Effect of Shadowing on Survival of Bacteria under Conditions Simulating the Martian Atmosphere and UV Radiation

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Spacecraft-associated spores and four non-spore-forming bacterial isolates were prepared in Atacama Desert soil suspensions and tested both in solution and in a desiccated state to elucidate the shadowing effect of soil particulates on bacterial survival under simulated Martian atmospheric and UV irradiation conditions. All non-spore-forming cells that were prepared in nutrient-depleted, 0.2-μm-filtered desert soil (DSE) microcosms and desiccated for 75 days on aluminum died, whereas cells prepared similarly in 60-μm-filtered desert soil (DS) microcosms survived such conditions. Among the bacterial cells tested, Microbacterium scheuferi and Arthrobacter sp. exhibited elevated resistance to 254-nm UV irradiation (low-pressure Hg lamp), and their survival indices were comparable to those of DS- and DSE-associated Bacillus pumilus spores. Desiccated DSE-associated spores survived exposure to full Martian UV irradiation (200 to 400 nm) for 5 min and were only slightly affected by Martian atmospheric conditions in the absence of UV irradiation. Although prolonged UV irradiation (5 min to 12 h) killed substantial portions of the spores in DSE microcosms (~5- to 6-log reduction with Martian UV irradiation), dramatic survival of spores was apparent in DS-spore microcosms. The survival of soil-associated wild-type spores under Martian conditions could have repercussions for forward contamination of extraterrestrial environments, especially Mars.

Several environmental factors must be considered when the ability of microbes to survive and proliferate in extraterrestrial environments is assessed (28). It has been postulated that survival and propagation of terrestrial microbes on Mars are possible only if the organisms can withstand extremely low water activity conditions, including complete desiccation (28).

The nutrient-deprived (oligotrophic), ultraclean, and desiccated (constant relative humidity) conditions maintained within spacecraft assembly areas limit the proliferation of microbial life in these environments. Rigorous maintenance procedures, such as regular cleaning (14, 30), HEPA air filtration, and constant humidity and temperature control, make these facilities inhospitable to microbial life. Much like similarly maintained facilities in the medical and pharmaceutical industries (7), these settings have been dubbed “extreme” with respect to microbial persistence (5, 49). Microbes isolated from the clean rooms are often found to be remarkably resilient and capable of tolerating a variety of harsh environmental conditions. A recent report demonstrated that not only spore-forming Bacillus species but also a diverse suite of equally “hardy,” physiologically flexible nonsporulating bacterial species persist in the inhospitable conditions of clean-room environments (22).

The endospores of several Bacillus species isolated from spacecraft assembly facilities have previously exhibited various levels of resistance to H2O2 treatment, desiccation, γ-radiation, and UV radiation (19, 24, 48), as well as simulated Martian conditions (32, 45). Furthermore, Bacillus subtilis spores have been shown to survive the conditions of low-Earth orbit for up to 6 years (15, 16). However, these spores could survive exposure for 6 years only with shielding from UV radiation (15, 17). The solar UV flux at the Martian surface is considerably less than that in interplanetary space (15, 44), and UV attenuation at the planet’s surface is dependent upon ever-changing atmospheric conditions (3, 12, 29).

On Mars, windblown dust has been hypothesized to attenuate solar UV irradiation, resulting in partial or complete shielding of viable bacteria (10, 13, 17, 27, 36, 44). These UV-shielding effects have not been limited to specific soil types or particle sizes. Simulated Martian dust (17, 27), Fe-montmorillonite (27), limonite (10, 13), crushed red sandstone (17), and terrestrial field soils (17, 36) have all been shown to effectively protect bacterial spores. When these reports were reviewed (44), the consensus was that covering B. subtilis spores with dust particles that were 2 to 8 μm in diameter resulted in total inactivation of spores after 1 h of UV exposure, while it took 8 h of exposure to UV irradiation to inactivate spores blanketed in dust particles that were up to 50 μm in diameter. Only a 0.5-mm contiguous layer of simulated Martian dust was able to protect B. subtilis from longer exposure to normal Martian UV flux (44). None of these studies tested the survival of vegetative bacterial cells under simulated Martian conditions.

The findings reported here are the findings of the first examination of the survival of non-spore-forming bacteria under
simulated Martian conditions. Also tested was the hypothesis that conditions resulting in a reduced cellular water content (e.g., vacuum and extreme desiccation) are major predisposing factors that would select for both microbial persistence in interplanetary space and subsequent proliferation on the Martian surface. A low-nutrient Atacama Desert soil sample was used to simulate Martian aeolian dust in an attempt to elucidate any effects of shielding from UV radiation, as measured by survival indices for bacterial cells and spores following exposure. Such information should prove to be invaluable for future risk assessment of forward contamination (41) and should increase our general understanding of the potential for microbial survival and proliferation on the surface of or within the subsurface of Mars.

MATERIALS AND METHODS

Isolation and identification of microbes from spacecraft and associated environments. Bacillus pumilus SAFR-032 was isolated from the Jet Propulsion Laboratory spacecraft assembly facility (19), and other bacterial strains were retrieved from various assembly facilities as described previously (22). With the exception of SAFR-032, all bacteria exhibited tolerance to high pH (pH 11.0). Bacterial small-subunit rRNA genes of purified strains were PCR amplified with the eubacterially biased B27F and B1492R primers, and PCR conditions described elsewhere were used (40). After purification with QIAquick columns (Qiagen), 16S rRNA gene amplicons were fully, bidirectionally sequenced. The accession numbers for their 16S rRNA gene sequences are shown in Table 1.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Straina</th>
<th>GenBank accession no.</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>No UV 200 J m⁻² 500 J m⁻² 1,000 J m⁻²</td>
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<tr>
<td>Non-spore-forming bacteria</td>
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<td>SAFR-032</td>
<td>AY167879</td>
<td>+ + +</td>
</tr>
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a JPL, Jet Propulsion Lab; LMA, Lockheed Martin Aeronautics; JSC, Johnson Space Center; KSC, Kennedy Space Center.
b +, growth after exposure; −, no growth.

TABLE 1. Qualitative analysis of the UV tolerance of several extremotolerant bacteria isolated from spacecraft assembly facilities.
The sample also contained low but measurable levels of organic materials, and \(\epsilon\)-amino acids and glycine were the most abundant amino acids. Furthermore, four Martian diurnal cycle simulations with Arequipa soil after the soil sample was spiked with \(\epsilon\)-alanine (\(\sim 40\%\)) was measured, which was indicative of highly reactive soil mineralogy.

Preparation of microcosms using Atacama Desert soil. One gram of Atacama Desert soil was suspended in 100 ml of sterile, molecular-grade Sigma water (Sigma, St. Louis, MO) and stirred thoroughly for 1 min. After settling, the resulting supernatant was poured plated (R2A medium) and incubated at 25°C for 14 days to enumerate the cultivable bacteria. In addition, a bead beating step was employed before a multisollow extraction method (18) was used to isolate DNA from 10 ml of the soil suspension. Culture-based analysis did not reveal the presence of any bacterial colonies after 14 days of incubation, and DNA extracts from the soil suspensions did not yield any PCR-amplifiable 1.5-kb 16S rRNA gene fragments (23). To prepare desert soil and a desert soil extract, 2 g of Atacama Desert soil was aseptically weighed and suspended in 2 liters of autoclaved, nanopure water (Millipore, Milford, MA), which was then stirred for 15 min. After large particulates were allowed to settle (2 h), the supernatant was carefully removed and aseptically filtered through sterile 0.2-µm filters. The resulting filtrate was designated desert soil (DS). The sterility of the DS and DSE solutions was evaluated by culturing in R2A medium and attempted extraction of DNA. As was the case previously, cultivable bacteria were not observed on R2A medium plates, nor were 16S rRNA genes amplified from either solution. The DS and DSE suspensions were divided into 100-ml aliquots before they were inoculated with spores or vegetative cells. In a similar manner, bacterial suspensions in sterile PBS or spore suspensions in sterile Sigma water were prepared without desert soil for comparative analyses.

UV254 resistance of Atacama Desert microcosms. The DS and DSE microcosms inoculated with spores and cells mentioned above were exposed to UV light (254 nm) in solution. As described above, bacterial suspensions were placed in uncovered 50-mm glass petri dishes containing a magnetic stir bar and exposed to UV254 resistance of Atacama Desert microcosms. After the lid was closed, the pressure in the chamber reached 7.0 \(\times\) 10\(^2\) Pa. This procedure was repeated three times to replace any water vapor adsorbed on the surface of the chamber. The pressure in the Mars simulation experiments the samples were irradiated for 12 h with a solar simulator under ambient conditions in air. The output of the deuterium lamp was measured with the Sciencetech UV sensor at the level of the samples. Integrated over the wavelength range from 200 to 400 nm, the deuterium lamp had an output of 3.4 W \(\text{m}^{-2}\). The spectrum of the deuterium lamp is shown in Fig. 1. Martian simulation experiment. Samples were irradiated for 5 min, 30 min, or 12 h with a solar simulator under ambient conditions in air. These samples, which were exposed to only Martian UV radiation, were designated MUV. The Mars simulation chamber that was used to expose samples to the Martian atmospheric conditions has been described elsewhere (Peeters et al., submitted). A brass box connected to copper loops fed pipes was used as a cooling stage. The top of the brass block was fitted with a highly polished flat copper plate to optimize the thermal conduction between the cooling stage and the aluminum coupons holding the samples. The cooling stage was placed in a small vacuum chamber with a background pressure of \(10^{-6}\) Pa. A small valve allowed introduction of CO\(_2\) (99.996%; Praxair) into the vacuum chamber. Above the cooling stage a UV-grade quartz window was mounted, which allowed admittance of light having wavelengths down to 200 nm into the Mars simulation chamber. For the Mars simulation experiments the samples were irradiated with the solar simulator. The output of the lamp as measured by the UV sensor was the same as the output used for the irradiation experiments outside the Mars simulation chamber.

The experimental setup was prepared by placing samples on the cooling stage in the Mars simulation chamber. After the lid was closed, the pressure in the chamber was adjusted to \(10^{3}\) Pa with a pump. The chamber was then filled with CO\(_2\) at a pressure of \(10^6\) Pa. This procedure was repeated three times to replace (dilute) the air in the chamber with a CO\(_2\) atmosphere, while a pressure that did not drop below the average Martian pressure (\(7 \times 10^3\) Pa) was maintained. The experiments started when the pressure in the chamber reached \(7 \times 10^3\) Pa. In the Mars simulation experiments, samples were exposed to solar simulator radiation for 5 or 30 min at room temperature (MUV samples); to 12 h of solar simulator radiation at room temperature, followed by incubation for 12 h at \(-60\°\text{C}\) without radiation (MUVP samples); or to 12 h without irradiation at room temperature, followed by 12 h of incubation at \(-60\°\text{C}\) (MUV sample). All experiments were performed with \(7 \times 10^3\) Pa CO\(_2\).
Microscopy. An Olympus (Nega, CA) phase-contrast microscope (BX-60) was used to determine the refractile nature of the spores. A field emission environmental scanning electron microscope (FE-SEM) (Philips XL30; FEI Co., Pottomac, MD) was used for nondestructive examination of spores and vegetative cells (see below). Specimen preparation procedures, which often lead to sample artifacts, are not necessary when the FE-SEM is used. In addition, a transmission electron microscope was used to examine the surface details and cross-sections of the spores and cells, respectively, by established methods (4). Briefly, the spores or cells were suspended in an equal volume of 5% glutaraldehyde in aqueous 100 mM HEPES buffer (pH 6.8) containing 2 mM MgCl₂ as a fixative. After several washes (three washes ranging from 10 min to overnight incubation) in the HEPES buffer fixative solution, spores or cells were allowed to sit for 2 to 4h at room temperature. The spore or cell pellets were then suspended in 1% osmium tetroxide for 2 to 4h at room temperature and washed with the HEPES buffer. The fixed spores or cells were embedded in agar, and the agar cubes were dehydrated using successive ethanol treatments (70% to 100% ethanol). The em- bedding and thin-sectioning procedures used have been described elsewhere (4).

An FE-SEM provides high spatial resolution combined with low electron beam accelerating voltage. The low beam voltage of the FE-SEM allows examination of electrical insulators without deposition of a surface-conducting (carbon or metal) layer to eliminate specimen charging, which can lead to a distorted and often completely unusable image. The deposition of a conducting material to control charging can complicate analysis of the results. In many situations, a low electron beam voltage intrinsically results in a much sharper image, especially for thin structures composed of elements with low atomic numbers. A Philips (Hillsboro, OR) FE-SEM (XL-50) was used to analyze the majority of the specimens. The FE-SEM is equipped with an energy-dispersive X-ray (EDX) analyzer. EDX analysis is based on analysis of the characteristic X rays emitted when an electron beam is incident on a sample. Unfortunately, the spatial resolution obtainable with EDX analysis is at best about 1 μm. The acceleration voltage used to analyze aluminum samples was ~10 to 20 kV. In the high-vacuum mode secondary electron images were acquired for both metals. Similar settings were maintained when different models or SEM instruments were used.

RESULTS

Identification of UV irradiation-resistant microbes. Of 17 alkalotolerant (pH 11.0) species identified, 13 (~76%) were gram positive and four strains were identified as the members of the Alpha- and Gamma-proteobacteria (Table 1). The actino- bacterial alkalotolerant isolates belonged to the genera Arthrobacter, Brachybacterium, Kocuria, Microbacterium, and Micrococcus. The alkalotolerant spore-forming rods were species of Bacillus, Oceanobacillus, and Exiguobacterium. Three alkalo- tolerant isolates represented novel bacterial species, based on 16S rDNA gene sequence dissimilarity, and were most closely related to species of Arthrobacter (KSC_Ak2i), Bacillus (JSC_Ak7-1), and Oceanobacillus (JPL_Ak1).

Survival of hydrated bacteria under UV₂₅₄ radiation. Of the 12 non-spore-forming strains that exhibited UVC resistance, 4 spacecraft-associated isolates were chosen for further study based on their enhanced survivability (elevated doses at which 90% of the spores or cells were inactivated) (data not shown). These isolates included two actinobacteria (Arthrobacter sp. strain KSC_Ak2i and Microbacterium schleiferi LMA-AkK1) and two gram-negative species (Brevundimonas diminuta KSC_Ak3a and Sphingomonas trueperi JSC_Ak7-3). In addition to these four strains, B. pumilus SAFR-032 was selected as a reference control since this strain has been used in numerous previous resistance studies (19, 25, 32). Survival indices resulting from exposure of PBS-hydrated spores or cells to UVC (254 nm) irradiation demonstrated, as expected, that spores were much more resistant to UVC (less than a 1-log reduction) than any of the vegetative cells tested were (see Fig. S1 in the supplemental material). Vegetative cells of two of the non-

spore-forming species, S. trueperi and B. diminuta, showed ~6-log reductions when they were exposed to 200 J m⁻² of UVC irradiation. M. schleiferi LMA_AkK1 and Arthrobacter sp. strain KSC_Ak2i exhibited greater resistance, showing only ~1- and ~3-log reductions, respectively. When spore- and vegetative cell-DSE microcosms were exposed to UVC, a de- clining trend in microbial viability was observed for all prepa- rations tested, including spores (Fig. 2A). For example, SAFR-032 spores showed a 2-log reduction in UVC-irradiated DSE microcosms (cumulative dose, 1,000 J m⁻²) (Fig. 2A), com- pared to the negligible reductions observed under similar UV- irradiated conditions for PBS-spore microcosms (see Fig. S1 in the supplemental material). Similarly, both M. schleiferi LMA_AkK1 and Arthrobacter sp. strain KSC_Ak2i showed at least a 1-log greater reduction in DSE microcosms. The comparatively low salt content of the DSE microcosms compared to the PBS microcosms could potentially have influenced the recovery of UV-damaged cells. In contrast to the DSE micro- cosms, the generally larger soil particles (~60 μm) in the DS microcosms appeared to have shielded both spores and cells from the biocidal effects of UV radiation (Fig. 2B). Continuous stirring with sterile magnetic beads was not adequate to elim- inate the shadowing effect in the DS suspensions. Further- more, the effect of the shadowing was even more pronounced in DS microcosms containing S. trueperi and B. diminuta, both of which yielded surviving cells following exposure to 1,000 J m⁻² UV, whereas in both the PBS and DSE microcosmolayer counterparts there were no survivors after exposure to 500 J m⁻² or a higher dose.

Atacama Desert soil characterization. Figure 3 shows electron micrographs of desiccated DSE (Fig. 3A) and DS (Fig. 3B) microcosms. When dissolved salts were dehydrated during desiccation, uniform crystals formed and were evident in DSE microcosms (Fig. 3A, inset). However, these crystals did not form aggregated clumps but rather dissociated from each other during desiccation. When spores (Fig. 3C) and cells (Fig. 3E, 4A, 4C, and 4E) were mixed with DSE, uniform monolayers were observed. Clumps and/or aggregates of spores or cells were seldom noticed. However, on the aluminum coupons that were seeded with DS microcosms and left to desiccate there were clumps of soil particles harboring spores and cells (Fig. 3 and 4). Even though 50% of an aluminum coupon was not covered with soil particles, spores and cells tended to associate with such particles, possibly due to the aggregation of biological materials with solids during desiccation.

Influence of desiccation on the survival of spores or cells. Ten percent of cells of M. schleiferi and S. trueperi survived after 15 days of desiccation in DSE microcosms, while less than 0.2% survival was observed for Arthrobacter sp. and B. diminuta (see Table S1 in the supplemental material). Invar- iably, all non-spore-forming cells died after prolonged desiccation (75 days) in nutrient-depleted DSE microcosms. As expected, ~10% (75 days) to 20% (15 days) survival was observed for B. pumilus SAFR-032 spores when they were desiccated in DSE microcosms. Vegetative cells generally exhibited similar levels of survival in both DS and DSE microcosms following 15 days of desiccation. However, par- ticles present in the Atacama Desert soil (DS) enhanced the survival of all spores or cells tested following 75 days of desiccation. Unlike DSE microcosms, with the exception of
**B. diminuta**, non-spore-forming cells exhibited only 4- to 5-log reductions when DS microcosms were desiccated for 75 days. The DS-B. diminuta microcosm contained no viable cells after 75 days of desiccation. With DS-spore microcosms, desiccation had no effect after 15 days (100% survival), and ~10% of the spores could be retrieved after 75 days of desiccation, as observed for DSE microcosms.

**Survival of desiccated spores or cells under UV254 radiation.** The results for UV254 irradiation of desiccated DSE microcosms on aluminum are shown in Fig. 5A. As expected, spores displayed enhanced resistance to UVC compared to most of the vegetative cells tested. However, among the non-spore-forming bacteria, *M. schleiferi* LMA_AkK1 and *Arthrobacter* sp. strain KSC_Ak2i cells exhibited unusually high resistance to UVC when they were mixed with DSE. As observed with spores, for *Arthrobacter* sp. seeded in DSE there was only a 3-log reduction in viability after exposure to 1,000 J m\(^{-2}\) UVC (Fig. 5A). The resistance observed for these non-spore-forming bacteria when they were in DSE and PBS microcosms was greater than that of vegetative cells of *B. pumilus* SAFR-032 (data not shown). Conversely, *S. trueperi* and *B. diminuta* showed ~4- to 5-log reductions after exposure to 200 J m\(^{-2}\) UVC irradiation. The difference between the two sets of bacteria may be a consequence the extremely high tolerance of *Microbacterium* and *Arthrobacter* species to desiccation (20).

The effect of the presence of DS on the viability of spores and cells in desiccating conditions is shown in Fig. 5B. Invariably, all DS microcosms dried onto aluminum coupons showed higher viability even after exposure to 1,000 J m\(^{-2}\) UVC, except for the *B. diminuta* cells, which proved to be nonculturable even after an exposed coupon was incubated in liquid medium. Spores in DS microcosms exhibited only a 1-log reduction, and all bacterial cells tested showed 2- to 4-log greater survival in DS desiccated microcosms than in DSE desiccated microcosms. However, the lethal effect of UVC was still apparent, as the *B. diminuta* desiccated cells mixed with DS were all killed after exposure to 1,000 J m\(^{-2}\) UVC irradiation. The phenomenon of increased viability following UVC exposure may be attributed to the shadowing effect of large soil particles and the inability of UVC to make direct contact with and lethally affect all of the bacteria tested. The higher survival...
indices observed for the *Sphingomonas* and *Bruvendimonas* cells inoculated into DS than for the *Sphingomonas* and *Bruvendimonas* cells inoculated into DSE were expected. Higher mineral and trace element concentrations, not to mention the much larger particles, present in the soil may have bolstered the survival of these cells, which otherwise would have been susceptible to desiccation-induced death. In addition, the retention of a soil moisture content higher than that of the DSE

FIG. 3. Environmental scanning electron micrographs of several bacteria and spores prepared in DSE and DS and desiccated on spacecraft-qualified aluminum 6061. The arrows indicate where spores or bacterial cells are present. Some spores or bacterial cells were exposed to the UV, and some were hidden under soil particles.
microcosms may also have bolstered the survival of these cells when they were DS associated.

Survival of desiccated spores or cells following deuterium lamp radiation. Integrated over the wavelength range from 200 to 400 nm, the deuterium lamp had an output of 3.4 W m^{-2}. None of the desiccated DSE or DS microcosms containing non-spore-forming cells exhibited growth after 12 h of irradiation with the deuterium lamp (see Table S2 in the sup-
The sporicidal effects of UV irradiation were observed for the *B. pumilus* spores when DSE-desiccated coupons were exposed to deuterium irradiation for 12 h. However, 0.2% of the 75-day DS-desiccated spores (initial spore concentration after 75 days of desiccation, $4.3 \times 10^6$ per coupon) survived UV irradiation with the deuterium lamp for 12 h.

Survival of spores under simulated Martian conditions. The elimination of non-spore-forming cells from desiccated aluminum coupons by simulated Martian conditions was inconsistent. Although prolonged (75 days) desiccation effectively killed non-spore-forming bacterial cells, in some cases sterility of the aluminum coupons was not observed after exposure for 12 h to Martian UV irradiation (total irradiation, $30 \, \text{W m}^{-2}$) and Martian atmospheric pressure ($7 \times 10^2 \, \text{Pa}$). It is possible that water molecules trapped in the uneven surfaces of the unpolished aluminum material (47) mediated the survival of a subset of bacterial cells. Hence, the lethal effect of simulated Mars UV irradiation and atmospheric conditions was not efficiently documented for the desiccated non-spore-forming cells. Cells of *M. schleiferi* LMA_AkK1 were consistently recovered from desiccated coupons (both DSE and DS microcosms) after exposure to Martian atmospheric conditions without UV (MUVM) both following incubation for 12 h at $-60^\circ\text{C}$ and following incubation for 12 h at room temperature ($25^\circ\text{C}$). As observed in other experiments, desiccated DS microcosms containing *M. schleiferi* exhibited at least 1-log-higher survival than their desiccated DSE counterparts (see Table S2 in the supplemental material).

In general and as expected, spores survived under simulated Martian conditions. Figure 6A shows the effect of simulated Martian conditions on spores desiccated in DSE microcosms. Simulated atmospheric conditions (8) without UV had a minimal effect on the survival of spores (less than a 1-log reduc-
Exposure to only Martian UV irradiation for 5 min resulted in only a 3-log reduction in the size of the spore population. Recovery of spores was not possible when the exposure to Martian UV irradiation was increased from 5 to 30 min or longer. However, the aluminum coupons were not completely sterile following exposure to only Martian UV irradiation, as *B. pumilus* SAFR-032 was cultured when the UV-irradiated coupons were placed in TSB. Observations were also recorded when Martian UV irradiation was combined with simulated atmospheric conditions (MUVP). More than 5 min of MUVP exposure proved to be 100% lethal, but growth was detected when the same coupons were incubated in liquid medium. Exposure of these DSE microcosms for 12 h to MUVP conditions, however, resulted in complete sterility, as verified by the lack of cultivable bacteria even in broth. The ability of the Atacama Desert soil slurry to protect spores from Martian UV irradiation and atmospheric conditions is shown in Fig. 6B. A dramatic increase in spore survival was observed.

FIG. 6. Survival of desiccated *B. pumilus* SAFR-032 spores on spacecraft-qualified aluminum 6061 under various simulated Mars conditions. MUVM, incubation in the Mars atmosphere without UV irradiation for 12 h at room temperature, followed by 12 h at −60°C; MUV-5, incubation with Mars UV irradiation in air for 5 min; MUV-30, incubation with Mars UV irradiation in air for 30 min; MUV-1440, incubation with Mars UV irradiation in air for 12 h; MUVP-5, incubation with Mars UV irradiation in the Mars atmosphere for 5 min; MUVP-30, incubation with Mars UV irradiation in the Mars atmosphere for 30 min; MUVP-1440, incubation in the Mars atmosphere for 12 h with Mars UV irradiation at room temperature, followed by 12 h with no UV irradiation at −60°C; +, not detected as CFU but detected by incubating an exposed coupon in liquid medium; −, not detected on solid medium or in liquid medium. All experiments were performed at room temperature, unless otherwise noted. Mars UV irradiation was provided by the solar simulator, and the Mars atmosphere used contained 7 × 10² Pa CO₂. N and N⁰ represent cfu of the samples without and with radiation, respectively.
in all microcosms even though prolonged UV irradiation killed substantial portions of the spore population. However, under similar environmental conditions and in the absence of DS particles (DSE conditions) (Fig. 6A), exposure to Martian UV irradiation for more than 5 min effectively reduced the sizes of spore populations by 5 to 6 logs. Here, for the first time, we show that recovery of spores from aluminum coupons is possible even after exposure to 12 h of UV irradiation and 24 h of a simulated Martian diurnal atmosphere.

**DISCUSSION**

The intimate association of bacterial endospores with spacecraft and assembly facility-associated surfaces has been well documented, as has the ability of these well-adapted microorganisms to resist UV-based methods of decontamination. Therefore, it is generally accepted that such endospores would be the most likely survivors of spacecraft disinfection and thus pose the greatest risk of forward contamination (22, 24, 32, 40, 43–45). Of the five bacterial species examined here, *B. pumilus* SAFR-032 spores have repeatedly tolerated UVC irradiation very well, as reported elsewhere (44), and demonstrated the greatest tolerance to simulated Martian UV and atmospheric conditions (Fig. 6B). It is presumed that this microbe’s remarkable resilience to severe stress, including heating, drying, and radiation (35), is a direct consequence of the maintenance of an ultralow water content in its spore cores (~10 to 25% of dry weight). By keeping the water content low, these spores minimize their interactions with deleterious electron-scavenging radicals, which result from splitting of water molecules (i.e., oxidative stress) (9). In addition, spores of *B. pumilus* are less susceptible to desiccation and are better preserved under reduced pressure (1 × 10^2 Pa) than under standard terrestrial atmospheric pressure (see Fig. S2 in the supplemental material). This could be a result of the more intense drying of the spore core due to the vacuum. It may be presumed that the 10 to 100% survival of *B. pumilus* SAFR-032 spores in DS or DSE after 75 days of desiccation at simulated Martian pressure (7 × 10^2 Pa) (Fig. 6A and 6B) is likely attributable to the lower spore core water content. Lower core water content would correlate with a lower occurrence of generated free radicals and thus less damage to core-housed nucleic acids, a hypothesis warranting further study.

While the effects of water content on stress resistance have been tested using pure cultures of microorganisms, relatively little is known about the extent to which the natural habitats of microbes can bolster or counteract this phenomenon. The enhanced UV resistance seen in *Bacillus* spores, for instance, may be partially due to differences in soil chemistry during sporulation. Nicholson and Law (34) isolated *Bacillus* spores directly from Sonoran Desert soil and found that they were much more UV resistant than spores of the standard laboratory strain, *B. subtilis* 168. However, much of the unusually high resistance was lost upon germination, propagation, and subsequent sporulation in culture. The sequestering of particulate-bound water in soils during and following sporulation may have a synergistic effect on the UV resistance of spores. This could explain the differences in UV resistance observed between wild-type bacterial strains isolated from extremely desiccated desert and clean-room environments and strains recultured in the laboratory in liquid media.

While spore hardiness may allow spores to persist in extraterrestrial environments, metabolic dormancy most likely would prevent proliferation upon deposition on the surface of Mars. Furthermore, it has been shown that the vegetative cells of a dosimetry strain, *B. subtilis* 168, were killed at UV_{254} doses of less than 100 J m^-2 and cells of highly UV-resistant *B. pumilus* strain SAFR-032 were completely eradicated at UV_{254} doses greater than 500 J m^-2 (32). The finding that non-spore-forming isolates (e.g., *Arthrobacter* sp. and *M. schleiferi*) were capable of surviving such extreme UV_{254} regimens was therefore of great significance. Indeed, *Arthrobacter* sp. and *M. schleiferi* cells were as UV resistant as *B. subtilis* spores in both liquid (32) and desiccated (45) states. It has been reported that the survival indices for multilayer microbes (10^6 cells sample^-1) following UV irradiation were 4 orders of magnitude greater than those for monolayer *B. subtilis* (10^6 cells sample^-1) (15). To ensure that the differences in UV resistance observed in these experiments were not due to cell layering effects, vegetative cells were initially screened for UV resistance in a liquid environment (PBS, DSE, and DS). Furthermore, the uniformity of the desiccated cellular and spore monolayers on the surfaces of aluminum coupons was validated by FE-SEM (Fig. 3 and 4).

As a soil dries out, stress due to starvation has a detrimental effect on resident microbes long before the loss of intracellular water becomes a concern (21). *Bacillus* and *Arthrobacter* are two well-known extremely desiccation-tolerant soil-inhabiting genera (20). *Arthrobacter* spp. are arguably the most starvation and desiccation tolerant of all non-spore-forming microbes and are the microorganisms most frequently cultivated from desert soils (20), Antarctic ice (11), the subsurface (46), and clean rooms (22). Early studies demonstrated that *Arthrobacter crystallopoites* can survive extended periods of starvation through slow, sparing catabolism of endogenous substrates (2, 6). It has been shown that, upon desiccation, *A. crystallopoites* exhibited 50% viability after 6 months in air-dried soil, at which time the endogenous catabolism was slowed to a rate at which it could be projected that 50% of cellular carbon would remain after 12 years (1). The desiccation tolerance (~75 days) exhibited by cells of *M. schleiferi* LMA_AkK1 and *Arthrobacter* sp. strain KSC_Ak2i during this study, coupled with their ubiquity in soil and general resilience in the presence of various environmental insults, makes these actinobacteria prime candidates for forward contamination of Mars.

While microbial responses to solute-induced low water activity are relatively well understood (26, 38, 50), studies addressing responses to desiccation-induced low water activities in natural environments remain sparse. It is clear that water limits microbial activity in and on desert soils and rocks (20, 31), yet it is not known how much water is sufficient to trigger the rare, brief periods of microbial proliferation in these environments. We attempted to bridge this knowledge gap by testing the hardest oligotrophic bacterial cells for tolerance to matrix-induced water stress and thereby establishing a true water threshold for survival in Martian regolith. When cells of *M. schleiferi* LMA_AkK1 (5.1 × 10^7 cells) that had been desiccated for 75 days were exposed to a simulated Martian atmosphere without UV irradiation for 24 h (12 h at room...
temperature, followed by 12 h at −60°C. ~40% of the population survived. This cellular response to low water activity was observed only in the presence of DS, while in DSE microcosms there were no survivors. Furthermore, similar to B. pumilus spores, desiccated cells of M. schleiferi exhibited survival after 24 h of exposure to a simulated Martian atmosphere (7 × 10⁻⁶ Pa; 12 h at room temperature, followed by 12 h at −60°C). This is the first account of survival of desiccated cells under simulated Mars atmospheric conditions in the absence of protection conferred by rock or salt crystal shielding.

The Martian atmosphere attenuates very little UV light, and calculations have posited that incoming solar radiation would kill the hardest terrestrial organisms within minutes (29). Thus, the persistence of any life near the surface depends, to a large extent, on how much UV radiation it receives. The results of this study support the interpretations described in previous investigations (32, 44, 45): the survival of spacecraft-borne microorganisms would be limited to, at best, a matter of hours on a surface exposed to full sun (Fig. 6A). However, subtle variations in small-scale spacecraft geometry, such as pits, trenches, and overhangs, can have a profound effect on the incident UV fluence rates and may result in “safe havens” for microorganisms and organic molecules. In this study, vegetative cells (not spores) that had been desiccated on aluminum exhibited inconsistent survival following 24 h of exposure to a simulated Martian diurnal atmosphere (see Table S2 in the supplemental material). This could have been a consequence of the uneven surface geometry of the aluminum (47), which may have resulted in small areas where there was UV shielding. Such a reduction in Martian UV flux was modeled to facilitate the persistence of hardy terrestrial microorganisms like B. pumilus SAFR-032 for several tens of Martian years (29).

Previous studies on the UV shadowing effect of Martian regolith soils have generated contradictory results (10, 13, 17, 27, 36, 44). The microbial reduction observed in these studies was attributed not to the thickness but rather to the quality of soil and organic matter depletion, 60–90% of the population support. Based on this study, it is presumed that the mitigation of UV radiation would be limited to, at best, a matter of hours on a surface exposed to full sun. This is the first account of survival of desiccated cells under simulated Mars atmospheric conditions in the absence of protection conferred by rock or salt crystal shielding.

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