Tolerances of *Deinococcus geothermalis* Biofilms and Planktonic Cells Exposed to Space and Simulated Martian Conditions in Low Earth Orbit for Almost Two Years

Corinna Panitz, Jan Frösler, Jost Wingender, Hans-Curt Flemming, and Petra Rettberg

**Abstract**

Fossilized biofilms represent one of the oldest known confirmations of life on the Earth. The success of microbes in biofilms results from properties that are inherent in the biofilm, including enhanced interaction, protection, and biodiversity. Given the diversity of microbes that live in biofilms in harsh environments on the Earth, it is logical to hypothesize that, if microbes inhabit other bodies in the Universe, there are also biofilms on those bodies. The Biofilm Organisms Surfing Space experiment was conducted as part of the EXPOSE-R2 mission on the International Space Station. The experiment was an international collaboration designed to perform a comparative study regarding the survival of biofilms versus planktonic cells of various microorganisms, exposed to space and Mars-like conditions. The objective was to determine whether there are lifestyle-dependent differences to cope with the unique mixture of stress factors, including desiccation, temperature oscillations, vacuum, or a Mars-like gas atmosphere and pressure in combination with extraterrestrial or Mars-like ultraviolet (UV) radiation residing during the long-term space mission. In this study, the outcome of the flight and mission ground reference analysis of *Deinococcus geothermalis* is presented. Cultural tests demonstrated that *D. geothermalis* remained viable in the desiccated state, being able to survive space and Mars-like conditions and tolerating high extraterrestrial UV radiation for more than 2 years. Culturability decreased, but was better preserved, in the biofilm consortium than in planktonic cells. These results are correlated to differences in genomic integrity after exposure, as visualized by random amplified polymorphic DNA–polymerase chain reaction. Interestingly, cultivation-independent viability markers such as membrane integrity, ATP content, and intracellular esterase activity remained nearly unaffected, indicating that subpopulations of the cells had survived in a viable but nonculturable state. These findings support the hypothesis of long-term survival of microorganisms under the harsh environmental conditions in space and on Mars to a higher degree if exposed as biofilm.

**Key Words:** Biofilms—Planktonic cells—Desiccation—Extraterrestrial UV radiation—Space—Mars—*Deinococcus geothermalis*—ISS—EXPOSE-R2. Astrobiology 19, 979–994.

1. Introduction

**Extreme environments have always aroused tremendous scientific curiosity. These habitats are characterized by extreme temperatures, pressures, radiation regimes, desiccation, salinity, pH, and redox potential (Rothschild and Mancinelli, 2001). Physical and chemical barriers to life that were once believed to be insuperable are now seen as yet another niche with the potential to harbor life. These life-forms possess various stress adaptation mechanisms that enable them to bypass multiple physical and chemical barriers for survival. Further, there have been reports of liquid water existing elsewhere in the Solar System (Martin-Torres and Zorzano, 2017; Dundas et al., 2018). In combination with the fact that on the Earth “where there is water there is life,” these findings suggest that life-forms may exist or might have existed on other planets and moons in the Universe.**

**Activities in the interdisciplinary scientific field of astrobiology focus on deciphering the key environmental parameters that have enabled the origin of life and its subsequent evolution and distribution on the Earth or elsewhere in the Universe (Horneck et al., 2016; Cottin et al.,**

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Space and the surface of Mars with its cycles of extreme temperatures, prolonged dryness, and intense solar radiation can be considered as yet another environment that is hostile to life. These harsh extraterrestrial environments are being used as a tool for testing its impact on the most resistant life-forms from the Earth, such as extremophiles, and therefore they have led to new data on the survivability of microbes under these conditions. Modeling studies of the potential for transfer of life between celestial bodies by ejection into space as a consequence of meteoritic impacts support the likelihood of interplanetary transfer of microorganisms within meteorites, phrased in the hypothesis of lithopanspermia (Nicholson et al., 2009; Horneck et al., 2010), and they suggest that life could be more common than previously believed.

In natural habitats, bacteria often live within matrix-embedded microbial communities, termed biofilms, which are now understood to be a major mode of microbial life (Carey et al., 2017). Since they are among the oldest signs of life on the Earth, documented as fossils in rocks throughout the 3.5 billion-year-old morphological fossil records (Westall et al., 2000), they may represent the most successful microbial survival strategy on the Earth (Des Marais et al., 2010), and they suggest that life could be more common than previously believed.

In biofilms, bacterial cells live as structured, frequently multispecies communities of microorganisms in close association with surfaces and interfaces, preferably at the boundary between the liquid medium and solid material. These aggregates bear aspects of multicellularity. This proclivity toward multicellularity makes bacterial cells similar to many other types of living cells: capable of unicellular existence and yet generally residing within multicellular communities (Davey and O’Toole, 2000). In biofilms they live encapsulated in a self-produced matrix of excreted extracellular polymeric substances (EPS) (Persat et al., 2015; Flemming, 2016; Nadell et al., 2016). The EPS consist of extracellular nucleic acids, polysaccharides, proteins, glyco-proteins, and glycolipids. Biofilms offer their member cells several benefits, of which protection from environmental insults and assaults is foremost (Davey and O’Toole, 2000).

In addition, however, there is also the form of individual cells floating in a liquid medium, the so-called planktonic form. In biofilms, the organisms have developed emergent properties that cannot be predicted by the study of single cells; therefore, biofilms are counted among the collective forms of life in biology, similar to bee hives, coral reefs, or forests (Flemming et al., 2016).

The discovery of the thermophilic microorganism Deinococcus geothermalis has given new insights into the field of radiation microbiology. It is a representative of the extremely radiation-resistant family Deinococcaceae. D. radiodurans has been reported to survive acute exposures to ionizing radiation (IR, 10 kGy), ultraviolet radiation (UV254nm, 1 kJ/m²), and desiccation (Daly et al., 2004; Makarova et al., 2007) and can even grow under chronic IR (60 Gyu/hour) (Daly, 2000).

Several factors have been suggested to contribute to the ability for the more than 20 distinct species (Lai et al., 2006) of the family Deinococcaceae to withstand adverse environmental conditions: Among them are highly efficient DNA repair systems (Blasius et al., 2008) and protection of macromolecules from oxidative damage by manganese ions (Daly et al., 2004, 2007) and carotenoids (Tian et al., 2007; Tian and Hua, 2010; Zhang et al., 2015). Moreover, their ability to deal with the lethal effects of IR such as DNA damage, lipid oxidation, and protein damage has made especially D. radiodurans a model system for astrobiology and a hypothetical interplanetary transmission of life (Rainwater and Rawat, 2017).

D. geothermalis was originally isolated from a hot pool at the Termi di Agnano, Naples, Italy (Ferreira et al., 1997). D. geothermalis is a moderate thermophile with growth in a temperature range from 45°C to 50°C (Ferreira et al., 1997). This heterotrophic bacterium has also been recognized for its propensity to form biofilms in addition to its planktonic growth (Kolari et al., 2002; Saarimaa et al., 2006). Since space technology provides the opportunity to expose microorganisms intentionally to the harsh space environment, or selected parameters of it and due to the combination of the aforementioned special characteristics, D. geothermalis was selected as an ideal candidate for the astrobiological investigations for the space experiment Biofilm Organisms Surfing Space (BOSS).

BOSS has allowed us to study the way in which the biofilm habitat of the organism contributes to its resistance to extreme conditions. The experiment was composed of six parts where an international consortium of scientists investigated the ability of a variety of biofilm formers such as D. geothermalis DSM 11300, further explored in this project, Bacillus hornaceae BH+, Halococcus morrhuae + Halomonas muralis, the cyanobacteria Gloeocapsa OU20, and Halomonas muralis

<table>
<thead>
<tr>
<th>BOSS</th>
<th>Investigator</th>
<th>Institute</th>
<th>Test organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P. Rettberg/E. Rabbow</td>
<td>DLR Cologne, Germany</td>
<td>Deinococcus geothermalis</td>
</tr>
<tr>
<td>2</td>
<td>H.-C. Flemming/J. Wingender/J. Frösler</td>
<td>Klinikum/RWTH Aachen, Germany</td>
<td>D. geothermalis</td>
</tr>
<tr>
<td>3</td>
<td>K. Venkateswaran/P. Vaishampayan</td>
<td>NASA/JPL, United States of America</td>
<td>Bacillus hornaceae</td>
</tr>
<tr>
<td>4</td>
<td>Ch. Cockell/J. Wadsworth</td>
<td>University of Edinburgh, United Kingdom</td>
<td>Gloeocapsa OU20</td>
</tr>
<tr>
<td>5</td>
<td>S. Leuko</td>
<td>DLR Cologne, Germany</td>
<td>Halomonas + Halococcus</td>
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<tr>
<td>6</td>
<td>H. Stan-Lotter</td>
<td>University of Salzburg, Austria</td>
<td>Chroococcidiopsis CCMEE 057, CCMEE 029 and CCMEE 064</td>
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BOSS = Biofilm Organisms Surfing Space.
and Chroococidiopsis sp. CCMEE 029 to survive in Space and on Mars as a function of their lifestyle (Table 1). The hypothesis in focus was to test whether microorganisms that are organized as a biofilm are superior to the same individually exposed microorganisms with regard to long-term survival in space or under Mars-like conditions. In several already performed missions, preparatory and complementary tests suitable strains or microorganism species and a suitable exposure material and set up were defined for the mission (Baque et al., 2013; Frösler et al., 2017). In these tests, the survival of D. geothermalis biofilms and planktonic cells with regard to cultivability as well as further culture-independent characteristics exposed to desiccation, vacuum and artificial martian atmosphere, and pressure individually or in selected combinations was investigated.

BOSS was performed within the European Space Agency (ESA) EXPOSE-R2 facility aboard the International Space Station (ISS) from July 24, 2014, to July 5, 2016. More details on the mission scenario are provided in the basic description of the EXPOSE-R2 mission (Rabbow et al., 2017). In this study, we present the results with D. geothermalis DSM 11300 as part of the space experiment BOSS (BOSS 1, see Table 1) exposed outside the ISS and the corresponding Mission Ground Reference (MGR) experiment performed in the Planetary and Space simulation facilities at Deutsches Zentrum für Luft- und Raumfahrt e.V. (DLR), Cologne (Germany).

2. Materials and Methods

2.1. Bacteria, experimental setup, and accommodation

D. geothermalis DSM 11300 (type strain) cells (Ferreira et al., 1997) obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) was used as the test organism in the BOSS 1 experiment. For sample preparation, the cells were incubated in R2A broth (yeast extract 0.50 g, Proteose Peptone [Difco no. 3] 0.50 g, Casamino acids 0.50 g, Glucose 0.50 g, Soluble starch 0.50 g, Na-pyruvate 0.30 g, K2HPO4 0.30 g, MgSO4 · 7H2O 0.05 g, distilled water 1000 mL; Fluka, Steinheim, Germany; Reasoner and Geldreich, 1985) grown from a single colony at 45°C in a shaker-incubator running at 150 rpm for ~25 h (early stationary phase).

To prepare biofilm and planktonic samples, cellophane disks (11.2 mm diameter; Bio-Rad, Munich, Germany) were used as a carrier material (Frösler et al., 2017). Biofilms were generated by inoculating the disks with ~1 × 10^7 cells/mL. Inoculated filters were placed on R2A agar and incubated for 2 days at 45°C. Planktonic cultures were grown under agitation (160 rpm) at 45°C in R2A broth. The planktonic cells were precipitated onto membranes in the similar cell concentration as calculated for the biofilm sample (Frösler et al., 2017). All samples and their respective controls were prepared, exposed, and analyzed in triplicate. The controls were kept in an air-dried, desiccated state in the dark under ambient laboratory conditions (temperature 20–2°C and relative humidity 33–5%). These non-exposed laboratory controls were subsequently analyzed in parallel to the space mission and the corresponding MGR experiment.

For the conduction of the space mission and the MGR experiment, these sample disks were arranged in stacks of 5 disks above each other for each of the 36 sample locations provided for D. geothermalis samples in compartment 4 of tray 1 and 2 of the EXPOSE-R2 facility. On the one hand, in this way of sample setup, the sample amount could be significantly increased (total of 360 for mission and MGR) in spite of the limited amount of sample sites for the BOSS experiment (Fig. 1). On the other hand, due to the partial UV absorption of the cellophane material covered with the biofilm or planktonic cells on top, it was possible to determine the effect of different fluences for each single sample site.

One batch of samples (18 locations), in Tray 1, was exposed to space conditions achieved by exposure beneath
short-wavelength-transparent MgF₂ windows and filters with the wavelength λ > 110 nm and evacuated to space vacuum. The second set from the same sample batch (18 locations) in Tray 2 was exposed in a simulated Mars atmosphere, a Mars-like gas mixture composed of 95.55% CO₂, 2.70% N₂, 1.60% Ar, 0.15% O₂, and 370 ppm H₂O (Praxair Deutschland GmbH) at a pressure of 980 Pa. These samples were covered with quartz windows and filters, allowing the transmission of solar electromagnetic radiation of wavelength λ > 200 nm, similar to the spectrum expected on the martian surface. Subsequent for all samples of the BOSS 1 experiment, the transmission was further reduced by appropriate neutral density filters with 0.1% transmission of the respective received UV fluence. This fluence attenuation was determined in the science verification test (SVT) before the mission to ensure the survival of the organisms even after the long-term EXPOSE-R2 mission (Frösler et al., 2017).

For the space and the MGR experiment, the samples were placed in the different compartments of the EXPOSE-R2 trays and stacked as: top (t) layer, where samples were exposed to the full solar radiation spectra beneath the windows and filters, middle (m) layer, and bottom (b) layer where no solar radiation could penetrate the samples. The stacked conformation of the sample carriers provided identical exposure of all samples to space conditions, such as vacuum, temperature, Mars atmosphere and pressure, except solar radiation (Fig. 3b, c). A detailed description of the hardware is given by Rabbow et al. (2012). The exposed biofilm and planktonic cells were retrieved after performance of the space and MGR experiment as described in the work of Frösler et al. (2017) and analyzed for culturability and the additional biological characteristics presented here.

2.2. EXPOSE-R2 mission protocol

After the accommodation of all four international astrobiological experiments of the EXPOSE-R2 mission: BOSS, BIOMEX, BIODIVERSITY, and PSS (Rabbow et al., 2017), the facility was launched from Baikonur to the ISS on July 23, 2014, with the Progress cargo spacecraft 56P. It was attached to the universal outside platform (URM-D) of the Russian Service Module Zvezda of the ISS during an extravehicular activity (EVA) on August 18, 2014. The outside space exposure of 531 days ended on February 3, 2016, including genuine outside conditions for 469 days in space and simulated Mars environment with coincidental extraterrestrial UV. The residence time of the samples in tray 1 to low Earth orbit (LEO) vacuum (1.33 × 10⁻³ to 1.33 × 10⁻⁴ Pa) was 672 days. Since tray 2 was already filled with the artificial Mars gas mixture (95.55% CO₂, 2.70% N₂, 1.60% Ar, and 0.15% O₂, 370 ppm H₂O; Praxair Deutschland GmbH; pressure 980 Pa) in the Planetary and Space Simulation Facilities at DLR, Cologne before the EXPOSE-R2 mission, a Mars gas period of 722 days was calculated. During this time, samples experienced an outside space vacuum period (valve open to valve closed) of 509 days, including an outside extraterrestrial UV exposure for 469 days. The final overall mission UV 200–400 nm fluence experienced by the EXPOSE-R2 samples calculated by RedShift (Redshift Design and Engineering BVBA, Belgium) with regard to the size and height (field of view) at sample site was not yet available at the time of preparation of this article.

A maximum UV fluence of 505 kJ/m² (Max. fluence of mission attenuated by the 0.1% neutral density filters) experienced by the BOSS 1 samples in Tray 1 (vacuum) and 2 (artificial Mars gas mixture) at the uppermost position (disk 1) was determined. Subsequently, this UV fluence was reduced by the transmission rate of the cell covered disks (2–4) to the lowest value experienced by the *D. geothermalis* on disk 5. All samples encountered temperatures between −20.9°C (limited by the heating system) and 57.98°C (highest temperature measured during mission). The total mission dose reached values up to 1 Gy (Berger et al., results presented during the EXPOSE-R2 Postflight Review, ESTEC, December 2016). Temperature, insolation, and galactic cosmic radiation data of EXPOSE-R2 were regularly sent via telemetry to the ground, or for UV radiation modeled by RedShift (UV radiation fluence data). After 696 days, total mission duration of Tray 1 + 2 from launch to return the BOSS samples was carried back to the Earth on June 18, 2016. A detailed description of the procedure and parameters of the EXPOSE-R2 mission and MGR was previously published (Rabbow et al., 2017).

2.3. MGR experiment

An MGR experiment with a flight identical hardware charged with biological samples from the same batch as the space experiment was performed at DLR (German Aerospace Centre) in Cologne (Germany) by Microgravity User Support Centre, according to the mission data either downloaded from the ISS (temperature data, facility status, inner pressure status) or provided by RedShift (calculated UV radiation fluence data for the wavelengths 200–400 nm) with a delay of a maximum of 2 months due to data analysis and reformatting processes relative to the space experiment.

To mimic the environmental conditions of the EXPOSE-R2 flight mission as closely as possible, a similar set of trays and the four astrobiology experiments, including BOSS in flight identical accommodation trays, were exposed to simulated environmental conditions according to the flight data, with respect to vacuum, temperature oscillations, and UV 200–400 nm radiation on ground in the Planetary and Space simulation facilities at DLR in Cologne. The MGR began on August 28, 2014 and ended on August 16, 2016. Thereafter, all samples were de-integrated under sterile conditions and distributed to the investigators for further analysis (Rabbow et al., 2017).

2.4. Sample analysis

For subsequent sample processing, the stacks were subdivided. Disks 1, 3, and 5 of each stack (Fig. 2) were analyzed by DLR, Cologne (culturability, DNA integrity). Disks 2 and 4 (Fig. 2) were analyzed at the University of Duisburg-Essen (total cell counts, culturability, and cultivation-independent parameters).

2.4.1. Sample rehydration and resuspension. Dried samples on cellophane discs (Fig. 3a) were allowed to rehydrate for 30 min at room temperature in 1 mL of phosphate-buffered saline (PBS), pH 7.4 (method described...
in Frösl er et al., 2017). Cells were then dislodged from the substrate by vigorous shaking for 2 min with glass beads (diameter 0.45–0.50 mm; ~300 mg per sample; B. Braun Biotech International, Melsungen, Germany). The cell suspensions obtained were used to determine total cell counts, culturability (plate counts), cells with intact and damaged membranes, ATP content, and esterase activity.

2.4.2. Total cell counts and culturability. For total cell counts, cells in suspensions prepared from disks 2 and 4 were stained with the DNA-intercalating fluorescent dye 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI; Sigma). For staining D. geothermalis cells immobilized on cellophane disks, 1 mL of five-fold diluted DAPI solution (5 μg/mL DAPI in 2% formaldehyde) was pipetted onto the support material and incubated for 20 min in the dark. The supernatant dye solution was removed by pipetting. Stained cells were enumerated by epifluorescence microscopy (Nikon Eclipse Ni-E, Plan Apo 100x/1.4 oil WD 0.13 mm; Nikon, Tokyo, Japan).

After 10-fold serial dilution of the resuspended biofilms and planktonic cells (method described in Frösl er et al., 2017) of D. geothermalis from the space and MGR experiment, the ability of the cells to undergo cell division and form macroscopic visible colonies on R2A agar plates was investigated after incubation for 48 h at 45°C. For comparability, total cell counts (in cells/mL) and plate counts (in colony-forming unit [CFU/mL]) refer to the surface area of the investigated samples. Thus, results were expressed in cells/cm² or CFU/cm², respectively (Frösl er et al., 2017) for disks 2 and 4. Survival rates were calculated as N/N₀, where N is the bacterial population derived from the number of CFUs per milliliter at each sampling time and N₀ is the initial bacterial population before the mission. Finally, survival rates were plotted against the individual exposure conditions, including the received UV fluence at sample site and atmospheric conditions.

2.4.3. DNA-extraction and integrity analysis by random amplified polymorphic DNA-polymerase chain reaction. To assess the genomic integrity status of D. geothermalis, random amplified polymorphic DNA–polymerase chain reaction (RAPD-PCR) was carried out. This method is based on the amplification of genomic DNA with a single primer of arbitrary nucleotide sequence that anneals to multiple regions of the template, thus generating a DNA fingerprint (Atienzar et al., 2002). Differences in band intensity as well as gain/loss of bands between treated cells compared with control cells allow for the visualization of DNA damage as a result of the treatment (Atienzar and Jha, 2006). DNA from cells exposed to mission and MGR parameters in dried UV-exposed and -unexposed samples was extracted after resuspension and consolidating the triplicates of each condition by using an off-the-shelf DNA extraction kit (QiAamp DNA Mini Kit; Qiagen, Germany) following the manufacturer’s protocols. DNA was finally dissolved in 40 μL buffer (10 mM Tris–Cl; 0.5 mM ethylenediaminetetraacetic acid; pH 9.0) and stored at −20°C until RAPD downstream processing. After extraction, the DNA concentration in the sample was estimated with a Nanodrop 2000c UV-Vis Spectrophotometer (Thermo Scientific, Wilmington).

RAPD was performed by using five different primers: Primer 1 (5′-GGT GCGGAA-3′), Primer 2 (5′-GTAGA CCCGT-3′), Primer 3 (5′-AAGCGGC AAC-3′), Primer 4 (5′-CCCGTCAGCA-3′) (Asker et al., 2011), and Primer 5 (5′-GGCGGGCCG-3′) in several test runs to determine a reproducible pattern of bands. Subsequent analysis was exclusively carried out with Primer 5 and 10 ng of extracted genomic DNA as template. PCR reactions were performed in 20 μL reaction volume containing 1 U Platinum Taq polymerase, 2 μM polymerase buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, and 1 μM of primer. PCR amplification was carried out with a thermal cycler (My Cycler BIO RAD) programmed for initial denaturation at 95°C for 3′, followed by 45 cycles of denaturation at 95°C for 30′, primer annealing at 40°C for 1′, strand extension at 72°C for 2′, and a final extension step at 72°C for 7′. Amplified PCR products were electrophoresed in a 2% (w/v) agarose gel in TAE buffer with 1 kb DNA ladder as a molecular size marker and run with 7 V/cm for 3 h. Gels were stained with SYBR Safe and documented on an ImageQuant LAS4000 (GE Health Care, Germany).

2.4.4. Membrane integrity. Cell membrane integrity was assessed with the LIVE/DEAD® BacLight™ Bacterial Viability Kit (Molecular Probes, Eugene, OR) by using the dyes SYTO® 9 and propidium iodide (PI) at a final

![FIG. 2](image_url). Representative compartment cut out of the EXPOSE-R2 facility with accommodated sample stack of five cellophane disks covered with *Deinococcus geothermalis* planktonic cells or biofilms (uppermost disk receiving highest UV fluence = disk 1, bottom disk receiving lowest UV fluence = disk 5) for exposure to space and martian conditions.
concentration of 5 and 0.3 μM, respectively (Frösl er et al., 2017). The cell density of the bacterial suspension to be stained was adjusted to *3–6·10⁵ cells/mL. One milliliter of suspension was mixed with 3 μL of LIVE/DEAD working dye solution (containing 100-fold diluted PI) and incubated for at least 15 min in the dark before adding 4 mL of PFD water. The cell suspension was filtered through a black Isopore™ polycarbonate membrane (30 mm diameter, 0.2 μm pore size; Millipore). Stained cells on the membrane surface were enumerated at 1000-fold magnification in 10 randomly chosen fields of vision of an epifluorescence microscope equipped with a 100 μm × 100 μm counting grid. PI-negative cells that were stained green were considered intact (viable), whereas PI-positive cells that were stained red were considered membrane-damaged/dead.

2.4.5. ATP content. Total ATP was quantified with the luciferase-based BacTiter-Glo™ Microbial Cell Viability Assay (Promega, Madison, WI). The assay was performed in opaque 1.5 mL micro centrifuge tubes and executed according to the manufacturer’s protocol, with an ATP standard curve ranging from 10 pM to 1 μM. Suspensions of *D. geothermalis* cells in 0.14 M sodium chloride solution corresponded to the sample solution. Luminescence was recorded with a Glomax 20/20 Luminometer (Promega GmbH, Mannheim, Germany). Results (in mol ATP/mL) were referred to total cell counts (in cells/mL).

2.4.6. Esterase activity. The fluorogenic substrate 5(6)-carboxyfluorescein diacetate (CFDA; Sigma) was used to determine cellular esterase activity. A 10 mM stock solution of CFDA in dimethyl sulfoxide was diluted in PBS to a concentration of 20 μM. After mixing 100 μL of 20 μM CFDA solution with 100 μL of cell suspension, fluorescence intensity was measured at 37°C over a period of 60 min by using a plate reader Infinite 200 PRO (Tecan, Switzerland). All data were corrected by blanks (sterile PBS). Relative fluorescence was plotted against time and the slope of the linear part of each curve, corresponding to the change of relative fluorescence unit (RFU) over time (ΔRFU/min) as a measure of enzyme activity, was calculated.

2.4.7. Statistical analysis. The surviving fraction of *D. geothermalis* cells was determined from the quotient *N/N₀*, with *N*= colony formers after UV exposure to space vacuum or Mars atmosphere and *N₀*= colony formers of the dried samples before the mission and MGR experiment. All data shown are expressed as averages ± standard deviations (n = 3). The results of the culturability experiments were compared statistically by using two-tailed Student’s t-tests. The results were analyzed in multigroup pairwise combinations, and differences with *p* ≤ 0.05 were considered statistically significant.

3. Results

The main objective of the BOSS experiment was a comparative study of the endurance of desiccated biofilms and planktonic cells after exposure to space and simulated Mars conditions at the exterior of the ISS as part of the ESA EXPOSE-R2 mission. Among extraterrestrial stressors, desiccation and UV radiation are especially deleterious; tolerances to both are distinguished characteristics of *D. geothermalis*. After completion of the mission, the trays with the BOSS samples, that is, the cellophane disks with *D. geothermalis* biofilms and planktonic cells exposed to space and artificial Mars conditions simultaneously exposed to UV radiation for 469 days, were returned to the Earth. After the handover of the samples of the mission-accompanying MGR test, all samples were analyzed at the same time together with the laboratory controls. The survival of the test organisms was assessed by cultivation and by cultivation-independent methods based on viability markers, including membrane integrity, ATP production, and esterase activity.
3.1. Determination of the UV fluences received at the individual BOSS sample sites in flight and MGR experiment

Before further downstream analysis of the retrieved samples, transmission scans (T in %, scan speed: 30 nm/min, sampling interval: 1 nm, wavelength range 200–400 nm) were conducted with a Hitachi U-3310 Spectrophotometer (Uwe Bninger Analytik, Germany) to determine the transmission reduction throughout the sample stacks caused by the absorption of the cell-covered cellophane disks (Figs. 2 and 3a). For every exposure condition, as in Mission Space and Mars and MGR Space and Mars, of all disks up to disk four transmission were measured at three different locations from one of the triplicate stacks. Based on the UV-fluence values for the biologically effective UV wavelength range from 200 to 400 nm for each compartment and tray calculated by RedShift (based on ISS flight data that were used to model solar irradiation of EXPOSE-R2) in MJ/m² and attenuated by the absorption of the respective top window and neutral density filter (T=0.1% for disk 1 and by the stacked samples for disks 2–5, the UV values for each stack position are shown in Table 2. The UV exposure from top to bottom of each stack allowed the investigation of a fluence effect for each single sample site. Non-irradiated samples were used as dark controls. From stack position 1 to 2, the fluence was reduced to 50% whereas the fluence in the lowest stack position 5 was reduced from 20% to 10%.

3.2. Survivability in space and on Mars: effect of space vacuum or simulated martian atmosphere and pressure on culturability of biofilms and planktonic cells of D. geothermalis

Besides the UV-exposed samples, both biofilms and planktonic cells of D. geothermalis were exposed to vacuum and Mars-like atmosphere in the dark in the corresponding dark layers of compartment 4 of both Trays 1 and 2. These samples showed a reduction in culturability of 2 (flight) to 3 (MGR) orders of magnitude for dark samples (N) and laboratory control after mission (N) in relation to laboratory control before mission (N₀) (Fig. 8). All results were normalized (N/N₀) by using dried and non-exposed laboratory controls as a reference. The loss in culturability as an effect of desiccation has already been demonstrated in the SVT. Here, a significant proportion of cells lost culturability after a desiccation time of about 60 days, whereas the fraction of culturable cells in biofilms seemed to be higher than in planktonic cell samples (Frössler et al., 2017).

3.3. Survivability in space and on Mars: effect of surrounding vacuum and extraterrestrial UV (>110 nm) or Mars-like atmosphere and UV (>200 nm) spectrum on culturability of biofilms and planktonic cells of D. geothermalis

To investigate the survivability in space and on Mars of D. geothermalis, biofilm and planktonic cells were exposed either to the surrounding vacuum and space UV or to simulated martian atmosphere and Mars UV spectrum by corresponding filters outside the ISS. As elucidated earlier, samples were exposed in stacks of five cellophane disks above each other, resulting in a decreasing UV exposure from top (disk 1) to bottom (disk 5) (Table 2). Beneath the UV-exposed upper layer of biofilm and planktonic cell-loaded disks, there was one layer of sample stacks kept in the dark. They experienced the same environmental conditions as the upper sample layer, except the insolation. An identical arrangement of samples was produced in the ground control trays that served for the MGR (Rabbow et al., 2017). In addition, laboratory controls were prepared at the same time and from the same batch of D. geothermalis cells as the flight and MGR samples. They were stored in the dark under ambient laboratory conditions (temperature 20±2°C and relative humidity 33±5%) and served as N₀. Figure 7a shows the colony-forming ability of the biofilm and planktonic cell flight samples determined on R2A agar plates and correlated with the fluence in space and Mars gas atmosphere in the corresponding stack position.

In general, a negative correlation between survival and fluence due to the use of stacks is possible, and, therefore, fluence effect curves can be recorded. Figure 7a and b show the fluence effect results of the culturability studies for positions 1–5 in the cellophane stack. As mentioned earlier, UV radiation decreased the culturability of D. geothermalis but none of the other viability

Table 2. UV₂₀₀–₄₀₀ nm Fluences Received by the Flight and Mission Ground Reference Samples at Sample Site (Values Determined by Photometric Measurements N=3) of the Disks Using the Calculated Ultraviolet Fluences for Each Sample Site

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Stack position, top to bottom</th>
<th>Flight</th>
<th>Mars</th>
<th>MGR</th>
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<tr>
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<td>Space fluence (kJ/m²)</td>
<td>Mars fluence (kJ/m²)</td>
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<td></td>
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MGR = Mission Ground Reference.
parameters. The deleterious effect on culturability increased with increasing UV fluence (Fig. 7a, b). Culturability of biofilms was superior to that of planktonic cells and was higher under Mars conditions compared with space conditions. Biofilms tolerated residence in space/Mars (mission; Fig. 7a) better than simulated space/Mars conditions (MGR; Fig. 7b).

3.4. Determination of the total cell counts, intact cell counts (membrane integrity), and plate counts of biofilms and planktonic cells of D. geothermalis

For total cell counts, disks 2 and 4 of each stack were rehydrated and the suspended cells were stained with DAPI fluorescent stain and enumerated by epifluorescence microscopy. In Fig. 4, results of disk 2 are referred to as UV (top) and disk 4 was labeled UV (bottom). Total cell counts were not affected by the exposure to UV radiation or the tested conditions. Irrespective of the test conditions, a significant proportion of the population of D. geothermalis remained membrane intact. Plate counts were generally significantly lower than cell counts. Exposure to UV radiation reduced the amount of culturable cells.

3.5. Measurement of intracellular and extracellular ATP of biofilms and planktonic cells of D. geothermalis

For further evaluation of the vitality status, the ATP content of suspensions of planktonic cells and biofilm cells detached from the cellophane disks was determined. The ATP concentration of the biofilms and planktonic cell samples remained relatively constant during the course of the experiment and regardless of the experimental conditions, between $10^{-18}$ and $10^{-19}$ mol/cell. High levels of extracellular ATP (eATP) could severely distort the analysis of intracellular ATP (iATP) content. However, the dried cells of D. geothermalis of the mission and MGR showed that the content of eATP is relatively small (~1%) and, therefore, negligible. The exposure to varying fluences of UV radiation had no significant effect on the iATP content compared with the nonexposed dark and laboratory control (Fig. 5).

3.6. Enzymatic activity of biofilms and planktonic cells of D. geothermalis under space and Mars conditions

The activity of nonspecific intracellular esterases is regarded as an indicator of the general cellular metabolic activity and cell integrity of bacteria (Joux and Lebaron, 2000). Diverse enzymes with esterolytic activity can be determined collectively in a single assay by using fluorogenic fluorescein diacetate-based enzyme substrates. Thus, CFDA has been described to be suitable to measure esterase activity in different bacterial species both in pure cultures as well as in natural bacterial communities of aquatic environments (Hoefel et al., 2003). In this study, CFDA was also found to be applicable for the determination of esterase activity in D. geothermalis. Enzymatic activity was detected in all samples of biofilms and planktonic cells after long-term exposure to space and Mars conditions with and without UV irradiation (Fig. 6). Generally, there were only minor differences in the esterase activities between biofilms and planktonic cells. Biofilms exposed during the EXPOSE-
FIG. 5. Intracellular ATP and extracellular ATP of biofilms and planktonic cells of D. geothermalis under space (a, c) and Mars-like (b, d) conditions in the EXPOSE-R2 mission (a, b) and the MGR experiment (c, d). n = 3.

FIG. 6. Esterase activity of biofilms and planktonic cells of D. geothermalis under space (a, c) and Mars-like (b, d) conditions in the EXPOSE-R2 mission (a, b) and the MGR experiment (c, d). n = 3.
R2 mission displayed esterase activities of similar levels as found in unexposed laboratory controls, whereas a trend of slightly higher esterase activities was observed in exposed biofilms compared with unexposed control samples in the MGR experiments. Exposure to UV irradiation did not result in significant changes in esterase activities of biofilms and planktonic cells compared with the non-exposed dark and laboratory control samples.

3.7. Investigation of differences of the integrity of genomic DNA of biofilms and planktonic cells of *D. geothermalis*

The *D. geothermalis* biofilm and planktonic sample triplicates from each parameter of the BOSS experiment were combined according to their individual parameters experienced in the flight and MGR experiment (three parallel disks), genomic DNA was extracted, and RAPD-PCR was employed. All samples yielded sufficient amounts of DNA (quantified by Nanodrop), and a reproducible band pattern (Data not shown, $n = 3$) could be obtained. In the fingerprints, it became evident that *D. geothermalis* exposed to high UV fluences experienced some loss of higher molecular bands in parallel to the occurrence of lower molecular bands corresponding to the degree of DNA damage induced by UV radiation (Figs. 9 and 10). This loss of high molecular DNA occurred in a descending order from dark samples to samples exposed to full UV, suggesting a negative fluence effect dependence of DNA integrity on stack position. The genomic fragmentation is more deleterious in the DNA from planktonic cells than in DNA from biofilms after exposure of up to 505 kJ/m² of extraterrestrial UV (200–400 nm). The band patterns of both sample types indicated a more deleterious effect under space conditions compared with simulated Mars conditions.

**FIG. 7.** Fluence effect of extraterrestrial UV radiation (a) and UV irradiation (corresponding mission based calculations by Redshift) (b) under space (circles) and Mars-like conditions (triangles) on the culturability of biofilms (closed symbols) and planktonic cells (open symbols) of *D. geothermalis*. $n = 3$.

**FIG. 8.** Culturability of biofilms (plain) and planktonic cells (pattern) of *D. geothermalis* exposed to space (light gray) and simulated martian conditions (white) and laboratory conditions in the dark after flight (a) and MGR (b). CP refers to Corinna Panitz and JF to Jan Fröhler who analyzed the respective samples. $n = 3$. 
4. Discussion

4.1. Effect of space vacuum or artificial Mars gas atmosphere and pressure on desiccated D. geothermalis cells

In this study, the D. geothermalis samples experienced exposure to space and Mars simulations in a dried state from biofilms or multi-layered planktonic cell samples in the dark layers of the corresponding trays during the time span of the EXPOSE-R2 mission. Exposure to LEO vacuum and artificial Mars gas and pressure lasted for almost 2 years. In general, exposure to vacuum causes rapid dehydration of bacterial cells, alteration of intracellular biomolecules, and changes in membrane permeability (Horneck et al., 2010). Previous desiccation, on the other hand, can also be beneficial to vacuum tolerance as demonstrated in storage experiments of the closely related D. radiodurans compared with storage at ambient air (Bauermeister et al., 2012).

FIG. 9. RAPD-PCR band profiles of biofilms and planktonic cells of D. geothermalis after flight and MGR test with a loss of high molecular DNA in a descending order from dark samples to samples exposed to full UV (negative fluence effect dependence of DNA integrity on stack position). n=3. RAPD-PCR, RAPD, random amplified polymorphic DNA-polymerase chain reaction.

FIG. 10. RAPD-PCR band profiles of biofilms and planktonic cells of D. geothermalis of the science verification test with or without addition of S-MRS Mars soil simulant. n=3. S-MRS, sulfatic Mars regolith stimulant.
Endurance of vacuum exposure has also been demonstrated for *D. radiodurans*, *D. aeuris*, and *D. aetherius* for 1 year with a 10- to 100-fold reduction in culturability (Kawaguchi et al., 2013). As already outlined in the previous papers (Frösler et al., 2017), exposure to vacuum and Mars gas and pressure did not seem to affect the colony-forming ability of *D. geothermalis* compared with a non-exposed laboratory control from the same batch being stored for the same period in ambient air in the dark. During the SVT conducted as a final rehearsal of the EXPOSE-R2 mission, this effect could already be observed for exposure duration up to 174 days (Frösler et al., 2017). The same was observed for the BOSS of mission and MGR even after being subjected to 672 days of vacuum and 722 days of artificial Mars gas.

Since the samples had already been air-dried up to the time of accommodating them inside their compartments and particular tray, it is supposed that the main water loss leading to a loss of culturability of 2–3 orders of magnitude compared with the initial viability of the dried cell samples at sample preparation time occurred in the course of the mission. The residual water content was maintained throughout the EXPOSE-R2 mission and MGR duration and seemed low enough to cause any additional deleterious effect of vacuum or Mars gas. The endurance observed in vacuum may be due to the low amount of residual water in *D. geothermalis* trapped in the glass-like cytoplasm and/or being tightly bound to intracellular biomolecules such as DNA or proteins as has been reported for *D. radiodurans* (26.9%) (Bauermeister et al., 2011) and the spores of *Bacillus subtilis* ~40.3% (Moeller et al., 2009).

As for the results of the previous experiments, the authors suggest that the oxygen-poor Mars gas (0.13%) did not impair cell viability, but it may have even been beneficial for survival as exogenous sources of oxidative stress (i.e., atmospheric oxygen) were limited (Fredrickson et al., 2008; Frösler et al., 2017). As already concluded for the preceding tests, *D. geothermalis* is very tolerant to low pressures in desiccated state since we showed here that both biofilms and planktonic cells were able to withstand space vacuum and the atmospheric conditions of Mars for almost 2 years, if protected against UV radiation.

### 4.2. Effect of space vacuum or martian atmosphere and pressure combined with extraterrestrial UV radiation

Further, *D. geothermalis* biofilms and planktonic samples were exposed to extraterrestrial UV radiation in space vacuum and Mars-like atmosphere and pressure in a Mars-relevant UV climate to scrutinize its specific endurance in space or on the surface of Mars respectively. The exposure for 469 days to extraterrestrial UV radiation during the EXPOSE-R2 mission had a high impact on the culturability of the *D. geothermalis* cells. Colony-forming ability on agar plates clearly decreased in correlation to the increasing UV fluences in dependence of the corresponding stack position. The highest UV fluence of 505 kJ/m² received by each sample type in combination with space vacuum caused a decrease in culturability of the planktonic cells up to seven orders of magnitude compared with the amount of culturable cells at the time of sample preparation before the mission.

From the highest fluence, the survival rate increased continuously to the lowest fluence and in the bottom most layers, where the top layers provided some UV screening to the subjacent layers, which was almost comparable to the survival of the corresponding dark specimens. Endurance from exposure to polychromatic UV radiance was generally higher under Mars conditions than in vacuum, (i.e., under space conditions). Exposure of *B. subtilis* spores, for instance, to the full extraterrestrial UV spectrum (λ > 110 nm) that included V-UV (λ < 200 nm) was more deleterious than exposure to simulated Mars UV radiation (λ > 200 nm) as was also shown by Wassmann et al. (2012). Especially short-wavelength UV radiation was identified as one of the most detrimental factors that leads to the inactivation of terrestrial microorganisms exposed to space and simulated martian surface conditions.

The biofilm samples survived up to an order of magnitude better than the corresponding planktonic samples under these conditions. As already outlined in the previous paper of the authors, it was attributed to the fact of evaporation of residual water from the samples (especially in the space vacuum conditions of BOSS experiment), minimizing UV radiation-generated reactive oxygen species. On the other hand, shielding by upper cell layers (stack arrangements) or in multi-cellular structures (biofilms in general) and by dust layers of only 0.5–1 mm in thickness (comparable to the SVT with sulfatic Mars regolith simulant [S-MRS]) can provide sufficient protection (Retberg et al., 2004; Panitz et al., 2015; Frösler et al., 2017). The lower survival of the same samples of the MGR test is currently being examined more closely and will be verified after the receipt of a corresponding mission-based final calculation report at sample site by Redshift.

We conclude that *D. geothermalis* could withstand extraterrestrial UV radiation present in space or Mars conditions, especially when living in the community of a biofilm. The survivability seems to be advantageous for sustainability under the circumstances of shielding from UV, for instance by incorporated or superimposed minerals (Frösler et al., 2017).

### 4.3. Genomic integrity after mission performance and MGR test

Subcellular damage, here DNA damage, was investigated in biofilms and planktonic cells after mission and MGR completion. The effects of exposure to space or simulated martian conditions on *D. geothermalis* was determined by analyzing the integrity of genomic DNA, since nucleic acids represent the prime target of radiation-induced damage (Honeck et al., 2010). In this study, we used DNA as template for a PCR (RAPD-PCR) where DNA damage is revealed by yielding altered PCR product fingerprints (Williams et al., 1990; Trombert et al., 2007). The pooled triplicate samples from stack positions 1, 3, and 5 exposed to space vacuum or martian atmosphere, pressure, and UV radiation ranging from the maximum 505 kJ/m² (position 1), 160 kJ/m² (position 3) to 99 kJ/m² (position 5) showed a band pattern of genomic DNA as in the corresponding dark samples. The visualization of the fingerprint patterns showed some loss of bands of higher molecular size and the occurrence of lower molecular size bands after exposure to
higher fluences. The fact that D. geothermalis cells formed colonies even when their genomic DNA was severely damaged is in line with the capability of other species of Deinococcus to repair extensive DNA damage. Carrying multiple copies of a chromosome enables the cells to better deal with DNA breakage. The need for polyploidy to tolerate radiation and desiccation has been shown earlier in D. radiodurans (Slade et al., 2009). A quantitative evaluation of the accumulated DNA damage in D. geothermalis exposed to the mentioned stressors by using real-time quantitative PCR was not performed in this study and awaits further investigation.

As described in the previous paper of the authors (Frösl et al., 2017) in the SVT, additional biofilm and planktonic samples were prepared by mixing cells with S-MRS (32% pyroxene, plagioclase, amphibole, ilmenite, 30% gypsum, 15% olivine, 13% hematite, 7% goethite, 3% quartz, all wt %; Böttger et al., 2012). Before biofilm formation, 0.03 g/mL of autoclaved S-MRS was added to the biofilm inoculate or to planktonic cells harvested after growth in liquid culture. Cells from S-MRS supplemented biofilms remained culturable up to a UV fluence of 550 MJ/m², with a reduction in culturability of five orders of magnitude compared with non-exposed samples of the SVT.

S-MRS supplementation did not increase the UV tolerance of planktonic cells significantly (Frösl et al., 2017). Though promising results, these samples could not be part of the BOSS EXPOSE-R2 experiment due to limited space in the flight hardware. Nevertheless, DNA from the SVT samples was isolated and the differences of the integrity of genomic DNA were investigated. The altered RAPD patterns highlighted DNA damage and the increasing polychromatic UV doses were paralleled by increasing alteration of RAPD patterns for the individual fluence.

Amplicons were consistently obtained at higher UV fluences for biofilms than for planktonic samples, compared with those samples without added S-MRS. This is an indication that the Mars regolith simulant significantly increased the UV radiation tolerance of D. geothermalis biofilms, presumably due to physical shielding from radiation. This is in line with previous reports on the protective effects of S-MRS (Gómez et al., 2010; Baqué et al., 2013) or other mineral additives (de la Vega et al., 2007) during irradiation and under comparable conditions (Billi et al., 2000, 2011; Baqué et al., 2014). In general, RAPD profiles were not affected by exposure to vacuum or artificial Mars gas mixture in the dark and led to unaltered PCR profiles compared with the dried or liquid laboratory controls.

4.4. Total cell counts, plate counts, and membrane integrity

In addition to the ability of D. geothermalis to form colonies on agar plates, other aspects of viability were investigated in both sample types to distinguish between dead cells and cells that were viable but nonculturable (VBNC) (Oliver, 2005; Hammes et al., 2011; Pienaar et al., 2016). For disks 2 and 4, the cultivation-independent viability markers membrane integrity, ATP, and esterase activity were analyzed. In addition, total cell counts were determined and compared with plate counts. After the exposure to different stressors, particularly desiccation and UV radiation, these markers indicated that D. geothermalis maintained a level of viability that would have been missed if survival had been determined by using plate counts only.

On exposure to desiccation, D. geothermalis exhibited significantly reduced culturability, demonstrated by the difference between significantly lower plate counts and total cell counts, while sustaining cultivation-independent viability. Exposure to UV radiation further reduced the amount of culturable cells, while sustaining cultivation-independent viability, demonstrating the extreme radiation tolerance of D. geothermalis (Makarova et al., 2007).

Membrane integrity, a widely used indicator for VBNC, was investigated in this study. During exposure of D. geothermalis to desiccation and UV radiation in the EXPOSE-R2 mission, membrane integrity remained high in both biofilms and planktonic cells, indicating that the organism remained viable during space travel. However, it must be mentioned that UVC radiation, as it is widely used for disinfection, can cause cell death without immediate detectable damage to the cell membrane (Hammes et al., 2011) by the induction of lethal DNA damage.

Alternatively, part of the exposed D. geothermalis population may have entered a VBNC state, contributing to an elevated stress tolerance to desiccation and UV irradiation as mentioned in the previous papers of the authors (Frösl et al., 2017). Similar findings were also obtained for Escherichia coli and Pseudomonas aeruginosa due to UV irradiation (Said et al., 2010; Zhang et al., 2015).

4.5. ATP content

Because the intracellular concentration of ATP corresponds to a cell’s metabolic activity and physiological status, ATP can be used as a target molecule for the assessment of microbial viability (Hammes et al., 2011). Non-stressed cells of D. geothermalis were found to contain ATP in the order of 10⁻⁷–10⁻¹⁹ mol/cell. These values are in the range of intracellular ATP concentrations of bacteria depending on the species and the environmental conditions; ATP levels ranging from 10⁻²¹ to 10⁻¹⁷ mol/cell have been described (Shama and Malik, 2013). As already previously outlined, regardless of the sample type, ATP levels of D. geothermalis remained within the same order of magnitude compared with a non-exposed control during exposure to the conditions of the mission simulation tests (Frösl et al., 2017). This could also be witnessed for iATP values and eATP values of D. geothermalis biofilms and planktonic cells after performance of the BOSS experiment in space for almost 2 years in a desiccated state.

Concentrations of ATP, either free or intracellular, remained steady during the BOSS experiment. The very low eATP content is observed for only a very small quantity of lysed cells under the respective conditions. These results are in line with the data regarding the membrane integrity described earlier. Though ATP is susceptible to biological degradation (Cowan and Casanueva, 2007), it was also shown by other authors to persist under irradiation with Mars-like UV for extended periods (Schuerger et al., 2008). The suitability of ATP as a marker for viability in D. geothermalis was verified in previous laboratory experiments. After inactivation by isopropl alcohol, D. geothermalis suffered a significant loss of ATP, showing that this test is
dependent on the type of stress that the organism is exposed to and that its significance is limited if using it as a single viability parameter (Hammes et al., 2011).

4.6. Esterase activity

Determination of esterase activity can be used to provide information on the metabolic state of bacterial cells (Joux and Lebaron, 2000). In this study, the fluorogenic substrate CFDA was used for measuring esterase activity as well as for evaluating cell-membrane integrity, which is required to retain the fluorescent hydrolysis product inside the cell, whereas dead or lysed cells will rapidly leak the fluorescent product (Joux and Lebaron, 2000). D. geothermalis biofilms and planktonic cells have maintained esterase activity after exposure to space and Mars conditions during the EXPOSE-R2 mission and the MGR experiments. The levels of enzyme activity did not significantly vary between samples that were exposed to UV irradiation and non-exposed dark and laboratory controls, indicating that UV irradiation had no inhibitory influence on esterase activity.

These results are in accordance with the observation of this study that desiccated biofilms and planktonic cells exposed to space and Mars conditions largely retain membrane integrity and ATP levels comparable to non-exposed dark and laboratory controls.

5. Conclusions

The BOSS experiment of the EXPOSE-R2 mission demonstrated that D. geothermalis grown as biofilms and planktonic cells remain viable in the desiccated state after an exposure to space vacuum and simulated martian conditions in combination with extraterrestrial UV radiation in LEO for almost 16 months. Culturability is better preserved in biofilm cells, confirming them to be even more stress tolerant than planktonic cells. In contrast, cultivation-independent viability markers, including membrane integrity, ATP content, and enzyme (esterase) activity, remained nearly unaffected, indicating that the cells had switched to the VBNC state as a response to the existing stresses characteristic of space and Mars environments. The findings contribute to our understanding of the opportunities and limitations of adaptations of life under extreme environmental conditions and give answers to the question as to whether, in harsh places including space and Mars, living in a biofilm is the key to survival.

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Author Disclosure Statement

No competing financial interests exist.

References


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Abbreviations Used

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