extremophilic microorganisms may use different adaptation mechanisms to survive prohibitive conditions of extreme environments which provide a unique perspective on the fundamental characteristics of biological processes. Although the exact adopted strategies are not entirely clarified, it is well known that these organisms have evolved specific macromolecules, such as enzymes and lipids capable of non-denaturation or degradation under temperature variations, pH, high salinity or pressure. In this context, extremophiles may be useful as test or model organisms to investigate the existence of life on other planets. For examples, eukaryotic microorganisms isolated from Antarctic cryptoendolithic microbial community, such as black fungi, were used as test organisms in astrobiological related studies (Onofri et al. 2007) to test the survivability of organisms after exposure to space and Mars-like conditions in space (Onofri et al. 2012, 2015, 2019). In addition, the green alga *Sphaerocystis* sp. CCCryo 101-99 and the lichens *Circinaria gyrosa* and *Buellia frigida*, were selected as organisms to expose in Low Earth Orbit outside the International Space Station (ISS) (De Vera et al. 2019). Microorganisms discovered in ice core of the subglacial Lake Vostok (Antarctica) may be utilized as tests for the search of life on the Jupiter's moon Europa. Likewise, hyperthermophilic microorganisms isolated in hot springs, hydrothermal vents and volcanic sites, both in terrestrial either marine habitats may simulate hypothetical life forms existing in other celestial bodies. Nowadays, one of the main focus of space agencies is Mars exploration, since it is thought that it may hosts or hosted suitable conditions for life; in its early stage was characterized by a warmer and wetter environment probably compatible for life as we know it. In this context, the study of endolithic organisms could provide support for the upcoming space exploration missions finalized to life-detection on Martian subsurface, because hypothetic organisms could find refuge in the subsurface, similarly to what occurs in hot and cold deserts on Earth.

6. Conclusions

Eukaryotic organisms are considered as one of the best organisms able to thrive the extreme conditions of some terrestrial habitats. Although there are few studies about adaptations mechanisms in extreme conditions of microbial eukaryotes, ground-based astrobiological studies demonstrated that these organisms could be utilized i) as test organisms for the search of life elsewhere in our Solar System and to ii) expand our knowledge about the limits of life. In addition, deepening knowledge about eukaryotic extremophiles could be useful i) to shed light on the stress responses and possible defense mechanisms in other eukaryotic cells including human cells and ii) to understand how extraterrestrial life may survive to space travel, iii) to treat the planetary protection issue.

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