Survival, DNA Integrity, and Ultrastructural Damage in Antarctic Cryptoendolithic Eukaryotic Microorganisms Exposed to Ionizing Radiation

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Abstract

Life dispersal between planets, planetary protection, and the search for biosignatures are main topics in astrobiology. Under the umbrella of the STARLIFE project, three Antarctic endolithic microorganisms, the melanized fungus *Cryomyces antarcticus* CCFEE 515, a hyaline strain of *Umbilicaria* sp. (CCFEE 6113, lichenized fungus), and a *Stichococcus* sp. strain (C45A, green alga), were exposed to high doses of space-relevant gamma radiation (⁶⁰Co), up to 117.07 kGy. After irradiation survival, DNA integrity and ultrastructural damage were tested. The first was assessed by clonogenic test; viability and dose responses were reasonably described by the linear-quadratic formalism. DNA integrity was evaluated by PCR, and ultrastructural damage was observed by transmission electron microscopy. The most resistant among the tested organisms was *C. antarcticus* both in terms of colony formation and DNA preservation. Besides, results clearly demonstrate that DNA was well detectable in all the tested organisms even when microorganisms were dead. This high resistance provides support for the use of DNA as a possible biosignature during the next exploration campaigns. Implication in planetary protection and contamination during long-term space travel are put forward. Key Words: Biosignatures—Ionizing radiation—DNA integrity—Eukaryotic microorganisms—Fingerprinting—Mars exploration. Astrobiology 17, 126–135.

1. Introduction

APRIORITY FOR Mars Science Laboratory (NASA), ExoMars (ESA), and upcoming Mars exploration missions is the search for organic molecules as biosignatures that indicate the presence of putative extinct or extant life. A task force is planned to explore the martian surface and subsurface and to return martian samples to Earth for comprehensive analyses (Bada, 2001). Therefore, a key step for interpretation of future data is to investigate the possibility of preservation of organic matter in the martian environment and space.

DNA sequences of halophilic archaea, 22,000 to 34,000 years old, can be detected in crystals of halite from Death Valley, California. PCR-based approaches detected DNA in even older halites, 10⁶ to 10⁸ years old (Sankaranarayanan *et al.*, 2014), while microbial nucleic acids hundreds of millions of years old were obtained from Siberian and Antarctic

permafrost (Willerslev *et al.*, 2004). The surface of Mars is continuously exposed to high levels of cosmic radiation because the planet lacks a thick atmosphere and a global magnetic field, and ionizing radiation is deleterious for both survival and persistence of molecular biosignatures such as DNA (Dartnell *et al.*, 2007). The effect of ionizing radiation on biological systems has to be taken into consideration when analyzing the survival of microorganisms and looking for stable biosignatures. Deleterious effects of radiation on DNA and other biological molecules can be induced either directly or indirectly; the energy can be absorbed directly by the key biomolecules, such as protein or nucleic acids. Alternatively, the energy can be absorbed first by other molecules, producing a radiation-induced radical that secondarily damages the biosignatures.

Several studies are addressed that were designed to test both microbial survival and persistence of biosignatures following irradiation. A comprehensive review of the survival of

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microorganisms in space was completed by Horneck *et al.* (2010), who investigated microbial survival under different extreme conditions. A great deal of interest in astrobiology has recently been devoted to different biomolecules, including DNA, to establish a suitable biosignature. Recent studies have proved the persistence of both carotenoids and DNA to polychromatic UV irradiation (Baqué *et al.*, 2016), while eukaryotic DNA remained well detectable after both polychromatic UV irradiation and cosmic rays in real space conditions (Onofri *et al.*, 2015; Pacelli *et al.*, 2016). The STARLIFE project (Moeller *et al.*, 2017, in this issue) aims to characterize the effect of ionizing radiation of astrobiological relevance on different microorganisms. In this frame, we analyzed survival, ultrastructural damage, and DNA persistence at different ^{60}Co irradiation doses, in three eukaryotic models from Antarctic cryptoendolithic communities widespread in the Antarctic desert of the McMurdo Dry Valleys, the closest terrestrial analogue for Mars. Our models were (i) *Cryomyces antarcticus* CCFEE 515, a black fungus; (ii) a hyaline fungus, strain CCFEE 6113, related to the lichenized genus *Umbilicaria*; and (iii) *Stichococcus* sp. strain C45A, a green alga. *Cryomyces antarcticus*, in particular, was already selected in the past for astrobiological studies for its exceptional stress resistance and long-term survival when exposed to actual space conditions and simulated martian conditions in space (Onofri *et al.*, 2012, 2015). From October 2014 to February 2016, *C. antarcticus* was exposed anew on the EXPOSE-R2 platform, outside the International Space Station, in the frame of the BIOMEX project (de Vera *et al.*, 2012). Less information is available on the resistance of the selected alga and *Umbilicaria* sp. Photosynthetic organisms are known to be very sensitive to ionizing radiation since it can directly interfere with photosynthesis along with other metabolic functions (Kovacs and Keresztes, 2002). Algal cells in *Cladonia verticillata* treated with 10,000 Gy ionizing radiation showed cytoplasmic breakup in 20–30% of cells and altered growth (de la Torre *et al.*, 2017, in this issue). Besides, both an Antarctic strain of *Stichococcus* sp. and a lichenized hyaline ascomycete were isolated from rocks colonized with cryptoendolithic communities after long-term permanence in outer space (Scalzi *et al.*, 2012). The responses of taxonomically and functionally distant organisms to the same treatment in terms of survival and DNA persistence are of astrobiological significance, giving indication on resistance to some space parameters and searching for biosignatures.

2. Materials and Methods

2.1. Sample preparation

Three different eukaryotic models from Antarctic cryptoendolithic communities were chosen for testing the effect of ionizing radiation, as follows: (i) *Cryomyces antarcticus* CCFEE 515, an Antarctic cryptoendolithic black yeast-like microcolonial fungus isolated from sandstone collected at Linnaeus Terrace (McMurdo Dry Valleys, Southern Victoria Land), Antarctic expedition 1980–1981. The fungus was described by Selbmann *et al.* (2005) as a new genus and species endemic to the Antarctic. (ii) A sterile hyaline fungus CCFEE 6113, hyphae 2.5–3.0 μm diameter. Based on internal transcribed spacer (ITS), small subunit (SSU), and large subunit (LSU) ribosomal DNA sequence comparison, it belongs to a possible new species in the lichen

genus *Umbilicaria*. (iii) A green one-celled alga, with nearly cylindrical cells, 11–12 $\times$ 5.0 μm , strain C45A; the BLASTn analysis based on ITS and SSU sequences indicated this strain as a new species in the genus *Stichococcus*. Both the *Umbilicaria* sp. and *Stichococcus* sp. were isolated from sandstone collected in the McMurdo Dry Valleys, Antarctica, during the Italian expedition of 2010–2011.

Samples were prepared as follows: cell suspensions (1000 colony-forming units, CFU) were spread on Petri dishes of MEA medium (malt extract agar: malt extract, powdered 30 g/L; peptone 5 g/L; agar 15 g/L; Applichem, GmbH). *Cryomyces antarcticus* was incubated at 15°C for 3 months, *Umbilicaria* sp. at 25°C for 1 month, and *Stichococcus* sp. at 15°C for 1 month. Once grown, colonies were dried under laminar flow in a sterile cabinet overnight and then irradiated.

2.2. Test facilities and exposure conditions

Dried samples were exposed to γ radiation from a ^{60}Co source (γ rays at 1.17 MeV, low linear energy transfer of 0.3 keV/ μm). The doses are reported in Table 1. Irradiation was performed at Beta-Gamma-Service GmbH (BGS, Wiehl, Germany) (Moeller *et al.*, 2017, in this issue). Tests were performed in triplicate.

2.3. DNA extraction and PCR reactions

DNA was extracted from colonies and rehydrated in 1 μL of physiological solution for 48 h, using NucleoSpin Plant kit (Macherey-Nagel, Düren, Germany) following the protocol optimized for fungi (Selbmann *et al.*, 2014a).

Internal transcribed spacer (ITS), LSU, and SSU amplifications were performed with BioMix (BioLine GmbH, Luckenwalde, Germany), adding 5 pmol of each primer and 20 ng of template DNA at final volume of 25 μL . The amplification was carried out with MyCycler Thermal Cycler (Bio-Rad Laboratories GmbH, Munich, Germany) equipped with a heated lid. The fungal rDNA regions were amplified according to the work of Selbmann *et al.* (2011). For *Stichococcus* sp. the SSU regions of different lengths (500, 1000, and 1500 bp) were amplified by using NS1-NS2, NS1-NS4, and NS1-18L, respectively. Conditions for amplification were as follows: first denaturation step at 94°C for 3 min followed by denaturation at 94°C for 45 s, annealing at 55°C for 60 s, extension at 72°C for 3 min. The last three steps were repeated 40 times, with a last extension at 72°C

TABLE 1. GAMMA RADIATION DOSES APPLIED FOR EACH SAMPLE

Sample	Applied/Received doses
DLR lab control	non-irradiated
Transport control	non-irradiated
Irradiated sample	^{60}Co : 6.66 kGy
Irradiated sample	^{60}Co : 12.72 kGy
Irradiated sample	^{60}Co : 19.04 kGy
Irradiated sample	^{60}Co : 27.15 kGy
Irradiated sample	^{60}Co : 55.81 kGy
Irradiated sample	^{60}Co : 81.11 kGy
Irradiated sample	^{60}Co : 117.07 kGy
Positive control	non-irradiated wet sample

TABLE 2. rDNA REGION LENGTHS AND RELATIVE PRIMER PAIRS

Samples	DNA region	Primers
<i>Cryomyces antarcticus</i>	ITS (700 bp)	ITS4-ITS5
	LSU (1600 bp)	ITS5-LR5
	LSU (2000 bp)	ITS5-LR7
<i>Umbilicaria</i> sp.	ITS (700 bp)	ITS4-ITS5
	LSU (1600 bp)	ITS5-LR5
	LSU (2000 bp)	ITS5-LR7
<i>Stichococcus</i> sp.	SSU (500 bp)	NS1-NS2
	SSU (1000 bp)	NS1-NS4
	SSU (1500 bp)	NS1-18L

for 5 min. Gene lengths amplified, primer pairs and primer sequences for each test organism are reported in Tables 2 and 3.

Band intensity was measured and compared by using Image J software (Schneider *et al.*, 2012).

2.4. Random amplification of polymorphic DNA (RAPD) assay

For both fungi, the RAPD protocol was according to the work of Selbmann *et al.* (2011). For *Stichococcus* sp., the protocol was optimized, using OPA13 primer, as follows: first denaturation step at 94°C for 4 min followed by denaturation at 96°C for 30 s, annealing at 49°C for 60 s, extension at 72°C for 30 s. The last three steps were repeated 40 times, with a last extension at 72°C for 6 min.

2.5. Clonogenic assay

Microorganism survival was determined by measuring colony-forming ability as percentages of CFU. For the test, three of the treated colonies were rehydrated for 72 h in 1 mL of physiological solution (NaCl 0.9%) and diluted to a final concentration of 3000 cells/mL; 0.1 mL of the suspension was spread on Petri dishes supplemented with MEA medium (five replicates). *Cryomyces antarcticus* was incubated at 15°C for 3 months; *Umbilicaria* sp. was incubated at optimal growth temperature (25°C) for 3 weeks; the alga was incubated at 15°C (temperature of isolation) for 1 month exposed to light 24 h/day. After incubation the colonies were counted. Means and standard deviations were calculated. Statistical analyses were performed by one-way

analysis of variance (ANOVA) and pairwise multiple comparison procedure (Tukey test), carried out using the statistical software SigmaStat 2.0 (Jandel, USA).

The dose responses for clonogenic survival were modeled by the linear-quadratic (LQ) formalism, which is a simple mechanistically plausible radiobiological model (Sachs *et al.*, 1997; Brenner *et al.*, 1998). According to this model, the cell surviving fraction S (relative to un-irradiated controls) can be described by the following equation, where d is radiation dose and α and r are adjustable parameters:

$$S = \exp\left(-\alpha d - \left(\frac{\alpha}{r}\right)d^2\right)$$

Parameter α is a linear dose response term, and parameter r quantifies the quadratic dose response component: low values of r generate a strongly “downwardly curving” dose response (on a log scale). Best-fit values of α and r were found by maximizing the log-likelihood for the binomial distribution where the number of plated cells represented the number of “trials” and the number of observed colonies represented the number of “successes” (Shuryak *et al.*, 2016). Uncertainties (95% confidence intervals, CIs) for parameter values were estimated by profile likelihood.

2.6. Ultrastructural damage

After rehydration, controls and colonies irradiated with the maximum irradiation dose (117.07 kGy) were prepared according to the protocol reported by Pacelli *et al.* (2016). Transmission electron microscope observations were performed with a JEOL 1200 EX II electron microscope at the Center for High Instruments, Electron Microscopy Section of the University of Tuscia (Viterbo, Italy). Micrographs were acquired with an Olympus SIS VELETA CCD camera and iTEM software.

3. Results

3.1. DNA integrity

The integrity of DNA template after ionizing irradiation was assessed by amplifying three different gene lengths and by fingerprinting analysis.

3.2. *Cryomyces antarcticus*

Amplicons were obtained both for ITS and for LSU regions, fragments of 700, 1600, and 2000 bp *C. antarcticus*

TABLE 3. PRIMER SEQUENCES

Primers	Sequence (5' → 3')	References
ITS4	TCCTCCGCTTATTGATATGC	White <i>et al.</i> , 1990
ITS5	GGAAGTAAAAGTCGTAACAAGG	White <i>et al.</i> , 1990
LR5	TCCTGAGGGAAACTTCG	Vilgalys and Hester, 1990
LR7	TACTACCACCAAGATCT	Vilgalys and Hester, 1990
NS1	GTAGTCATATGCTTGTCTC	White <i>et al.</i> , 1990
NS2	GGCTGCTGGCACCAGACTTGC	White <i>et al.</i> , 1990
NS4	CTTCCGTCAATTCCTTTAAG	White <i>et al.</i> , 1990
18L	CACCYACGGAACCTTGTTACGACTT	Hamby <i>et al.</i> , 1988
(GGA) ₇	GGA GGA GGA GGA GGA GGA GGA	Kong <i>et al.</i> , 2000
OPA 13	CAGCACCCAC	Ho <i>et al.</i> , 1995

FIG. 1. Assessment of DNA damage in *C. antarcticus* irradiated with γ ray doses (^{60}Co): single-gene PCR (a) 700 bp, (b) 1600 bp, (c) 2000 bp, and (d) RAPD genomic fingerprinting.

DNA, respectively, after irradiation treatments (Fig. 1a, 1b, 1c). All bands were well preserved in 700 and 1600 bp gene length; the gel analysis for ITS measured 100% relative density of the band till the highest irradiation dose, while a reduction ranging from 34% to 31% was measured in LSU from 55.8 to 117.07 kGy. For 2000 bp fragments, a reduction up to 50% was measured starting from 19.04 kGy with a progressive decrease; besides, 20% of intensity was still maintained at 117.07 kGy (Fig. 1c). The RAPD profiles were mostly preserved in all the conditions tested with a

visible reduction of the highest molecular weight (MW) band at the two last irradiation doses. Yet a reduction was also recorded at 27.15 kGy (Fig. 1d).

3.3. *Umbilicaria* sp.

All amplifications worked out for all the gene lengths for *Umbilicaria* sp. (Fig. 2a, 2b, 2c).

Band intensities were well preserved (around 100%) in 700 bp fragments with a relative density reduction of 8% at

FIG. 2. Assessment of DNA damage in *Umbilicaria* sp. irradiated with γ ray doses (^{60}Co): single-gene PCR (a) 700 bp, (b) 1600 bp, (c) 2000 bp, and (d) RAPD genomic fingerprinting.

the highest dose only. In the LSU gene (1600 bp), a decrease of 23% in relative density was measured starting from 55.81 kGy, and 50% was still measured at maximum dose. For 2000 bp fragments, the DNA was more affected by treatments, and almost no signal was present at maximum dose (around 1% only of relative density was maintained, Fig. 2c). The RAPD profiles were well preserved till 55.81 kGy, while the disappearance of the highest MW band was observed at 81.11 and 117.07 kGy doses (Fig. 2d).

3.4. *Stichococcus* sp.

The effect on single genes on DNA of the alga was tested by using three different lengths of the SSU portion of ribosomal genes: perfectly visible single bands were obtained at all the exposures (Fig. 3). Band intensities were well preserved (around 100%) for the shortest gene lengths, while a reduction up to 50% was measured for the medium gene length starting from 55.81 kGy till the maximum dose applied (Fig. 3a, 3b). The highest effect was observed for the longest genes where a decrease of 88% was measured at 55.81 kGy and an almost complete disappearance of the band at 117.07 kGy where 5% only of band relative intensity was recorded (Fig. 3c). The RAPD profiles preserved in all the conditions tested as expected for an intensity reduction of the highest MW band starting from 27.15 kGy onward (Fig. 3d).

3.5. Clonogenic survival

The cultivation tests indicated that the melanized *C. antarcticus* maintained the highest capacity to multiply after the irradiation treatment with no statistically significant decrease after the first irradiation dose; a progressive decrease was observed until 55.81 kGy, where 12% survival was still maintained (Fig. 4a). *Umbilicaria* sp. showed a statistically significant decrease in CFU formation at the first

irradiation dose already. In general, CFU declined more rapidly with the increase of irradiation compared to *C. antarcticus*; 20% of survival was still present at 27.15 kGy, but a few colonies were still recorded at 55.81 kGy already (Fig. 4b). *Stichococcus* sp. exhibited much more sensitivity to the treatment with a reduction of CFU to 63% at the first irradiation dose. The decrease of survival was rather sharp with a few colonies at 27.15 kGy already (Fig. 4c). The data and LQ model fits to clonogenic survival dose responses for all three organisms are shown in Fig. 4d. The LQ model described the data reasonably—substantial discrepancies between best-fit model predictions and observed data occurred only at high doses/low surviving fractions for each organism. This probably occurs because the LQ model is a simplified approximation of more detailed DNA repair models (Sachs *et al.*, 1997; Brenner *et al.*, 1998); therefore, its accuracy tends to decrease at high doses/low surviving fractions (Garcia *et al.*, 2006; Kirkpatrick *et al.*, 2008).

Cryomyces antarcticus was the most radioresistant of the tested organisms (Fig. 4d), and its best-fit dose response parameters were $\alpha = 6.92$ (95% CIs: $5.57, 8.28$) $\times 10^{-3}$ kGy $^{-1}$, $r = 11.03$ (8.39, 14.0) kGy. *Umbilicaria* sp. was somewhat more sensitive and had a more strongly curved dose response shape: $\alpha = 2.39$ (95% CIs: 0.00, 5.07) $\times 10^{-3}$ kGy $^{-1}$, $r = 1.21$ (0.51, 2.74) kGy. *Stichococcus* sp. was the most sensitive: $\alpha = 34.17$ (95% CIs: 28.98, 39.40) $\times 10^{-3}$ kGy $^{-1}$, $r = 10.60$ (8.22, 13.50) kGy.

3.6. Ultrastructural damage

Figure 5 shows the ultrastructural damage observed by transmission electron microscopy (TEM) in samples exposed at maximum doses compared to untreated controls. All the control cells (Fig. 5a, 5c, 5e) maintained cell membrane and wall integrity with a well-organized cytoplasm even after dehydration. Irradiated samples of *C. antarcticus* showed a number of empty cells and dispersed cell wall fragments

FIG. 3. Assessment of DNA damage in *Stichococcus* sp. irradiated with γ ray doses (^{60}Co): single-gene PCR (a) 500 bp, (b) 1000 bp, (c) 1500 bp, and (d) RAPD genomic fingerprinting.

FIG. 4. Clonogenic test after ionizing irradiation (^{60}Co), CFU% of (a) *C. antarcticus*, (b) *Umbilicaria sp.*, (c) *Stichococcus sp.* Significant differences were calculated by Tukey test with $**=p>0.001$. (d) Clonogenic survival for *C. antarcticus* (CA), *Umbilicaria sp.* (Hy), and *Stichococcus sp.* (Al). Symbols represent data points, and error bars represent 95% CIs. Curves represent LQ model fits.

(Fig. 5b, white arrows); besides, some cells maintained an appreciable membrane integrity even if survival was not observed in the clonogenic tests (Fig. 5b, dark arrow). Damaged cells were present in both the irradiated *Umbilicaria sp.* and *Stichococcus sp.*, with broken cells discharging cytoplasm (Fig. 5d, white arrow) and irregularly shaped cells with disorganized cytoplasm (Fig. 5f), respectively.

4. Discussion

Survival capacity and DNA damage of organisms in space are of high astrobiological significance with regard to speculation as to life dispersal between planets, planetary protection, and the search for biosignatures.

In the frame of the STARLIFE consortium, which aims to compare the response of different astrobiological model systems to different types of ionizing radiation, three eukaryotic cryptoendolithic Antarctic microorganisms were irradiated, while dried, with high ionizing irradiation doses of γ rays that represent part of the galactic cosmic radiation spectrum.

Survival, ultrastructural damage, and DNA stability were tested by cultivation test, TEM observations, and PCR ap-

proaches, respectively; the last was based on the fact that damaged DNA is not suitable for amplification (Selbmann *et al.*, 2011).

The black fungus *C. antarcticus* showed the highest survival ability both in terms of CFU% and γ ray dose resistance; in fact, 12% survival was still recorded at 55.81 kGy, while *Umbilicaria sp.* and *Stichococcus sp.* were barely able to multiply at 55.81 and 27.15 kGy, respectively. Even the LQ model, which was applied to analyze the survival dose responses, confirmed the highest radioresistance of *C. antarcticus* among the tested organisms (Fig. 4d). None of the tested organisms were able to multiply after a maximum dose of irradiation (117.07 kGy), confirming what was observed with TEM, where consistent ultrastructural damage in all the irradiated test organisms was observed (Fig. 5). The scattered intact cells in *C. antarcticus* (Fig. 5b) most likely accumulated other damage that limited their vitality, since no growth was observed in samples irradiated at the maximum dose.

This higher survival is likely related to the conspicuous amount of melanin in the thick cell wall of *C. antarcticus* (Fig. 4a), the presence of which confers on the fungus a notable capacity to withstand ionizing radiation (Pacelli *et al.*, unpublished data). Melanins in black fungi are different types

FIG. 5. Transmission electron micrographs: comparison of untreated colonies (controls) and colonies irradiated at maximum γ irradiation dose (117.07 kGy). *Cryomyces antarcticus* (a) control, (b) irradiated sample, empty cells, and dispersed cell wall fragments (white arrows); cell with an appreciable membrane integrity (dark arrow). *Umbilicaria* sp. (c) control, (d) irradiated sample, broken cell discharging cytoplasm (white arrow). *Stichococcus* sp. (e) control, (f) irradiated sample, irregularly shaped cell with disorganized cytoplasm.

of high-molecular-weight pigments produced by enzymatic coupling of phenolic units reported as 1,8-dihydroxynaphthalene (Kogej *et al.*, 2004); they can play many different roles in microorganisms because of their unique physical-chemical properties. These pigments are responsible for the typical dark green to brown or totally black color of these fungi and confer on them the ability to survive a number of different external pressures, such as excessive heat or cold, extreme pH or osmotic conditions, polychromatic UV radiation; melanins also seem to mediate tolerance toward metals (Selbmann *et al.*, 2014b). Many melanized fungi are also very radio-resistant, requiring radiation doses exceeding 5 kGy to reduce cell survival to 10% (Dadachova *et al.*, 2008). Such doses are

roughly 1000-fold higher than the lethal dose for humans, which shows that extreme radioresistance is not limited to prokaryotes such as *Deinococcus radiodurans* and can be achieved by eukaryotic cells. Dadachova *et al.* (2007) even reported the surprising results that low levels of γ radiation stimulate growth in melanized fungi.

Stichococcus sp. was the most affected with a rapid decrease of survival even at the lowest dose: no CFU were recorded starting from 55.81 kGy. This high susceptibility of eukaryotic phototrophs was already reported for the photobionts of the lichen *Circinaria gyrosa* showing a sudden reduction of photosynthetic activities at the same irradiation dose (de la Torre *et al.*, 2017, in this issue).

The analyses on DNA stability confirmed the data observed in the cultivation test; *C. antarcticus* DNA showed the highest stability, maintaining 20% of band relative density even at the maximum dose applied and the longest gene considered. The DNA of *Umbilicaria* sp. was also well preserved, but a relative density reduction of the bands was already well visible in the second gene length tested (LSU) at high doses, while the SSU amplicons completely disappeared at the maximum irradiation dose applied.

The most susceptible to the treatment was *Stichococcus* sp., which showed a reduction up to 50% for the medium gene length starting from 55.81 kGy and an almost complete disappearance of the highest MW bands starting from 55.81 kGy.

Except for the disappearance of the highest MW bands at high irradiations, the RAPD profiles were well preserved in all the tested organisms. These results are in concordance with the work of Atienzar *et al.* (2002), who reported that DNA damage is visible in the highest MW amplicons first, because of the higher probability to accumulate mutations.

Notably, in our experiments DNA could be easily detected in all the tested organisms even after high irradiation treatment and even when microorganisms lost their viability and ultrastructural damage was extended. A suitable biosignature for life detection must have both high specificity and high intrinsic stability; DNA would provide unequivocal proof of the presence of extant life, or at least its presence in the recent past (Aerts *et al.*, 2014). Here, we found that DNA also has a high intrinsic stability and could be regarded as a biosignature even after life extinction.

Previous criticisms of the use of such biosignatures asserted that life on other planets does not have to be based on an exact replica of terrestrial DNA, and common gene sequences used as primer targets for Earth organisms may not be present in extraterrestrial DNA (Röling and Head, 2005), which would make it difficult to detect putative extraterrestrial DNA. The use of primers with very low specificity could overcome this problem and allow the amplification of short, repeated sequences of eukaryotic genome.

The discovery of nucleotide bases in the Murchison meteorite lends credence to the possibility of life emerging on another planet and traveling via meteorite to Earth (Martins *et al.*, 2008). The detection of nucleobases in meteorites indicates that fundamental building blocks of complex biopolymers are present beyond Earth and could potentially be involved in the evolution of life on other planets (Aerts *et al.*, 2014). There is a high potential for material to spread from one planet to another, due to the large number of meteorites within our solar system, theoretically paving the way for an interplanetary ancestor of all life (Mileikowsky *et al.*, 2000a, 2000b).

One concern is the life span of biomolecules. The timescales that DNA can persist in the fossil record are still under debate, although lifetimes of at least several tens of thousands to a hundred thousand years (Lindahl, 1993; Wayne *et al.*, 1999) are generally accepted (Aerts *et al.*, 2014). Critics assume that reports of ancient DNA dating from millions of years ago are the result of flawed experiments or contamination (Pääbo *et al.*, 2004; Willerslev *et al.*, 2004). Nonetheless, certain conditions, such as halite crystals, permafrost, and marine sediments, could improve

the conservation of ancient DNA (Sankaranarayanan *et al.*, 2014).

The low temperatures in space, of about 80 K, reduce DNA breaks by half as compared to ambient Earth temperatures (Lindahl, 1993), and the dry space environment preserves DNA (Lyon *et al.*, 2010). These findings encourage consideration that the low temperatures and dry conditions on Mars may better preserve DNA, even in the long term, than do conditions on Earth (Kanavarioti and Mancinelli, 1990; Sephton, 2010). Other studies on *C. antarcticus* DNA damage after exposure to a Mars-like atmosphere have demonstrated that fungal DNA is not affected and remains perfectly detectable by PCR (Pacelli *et al.*, 2016).

This preliminary study on survival and DNA resistance of eukaryotic extremophiles to gamma radiation, which constitutes part of the cosmic radiation spectrum, is a first step in the investigation of the fate of microorganisms and their biomolecules in space. The survival ability of microorganisms to space conditions and radiation in particular is of great interest in space biological research and the evaluation of potential contamination risks in the frame of planetary protection. Fungi, in particular, are of critical importance due to their potential to adapt to the human body and act as opportunists that could endanger the health of a crew. It is also true that fungi possess a strong metabolic and degradative resilience such that they could affect the integrity of spacecraft materials during long-distance spaceflight.

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Abbreviations Used

CFU = colony-forming units

CIs = confidence intervals

ITS = internal transcribed spacer

LQ = linear-quadratic

LSU = large subunit

MW = molecular weight

RAPD = random amplification of polymorphic DNA

SSU = small subunit

TEM = transmission electron microscopy