Surviving Mars: new insights into the persistence of facultative anaerobic microbes from analogue sites

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Abstract

Mars analogue environments are some of the most extreme locations on Earth. Their unique combination of multiples extremes (e.g. high salinity, anoxia and low nutrient availability) make them valuable sources for finding new polyextremophilic microbes, and for exploring the limits of life. Mars, especially at its surface, is still considered to be very hostile to life but it probably possesses geological subsurface niches where the occurrence of (polyextremophilic) life is conceivable. Despite their well-recognized relevance, current knowledge on the capability of (facultative) anaerobic microbes to withstand extraterrestrial/Martian conditions, either as single strains or in communities, is still very sparse. Therefore, space experiments simulating the Martian environmental conditions by using space as a tool for astrobiological research are needed to substantiate the hypotheses of habitability of Mars. Addressing this knowledge gap is one of the main goals of the project MEXEM (Mars EXposed Extremophiles Mixture), where selected model organisms will be subjected to space for a period of 3 months. These experiments will take place on the Exobiology facility (currently under development and implementation), located outside the International Space Station. Such space experiments require a series of preliminary tests and ground data collection for the selected microbial strains. Here, we report on the survivability of Salinisphaera shabanensis and Buttiauxella sp. MASE-IM-9 after exposure to Mars-relevant stress factors (such as desiccation and ultraviolet (UV) radiation under anoxia). Both organisms showed survival after anoxic desiccation for up to 3 months but this could be further extended (nearly doubled) by adding artificial Mars regolith (MGS-1S; 0.5% wt/v) and sucrose (0.1 M). Survival after desiccation was also observed when both organisms were mixed before treatment. Mixing also positively influenced survival after exposure to polychromatic Mars-like UV radiation (200–400 nm) up to 12 kJ m⁻², both in suspension and in a desiccated form.

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Introduction

Earth hosts many natural extreme environments, characterized by e.g. extremes in temperature, pH, salinity and hydrostatic pressure, as well as in the availability of water, nutrients and oxygen. Nevertheless, these environments are colonized by specifically adapted and very successful life forms, also called extremophilic organisms or extremophiles (Rothschild and Mancinelli, 2001; Rampelotto, 2013).

Taking these extreme conditions into consideration and based on their similarities with conditions prevailing on other parts of our Solar System, some of these extreme sites were defined as analogue environments. A significant focus has been specifically placed on Mars analogue environments, with many locations highlighted across the world, namely in Tibet, USA, Argentina, Antarctica and India, just to name a few (e.g. Szponar et al., 2013; Forte et al., 2016; Xiao et al., 2017; Pandey et al., 2019; Favaro et al., 2020). European Mars analogue environments were selected and investigated in detail in the project MASE (Mars Analogues for Space Exploration) with their characterization from a microbial, physical, mineralogical and chemical point of view (Cockell et al., 2018; Bashir et al., 2021).

One of these sites is the Islinger Mühlbach in the Sippenauer Moor, located in southern Germany and hosting several cold sulphidic springs. These are part of a network of springs that emanate from the subsurface, and are characterized by low organic content, low temperatures, anoxia and having sulphur compounds as possible energy sources. The springs have been extensively investigated from a microbiological point of view for the past 20 years, illustrating their relevance (e.g. Moissl et al., 2002; Rudolph et al., 2004; Probst et al., 2014; Cockell et al., 2018). More recently, several new organisms have been isolated from this site during the MASE project (Cockell et al., 2018), including Buttiauxella sp. MASE-IM-9 (abbreviated as Buttiauxella sp. hereinafter). Buttiauxella sp. is a Gram-negative, facultative anaerobic and motile bacterial species within the family Enterobacteriaceae, and consists of rod-shaped cells. This strain has been tested under diverse Martian-relevant stress factors, such as desiccation, ionizing radiation and oxidizing compounds such as perchlorates (Beblo-Vranesevic et al., 2018, 2020).

An additional interesting analogue site is provided by deep-sea brine pools. Given their unique combination of high salinity, anoxia and varying levels of hydrothermal input (Antunes et al., 2011a), they have recently been identified as new analogue environments of relevance for astrobiological studies (Antunes et al., 2020). The deep-sea brines are further characterized by very sharp brine–seawater interfaces, with drastic transition in environmental conditions and significant particle accumulation, fuelling unique ecosystems and harbouring several new organisms (e.g. Antunes et al., 2011a; Oliver et al., 2015; Kaartvedt et al., 2016). One of the most striking new microbes from these locations is Salinisphaera shabanensis, isolated from the brine–seawater interface of the Shaban Deep, in the northern Red Sea (Antunes et al., 2003). S. shabanensis belongs to a deeply branching lineage within...
the γ-Proteobacteria, and is described as a Gram-negative, facultative anaerobic and monotrichous cocc. This species was shown via lab-based testing and genome-based analysis to be particularly versatile and resilient, likely an adaptation and advantage for living in the brine–seawater interface (Antunes et al., 2003, 2011b; Vetriani et al., 2014).

The harsh conditions on the surface of Mars are characterized by low water and nutrient availability, high salinity (including oxidizing compounds), high ultraviolet (UV) radiation influx, low ionizing radiation influx and the presence of Mars regolith itself. Although the Mars solar constant is, on average, only 43% of that observed on Earth, the biologically effective fluence rate of UV irradiation on the Martian surface is up to three orders of magnitude higher than on the surface of Earth due to its atmosphere, which is a thin, carbon dioxide (CO2)-dominated atmosphere, with only trace amounts of oxygen and the lack of a protecting ozone layer. Thus, and in contrast to Earth, biologically damaging UV (200–400 nm) radiation reaches the surface of Mars at relatively high levels. The total polychromatic UV fluence rate for low dust scenarios on the surface of equatorial Mars ranges between 42 and 55 W m$^{-2}$ (Schuerger et al., 2006). Considering the theoretical surface flux model as described in Patel et al. (2003) the UV flux in summer at the destined latitude of 60 lies around 38 W m$^{-2}$. In general, the UVC fluence rates were estimated to be approximately 8–10% of the total UV flux. Based on these models, between 3.2 and 5.5 W m$^{-2}$ of UVC irradiation is likely to reach the surface of equatorial Mars under clear-sky conditions (Schuerger et al., 2006). However, the Martian regolith and dust particles can contribute to a UV-protection scenario on the surface of Mars today (Vicente-Retortillo et al., 2020). Due to the lack of a magnetic field, on Mars there is also an increased dose of ionizing radiation. Indeed, the radiation dose rate of ionizing radiation on the surface of Mars was measured and calculated to be up to 0.21 mGy day$^{-1}$ (Matthiä et al., 2016) approximately ten times higher than on Earth.

From previous missions to Mars, the composition of Martian regolith is quite well known for different locations, permitting the description of different Mars soil simulants which are commercially available for purchase (Cannon et al., 2019). One of them is the Mars Global Simulant (MGS-1) standard mixture, resembling basaltic soils on Mars, and based on quantitative mineralogical data obtained from the Curiosity rover/Mars Science Lab mission (Grotzinger et al., 2012). MGS-1 is designed to replicate the Rocknest windblown soil in Gale crater on Mars, which is chemically similar to other basaltic soils at disparate landing sites and thus constitutes a ‘global’ basaltic soil composition. MGS-1S is a modified version of the MGS-1 simulant and is enriched in the polyhydrated sulphate gypsum.

Current knowledge on the capability of (facultative) anaerobic microbes to withstand extraterrestrial conditions is still very sparse and is one of the main goals of the project MEXEM (standing for Mars EXposed Extremophiles Mixture). The proposal for the space experiment MEXEM was selected by ESA in 2014, and at the moment the implementation is foreseen for 2025 at the new Exobiology facility that will be installed on the upcoming Bartolomeo platform outside the European Columbus module on the International Space Station (ISS). It is worth noting that space experiments often have long lead times, so it is even more important to prepare the experiments accurately. With MEXEM the long and successful tradition of passive exposure experiments on satellites and on the outside of the ISS will be continued. Thereby, biological samples will be exposed to a simulated anoxic Martian environment, i.e. a combination of Mars-like UV radiation from the Sun, simulated Martian atmosphere and Martian atmospheric pressure. Bacterial, archaeal and eukaryotic single species as well as natural communities, and mixtures will be evaluated. Besides other bacterial strains, Buttiauxella sp. and S. shabanensis were selected a part of the ‘passenger list’. The general aim of the space exposure experiments is to determine and analyse in detail the limits of terrestrial life. The results of MEXEM will contribute to our understanding of the capability of life to persist in extreme environments on Earth, on other planets (e.g. Mars), and moons of our Solar System.

According to the literature, past attempts to investigate the response of microorganisms to Martian extremes have generally been focused on aerobic organisms adapted to life under oxic conditions. Only a few studies have been conducted on (facultative) anaerobic microorganisms obtained from, and are thereby adapted to, Mars analogue environments (e.g. Serrano et al., 2019). This will be the first time...
that anaerobic polyextremophilic microbial isolates, i.e. bacteria, archaea, ciliates and viruses, will be investigated utilizing space as a tool for astrobiology.

The direct comparison of the survival strategies of phylogenetically different microbial species will also give new insights into the adequacy of actual planetary protection measures and may support the development of new life detection technologies for space application. When we know which terrestrial microorganisms can survive Martian conditions or can even live under these conditions, we can further improve our efforts and refine control protocols to prevent contamination of Mars and other planets during future missions.

The overall aim is the assessment of the survivability of microorganisms from extreme environments on Earth using the space environment as a tool. The purpose of the present work is a general data collection and detailed analysis of the desiccation and radiation tolerance of two new model organisms in the presence of added protective substances. The generated data serve on the one hand as an accurate preparation for the implementation of the space experiment itself and on the other hand the data are also essential information for future comparison with the results of the space experiments.

**Materials and methods**

**Cultivation of the strains**

In this study, *Buttiauxella* sp. MASE-IM-9 (DSM 105071) and *S. shabanensis* E1L3A\(^{T}\) (DSM 14853) were investigated. Both strains were cultivated under microoxic conditions in liquid medium (less than 1% v/v \(O_2\)). Therefore, the protocol by Miller and Wolin (1974) was used with the adaptation, that the medium was portioned (20 ml) in 120 ml serum bottles without prior addition of a reducing agent within the anaerobic chamber (Coy Laboratory Products Inc.; \([O_2] < 5 \text{ ppm, relative humidity } 13 \pm 0.5\%\)). The headspace was filled with \(N_2/CO_2 (80 : 20 \text{ v/v})\) with a pressure of 1 bar. *Buttiauxella* sp. was grown in Sigma Aldrich tryptic soy broth No. 2 (TSB), *S. shabanensis* was grown in BD Difco™ marine broth 2216 (MB) adjusted by the addition of sodium chloride (NaCl) to a final concentration of 10% wt/v. Alternatively, the cells were cultivated on tryptic soy agar plates for *Buttiauxella* sp. and marine agar with 10% NaCl wt/v for *S. shabanensis*. The cultivation temperature, either in liquid or on plates was 30°C, the optimal growth temperature for both strains. The strains were grown in broth until reaching the stationary growth phase (24 h for *Buttiauxella* sp. and 24–48 h for *S. shabanensis*); the plates remained in the incubator for up to 1 week.

**Added substances**

In this study, three different substance classes were added to the liquid medium/cultures: sugars (sucrose and trehalose), low-molecular substances (ectoine and betaine) and different Mars regolith simulants. The sucrose, trehalose, ectoine and betaine were prepared as aqueous stock solutions, gassed with \(N_2/CO_2 (80 : 20 \text{ v/v})\) and sterile filtered under anoxic conditions. The appropriate amount of added substances (end concentrations 0.1, 0.5 and 1 M) was added to the liquid medium/culture within the serum bottle via injection with a syringe.

Three Martian regolith simulants (MGS-1, MGS-1S and MGS-1C) were used in this study; detailed description of their components can be found at the homepage of CLASS Exolith Lab (Center for Lunar and Asteroid Surface Science at the University of Central Florida; [https://sciences.ucf.edu/class/exolithlab/](https://sciences.ucf.edu/class/exolithlab/)). MGS-1S, a modified version of MGS-1 (with a grain size of \(>0.04–600 \mu\text{m}\)), is described in detail by Cannon et al. (2019). According to the suppliers, MGS-1S has the following mineralogy in % (wt) as mixed: gypsum 40.0, plagioclase 16.4, basaltic glass 13.7, pyroxene 12.2, olivine 8.2, epsomite 2.4, ferrihydrite 2.1, hydrated silica 1.8, magnetite 1.1, anhydrite 1.0, siderite 0.8 and haematite 0.3. Additionally, MGS-1S has the following bulk chemistry by % (wt), as measured by X-ray fluorescence: SiO\(_2\) 29.3, TiO\(_2\) 0.3, Al\(_2\)O\(_3\) 6.4, Cr\(_2\)O\(_3\) 0.1, FeO\(_T\) 11.3, MnO 0.1, MgO 16.2, CaO 14.8, Na\(_2\)O 2.1, K\(_2\)O 0.5, P\(_2\)O\(_5\) 0.3 and SO\(_3\) 18.7.
Prior to being used in the experiments, Martian regolith was portioned and autoclaved under oxic conditions. The addition of regolith to the liquid medium/cultures in the appropriate quantities (end concentration of 0.1, 0.5 and 1% wt/v) was done under anoxic conditions within the anaerobic chamber. Therefore, the sterile serum bottles containing the medium/culture were opened under forming gas in the anaerobic chamber, regolith (MGS-1/MGS-1S/MGS-1C) was added, the serum bottles were closed again and the gas headspace was changed again to N₂/CO₂ (80 : 20 v/v).

**Microscopic observations**

Light and fluorescence microscopy was performed with 4′,6-diamidino-2-phenylindole (DAPI)-stained cells on a Zeiss Axio Imager.M2 including a DAPI filter set. For the fluorescent dye, we used the protocol previously described by Küper et al. (2010) (adapted from Huber et al., 1985). Briefly, prior to microscopy observations, 8 μl of the grown cultures were mixed with 1 μl of DAPI-staining solution, and incubated for 1 h at room temperature (RT).

Scanning electron microscopy was used to analyse the granular structures of the simulated Martian regolith. Samples were examined using a Hitachi TM3000 tabletop microscope with an accelerating voltage of 15 kV. Energy-dispersive spectroscopy (EDS) was performed using the Quantax70 program (Bruker).

**Desiccation of the cells**

For all experiments described below the strains were cultivated until early stationary phase (24 h for Butiauxella sp., 48 h for S. shabanensis). Growth and cell concentrations before the exposure experiments were determined microscopically by cell counting using a Thoma counting chamber (depth: 0.02 mm). The desiccation experiment was carried out as described before (Beblo et al., 2009). Briefly, the cell suspension (1 ml) was used directly without any washing or concentration steps, applied evenly on five glass discs (Ø 1.2 mm) and allowed to dry overnight at RT inside the anaerobic chamber (% O₂ < 5 ppm, relative humidity 13 ± 0.5%, 10⁵ Pa). The desiccation experiment was set up for 5 months (day 140), after 24 h of desiccation the first dataset (day 1) was evaluated. For the evaluation of the surviving fraction, the five glass discs with the attached dried cells were placed into 5 ml sterile anoxic phosphate-buffered saline (PBS). The PBS solution for Butiauxella sp. had the following composition: NaCl 8 g, KCl 0.2 g, Na₂HPO₄ 1.44 g, KH₂PO₄ 0.24 g per litre, while the modified PBS solution for S. shabanensis consisted of: NaCl 100 g, KCl 0.2 g, Na₂HPO₄ 1.44 g, KH₂PO₄ 0.24 g per litre. The cells were resuspended from the glass discs by shaking at RT. Afterwards, the cells were diluted in the corresponding PBS and plated on strain-specific agar plates (N). Control cells from liquid cultures were treated the same way: 1 ml of the grown culture was diluted in 5 ml of the corresponding sterile PBS, diluted further and plated on strain-specific plates (N₀). After incubation of all plates the number of grown colonies was noted.

**Mixture and desiccation experiments**

Both strains were cultivated under optimal cultivation conditions in their corresponding liquid medium. The cell concentration of both strains was determined before aliquots of the two liquid cultures were mixed and stored at RT for 24 h. Additionally, 1 ml of the mixture was desiccated on glass discs under anoxic conditions as described above. After 24 h, the cell numbers of both strains present in the liquid mixture and in the desiccated mixture was investigated. Thereby, the desiccated cells on glass discs were resuspended in PBS (adjusted to a final NaCl concentration of 5% wt/v), further diluted and plated on the corresponding agar plates of the strains. Preliminary tests confirmed that each strain was only able to grow on their strain-specific agar plates.
Irradiation treatment

Exposure to polychromatic UV (200–400 nm) in liquid
To avoid any absorbance due to the medium, or any of the individual medium components (e.g. organic material), cells were diluted 1 : 10 within the anaerobic chamber, and in the corresponding anoxic PBS, before the radiation treatment (Table 1). The absorption was measured with a Hitachi spectrophotometer (U-3310, Hitachi High Technologies America Inc.).

The polychromatic UV exposure was performed with the SOL2 lamp and conducted in UV transparent hermetically closed quartz cuvettes (type 1/GL14/S; Starna GmbH), including a magnetic stir bar. The SOL2 lamp was equipped with a UV 500S irradiation source (Dr Hönle AG, UV-Technologie). A detailed description of the irradiation facility is given in Rabbow et al. (2005). The biologically relevant spectrum for cellular damage (200–400 nm) was used to calculate the fluence rate and the exposure time to reach predetermined fluence values up to 12 000 J m\(^{-2}\). For the polychromatic UV experiments described here, a measured fluence rate of 10.35 W m\(^{-2}\) was used with a distance of 128 cm between the UV source and the sample. To ensure a homogenous irradiation, a magnetic stirrer was installed and the cultures were mixed during irradiation. All steps were done under anoxic conditions and at RT. After the irradiation with an appropriate fluence an aliquot of the irradiated cells was taken, further diluted in the corresponding PBS, plated on strain-specific plates, incubated and the grown colonies were counted \((N)\). A non-irradiated dark control was processed in the same way as a reference \((N_0)\).

Exposure to monochromatic UVC and ionizing radiation in liquid
Cells were treated for the exposure with monochromatic UVC (254 nm) in the same way as described above. For the anoxic UVC irradiation a low-pressure mercury lamp (model NN 8/15 Heraeus) with a major emission line at 254 nm (fluence rate 86.7 W m\(^{-2}\)) was used at RT as described by Beblo et al. (2011).

The exposure to ionizing radiation was performed with the X-ray source Gulmay RS 225A (Gulmay Medical Ltd.) at 200 kV and 15 mA following Beblo et al. (2011). Microbial cell cultures were transferred anoxically into high-performance liquid chromatography vessels and irradiated at a distance of 19.5 cm below the X-ray source with approximately 20 Gy min\(^{-1}\) up to 500 Gy. The dose rate was measured with a UNIDOS dosimeter (PTW Freiburg GmbH) and all irradiation experiments were performed under anoxic conditions at RT.

Exposure of dried cells to polychromatic UV (200–400 nm)
For the combination of desiccation and polychromatic irradiation, cells were treated as described above for the desiccation experiments under anoxic conditions. After 24 h of desiccation, the glass discs were transferred within the anaerobic chamber into the transport and exposure box (Trex-box; Beblo-Vranesvic et al., 2017a). The Trex-box was covered with a UV transparent quartz window and hermetically sealed. The exposure to polychromatic UV radiation was done under anoxic conditions at RT within the Trex-box.

Evaluation of the survival fraction
All experiments were repeated independently at least three times to represent biological replicas for each time point, fluence, dose and strain, respectively. \(N_0\) (non-desiccated/non-irradiated liquid control) and \(N\) (desiccated and/or irradiated sample) were determined from the number of counted colonies on the plates. The mean of the data with standard error was calculated. Within the graphs the survival fraction \((S = N/N_0)\) is given. The graphs and survival curves were created with Sigmaplot 13.0. Additionally, the \(F_{10}\) values/\(D_{10}\) values were calculated from regression lines of the exponential slopes of the survival curves as previously described (Harm, 1980). They provide the fluence/dose where the survival after treatment is reduced by one order of magnitude.
**Results**

**Effects of added substances on desiccation tolerance**

In a pretest series, we examined three different Mars regolith simulants (MGS-1, MGS-1S and MGS-1C), two sugars (sucrose and trehalose), as well as two low-molecular substances (ectoine and betaine) which are known to possibly influence the desiccation tolerance as intracellular accumulated compatible solutes. These substances were added to the cultures in different concentrations (0.1, 0.5, 1% wt/v for regolith, and 0.1, 0.5, 1 M for the other substances) both before and after inoculation (data not shown). The combination of MGS-1S (0.5% wt/v) and sucrose (0.1 M) revealed the greatest influence on desiccation tolerance after 24 h in *Buttiauxella* sp. and in *S. shabanensis*. Therefore, this combination was used for the evaluation of the long-term desiccation tolerance of both strains under anoxic conditions.

The desiccation tolerance under anoxic conditions of standard cultivated *Buttiauxella* sp. cells could be reproduced (Beblo-Vranesevic et al., 2018). Additionally, a general tolerance to desiccation was determined for standard cultivated cells of *S. shabanensis* under anoxic conditions up to 88 days (Fig. 1). After 88 days the survival rate of *S. shabanensis* was reduced by six orders of magnitude.
However, under standard conditions no growth after reactivation could be shown for both strains after 98 days of desiccation. For both strains, the desiccation tolerance under anoxic conditions was influenced by the addition of 0.5% (wt/v) MGS-1 and 0.1 M sucrose. In the presence of Martian regolith and sugar both strains survived at least 140 days (longer time periods were not tested in this experiment). The survival rate for *Buttiauxella* sp. decreased by only one order of magnitude over the time of 5 month ($S = 0.6 \times 10^{-1}$), while for *S. shabanensis* this decrease was about two orders of magnitudes ($S = 1.3 \times 10^{-2}$) for the same time span.

**Desiccation tolerance of the mixed cultures**

The storage of the mixed strains together, as an artificial community, slightly influences their absolute cell numbers. The survival of *Buttiauxella* sp. and *S. shabanensis* after mixture and after desiccation (24 h) was in the range of the survival of the strains alone or even slightly better (*Buttiauxella* sp.: $S = 6.1 \times 10^{-2}$ (alone) versus $S = 1.5 \times 10^{-1}$ (mixed); *S. shabanensis*: $S = 1.6 \times 10^{-2}$ (alone) versus $S = 7.9 \times 10^{-3}$ (mixed)) (Fig. 2).

![Fig. 1. Survival of Buttiauxella sp. (circles) and Salinisphaera shabanensis (triangles) after anoxic desiccation up to 5 months. Strains were cultivated either under standard conditions (open symbols) or in the presence of Martian regolith (MGS-1S; 0.5% wt/v) and sugar (sucrose; 0.1 M) (filled symbols) before desiccation. *No survival was detectable. Trendlines were fitted by hand.*](https://www.cambridge.org/core/journals/international-journal-of-astrobiology)
Survival after polychromatic UV in liquid and after desiccation

Survival of cells in a liquid suspension (Fig. 3(a)) and in a desiccated state (Fig. 3(b)) was determined after irradiation with Mars-relevant polychromatic UV radiation (200–400 nm). The cells were treated under standard conditions, as well as in the presence of Mars regolith (MGS-1S 0.5% wt/v) and sugar (sucrose). In general, the same concentrations were used as in the long-term desiccation experiments.

Survival of *Buttiauxella* sp. in liquid after irradiation with polychromatic UV was visible in all cases up to 12 kJ m$^{-2}$, the highest tested fluence (Fig. 3(a)) and displaying typical shouldered survival curves. For *S. shabanensis* survival up to the highest tested fluence of 10 kJ m$^{-2}$ was recognizable and the curves were less shouldered than those of *Buttiauxella* sp. (Fig. 3(c)). Nevertheless, the survival curves of both organisms bundle together. There is a marginal difference in the survival curves if MGS-1S was present. In the presence of Martian regolith, as well as in the presence of MGS-1S and sugar the survival of *Buttiauxella* sp. and *S. shabanensis* in liquid was about one order of magnitude higher than in the standard samples or in the samples with sugar only (Figs 3(a) and (c)).

In general, the elevated survival after desiccation in the presence of Martian regolith and sugar is visible here (Fig. 3(b)). The addition of sucrose or MGS-1S alone seems to have a minor effect on drought tolerance. More obvious is the difference in the presence of the added substances after irradiation at higher fluences and desiccation. In the samples of *Buttiauxella* sp. with Martian regolith either alone or in combination with sugar the survival rate is two orders of magnitude higher than in the standard samples (Figs 3(b) and (d)).

Survival after exposure to UVC (254 nm) and ionizing radiation in liquid

The survival after exposure to UVC and to ionizing radiation is shown here via the calculated $F_{10}$ values (254 nm; 200–400 nm) and $D_{10}$ values of *Buttiauxella* sp. MASE-IM-9 and *S. shabanensis* (Table 2). These values were compared with other spaceflight relevant strains, such as *Bacillus subtilis*,

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**Fig. 2.** Cell numbers of *Buttiauxella* sp. (black) and *S. shabanensis* (grey) after cultivation under optimal conditions separately for 24 h (first columns). Cells numbers of both strains after being mixed for 24 h at RT (second columns). Cells numbers after desiccation of the mixed cultures for 24 h at RT under anoxic conditions (third columns). Cell numbers after desiccation of the strains separately (fourth columns). Cell concentration was evaluated by plating on strain specific solidified medium.
as spores and as vegetative cells, *Deinococcus radiodurans*, and other slightly halophilic/aquatic microorganisms (*Aquifex pyrophilus* and *Hydrogenothermus marinus*).

The $F_{10}$ values and therefore the tolerance to monochromatic UVC and polychromatic UV of *Buttiauxella* sp. and *S. shabanensis* are comparable and were in the range of other vegetative cells such as *B. subtilis* and *Escherichia coli*. The $D_{10}$ value of *Buttiauxella* sp. is in the range of *E. coli*, which is also affiliated to the Gammaproteobacteria. The $D_{10}$ values of *S. shabanensis* (also a gammaproteobacterial) is even higher than the $D_{10}$ value of *B. subtilis* spores, but clearly lower than

![Fig. 3.](image-url)
those of the halophilic archaeon *Halobacterium salinarum* and the radiation tolerant model organism *D. radiodurans*.

**Microscopic observations**

Survival after UV irradiation in the presence of Martian regolith was drastically increased in both organisms. In the following, the distribution of microbes in samples with Martian regolith was analysed microscopically to assess the potential shielding by mineral grains, especially in case the microbes would attach to the grains, and by potential aggregate formation where outer cells would shield inner cells from UV radiation.

The cells were stained with DAPI, a fluorescence dye which binds to adenine- and thymine-rich areas in the DNA and is indicating thereby the location of the DNA-containing cells on particles. To avoid technical errors a DAPI-staining protocol without centrifugation steps was used for our experiments.

The Martian regolith simulant MGS-1S has a granular structure with particles of different sizes (Figs 4(a)–(c)). EDS measurements of the regolith samples identified silicon in a significant amount, present in the form of diatomaceous earth, also known as diatoms or kieselgur particles with characteristic shapes (arrow in Fig. 4(c)) visible even with phase contrast microscopy (arrow in Fig. 4(d)). Cells of *Buttiauxella* and *Salinisphaera* cultivated in the presence of MGS-1S were observed as either swimming separately in broth or clustering together on particles including the kieselgur particles (arrow in Fig. 4(e)). This extensive clustering or even colony/biofilm-like growth is especially visible after DAPI-staining of the cells of both strains (Figs 4(e), (h), (f) and (i)). However, the particles themselves do not stain with DAPI and a fluorescence of the particles could be excluded (arrow in Fig. 4(d) and arrow in Fig. 4(g)).

**Discussion**

The conditions on the surface of Mars seem to be very harmful or even deadly for terrestrial life. To recap a few, the anoxia, the aridity, the Martian regolith itself, the unfiltered short wavelength radiation and the cosmic radiation influx. In the following, the results of the stress tests with the two polyextremophilic organisms *Buttiauxella* sp. and *S. shabanensis* will be discussed with respect to desiccation, Martian-relevant radiation in combination with Martian regolith.

The detailed analysis of the survivability under the different conditions mentioned above is the basis for the preparation of the MEXEM space experiment.

**Both strains showed desiccation tolerance under anoxic conditions**

First of all, it should be noted that both studied organisms are facultative anaerobes and they can cope with the reduced oxygen levels in the experiments conducted under anoxic conditions. The known tolerance of *Buttiauxella* sp. under standard conditions to desiccation was confirmed (Beblo-Vranesevic et al., 2018). Additionally, the newly investigated non-spore forming bacterium *S. shabanensis* showed desiccation tolerance, although there are no periods of drought in its natural habitat. It is surprising that organisms originating from aqueous environments possess the ability to survive periods of water loss, as it was already shown for other organisms such as *Yersinia intermedia* MASE-LG-1 (Beblo-Vranesevic et al., 2017a). One possible explanation for this phenomenon in *S. shabanensis* can be the very high salinity (between 21.2 and 23%), found in the brine immediately below the brine–seawater interface of Shaban Deep, in the Red Sea, where *S. shabanensis* has been isolated (Antunes et al., 2003). Previous studies have already shown that osmotic stress has a positive influence on the desiccation tolerance of bacteria and archaea (Beblo-Vranesevic et al., 2017b). Here, the role of compatible solutes is discussed in particular (Galinski et al., 2014; Zeidler and Müller, 2019). *S. shabanensis* was reported as capable of accumulation of remarkably high concentrations of the compatible...
Fig. 4. Scanning electron microscopic pictures of the used Martian regolith simulant MGS-1S (a–c). Phase contrast light microscopic pictures of grown cultures (d, g), DAPI-stained cultures (e, h) and merged multi-channels picture (f, i) of Buttiauxella sp. (d–f) and S. shabanensis (g–i) grown in the presence of Martian regolith simulant MGS-1S.
solutes ectoine and betaine (above 4 M); additionally, it represented the first report of a prokaryote capable of accumulating glycerol (Antunes et al., 2003).

The drought tolerance of Buttiauxella sp. and S. shabanensis was also detectable and even slightly elevated when the two strains were previously mixed and dried together. This is of particular interest because communities are foreseen to be exposed in the space experiment MEXEM. This experiment should show whether the individual strains of the community can support or even protect each other during exposure under Martian conditions and thus survive better than the individual strains alone. For example, for the halophilic archaeon Halococcus morrhuae and the biofilm-forming bacterium Halomonas muralis, co-existing in natural environments, it could be shown that the biofilm of H. muralis can protect H. morrhuae in space exposure experiments. A mixture of both strains was advantageous only for the survival of H. morrhuae, as shown when growing this microbe exposed independently and in combination with H. muralis to space conditions outside of the ISS for 534 days during the EXPOSE-R2 mission (Leuko et al., 2020). For other halophilic Archaea co-entombment in salt crystals showed improved viability of mixed-cultures versus isolated ones (Gramain et al., 2011).

If this protective effect of co-exposure of two strains is also true after irradiation treatment, as it was shown for Bacillus strains, it should be further investigated in the future. Thereby, spores of different Bacillus species were shown to have different survival pattern after polychromatic UV irradiation (200–400 nm), with Bacillus pumilus being more resistant than B. subtilis. The tolerance of B. subtilis was higher if the strains were irradiated together in equal amounts (Newcombe et al., 2005).

Martian regolith and sugar are influencing the survivability of both strains after desiccation positively

If we are thinking of the habitability of Mars, there is of course the Martian regolith itself which could be influencing possible life there. In the past, different (negative, neutral and positive) effects of Martian regolith on various life forms could be shown. For example, plants grow worse in MGS-1 than in other artificial Martian soils (Eichler et al., 2021). In other experiments, it has been shown that basalt as a Mars analogue regolith and also silicates in combination with a movement that mimics wind-driven saltation can have a negative or even deadly influence on microorganisms (Bak et al., 2017, 2019). A slight negative influence of Martian regolith for the fungal species Cryomyces antarcticus was recently demonstrated by Aureli et al. (2020). There, cells exhibited a reduced growth capability after exposure to higher doses of heavy ion particles (iron) up to 1 kGy when mixed with different Martian regolith analogues. This effect would be explainable with the production of secondary particles which are produced during irradiation of the regolith material (Dartnell et al., 2007).

On the other hand, there are studies where the Martian regolith did not influence microbial life negatively. For example, an aqueous extract of a Mars analogue regolith (including significant amounts of perchlorates) did not inhibit the germination rate of spores of B. subtilis and B. pumilus (Nicholson et al., 2012). Another relevant case is the survival after desiccation (400 days) of methanogenic Archaea in the presence of regolith analogues which was investigated by Schirmack et al. (2015). However, there can also be a positive effect of Martian regolith analogues on different organisms. A positive influence on the survivability of fungi has been shown in the EXPOSE-R2 mission with different Martian and Lunar rock analogues (Onofri et al., 2019; Pacelli et al., 2020). The tolerance to anoxic desiccation in our experiments could be increased in both organisms by adding Martian regolith and sugar. In both strains, this results in only slightly reduced survival rate over months, therefore making the strains suitable for the 3 month space exposure experiments as planned and specified by us in the MEXEM mission. An increased survival in the presence of Martian regolith was also simultaneously shown for some human-associated microbial species (Pseudomonas, Klebsiella, Serratia and Burkholderia). Here, the desiccation-sensitive organisms survived periods of dehydration better in the presence of MGS or even only survived desiccation in the presence of Martian regolith (Zaccharia, 2021). One reason for the positive effect could be the water retention potential of some of the regolith particles (Jänchen et al., 2014).
This is of particular interest when thinking about various future Mars missions. Humans are surrounded by microorganisms and if these, including drought-sensitive organisms, are allowed to reach Martian soil and are eventually covered with Martian regolith by the ever-present Martian wind, they could survive the prevailing arid conditions.

It is known for some time that the addition of sugar increases the drought tolerance for example in *E. coli* and *Bradyrhizobium japonicum* (Louis *et al.*, 1994; Streeter, 2003). This effect could be confirmed in our experiments, even if only in combination with MGS as a long-term experiment. The reason behind this could be that the sugars protect the membranes and proteins in bacteria during drying as described by Leslie *et al.* (1995). Additionally, it has recently been shown that lipid bilayers are also stabilized by sucrose during desiccation (Stachura *et al.*, 2019), so this could also be a relevant aspect.

**Both strains are able to withstand desiccation combined with polychromatic UV radiation**

*Buttiauxella* sp. and *S. shabanensis* were able to survive polychromatic irradiation in a dried form and in liquid. For *D. radiodurans*, it has been shown that their cell withstand radiation better when in the dried state and in a monolayer under standard conditions rather than when in liquid and irradiation with polychromatic wavelength (Bauermeister *et al.*, 2011). This phenomenon was not confirmed in the model organisms studied here. This is more an additive effect of drying and radiation than a synergistic/interactive one.

The addition of sucrose had only a marginal influence on the survival of *Buttiauxella* sp. and *S. shabanensis* after polychromatic irradiation in liquid. However, for *Halococcus hamelinensis* it has been shown that the addition of 100 mM trehalose to the growth medium elevated the tolerance to polychromatic UV and the *F*<sub>10</sub> value tripled (Leuko *et al.*, 2015). The addition of the powdery, fine-grained Martian regolith increases the tolerance to radiation in liquid medium in both strains. Comparable results have been shown in experiments with other materials such as ores or desert soil (Osman *et al.*, 2008; Beblo *et al.*, 2011).

The addition of the two additives also increases the survival rate after the combination of desiccation and radiation, probably due to physical protection properties of the regolith material. The added Martian regolith protects against polychromatic radiation, as it would also occur on the surface of Mars. UV radiation can only penetrate a few hundred micrometres deep into the soil (Cockell *et al.*, 2000; Mancinelli and Klovstad, 2000). This means that organisms that would present a little deeper in the soil are not only protected from the radiation, but are also better protected from desiccation due to the Mars regolith. Similar effects could also be shown with *E. coli*. There too, components of the Martian soil were added to the cultures and it was concluded that its cells may be able to survive, but not grow, in surficial soils on Mars (Berry *et al.*, 2010).

Previous space experiments have shown the protective properties of powdered material (rock, sandstone, meteorite and simulated Martian regolith) on spores exposed to solar UV radiation (Rettberg *et al.*, 2004). Additionally, it was shown that none of these substances was toxic to the spores when kept in the dark or in vacuum, as demonstrated by the nearly 100% survival of the flight dark samples as well as the lab and ground controls (Rettberg *et al.*, 2004). In contrast, for *D. radiodurans* the UV irradiation was very efficient in reducing the microbial populations under the investigated surface conditions. Neither the strain, whether mixed with nano-crystalline haematite or not, survived the exposure to Mars-like solar radiation. Viability rates were only measured when Goldenrod B-6090, a commercial mineral pigment produced by blending red haematite and yellow goethite pigments, was added to the bacteria (Morris and Golden, 1998; Pogoda De la Vega *et al.*, 2007).

Both tested model organisms, *Buttiauxella* sp. and *S. shabanensis*, have an average to high tolerance to ionizing radiation. With a dose of 0.21 mGy day<sup>−1</sup> (Matthiä *et al.*, 2016) the majority of microbial life will be affected only in longer timescales such as decades. However, a potential influence of the Martian regolith in combination with ionizing radiation was not tested until now. It was shown that *B. subtilis* spores covered with Martian regolith were significantly more sensitive to X-rays than uncov ered spores, which is mainly due to the interaction of X-rays with artificial Martian regolith resulting in the formation of secondary electrons and reactive oxygen species (Moeller *et al.*, 2010).
The expected radiation environment of the MEXEM experiment

In space exposure experiments, mounted on the outside of the ISS, high fluences of UV radiation and rather small doses of ionizing radiation occur. For comparison, in the EXPOSE-R2 experiment with a mission duration of 469 days (approximately 17 months) $536 \pm 116 \text{ MJ m}^{-2}$ ($5.36 \times 10^8 \text{ J m}^{-2}$) in the wavelength range of 200–400 nm were modelled. Additionally, during this mission values up to 1 Gy were detected (Rabbow et al., 2017). Taking the UV fluences measured in the EXPOSE-R2 mission into account, and even though the MEXEM mission will only be exposed for 3 months, the estimated UV (200–400 nm) fluence will be about $9.5 \times 10^7 \text{ J m}^{-2}$. Therefore, the need of attenuation of the incoming UV with neutral filters is unavoidable as it is proposed in the mission description. This is still necessary even if the cells are mixed with protecting Martian regolith. The ionizing radiation that will occur in a 3 month exposure experiment in low Earth orbit plays a negligible role for microorganisms due to the low dose rate and total dose.

Conclusion

In general, the strategy of searching for new polyextremophilic and resistant organisms in extreme environments on Earth has proven successful for finding microbes capable of surviving Martian environmental conditions. Buttiauxella sp. had been previously shown to be tolerant of Mars relevant conditions such as perchlorate exposure and Martian gas composition and pressure (Beblo-Vranesevic et al., 2020). S. shabanensis was also tolerant to Martian atmospheric conditions and pressure over 5 months even when combined with exposure to UV at an altitude of 36 km (Cortesão et al., 2021).

Our experiments further support that Buttiauxella sp. and S. shabanensis are two promising candidates that are suitable for the MEXEM space experiment.

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References

Antunes A, Eder W, Fareleira P, Santos H and Huber R (2003) Salinisphaera shabanensis gen. nov., sp. nov., a novel, moderately halophilic bacterium from the brine–seawater interface of the Shaban Deep, Red Sea. Extremophiles 7, 29–34.

Antunes A, Ngugi DK and Stingl U (2011a) Microbiology of the Red Sea (and other) deep-sea anoxic brine lakes. Environmental Microbiology Reports 3, 416–433.

Antunes A, Alain I, Bajic VB and Stingl U (2011b) Genome sequence of Salinisphaera shabanensis, a gammaproteobacterium from the harsh, variable environment of the brine–seawater interface of the Shaban Deep, Red Sea. Journal of Bacteriology 193, 4555–4556.

Antunes A, Olsson-Francis K and McGenity TJ (2020) Exploring deep-sea brines as potential terrestrial analogues of oceans in the icy moons of the outer solar system. Current Issues in Molecular Biology 38, 123–162.

Arrage AA, Phelps TJ, Benoit RE and White DC (1993) Survival of subsurface microorganisms exposed to UV radiation and hydrogen peroxide. Applied and Environmental Microbiology 59, 3545–3550.

Aureli I, Pacelli C, Cassaro A, Fujimori A, Moeller R and Onofri S (2020) Iron ion particle radiation resistance of dried colonies of Cryomyces antarcticus embedded in Martian regolith analogues. Life (Chicago, Ill.) 10, 306.

Bak EN, Larsen MG, Moeller R, Nissen SB, Jensen LR, Nørnberg P, Jensen SJK and Finster K (2017) Silicates eroded under simulated Martian conditions effectively kill bacteria – a challenge for life on Mars. Frontiers in Microbiology 8, 1709.

Bak EN, Larsen MG, Jensen SK, Nørnberg P, Moeller R and Finster K (2019) Wind-driven salination: an overlooked challenge for life on Mars. Astrobiology 19, 497–505.

Bashir AK, Wink L, Duller S, Schwendener P, Cockell C, Retberg P, Mahnert A, Beblo-Vranesevic K, Bohmeyer M, Rabbow E, Gaboyer F, Westall F, Walter N, Cabezás P, Garcia-Descalzo L, Gomez F, Malki M, Amils R, Ehrenfreund P, Monaghan E, Vannier P, Marteinsson V, Erlacher A, Tanski G, Strauss J, Bashir M, Riede A and Moissl-Eichinger C (2021) Taxonomic and functional analyses of 3 intact microbial communities thriving in 4 extreme, astrobiology-relevant sites. Microbiome 9, 1–16.

Bauermister A, Moeller R, Reitz G, Sommer S and Retberg P (2011) Effect of relative humidity on Deinococcus radiodurans’ resistance to prolonged desiccation, heat, ionizing, germicidal, and environmentally relevant UV radiation. Microbial Ecology 61, 715–722.

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Beblo-Vranesevic K, Bohmeier M, Pernas AK, Schwendner P, Rabbo E, Moissl-Eichinger C, Cockell CS, Pukall R, Vannier P, Marteinsson VT, Monaghan EP, Ehrenfreund P, Garcia-Descalzo L, Gómez F, Malik M, Amils R, Gaboyer F, Westall F, Cabezas P, Walter N and Retting P (2017a) The responses of an anaerobic microorganism, *Versinia intermedia* MASE-LG-1 to individual and combined simulated Martian stresses. *PLoS One* **12**, e0185178.

Beblo-Vranesevic K, Galinski EA, Rachel R, Huber H and Retting P (2017b) Influence of osmotic stress on desiccation and irradiation tolerance of (hyper)-thermophilic microorganisms. *Archives of Microbiology* **199**, 17–28.

Beblo-Vranesevic K, Bohmeier M, Pernas AK, Schwendner P, Rabbo E, Moissl-Eichinger C, Cockell CS, Vannier P, Marteinsson VT, Monaghan EP, Ehrenfreund P, Garcia-Descalzo L, Gómez F, Malik M, Amils R, Gaboyer F, Westall F, Cabezas P, Walter N and Retting P (2018) Lack of correlation of desiccation and radiation tolerance in microorganisms from diverse extreme environments tested under anoxic conditions. *FEMS Microbiology Letters* **365**, fny044.

Beblo-Vranesevic K, Bohmeier M, Schleumer S, Rabbo E, Pernas AK, Moissl-Eichinger C, Schwendner PC, Cockell CS, Vannier P, Marteinsson VT, Monaghan EP, Riedo A, Ehrenfreund P, Garcia-Descalzo L, Gómez F, Malik M, Amils R, Gaboyer F, Hickman-Lewis K, Westall F, Cabezas P, Walter N and Retting P (2020) Impact of simulated Martian conditions on (facultatively) anaerobic bacterial strains from different Mars analogue sites. *Current Issues in Molecular Biology* **38**, 103–122.

Beblo K, Rabbo E, Rachel R, Huber H and Retting P (2009) Tolerance of thermophilic and hyperthermophilic microorganisms to desiccation. *Extremophiles* **13**, 521–531.

Beblo K, Douki T, Schmalz G, Rachel R, Wirth R, Huber H, Reitz G and Retting P (2011) Survival of thermophilic and hyperthermophilic microorganisms after exposure to UV-C, ionizing radiation and desiccation. *Archives of Microbiology* **193**, 797–809.

Berry BJ, Jenkins DG and Schuerger AC (2010) Effects of simulated Mars conditions on the survival and growth of *Escherichia coli* and *Serratia liquefaciens*. *Applied and Environmental Microbiology* **76**, 2377–2386.

Cannon KM, Britt DT, Smith TM, Fritsche RF and Batcheldor D (2019) Mars global simulant MGS-1: a Rocknest-based open standard for basaltic Martian regolith simulants. *Icarus* **317**, 470–478.

Clavero MRS, Monk JD, Beuchat LR, Doyle MP and Brackett RE (1994) Inactivation of *Escherichia coli* 0157:H7, Salmonellae, and *Campylobacter jejuni* in raw ground beef by gamma irradiation. *Applied and Environmental Microbiology* **60**, 2069–2075.

Cockell CS, Catling DC, Davis WL, Snook K, Kepner RL, Lee P and McKay CP (2000) The ultraviolet environment of Mars: biological implications past, present, and future. *Icarus* **146**, 343–359.

Cockell CS, Schwendner P, Pernas R, Rettberg P, Beblo-Vranesevik K, Bohmeier M, Rabbo E, Moissl-Eichinger C, Wink L, Marteinsson V, Vannier P, Gomez F, Garcia-Descalzo L, Ehrenfreund P, Monaghan EP, Westall F, Gaboyer F, Amils R, Malik M, Pukall R, Cabezas P and Walter N (2018) Anaerobic microorganisms in astrobiological analogue environments: from field site to culture collection. *International Journal of Astrobiology* **17**, 314–328.

Cortesão M, Siems K, Koch S, Beblo-Vranesevik K, Rabbo E, Berger T, Lane M, James L, Johnson P, Waters SM, Verna SD, Smith DJ and Moeller R (2021) MARSBOx: fungal and bacterial endurance from a balloon-flo wn analog mission in the stratosphere. *Frontiers in Microbiology* **12**, 177.

Dartnell LR, Desorgher L, Ward JM and Coates A (2007) Martian sub-surface ionising radiation: biosignatures and geology. *Biogeosciences Discussions (Online)* **4**, 455–492.

Eichler A, Hadland N, Pickett D, Masaitis D, Handy D, Perez A, Batcheldor D, Wheeler B and Palmer A (2021) Challenging the agricultural viability of Martian regolith simulants. *Icarus* **354**, 114022.

Favaro EA, Hugenholz CH, Barchyn TE and Gough TR (2020) Wind regime, sediment transport, and landscape dynamics at a Mars analogue site in the Andes Mountains of Northwestern Argentina. *Icarus* **346**, 113765.

Forte E, Dalle Fratte M, Azzaro M and Guglielmin M (2016) Pressurized brines in continental Antarctica as a possible analogue of Mars. *Scientific Reports* **6**, 1–10.

Galinski EA, Tanne C, Meffert A, Golovina EA and Hoekstra FA (2014) Glass-forming property of hydroxyectoine is the cause of its superior function as a desiccation protectant. *Frontiers in Microbiology* **5**, 150.

Gramain A, Diaz GC, Demergasso C, Lowenstein TK and McGinity TJ (2011) Archaeal diversity along a subterranean salt core from the Salaf Grande (Chile). *Environmental Microbiology* **13**, 2105–2121.

Grotzinger JP, Crisp J, Vasavada AR, Anderson RC, Baker CJ, Barry R, Blake DF, Conrad P, Edgett KS, Ferdowski B, Gellert R, Gilbert JB, Golombek M, Gómez-Elvira J, Hassler DM, Jandura L, Litvak M, Mahaffy P, Maki J, Meyer M, Malin MC, Mitrofanov I, Simmonds JJ, Vaniman D, Welch RV and Wiens RC (2012) Mars Science Laboratory mission and science reports. *Science* **338**, 170, 5–56.

Harm W (1980) *Biological Effects of Ultraviolet Radiation*. Cambridge: Cambridge University Press.

Huber H, Huber G and Stetter KO (1985) A modified DAPI fluorescence staining procedure suitable for the visualization of lithotrophic bacteria. *Systematic and Applied Microbiology* **6**, 105–106.

Jänchen J, Bauermeister A, Feyh N, de Vera JP, Retting P, Fleming HC and Szewczyk U (2014) Water retention of selected Martian soil simulants under close to Martian environmental conditions. *Planetary and Space Science* **98**, 163–168.

Kaartvedt S, Antunes A, Røstad A, Klevjer TA and Vestheim H (2016) Zooplankton at deep Red Sea brine pools. *Journal of Plankton Research* **38**, 679–684.

Kottemann M, Kish A, Iloanusi C, Bjork S and DiRuggiero J (2005) Physiological responses of the halophilic archaeon *Halobacterium* sp. strain NRC1 to desiccation and gamma irradiation. *Extremophiles* **9**, 219–227.
Küper U, Meyer C, Müller V, Rachel R and Huber H (2010) Energized outer membrane and spatial separation of metabolic processes in the hyperthermophilic Archaeaen *Halogeometrica halophila*. *PNAS* **107**, 3152–3156.

Leslie SB, Israeli E, Lighthart B, Crowe JH and Crowe LM (1995) Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying. *Applied and Environmental Microbiology* **61**, 3592–3597.

Leuko S and Retberg P (2017) The effects of HZE particles, γ and X-ray radiation on the survival and genetic integrity of *Halobacterium salinarum* NRC-1, *Halococcus halamehensis*, and *Halococcus morrhuae*. *Astrobiology* **17**, 110–117.

Leuko S, Neilan BA, Burns BP, Walter MR and Rothschild LJ (2011) Molecular assessment of UVC radiation-induced DNA damage repair in the stromatolitic halophilic archaeon, *Halococcus halamehensis*. *Journal of Photochemistry and Photobiology B: Biology* **102**, 140–145.

Leuko S, Domingos C, Parpart A, Reitz G and Retberg P (2015) The survival and resistance of *Halobacterium salinarum* NRC-1, *Halococcus halamehensis*, and *Halococcus morrhuae* to simulated outer space solar radiation. *Astrobiology* **15**, 987–997.

Leuko S, Stan-Lotter H, Lamers G, Sjöström S, Rabbow E, Parpart A and Retberg P (2020) Resistance of the archaeon *Halococcus morrhuae* and the biofilm-forming bacterium *Halomonas muralis* to exposure to low earth orbit for 534 days. In Seckbach J and Stan-Lotter H (eds), *Extremophiles As Astrobiological Models*. Scrivener Publishing LLC, pp. 221–236.

Luis P, Trüper HG and Galinski EA (1994) Survival of *Escherichia coli* during drying and storage in the presence of compatible solutes. *Applied Microbiology and Biotechnology* **41**, 684–688.

Mancinelli RL and Klovstad M (2000) Martian soil and UV radiation: microbial viability assessment on spacecraft surfaces. *Planetary and Space Science* **48**, 1093–1097.

Matthäi D, Ehresmann B, Loth H, Köhler J, Zeitlin C, Appel J, Sato T, Slaba T, Martin C, Berger T, Boehm E, Boetcher S, Brinza DE, Burmeister S, Guo J, Hassler DM, Posner A, Rafkin SCR, Reitz G, Wilson JW and Wimmer-Schweingruber RF (2016) The Martian surface radiation environment – a comparison of models and MSL/RAD measurements. *Journal of Space Weather and Space Climate* **6**, A13.

Miller TL and Wolin MJ (1974) A serum bottle modification of the Hungate technique for cultivating obligate anaerobes. *Applied Microbiology* **27**, 985–987.

Moeller R, Stackebrandt E, Reitz G, Berger T, Retberg P, Doherty AJ, Horneck G and Nicholson WL (2007) Role of DNA repair by nonhomologous-end joining in *Bacillus subtilis* spore resistance to extreme dryness, mono- and polychromatic UV, and ionizing radiation. *Journal of Bacteriology* **189**, 3306–3311.

Moeller R, Rohde M and Reitz G (2010) Effects of ionizing radiation on the survival of bacterial spores in artificial Martian regolith. *Icarus* **206**, 783–786.

Moissl C, Rudolph C and Huber R (2002) Natural communities of novel archaea and bacteria with a string-of-pearls-like morphology: molecular analysis of the bacterial partners. *Applied and Environmental Microbiology* **68**, 933–937.

Morris RV and Golden DC (1998) Goldenrod pigments and the occurrence of hematite and possibly goethite in the Olympus – Amazonis region of Mars. *Icarus* **134**, 1–10.

Newcombe DA, Schuerger AC, Benardini JN, Dickinson D, Tanner R and Venkateswaran K (2005) Survival of spacecraft-associated microorganisms under simulated Martian UV irradiation. *Applied and Environmental Microbiology* **71**, 8147–8156.

Nicholson WL, McCoy LE, Kerney KR, Ming DW, Golden DC and Schuerger AC (2012) Aqueous extracts of a Mars analogue regolith that mimics the Phoenix landing site do not inhibit spore germination or growth of model spacecraft contaminants *Bacillus subtilis* 168 and *Bacillus pumilus* SAFR-032. *Icarus* **220**, 904–910.

Oliver PG, Vestheim H, Antunes A and Kaartvedt S (2015) Systematics, functional morphology and distribution of a bivalve (*Apachecorbula muriatica* gen. et sp. nov.) from the rim of the ‘Valdivia Deep’ brine pool in the Red Sea. *Journal of the Marine Biological Association* **95**, 523–535.

Onofri S, Selbmann L, Pacelli C, Zucchini L, Rabbow E and de Vera JP (2019) Survival, DNA, and ultrastructural integrity of a cryptoendolithic Antarctic fungus in Mars and Lunar rock analogs exposed outside the International Space Station. *Astrobiology* **19**, 170–182.

Osman S, Peeters Z, La Duc MT, Mancinelli R, Ehrenfreund P and Venkateswaran K (2008) Effect of shadowing on survival of bacteria under conditions simulating the Martian atmosphere and UV radiation. *Applied and Environmental Microbiology* **74**, 959–970.

Pacelli C, Cassaro A, Baqué M, Selbmann L, Zucchini L, Maturilli A, Botta L, Saladino R, Böttger U, Demets R, Rabbow E, de Vera J-PP and Onofri S (2020) Fungal biomarkers are detectable in Martian rock-analogues after space exposure: implications for the search of life on Mars. *International Journal of Astrobiology* **20**, 1–14.

Pandey S, Clarke J, Nema P, Bonaccorsi R, Som S, Sharma M, Phartiayl B, Rajamani S, Mogul R, Martin-Torres J, Vaishampayan P, Blank J, Steller L, Srivastava A, Singh R, McGuirik S, Zorzano M-P, Güttler JM, Mendaza T, Soria-Salinas A, Ahmad S, Ansari A, Singh VK, Mungi C and Bapat N (2019) Ladakh: diverse, high-altitude extreme environments for off-earth analogue and astrobiology research. *International Journal of Astrobiology* **19**, 78–98.

Patel MR, Berces A, Kolb C, Lammer H, Retberg P, Zarnecki JC and Selsis F (2003) Seasonal and diurnal variations in Martian surface ultra-violet irradiation: biochemical and chemical implications for the Martian regolith. *International Journal of Astrobiology* **2**, 21–34.

Pogoda De La Vega U, Retberg P, Douki T, Cadet J and Horneck G (2005) Sensitivity to polychromatic UV-radiation of strains of *Deinococcus radiodurans* differing in their DNA repair capacity. *International Journal of Radiation Biology* **81**, 601–611.

Pogoda De La Vega U, Retberg P and Reitz G (2007) Simulation of the environmental climate conditions on Martian surface and its effect on *Deinococcus radiodurans*. *Advances in Space Research* **40**, 1672–1677.
Probst AJ, Weinmaier T, Raymann K, Perras A, Emerson JB, Rattei T, Wanner G, Klingl A, Berg IA, Yoshinaga M, Viehweger B, Hirnrichs KU, Thomas BC, Meck S, Auerbach AK, Heise M, Schintlmaster A, Schmid M, Wagner M, Gribaldo S, Banfield JF and Moissl-Eichinger C (2014) Biology of a widespread uncultivated archaeon that contributes to carbon fixation in the subsurface. *Nature Communications* **5**, 1–13.

Rabbow E, Retterberg P, Panitz C, Drescher J, Horneck G and Reitz G (2005) SSIOUX – space simulation for investigating organisms, evolution and exobiology. *Advances in Space Research* **36**, 297–302.

Rabbow E, Retterberg P, Parpart A, Panitz C, Schulte W, Moller F, Jaramillo E, Demrets R, Weiß P and Willnecker R (2017) EXPOSE-R2: the astrobiological ESA on board of the International Space Station. *Frontiers in Microbiology* **8**, 1533.

Rampelotto PH (2013) Extremophiles and extreme environments. *Life (Chicago, Ill.)* **3**, 482–485.

Retterberg P, Rabbow E, Panitz C and Horneck G (2004) Biological space experiments for the simulation of Martian conditions: UV radiation and Martian soil analogues. *Advances in Space Research* **33**, 1294–1301.

Rothschild LJ and Mancinelli RL (2001) Life in extreme environments. *Nature* **409**, 1092–1101.

Rudolph C, Moissl C, Henneberger R and Huber R (2004) Ecology and microbial structures of archaeal/bacterial strings-of-pearls communities and archaeal relatives thriving in cold sulfidic springs. *FEMS Microbiology Ecology* **50**, 1–11.

Schirmack J, Alawi M and Wagner D (2015) Influence of Martian regolith analogs on the activity and growth of methanogenic archaea, with special regard to long-term desiccation. *Frontiers in Microbiology* **6**, 210.

Schuereger AC, Richards JT, Newcombe DA and Venkateswaran K (2006) Rapid inactivation of seven *Bacillus* spp. under simulated Mars UV irradiation. *Icarus* **181**, 52–62.

Serrano P, Alawi M, de Vera JP and Wagner D (2019) Response of methanogenic archaea from Siberian permafrost and non-permafrost environments to simulated Mars-like desiccation and the presence of perchlorate. *Astrobiology* **19**, 197–208.

Shahmohammadi HR, Asgarani E, Terato H, Saito T, Ohyama Y, Gekko K, Yamamoto O and Ide H (1998) Protective roles of bacterioruberin and intracellular KCl in the resistance of *Halobacterium salinarum* against DNA-damaging agents. *Journal of Radiation Research* **39**, 251–262.

Stachura SS, Malajczuk CJ and Mancera RL (2019) Does sucrose change its mechanism of stabilization of lipid bilayers during desiccation? Influences of hydration and concentration. *Langmuir* **35**, 15389–15400.

Streeger JG (2003) Effect of trehalose on survival of *Bradyrhizobium japonicum* during desiccation. *Journal of Applied Microbiology* **95**, 484–491.

Szponar N, Brazelton WJ, Schrenk MO, Bower DM, Steele A and Morrill PL (2013) Geochemistry of a continental site of serpentinitization, the Tablelands Ophiolite, Gros Morne National Park: a Mars analogue. *Icarus* **224**, 286–296.

Vetriani C, Crespo-Medina M and Antunes A (2014) The family *Salinisphaeraceae*. In Rosenberg E, DeLong E, Lory S, Stackebrandt E and Thompson F (eds), *The Prokaryotes - Gammaproteobacteria*. Berlin: Springer, pp. 591–596.

Vicente-Retortillo A, Martinez GM, Rennó NO, Lemmon MT, de la Torre-Juárez M and Gómez-Elvira J (2020) The family *Acinetobacter baumannii*. *Microbiologyopen* **8**, e00740.

Wassmann M, Moeller R, Reitz G and Retterberg P (2010) Adaptation of *Bacillus subtilis* cells to Archean-like UV climate: relevant hints of microbial evolution to remarkably increased radiation resistance. *Astrobiology* **10**, 605–615.

Wassmann M, Moeller R, Reitz G and Retterberg P (2011) Growth phase-dependent UV-C resistance of *Bacillus subtilis*: data from a short-term evolution experiment. *Archives of Microbiology* **193**, 823–832.

Weinmaier T, Raymann K, Probst AJ, Prosperetti M, Kockel CS and Retterberg P (2012) Survival of spores of the UV-resistant *Bacillus subtilis* strain MW01 after exposure to low-earth orbit and simulated Martian conditions: data from the space experiment ADAPT on EXPOSE-E. *Astrobiology* **12**, 498–507.

Xia L, Wang J, Sang Y, Cheng Z, Huang T, Zhao J, Xu Y, Huang J, Xiao Z and Komatsu G (2017) A new terrestrial analogue site for Mars research: the Qaidam Basin, Tibetan Plateau (NW China). *Earth-Science Reviews* **164**, 84–101.

Zeidler S and Müller V (2019) The role of compatible solutes in desiccation resistance of *Acinetobacter baumannii*. *Microbiologyopen* **8**, e00740.