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Radiation resistance of the extremophiles: Implications for astrobiology

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Summary. — The extremophiles are microorganisms able to thrive in extreme environmental conditions and include bacterial species belonging to both *Bacteria* and *Archaea* domains of life. The ability to resist several harsh chemical and physical conditions make them of great interest for astrobiology, *i.e.*, the multidisciplinary approach to the study of the origin and evolution of life on Earth and in the universe. The resistance to radiations like ultraviolet radiations or ionizing radiations, the main components of the cosmic rays, is one of the main issues in astrobiology in relation to the search for bacterial species that, according to the *panspermia* theory, could adapt to life in space or that could have survived the interstellar transport. Here we present an overview of recent researches concerning the ability of extremophilic bacteria to resist solar and ionizing radiations. The relevance for the study of adaptability of terrestrial life forms in space environments or exoplanets, is also discussed.

1. – Introduction

Astrobiology is a very recent discipline that has been accepted only in 1979 by the International Astronomical Union (IAU). Astrobiology addresses the question of the origin, the evolution and distribution of life in the universe. One of main issues in astrobiology is the exploration of the limits at which life can occur on Earth or in the universe. In particular, the problem of life and habitability, *i.e.*, the search for life and habitable locations outside Earth is addressed by missions in space or by simulation experiments by means of laboratory facilities [1].

The study of extremophiles [2], *i.e.*, those microorganisms that are able to live and thrive on Earth in several extreme environments, is significantly relevant in astrobiology

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since the environmental conditions in space, and in other Solar System bodies, are closely similar to those of severe environments on Earth in which extremophiles can be found. For these reasons extremophiles are considered as valuable biological models for astrobiology studies, as confirmed by several recent findings that confirmed that these organisms could resist to the interstellar transport from or to Earth.

Indeed, the terrestrial environments harbouring the extremophiles are characterized by harsh chemical and physical parameters like extremely high or low temperatures, high acidity or alkalinity, high salinity, absence of water or oxygen, extreme pressure, solar radiation and cosmic rays [3].

With particular regard to the radiation, the existence of microorganisms that are able to resist such stressors is of great interest for astrobiology since the exposure to cosmic rays and solar space radiations are some main extreme parameters characterizing both space travelling and exoplanet environments. The solar radiation is constituted by radiations whose wavelengths range from the energetic X-rays, UV and gamma rays to visible light up to the radio waves. The most energetic ones, X- and γ-rays besides UVC rays, *i.e.*, ultraviolet radiations with wavelengths lower than 280 nm are filtered by the Earth's atmosphere but are not shielded in space or on exoplanets lacking an Earth-like atmosphere. Moreover, possible living organisms were exposed in the space environment cosmic rays. The cosmic rays are a continuous radiation generated by events like supernova explosions, and are composed of 85% protons, 14% α-particles and 1% heavier nuclei: the latter are also referred to as particles of high charge Z and high energy E (HZE particles) [4]. Therefore, assessing the resistance to radiations is relevant to identify those terrestrial species that could adapt to life on exoplanets or that could resist space transfers. Several bacterial extremophilic species that are resistant to solar high energetic radiations, have been identified. Their distribution and relevance to astrobiology will be the topic of this paper.

2. – The extremophilic bacteria: classification and habitats

The extremophilic organisms are distributed in all the domains of life. With particular regard to the extremophilic bacteria, they belong to both the *Bacteria* and the *Archaea* domains and are considered among the prokaryotic microorganisms that first colonized the Earth's biosphere and that have evolved, and still continue to evolve, in a variety of extreme conditions. Extremophiles not only have adapted to live in one or more extreme conditions, but they also often require them for their growth [1].

Based on the conditions in which they survive and optimally grow, the extremophiles can be classified as follows: thermophiles and hyperthermophiles, *i.e.*, microorganisms living at high or very high temperatures; psychrophiles, *i.e.*, living at low temperatures; acidophiles and alkaliphiles, *i.e.*, living at acidic or basic pH values, respectively; halophiles, *i.e.*, living in the presence of high NaCl concentrations; anaerobes, *i.e.*, living in the absence of oxygen; barophiles or piezophiles, *i.e.*, living under high pressure. A more detailed description of the extreme conditions in which the extremophiles can live and thrive is reported in table I.

In addition, some extremophiles are able to resist simultaneously to different stresses, *i.e.*, the so-called polyextremophiles. Some remarkable examples are *Sulfolobus solfataricus* that thrives at high temperature and low pH, some species belonging to the genus *Halomonas* that tolerate high salt's concentration and alkaline pH, or finally *Deinococcus radiodurans* whose vegetative cells resist cold, desiccation, vacuum and radiations.

The extreme ecosystems on Earth harbouring extremophiles are hence characterized

Table I. – *Classification of extremophiles based on the environmental conditions for optimal growth.*

Extremophile's type	Parameter	Range
Hyperthermophile	Temperature	$T > 80\,^{\circ}$C
Thermophile		$60\,^{\circ}\text{C} < T < 80\,^{\circ}$C
Psychrophile		$T < 15\,^{\circ}$C
Acidophiles	pH	$p\text{H} < 3$
Alkaliphiles		$p\text{H} > 9$
Halophiles	Salt concentration	2–5 M NaCl
Anaerobes	O_2 tension	No oxygen
Microanaerobes		Tolerate oxygen
Barophiles or *piezophiles*	Pressure	130 MPa
Radiation resistant	Electromagnetic radiation	X-rays, γ-rays, UV rays

by one or more of the harsh conditions listed in table I. Indeed, the environments in which extremophiles have been found include submarine hydrothermal vents, hot springs or active volcanoes with high temperatures up to 122 °C, solar-heated surface soils with temperatures up to 65 °C or finally artificial hot environments like, for example, the composting sites [5]. Psychrophiles have been isolated from cold environments like permafrost in Antarctica, rocks and cold seas in both Arctic or Antarctic regions, freshwater sediments or from some cold-blooded higher organisms [6-9]. Alkaliphiles can be isolated from soda lakes [6] while acidophiles can be found mainly in soils of volcanic origin or hot springs that are rich in sulphuric compounds like the solfataras, or in artificial acidic habitats like mines or contaminated rivers and lakes [10, 11]. Halophiles have been isolated from hypersaline soda lakes, from ocean and hypersaline lakes of oceanic origin and solar salterns [12]. Barophiles are generally found at high depths both inside the Earth's crust or in the ocean depths. Anaerobes can be found in several environments where also other extremophiles have been isolated such as marine ecosystems or artificial environments generated from human activities like composting sites. Finally, some radiation resistant (or radioresistant) microorganisms have been isolated from areas in which the Earth's atmosphere is thin like some areas near the Poles or the equator line [6], and from extremely dry areas like some Antarctica sites or the hot desert Atacama in Chile. The radioresistant bacteria's group is discussed in detail in the next section.

3. – The radioresistant extremophiles: environmental distribution and strategies of adaptation

The extremophilic species able to resist different kinds of radiations have been identified in several *genera* from both the *Archaea* (*i.e.*, *Thermococcus, Pyrococcus, Halobacterium*) or *Bacteria* (*i.e.*, *Deinococcus*, *Halomonas*, *Rubrobacter*, *Bacillus*, *Parageobacillus*, etc.) domains of life [13, 14]. They can be found on Earth in both natural and artificial environments. Several species able to resist radiations have been isolated from volcanic areas, hydrothermal vents, geothermal springs, arid soils like deserts in China, Chile and Africa, stratosphere samples, radioactive hot springs like Misasa town in Japan [14, 15]. Many artificial environments are also source of radioresistant extremophiles. Infact, they

TABLE II. – *D_{10} values for spores and cells of bacterial species resistant to γ-rays exposure. Legend: Miers Valley Transect (MVT), Miers Valley (MV), within the McMurdo Dry Valleys in Antarctica. All data reported are from* [17] *and* [18].

Species	D_{10} (kGy)
Bacillus pumilus	1.26
Bacillus subtilis	1.5
Bacillus thuringiensis	2.0
Bacillus cereus	3.0
Bacillus megaterium	2.5
Bacillus subtilis luxAB	2.5
Salibacillus marismortui	2.0
Parageobacillus thermantarcticus	>2.5
Halomonas sp. MVT 161	2.66
Halomonas sp. MVT 463	1.80
Halomonas sp. MVT 464	1.90
Halomonas sp. MVT 468	2.50
Brevundimonas sp. MV7	1.26
Rhodococcus sp. MV10	0.55
Deinococcus radiodurans	50

been found on the International Space Station (ISS), in radiation-polluted sites or in canned meat samples treated with γ-rays as standard sterilization procedures for bacteria's removal.

The solar UV radiations or the ionizing radiations like γ-rays and X-rays and HZE particles radiations, can cause damages to all the cellular components like DNA, proteins, carbohydrates and lipids [14]. The effect of the radiation can be direct or indirect: in the case of UV radiations, they are able to induce the generation of DNA photoproducts like cyclobutane-type pyrimidine between adjacent thymine residues on the same DNA strand [16]. On the other hand, the effect of ionizing radiations like γ-rays, X-rays or HZE particles is mediated by the production of reactive oxygen species (ROS) that, in turn, cause molecular damages at the level of DNA and proteins. The ROS are generated by the reaction of water that is converted into species like, for example, hydroxyl radicals that react with proteins, DNA and other cellular components thus impairing their structure and function.

The radioresistant bacteria are microorganisms that have developed cellular systems that make them able to resist and to adapt to radiations' effects. These microorganisms can be classified on the basis of the so-called D_{10} dose, *i.e.*, the radiation dose required to inactivate 90% of the initial cellular population. In table II, is listed the D_{10} of the spores of some species belonging to the *genera Bacillus*, *Salibacillus, Parageobacillus*, and of the cells from the *genera Halomonas, Brevundimonas, Rhodococcus* [17, 18]. For comparison, the inactivation's dose for the cells of the species *Deinococcus radiodurans*, one of the most radioresistant species identified, is also reported.

The species belonging to the genus *Deinococcus*, that are among the most radioresistant bacteria, are able to resist high radiation doses of γ-rays and UV thanks to their efficient DNA repair systems. The vegetative cells of the species *D. radiodurans*, identified for the first time in samples of sterilized meat, have been the object of several studies in consideration of their ability to resist high radiations doses of γ-rays. *D. radiodurans* has been shown to possess a peculiar DNA-repair strategy based on different factors like homologous recombination, post-irradiation DNA degradation that provides single-stranded DNA for recombination, regulation of DNA's replication and post-irradiation DNA degradation, export of damaged nucleotides from the cell to avoid mutation [19].

Instead, other bacterial species resist radiations by forming endospores: a remarkable example is represented by the species belonging to the genus *Bacillus* and the closely related *genera Geobacillus* and *Parageobacillus* [20]. The formation of spores is the strategy that some bacteria adopt to survive in response to adverse environmental conditions. Indeed, cells of the species belonging to the *genera Bacillus*, *Salibacillus, Parageobacillus* undergo a sporulation process upon exposure to radiations. The spores are usually more resistant to radiations then the respective vegetative cells. The radioresistance of spores depends upon their structure and chemical composition. Briefly, a spore is made up of layers that, going from the outside to the interior, include the exosporium, the coats, the outer membrane, the cortex, the germ cell wall, the inner membrane and the central core [21]. The spores' DNA is located in the central core whose main chemical components are the pyridine-2,6-dicarboxylic acid (dipicolinic acid, DPA), RNA and proteins like the α-/β-type small acid-soluble spore proteins (SASP). Different studies have shown that the resistance to UV radiations and to HZE particles irradiation is mainly related to the saturation of DNA with SASP, the low water content and the high DPA amount in the core [4, 16]. Similarly, the presence of DPA could be one of the main factors determining the resistance to gamma radiations [17].

4. – The relevance of radioresistance in astrobiology

The resistance to solar and cosmic galactic radiations is a key point for one of the main issues of astrobiology, *i.e.*, the search for conditions sustaining or limiting life in the Solar System outside the Earth. Indeed, the solar UV radiations or the ionizing radiations like γ-rays, X-rays and the HZE particles are among the main lethal factors that could hamper the emergence of life as we know it out of the Earth. The identification of species able to resist either the space travelling or the stay on exoplanets is therefore crucial. The extremophiles are a group of microorganisms that are found on Earth in extreme environments which in most cases closely resemble the space and exoplanets environments. Indeed, many of them have shown to be able to resist a variety of harsh conditions simulating the space environments like the exposure to ionizing radiations. The ability to resist radiations could be important for two astrobiology aspects, *i.e.*, the study of origin and the definition of boundaries of life in the universe. With regard to the first aspect, one of the most popular hypotheses concerning the emergence of life is the so-called *panspermia* theory [14]. According to this theory, at least some primordial terrestrial life forms could have originated from meteoroids that impacted the Earth's surface thus transferring bacterial life forms originated elsewhere in the space. Such a theory would be in accordance with the existence on the earth of radiation-resistant bacteria that apparently would take no advantages from this feature since high fluxes of radiations are not present on our planet. Instead, the radioresistant phenotype would be a reminiscence of life forms from other planetary bodies where higher fluxes of ionizing radiations are present [14]. With regards to the definition of life boundaries and, therefore, to the identification of terrestrial life forms that could adapt to life in the space, the bacterial species that could resist the exposure to radiation could also resist the interstellar transport that is crucial either for future space missions or for the identification of habitable planets. In this frame, the extremophiles that are considered to be among the first life forms that colonized the Earth, could be play a role as possible candidate species for these purposes since, as reported from several researches, they are able to resist a variety of extreme conditions that are typical of space environments like ionizing radiations fluxes.

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