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Laboratory simulation of interplanetary ultraviolet radiation (broad spectrum) and its effects on *Deinococcus radiodurans*

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ABSTRACT

The radiation-resistant bacterium *Deinococcus radiodurans* was exposed to a simulated interplanetary UV radiation at the Brazilian Synchrotron Light Laboratory (LNLS). Bacterial samples were irradiated on different substrates to investigate the influence of surface relief on cell survival. The effects of cell multi-layers were also investigated. The ratio of viable microorganisms remained virtually the same (average 2%) for integrated doses from 1.2 to 12 kJ m^{-2}, corresponding to 16 h of irradiation at most. The asymptotic profiles of the curves, clearly connected to a shielding effect provided by multi-layering cells on a cavitary substrate (carbon tape), means that the inactivation rate may not change significantly along extended periods of exposure to radiation. Such high survival rates reinforce the possibility of an interplanetary transfer of viable microbes.

1. Introduction

The hypothesis predicting that living organisms may stand viable and be transferred from one planet to another by means of natural processes (*panspermia*) is still a matter of debate. It is currently thought to be a process by which any living form, most probably microbial in nature, survives the following three steps: (i) the escape step, *i.e.* ejection of contaminated planetary material towards space, normally caused by a large impact on the parent planet; (ii) the journey in space through time scales comparable with those experienced by the Martian meteorites (estimated as 1–15 million years); and (iii) the landing process in a manner to afford non-destructive deposition of the biological material on a recipient planet (Horneck et al., 2003).

Escape and re-entry steps of this process are critical due to the high amounts of energy to which organisms may be exposed within a short period of time so recent studies have focused on these steps (Burchell et al., 2004; Cockell et al., 2007; De la Torre et al., 2009; Fajardo-Cavazos et al., 2009; Horneck et al., 2008; Möeller et al., 2008; Stoffler et al., 2007). Various types of microorganisms, such as bacterial or fungal spores and viruses, as well as biomolecules, such as DNA, amino acids and liposomes, have been exposed to selected and combined space conditions outside Earth's magnetic field (Apollo 16) or in low Earth orbit onboard missions Spacelab 1, Spacelab D2, ERA on EURECA, LDEF, BIOPAN on FOTON and EXPOSE on the International Space Station (Olsson-Francis and Cockell, 2010). Extraterrestrial parameters, such as high vacuum, intense solar ultraviolet radiation, different components of the cosmic radiation field and temperature extremes affected the genetic stability of the organisms in space, leading to increased mutation rates, DNA damage and inactivation (Horneck, 1999). Extraterrestrial solar ultraviolet (UV) radiation was shown to be the most lethal factor to naked samples. When shielded against the influx of solar UV, spores of *Bacillus subtilis* survived for more than 5 years in space (Horneck et al., 1994).

Recently, Horneck et al. (2008) have tested the first step of the panspermia hypothesis by exposing spores of *B. subtilis*, cells of *Chroococcidiopsis*, and thalli and ascocarps of the lichen *Xanthoria elegans* to shock pressures in the range 5 to ~40 GPa. Their results support the hypothesis that biological material could be successfully ejected from planets in a way that seeding of early Earth might have ensued.

Saffary et al. (2002) exposed cells of *Bacillus sp.* and *Deinococcus radiodurans*, one of the most radiation-resistant living

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beings known to exist on Earth, to extreme ultraviolet (EUV) radiation (λ=30.4 nm) during a rocket flight. The exposition to EUV radiation decreased the survival of both organisms by an extra order of magnitude below their desiccation tolerance. Since this type of radiation strongly interacts with biological targets, the authors attributed such inactivation to rely on superficial cellular damages other than DNA, most likely on membrane and surface proteins. On the other side, lichens were capable to fully restore their colonization ability and photosynthetic activity after an uninterrupted 16-day exposure to real outer space conditions in the Biopan-5 facility (Sancho et al., 2007).

In view of the surprisingly positive results reported above in support to the concept of *panspermia*, the critical re-entry step of the process was solely addressed by the results of the ESA's STONE experiment (Cockell et al., 2007). These authors demonstrated that the endolithic photosynthetic organism *Chroococcidiopsis sp*. inoculated in a gneissic rock sample did not resist the speedy re-entrance into the Earth atmosphere since extreme heating reached down to ~5 mm depth of the rock.

Shielding from deleterious ionizing radiation has been expected to provide viability of viruses, bacterial and fungal dried spores, thus alleviating radiation-imposed constraints to any putative interplanetary transfer step. Calculations by Mileikowsky et al. (2000) have predicted that microorganisms such as *D. radiodurans* and *Bacillus sp.* should be shielded against space radiation inside rocks of the order of 0.33 m, to keep a viable minimal population during time ranges suitable to afford Mars–Earth interplanetary travel (~1 million years). Those authors also consider that more than 1 billion fragments with temperatures below 100 °C were ejected from Mars and landed on Earth within the last 4 billion years. Early Earth has witnessed the infall of about 10-fold more extraterrestrial matter during the "heavy bombardment" period. Indeed the ~40 Martian meteorites discovered so far on Earth represent a tiny fraction of those imported from Mars during Earth's history (Fritz et al., 2005).

The discovery of Martian meteorites on Earth (Dreibus and Wanke, 1985) implies that rock fragments can escape from planetary bodies and that interplanetary transfer of matter is possible to occur in the solar system (Okeefe and Ahrens, 1986). At least five out of about 40 known Martian meteorites, might not have experienced sterilizing temperatures during ejection from Mars and re-entry into the Earth's atmosphere (Shuster and Weiss, 2005). However, it is still an open question whether living beings could be transported between planets by means of such fragments, withstanding the harsh in-between environment (Table 1). It is tempting to think of the biological features of microbial extremophilic species (Rothschild and Mancinelli, 2001) as those necessary to cope with extraordinary environments as those found in the interplanetary space.

The present study has focused on the harsh environment that could challenge an extremophile microbe during the long time of an interplanetary migration step of *panspermia*. Survival of *D. radiodurans* to conditions similar to those present in the interplanetary space was investigated in an experimental simulation apparatus. It includes a synchrotron beamline equipped with a toroidal grating monochromator (TGM) present at the Brazilian Synchrotron (Cavasso Filho et al., 2007) and a vacuum chamber. The TGM beam spectrum comprises the vacuum-ultraviolet (VUV) down to the infrared (IR) region ranging between 0.1 and 21.6 eV (Fig. 2). Additionally, exposure to a hydrogen lamp emitting a strong Lyman-alpha photon flux (Ly-α, 10.2 eV, λ=121.6 nm) was performed to evaluate the impact of this Sun's intense VUV emission on cell viability. Replicate experiments were performed to test the viability of dehydrated non-sporeforming *D. radiodurans* vegetative cells following several doses of radiation. Comparative irradiances of TGM beamline and those at Earth and Mars orbits are summarized in Table 2.

Table 1
Physical conditions prevailing in the interplanetary space and at low Earth orbit in comparison to the conditions cast in the simulated experimental setup (modified from Nicholson et al., 2000).

Parameter	Interplanetary space	Low Earth orbit (≤ 500 km)	TGM	Hydrogen lamp
Pressure (Pa)	10^{-14}	10^{-6}–10^{-4}[a]	10^{-5}–10^{-4}	60
Irradiance (W m^{-2})	–[b]	1360	0.22	16.34[c]
Spectral range (nm)	Continuum	Continuum	57.6–12,900	121.6
Temperature (K)	> 4[b]	Wide range[b]	~295	~295

[a] Values in Earth orbit depend on outgassing of the spacecraft.
[b] Values differ depending on orientation and distance to the Sun.
[c] Based on direct measurements of the narrow peak emission (121.6 nm) using photodiodes.

Table 2
Solar irradiances at Earth and Mars orbits in comparison to the TGM beamline.

Spectral ranges (nm)	Earth solar constant (W m^{-2})[a]	Mars solar constant (W m^{-2})[a]	TGM (W m^{-2})[b]
Vacuum UV (57.6–200)	0.014	0.006	0.2000
UVC (200–280)	7.390	3.180	0.0098
UVB (280–315)	19.490	8.380	0.0022
UVA (315–400)	89.280	38.390	0.0031
Total UV (200–400)	116.16	49.950	0.0150
VIS (400–700)	520.28	223.73	0.0028
NIR (700–1100)	448.74	141.90	0.0007
MIR (1100–3000)	259.05	162.48	0.0006
Total IR (700–2500)	707.79	304.38	0.0014
Total irradiance (57.6–2500)	1344.2	578.06	0.2200

[a] Based on Schuerger et al. (2003).
[b] Based on direct measurements at TGM beamline using photodiodes.

2. Methods

2.1. Sample preparation

Cultures of *D. radiodurans* R1 wild type and a *rec*A670-deficient strain were obtained at Instituto de Radioproteção e Dosimetria, Rio de Janeiro, Brazil. They were cultivated in TGY broth—1.0% tryptone, 0.6% yeast extract, 0.2% glucose (Anderson et al., 1956) with shaking at 200 rpm (Innova 4080, New Brunswick Scientific, Edison, NJ, USA), at 32 °C for 10 h, or until reach the early stationary phase. After this period, the optical density at $\lambda = 600$ nm (OD_{600}) of the culture was spectrophotometrically measured and an aliquot was taken for direct cell counting under the microscope.

2.2. Synchrotron irradiation at the Toroidal grating monochromator (TGM) beamline

Thirty-six circular carbon tape (Shinto Paint Co) with 5 mm in diameter, covered or not by a polycarbonate filter smooth surface were distributed on screws upraising from a hexagonal copper-made sample holder core. After the estimation of the cell concentration using a microscope, screws were loaded with 1 μL of the fresh culture each (up to 10^6 cells per sample in a monolayer). Observation of the screws under scanning electron microscopy confirmed that the optimized concentration of cells resulted in a single layer (Fig. 1a). However, cells deposited on rough surfaces could be multi-layered (Fig. 1b) or could be shielded by surrounding matrix formed by organic material of the culture medium (Fig. 1c). The sample holder was placed inside a −80 °C freezer for at least 30 min, following immediate exposure to low vacuum (600 Pa) in a lyophilizer for at least 5 h. After this period, the sample holder was aseptically transported to the experimental hall of the LNLS where it was placed inside the irradiation chamber at the TGM beamline workstation. The available experimental assembly included a differential pumping system that allowed the use of a neon filter to attenuate the X-ray portion of the synchrotron radiation spectrum (Fig. 2). The irradiation experiment was designed to allow a complete triplicate assay in a single irradiation run, using a rotary manipulator device to change sample positions. Measurements of the photon flux were taken using a photodiode and a golden grid to check for electrical current variations in real-time. The chamber pressure was kept between 10^{-4} and 10^{-5} Pa during all experimental assays. Survival curves were plotted for six different doses of irradiation plus the non-irradiated control. Samples were exposed to 0, 1, 10, 50, 100, 500 and 1000 min of UV plus VUV (wavelength ranging between 57.6 and 400 nm), which resulted in integrated doses of (kJ m^{-2}) 0, 0.012, 0.12, 0.6, 1.2, 6.0 and 12.0, respectively. All irradiation assays were performed at room temperature. Cells were reconstituted in TGY broth and the survival fraction was determined according to their colony-forming ability. The percentage values for survival have been calculated as follows: $(N_t/N_0) \times 100$, where: N_t is the number of colonies per aliquot after the exposure time t, N_0 is the number of colonies per aliquot of the non-irradiated culture. Detachment of cells from the substrates after extreme dehydration, which also reflects some loss of viability due to lyophilization and vacuum exposure (between 20% and 50%) and were computed while scoring non-irradiated controls (N_0). For each irradiation experiment, N_0 was fixed as the mean value of three replicates after lyophilization, vacuum exposure and cell recovery.

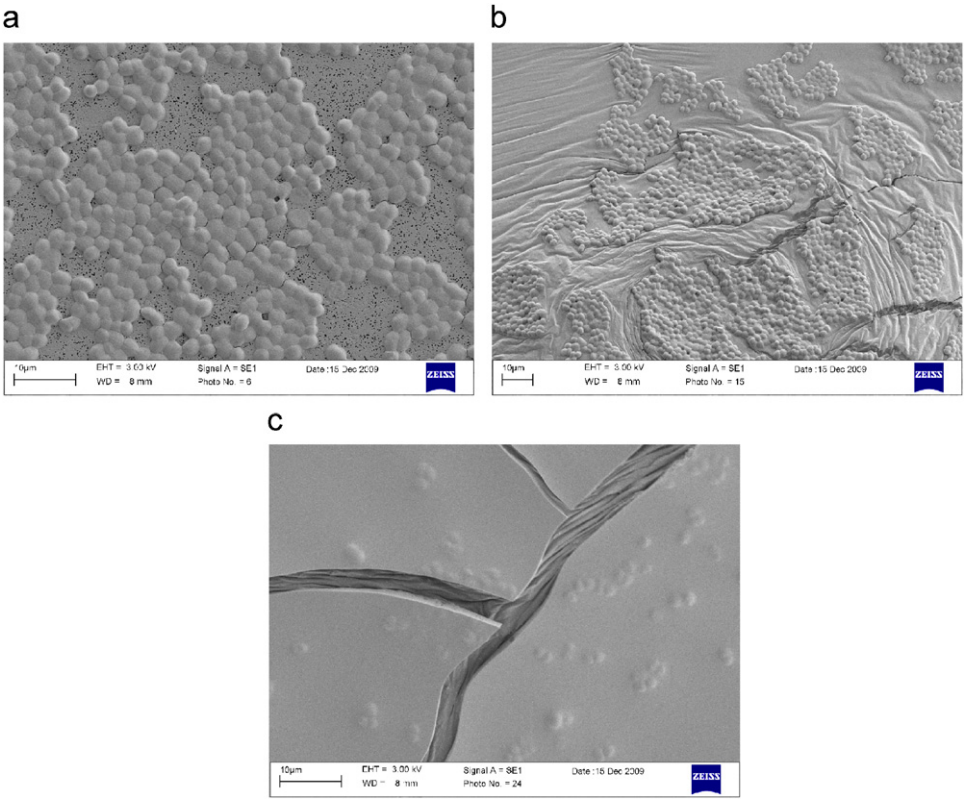

Fig. 1. Cells deposited on substrates showing three different situations: (a) cell monolayer formed over Millipore filters; (b) carbon tape surface with typical cell clumps; (c) cells embedded in a complex matrix of nutrients formed after extreme dehydration.

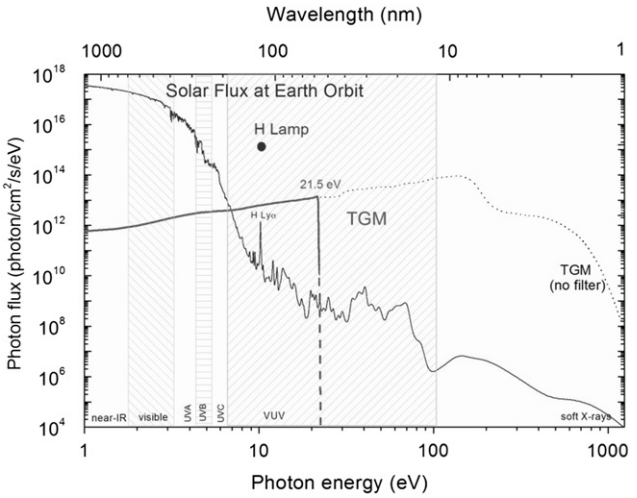

Fig. 2. TGM beamline spectrum with the harmonic gas cutting-off energies above 21.6 eV. For comparison, the dot named "H" refers to the intensity of the Ly-α line ($\lambda = 121.6$ nm) emitted from the hydrogen lamp used in this study.

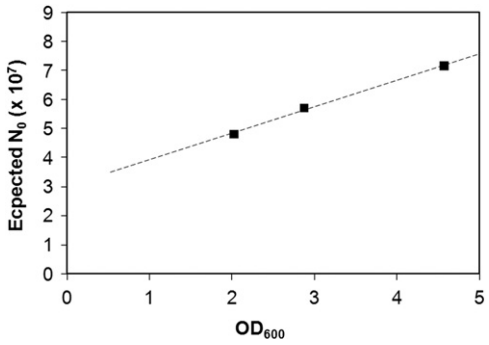

Fig. 3. Calibration curve plotting the initial optical density and viability of cell powder after sample rehydration.

2.3. Vacuum ultraviolet (VUV) inactivation assay using the hydrogen (H) lamp

Early stationary phase cultures of *D. radiodurans* were filtered on 25-mm-diameter and porosity of 0.45 μm, polycarbonate (smooth) surface. Observation of the filters under the microscope confirmed that 10^8 cells per sample resulted in a single layer of cells. Filters were fixed onto a metal sample holder, and the control samples were exposed to the same conditions, except for the irradiation. The sample holder was introduced inside the vacuum chamber at a distance of 8.0 cm away from the light source. The photon flux of the lamp (Opthos Instruments, Inc.), measured using a photodiode, was $10^{15}\,cm^{-2}\,s^{-1}$ (Fig. 2), corresponding to an irradiance of $16.34\,W\,m^{-2}$, for an internal atmosphere composed by 90% argon and 10% hydrogen at 60 Pa ($I/I_0 = 99.86\%$). Taking into account that biological effects of radiation are cumulative, several doses were given by increasing exposure times. To irradiate cells on the carbon tape (rough) surface, early stationary phase cultures ($OD_{600} > 1.0$) were lyophilized and the resulting powder was spread onto circular (25 mm in diameter) carbon tape, resulting in cell multi-layers. Survival curves were plotted for seven different doses of irradiation plus the non-irradiated control. Samples were exposed at room temperature to 0, 1, 5, 13, 51, 105, 227 and 771 min of Lyman-alpha radiation (10.2 eV), which resulted in doses of ($kJ\,m^{-2}$) 0, 1, 5, 12.5, 50, 103, 223 and 756, respectively. After the irradiation treatments, cells were reconstituted in TGY broth and the OD_{600} was measured. As the amount of powder varied between the samples, and taking into account that optical density reflects the number of cells, several OD_{600} measurements were performed for cell suspensions differing in turbidity. A calibration curve was plotted correlating the initial OD600 for non-irradiated samples to cell viability after recovery in medium (Fig. 3). It made possible to estimate the expected N_0 for different reconstituted powders differing in turbidity without knowing the initial number of cells. After calculating the expected N_0 for all samples, the percentage values of survival have been determined according to their colony-forming ability, as described for the synchrotron irradiation experiments.

3. Results

3.1. Synchrotron irradiation

The first experimental approach consisted in spreading dehydrated cells (10^6 cells per sample) on two different surfaces and exposing them to several doses of synchrotron radiation in order to examine the influence of the substrate on cell survival. The average roughness of the two surfaces was measured in a perfilometer as 7 μm for a rough carbon tape and 0.7 μm for a smooth Millipore filter. An inactivation curve entailing a two-component behavior was observed, with an initial exponential decrease followed by an asymptotic trend when cells were deposited on the rough surface (Fig. 4). The ratio of viable microorganisms remained virtually the same (average 2%) for integrated doses from 1.2 to 12 $kJ\,m^{-2}$, consisting of up to 16 h of uninterrupted irradiation. The asymptotic profile of the curve means that the inactivation rate may not change significantly along extended periods of exposure to radiation.

If the rough surface provided shielding to viable cells against radiation, then it should be also effective in shadowing the extremely radiation sensitive, *rec*A670-deficient strain of *D. radiodurans*. Indeed similar to what was observed for the wild-type strain, an average 1% cells of the deficient strain was seen to survive on the rough surface after exposition to up to 1.4 $kJ\,m^{-2}$

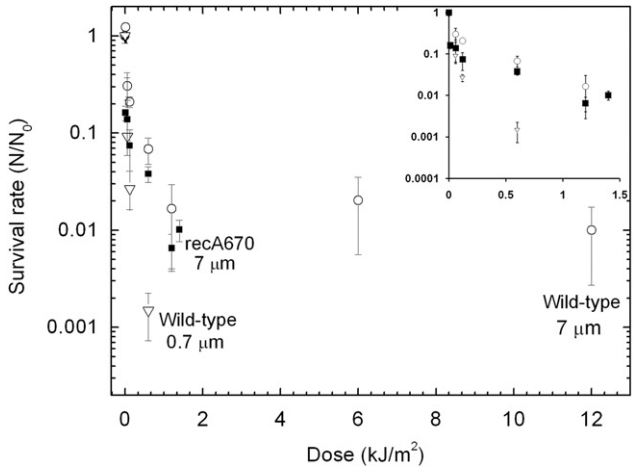

Fig. 4. Survival curves of *D. radiodurans* deposited on surfaces with two different levels of roughness (0.7 and ~7 μm) following several integrated doses of synchrotron radiation. The white circles represents survival of the wild type strain on a rough carbon tape; black squares, the same for the DNA repair deficient *rec*A670 strain of *D. radiodurans* and white triangles, wild type strain on the smooth surface. The error bars represent one standard deviation determined for each triplicate assay. Inset shows a better resolution for the points corresponding to the lower doses.

of synchrotron radiation (Fig. 4). However, these conclusions must be drawn only for the exponential phase of the inactivation curve of *rec*A670 (see detail in Fig. 4) because it was not exposed to higher doses due to beamtime constraints. When placed on the smooth surface this extremely radiation sensitive strain was annihilated by any tested dose.

The 7-μm cavities of the rough surface could cause layers of bacterial cells to pile, with superficial dead cells providing shielding to underneath ones which remained non-irradiated (Fig. 1b). To have such possibility checked, different cell densities were prepared by depositing 10^4–10^7 cells per sample on the carbon tape and irradiated at the same integrated dose of 0.6 kJ m^{-2} each. A clear enhancement of the survival rate was seen when concentrations above 10^6 cells per sample were irradiated (Fig. 5). Thus dead cell layers near the surface seem to be capable to shield underneath cells against UV and VUV photons, with fluxes comparable to that found at Earth orbit (Fig. 2).

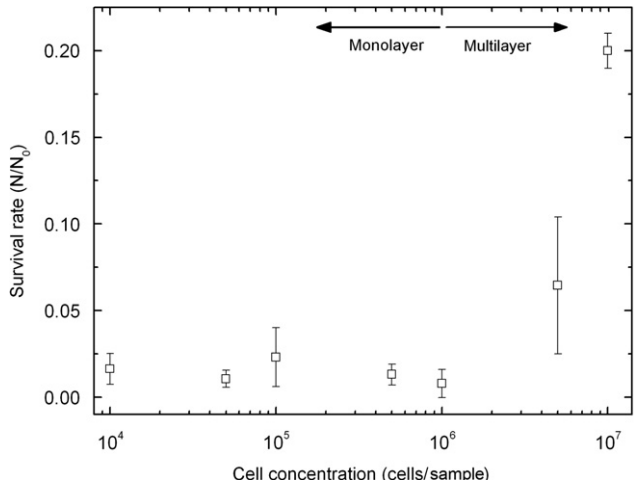

Fig. 5. Influence of cell concentration on the survival rate after 0.6 kJ m^{-2} exposure to VUV–UV radiation. Samples consisting of 10^4, 5×10^4, 10^5, 5×10^5, 10^6, 5×10^6 and 10^7 cells μL^{-1} per exposed surface have been irradiated with synchrotron light. Note that piling up multiple layers (above 10^6) cause a sharp increase in cell survival. The error bars represent one standard deviation determined for each triplicate assay.

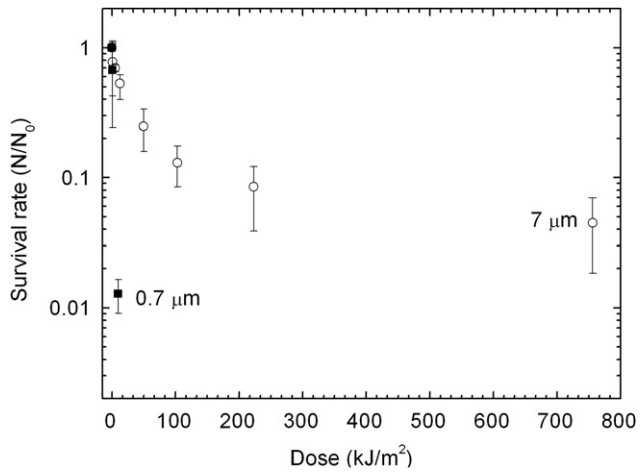

Fig. 6. Survival curves of wild-type *D. radiodurans* retrieved from surfaces with average cavities of 0.7 μm (smooth Millipore filter) or 7 μm (rough carbon tape) after irradiation with a Ly-α-emitting hydrogen lamp (λ=121.6 nm). The error bars represent one standard deviation determined for each triplicate assay.

3.2. Irradiation with hydrogen lamp (H lamp)

If arrival of bioactive material to any solar system's planet might have ever been possible, particularly relevant to the *panspermia* hypothesis is the resistance to the intense VUV Ly-α emission line by the Sun. A Ly-α emitting H lamp of high photon flux at λ=121.6 nm was used to determine cell survival. One-hundred million (10^8) cells were used in each assay. Even exposed to radiation in multi-layers, doses greater than 50 kJ m^{-2} reduced the viable cell population to values below the detection limit of the method (near sterilization) when placed on the smooth surface. On the other hand, the similar two-component behavior was otherwise observed for inactivation by the TGM source was seen in the case of H lamp if cells were deposited on the rough surface (Fig. 6).

4. Discussion

We report the survival of the non-sporeforming radiation-resistant bacteria *Deinococcus radiodurans* to synchrotron radiation mimicking the solar spectrum if trapped into a porous carbon tape and shielded by cell multi-layers surrounded by organic matrix material (Fig. 4). *D. radiodurans* has already been tested under several laboratory conditions and also assessed in space conditions when the Exobiology and Radiation Assembly (ERA) flew on the ESA's Eureca mission. Dose et al. (1995a) reported that although survival could not be scored due to storage conditions before and after the mission, the amount of DNA double strand breaks (DSB) per chromosome could be determined. If exposed to solar light ($>$170 nm; 4×10^8 J m^{-2} with respect to the range from 175 to 340 nm) in layers of about 1.5 mm, only the upper tenths on top 1 mm layer were affected, where more than 12 DSB were found in comparison to 8 DSB for the dark control. Control experiments have shown that *D. radiodurans* survives relatively well (15–35% survival rates, depending on the strain) if kept in dry argon for 17 months, but survival in humid argon (above 20% relative humidity) is less than 0.01% when exposed for the same period of time. Survival can be improved by up to 100% if the cells are dried in the presence of organics (tryptone–yeast extract medium).

Other microorganisms have also been tested in space. According to Dose et al. (1995b), monolayer of spores, fungal conidia or vegetative cells (*D. radiodurans*) are extremely sensitive to space conditions (vacuum and solar radiation). Multi-layers or macroscopic clusters, however, may resist for months or years, even if exposed to full solar light. Although the top cell layers become inactivated, they kept protecting interior cells from UV damage and also partially from dehydration.

In the experiments reported in this paper, *D. radiodurans* samples were exposed to vacuum and subjected to lyophilization, which resembles a fast dehydration process that biological material might undergo if ejected into space (Horneck et al., 2003). Early-stationary phase cells were spread out on the irradiation surfaces and lyophilized within nutrient liquid media (Fig. 1c). Microorganisms are not usually in logarithmic growth in the environment, and are often immersed in organic matter. Sugars and polyalcohols are known to help stabilize the structure of cellular macromolecules during vacuum-induced dehydration (Horneck et al., 2003), which could have contributed to the high survival rates observed in our experiments. In fact, the lyophilization process may lead to physical alterations similar to water loss during a process of mass ejection into space. Under such conditions, metabolizing cells are unlike to occur in the space environment and cellular repair mechanisms would not take place unless they re-entry a watery environment.

Comparing effects of the integrated doses from the two radiation sources, 1% cell survival was attained after exposition to 2 kJ m^{-2} of synchrotron radiation or 1000 kJ m^{-2} monochromatic Ly-α emission. Because different biomolecules have different absorption bands, it is expected that the wider TGM emission spectra will impact more cell targets and viability. Moreover, Ly-α irradiance from the source used here was a thousand times stronger than present solar Ly-α at Earth orbit, which is 0.01 J m^{-2} s^{-1} (Floyd et al., 2004). Yet cells were shown to be shielded even from such acute irradiation. This result suggests that micro-sized particles could prevent extremophilic microorganism from accumulating UV radiation damage. In agreement with our results, Osman et al. (2008) observed significant survival of spore-forming bacteria after irradiation with full spectrum Martian UV irradiation if shielded by micro soil particles (<60 μm) from the Atacama Desert. In addition, Pogoda de la Vega et al. (2007) have found that even nano-particles can afford survival of *D. radiodurans* upon UV irradiation under simulated Martian conditions.

In our experiments the fluxes of vacuum ultraviolet were much higher than those found in the interplanetary environment. Considering that dehydrated cells are metabolically inactive, dissimilar, unnatural dose rates are not expected to impact survival. In respect to this, fungal cells of *Neurospora* sp. irradiated in liquid culture at room temperature displayed 4-fold many mutants after acute UV in comparison to the same dose given at chronic rates (Stadler and Macleod, 1984). Interestingly, chronic UV at 0° incidence on the sample resulted in as many mutations as acute UV (Stadler and Macleod, 1984). Active repair during liquid holding at 22 °C was thought to prevent mutations to appear. For non-metabolizing cells it is therefore not expected that differences in mutation or survival rates may occur after the same total dose given either acute or chronically. Unfortunately, there is lack of experimental data about dose-rate effects on non-metabolizing cells.

As particular targets concerning recovery of genetic material after radiation, proteins belonging to DNA repair machinery were proven to critically affect cellular sensitivity following physical and chemical DNA damage (Daly et al., 2007). This is particularly true for longer UV wavelengths, which are known to overproduce free radicals by photoionization and photodissociation of water, the cell's main component. This situation was minimized in the present experimental setup with cells previously dehydrated and maintained in such state along the procedures allowing most proteins to be kept potentially active.

Considering that intact cells are exposed, Ito et al. (1980) remark that the penetrability of VUV radiation appears to be the key factor in determining the distribution pattern of damages. Accordingly, Munakata and Ito (1979) have found that sufficient synchrotron broad-band VUV light reaches *Bacillus* spores genetic material to induce mutation and killing. Nucleic acids were shown to be far-UV chromophores in *Streptomyces* conidia by Jagger et al. (1967). Altogether those data agree with physical estimates of the penetration depth of the VUV light (Wilkinson and Johnston, 1950; Watanabe and Zelikoff, 1953; Sowers et al., 1972), although no VUV chromophore could be identified so far.

The occurring extreme low temperatures and dehydration in the interplanetary space are conceivably protective regarding the formation of free radicals from water molecules. Noteworthy, microorganisms such as *D. radiodurans* and *B. subtillis* were shown to better survive UV if irradiated under extreme low temperatures relatively to the survival rates following irradiation under room temperature (Weber and Greenberg, 1985; Ashwood et al., 1968). Results of Weber and Greenberg (1985) showing increased UV–VUV survival of *B. subtilis* spores in low temperature and vacuum lend support to these conclusions.

The ability of extremophile microorganisms to thrive in extreme environmental conditions, such as high and low temperatures, desiccation, radiation, pressure and pH strengthens the probability that they could as well survive transport through the interplanetary (Horneck et al., 2008) or even the interstellar medium (Valtonen et al., 2009).

Extra-solar dust particles have been detected in the solar system by space probes (Frisch et al., 1999) and larger extra-solar meteoroids (5–35 μm) have most likely been detected in the upper Earth atmosphere by meteor-tracking radar facilities (Baggaley et al., 1994). Because of the small dimensions of these meteoroids, the bystander effects of high-energy charged (HZE) particles caused by the production of secondary electrons, shock waves or thermophysical events would probably be far less than those occurring in larger meteoroids. Therefore, hypothetical microbes within them would be mostly inactivated by direct hits. However, because of their low flux (e.g., 1 Fe ion per μm^2 per 10^5–10^6 years), it is predictable that damages may be localized to superficial layers, and few microorganisms would suffer hits within time scales of an interplanetary journey (Horneck et al., 2010).

Theoretical models of temperature variation alongside different altitudes above Earth atmosphere indicate that 10 μm diameter particles can withstand rapid deceleration without significant ablation (Coulson, 2004). In fact, Duprat et al. (2007) recovered highly friable micrometeorites from surface snow layers near the French–Italian station CONCORDIA in Antarctica, suggesting that those particles have experienced non-sterilizing heating upon atmospheric entry. Moreover, experimental evidence shows that small particles containing bacteria can survive the temperature regimens imposed during entry into Earth's lower atmosphere (Coulson, 2004).

The mechanisms by which microorganisms might infect interstellar dust particles are unknown. However, Napier (2004) suggests that boulders ejected putative life-bearing planets in the galaxy may be destroyed through erosion and fragmentation by impacting zodiacal light dust particles. Within less than a few thousand years, the fragmentation process leads to meteoroids of micrometer size that are ejected from the planetary system by radiation pressure. He estimates that about (10^{14}) life-bearing particles originating from the Earth leave the Solar system per year. To avoid the long travel times to other planetary systems, Napier (2004) suggests that contamination in fact occurs when the Sun passes through molecular clouds in the galaxy. Proto-planetary-forming systems within them will thus receive a certain number of Earth-born microorganisms. Supposing that the ejection mechanism occurs in all planetary systems harboring microbial life, Earth could have been in turn seeded from elsewhere. However, we should keep in mind that these numbers are highly arguable because the number of life-bearing rocks ejected into the solar system is actually unknown, as it is the density of living organisms within those rocks, their location relative to the surface, the erosive potential of micrometeorites on these rocks, etc.

The Sun and its accompanying planetary system were formed about 4.5 billion years ago. It has been shown to travel around the galactic center in a rough circular orbit with a linear rotation speed slightly greater than the local co-rotation speed. Thanks to such movement, each ~70–140 million years the solar system traverses a spiral arm containing dense ($n \geq 10^4$ cm^{-3}) interstellar clouds. Since its formation, the solar system has thus probably crossed tens of times such regions of much higher stellar and gas density. The flux of solid material arriving on Earth from nearby stars at the present distances was calculated in detail by Murray et al. (2004). They examined three possible sources of large extra-solar

meteoroids: Asymptotic Giant Branch stars (AGB), Young Stellar Objects (YSOs) and debris disks. Fluxes from AGB are the strongest ones arriving on Earth, approaching 8 particles $yr^{-1}\,km^{-2}$. The flux of dust and gas of extra-solar origin arriving on top of terrestrial atmosphere will increase by many orders of magnitude at each crossing of the sun through a spiral arm. The local density of stars in the solar neighborhood will also be increased by a factor up to ten.

An interesting possibility that extends the panspermia concept is enhanced from the discussion presented in this paper. Microbes from other places in the galaxy could benefit of the shielding effect operated by microparticulate material against UV–VUV radiation of the interplanetary space. As a matter of fact, living organisms could have more intensively seeded Earth during crossings of the solar system through dense galactic regions because of shorter times required for any organism to reach Earth. This process could thus advantageously overcome the following issues considered in the lithopanspermia theory: (i) intense heating during atmospheric entry, (ii) radioactive decay of heavy elements present in large rocks and (iii) the bystander effects of HZE particle radiation. Further experiments are currently being performed in order to address the effects of ionizing radiation (electromagnetic and charged particles) on radio-resistant microbes under this micro-shielding scenario.

Authors' contributions:

I.G.P.L. collected, processed and analyzed data, was involved in study design and wrote the manuscript; S.P. was involved in the experimental design, collected and processed data. E.J.P. was involved in data analysis and proposed the astronomical meaning to the primary data; A.N.B., J.A.R.G and A.A.C.L. were involved in advising experimental details and data analysis, provided the facilities and contributed equally to the study. C.A.S.L. designed the study, was involved in data analysis and revised the manuscript. All authors discussed the results and commented on the manuscript.

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