

Review

The solar UV environment and bacterial spore UV resistance: considerations for Earth-to-Mars transport by natural processes and human spaceflight

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Abstract

The environment in space and on planets such as Mars can be lethal to microorganisms because of the high vacuum and high solar radiation flux, in particular UV radiation, in such environments. Spores of various *Bacillus* species are among the organisms most resistant to the lethal effects of high vacuum and UV radiation, and as a consequence are of major concern for planetary contamination via unmanned spacecraft or even natural processes. This review focuses on the spores of various *Bacillus* species: (i) their mechanisms of UV resistance; (ii) their survival in unmanned spacecraft, space flight and simulated space flight and Martian conditions; (iii) the UV flux in space and on Mars; (iv) factors affecting spore survival in such high UV flux environments.

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1. Introduction

This review is concerned with assessing how the solar ultraviolet (UV) radiation environment limits the survival of bacterial spores during interplanetary transfer by either natural processes or human activities—and, conversely, how bacterial spores resist the lethal and mutagenic effects of solar UV in order to maximize their survival. The issue is important for two main reasons. First, bacterial spores are ubiquitous in the environment and are found on or within most natural and human-fabricated materials destined for interplanetary travel. Second, solar UV is by far the most lethal component of the space radiation environment for microorganisms, and spores are notoriously resistant to UV. As we will see below, spore survival in the solar UV environment has important implications both for natural interplanetary transport (panspermia) and planetary protection from forward contamination.

1.1. Panspermia by natural processes

The theory of panspermia, that viable organisms could arise anywhere in the universe where condi-

tions are favorable and be transported to distant locations, was postulated about 100 years ago independently by Richter, Thomson (Lord Kelvin), and Arrhenius [1–3]. A specialized version of the concept, dubbed “lithopanspermia”, has gained support in recent years as a result of the discovery on Earth of lightly shocked meteorites of Martian and Lunar origin, coupled with advances in our understanding of the physics of planetary impact processes and recognition of the high numbers of microbes inhabiting the Earth’s crust [4,5]. For experimental convenience, the process of lithopanspermia is divided into three distinct phases: (i) impact-mediated launch of ejecta from the donor planet; (ii) transit through space; (iii) entry and deposition onto the recipient planet [4,5]. As we will see below, exposure of living cells to solar UV radiation during transit through space is considered the major lethal factor in the space environment, although UV exposure can be mitigated by minimal shielding. Current lithopanspermia models admit the possibility of interplanetary transport of endolithic (literally, “inside rock”) microbes between terrestrial planets in our own solar system. It should be stressed, however, that the subject is controversial—some analyses conclude a

high probability of transfer [4] whereas others conclude transfer to be an unlikely event [6].

1.2. Human-directed panspermia: planetary protection

Studies of the current collection of Martian meteorites indicate that transit times between Mars and Earth can be rather long, on the order of 10^5 – 10^7 years [4,7]. More direct routes with short transit times, on the order of a few years, are theoretically possible, but only a small fraction of ejecta from an impact could be boosted into such fortuitous fast-track transit orbits [8,9]. In sharp contrast, human space probes routinely leave Earth on trajectories carefully calculated to intersect and land upon other planetary bodies with short transit times. Although a great deal of effort is expended on spacecraft disinfection during fabrication and assembly, it is nearly impossible to completely sterilize these devices; thus, each probe destined for another planet carries a finite microbial bioload which is considered a potential “forward contaminant” of the pristine environment of the target planet. As we shall see below, the UV radiation environments in space and on the target planet itself are important aspects to be considered in calculating the survival and possible proliferation of potential forward contaminants transferred from Earth on spacecraft.

1.3. Microbial bioloads of launched unmanned spacecraft

During the Apollo era, extensive studies were conducted to characterize the microbial contamination on unmanned spacecraft launched to both Mars and the Moon [10–19]. Species of *Alternaria*, *Aspergillus*, *Botrytis*, *Candida*, *Cladosporium*, *Fusarium*, and *Penicillium* were the most prevalent fungi; species of *Achromobacter*, *Acinetobacter*, *Alcaligenes*, *Bacillus*, the *Brevibacterium*–*Corynebacterium* group, *Enterococcus*, *Micrococcus*, *Pseudomonas*, *Staphylococcus*, and *Streptococcus* were the most prevalent bacteria recovered from these systems. Microorganisms recovered from manned and unmanned vehicles appeared to be very similar in species diversity [11,15,16,18], but manned spacecraft appeared to have slightly higher levels of microbial biomass per vehicle [16]. Microbial species indigenous to humans comprised approx-

imately 70% of the total microbial bioloads of these vehicles [15,16], while microbial species indigenous to soils comprised approximately 20% of the isolates recovered [12,16]. Microbial species comprising the balance recovered from spacecraft were often not identifiable with the cultural procedures used at the time. Most of the recovered bacteria (>80%) were described as mesophilic heterotrophic prokaryotes [11,16]. Spore-forming species from the genus *Bacillus* generally averaged 10% of the total bioloads of spacecraft and ranged from <1% to as high as 36% per vehicle [19]. However, most of these studies were conducted prior to the development of modern molecular techniques of microbial identification, and it is likely that many additional species of psychrophilic, halophilic, photoautotrophic, and non-culturable species were present on unmanned spacecraft at launch but were not identified. For example, recent microbial studies of spacecraft using 16S rDNA sequence and DNA–DNA hybridization analyses have widened the list of microorganisms recovered from unmanned spacecraft to include members of the genera *Bradyrhizobium*, *Deinococcus*, *Methylobacterium*, *Methylococcus*, *Nocardiopsis*, *Planococcus*, *Ralstonia*, *Rhizobium*, and *Variovorax* [20–23].

Both manned and unmanned spacecraft are assembled under strict isolation to reduce the risks of soil or dust particulates contaminating or damaging spacecraft components. During the 1960s and 1970s, many American spacecrafts were launched for lunar and Mars exploration. The estimated total bioloads (i.e., non-spore and spore formers) of these vehicles at launch ranged from a low of 5×10^2 viable cells/m² (Lunar Orbiter 1) to a high of 2×10^6 cells/m² (Surveyor 2) [11,19]; approximately 10% of the bioloads were spore-forming species in the genus *Bacillus* [19]. Based on recommendations from the Committee on Space Research (COSPAR), an International Space Science Community Policy Board (see reviews by DeVincenzi and Klein [24] and Rummel [25]), the currently accepted bioburden at launch for robotic surface missions to Mars without life-detection experiments is 300 spores/m² and 3×10^5 spores/vehicle [26]. However, this requirement can be lowered significantly when life-detection experiments or sample-return missions are launched. For example, the total bioloads estimated on the Viking landers prior to dry-heat sterilization were approximately 2.5×10^3 aerobic cells/m² and 1.4×10^2 aerobic spores/m² [16]. The

Viking 1 and 2 landers and their four biology experiments were terminally dry heat-sterilized at 112 °C just prior to launch for 30 and 23 h, respectively [16,24]. It was calculated that the Viking microbial bioloads were likely lowered as much as an additional 4 decades during the prelaunch heat-sterilization procedures [16,27], and thus the landers were for all practical purposes essentially sterile upon launch. Thus, of all unmanned spacecraft launched to Mars, the Viking landers had the lowest bioloads at the time of launch ($<1 \times 10^{-2}$ spores/m²), and recent spacecraft have averaged less than 3.0×10^5 spores/vehicle [26]. The emphasis on spore-forming species is based on the generally accepted assumption that sanitation and sterilization protocols that reduce the numbers of recovered spores will concomitantly reduce the numbers of non-spore forming species by similar or greater degrees.

2. The solar radiation environment

Due to its high energy and efficient absorption by biological macromolecules (proteins, nucleic acids, and lipids), solar UV is considered the component of solar radiation most immediately lethal to microorganisms. It is therefore relevant to understand the nature of solar UV. Our Sun is a G-type star whose radiant spectrum roughly matches that of a 5500–6000 °C blackbody. In space the Sun emits photons of wavelengths ranging from ~ 1 nm (soft X-rays) to $\sim 10^5$ nm (radio waves); however, most of the radiant energy emitted encompasses infrared ($\sim 37\%$), visible ($\sim 43\%$), and UV ($\sim 7\%$) wavelengths. The total amount of solar radiation a body in space receives is defined by the solar constant (S), the amount of energy, which falls on an area above the atmosphere at a vertical angle. A planet's S value varies inversely with the square of the distance of a body from the Sun as a function of the surface area of a sphere. At the mean Earth–Sun distance (1 astronomical unit or 1 a.u.), $S = 1371$ W/m², while at the mean Mars–Sun orbital distance (1.524 a.u.), $S = 590$ W/m² (for a spectral range of 200 nm–40 μ m) [28]. In addition, the solar constant for Mars can vary from 493 to 718 W/m² between aphelion (closest approach to the Sun) and perihelion (furthest distance from the Sun), respectively [28].

The UV portion of the solar spectrum spans wavelengths from ~ 10 to ~ 400 nm. While in reality a con-

tinuum, solar UV has been divided into rather arbitrary categories. One system (the Global Solar UV Index based on a “photobiological” definition of UV) [29,30] divides UV into three categories: UVC (200–280 nm), UVB (280–315 nm), and UVA (315–400 nm). In addition, the term “vacuum UV” can be used to refer to the UV flux found in interplanetary space at wavelengths shorter than UVC, and thus represents the UV flux in the solar beam that is not attenuated by the atmospheres of either Earth or Mars. For this review, we will use the nomenclature of the Global Solar UV Index, and include the use of “vacuum UV” to represent the UV flux <200 nm. Based on the UV models of Mars by Appelbaum and Flood [28], Cockell and Andradý [31], Kuhn and Atreya [32], and Patel et al. [33], fluence rates for UVC, UVB, and UVA for S at the mean orbital distance for Mars were recently estimated as 3.18, 8.38, and 38.39 W/m², respectively [34].

Solar UV light originates in the upper photosphere, chromosphere, and corona of the Sun. Due to solar dynamics in these regions, variability in the solar UV portion of the spectrum is far greater than variability in total solar output. To monitor daily solar UV output and spectrum from space, NASA and the Naval Research Laboratory sponsor a Solar Ultraviolet Spectral Irradiance Monitor (SUSIM) instrument on a satellite in near-Earth orbit [35]. The daily solar UV spectrum in space can be viewed at <http://www.solar.nrl.navy.mil/susim.html>.

The surfaces of planetary bodies which lack atmospheres (such as asteroids or the Earth's Moon) are illuminated by the same intensity and spectrum of solar UV as encountered in interplanetary space. Because these bodies rotate relative to the Sun and are roughly spherical, the incident UV flux at any point on the surface varies due to a number of factors including orbital position, Sun elevation angle, latitudinal changes, rotational periods, positions on the “day” or “night” sides of objects, shading by location within pits or involuted surfaces, and reflection of UV off nearby surfaces [6]. The net result is that an object on the surface of an airless body can, depending on its location, be exposed to a UV flux ranging from zero to a value actually exceeding the solar constant. The greatest contribution to increasing the UV flux above S is the level of UV radiation reflected off of spacecrafts and planetary surfaces. Many of these surfaces are fundamentally lambertian in nature, and thus reflect light isotropically.

Therefore, specific locations on spacecraft or planetary surfaces can be impinged upon by direct UV, diffuse UV (if an atmosphere is present) and reflected UV radiation from all directions. The increased UV flux from the surrounding terrain can approach 70% of S (R. Tanner, personal communication), and can thus approximately double the UV flux falling on a surface.

The situation is further confounded on planetary bodies that contain atmospheres. In addition to the factors described above (orbital position, solar elevation, latitude, time of year, ground reflection, etc.), the presence of an atmosphere causes a general attenuation of the amount of UV reaching the surface, and also can exert a strong influence on the spectral quality of UV radiation that reaches the surface. Atmospheres are generally composed of various gases, liquid vapor droplets, solid particulate material (i.e., dust), and suspended ices. These components variously absorb, reflect back to space, and scatter solar UV. Because both planets and atmospheres are dynamic systems, the amount, spectrum, or angle of UV striking a surface is a constantly changing quantity.

The amount of absorption, reflection, and scattering is a direct function of the atmospheric density of a planet. For example, Earth's atmospheric pressure averages 1013 mbar (10^5 Pa) at sea level, which is over 100 times the density of the average Martian atmosphere (~ 7 mbar or 710 Pa) [36]. Thus, the UV flux on the Martian surface is much less attenuated by the atmosphere than is the UV flux on the Earth's surface. In addition, due to the extremely cold surface temperatures on Mars, a significant percentage of the Martian CO_2 atmosphere freezes into solid CO_2 at the poles during winter months and then resublimates in the spring and summer, resulting in seasonal swings of $\sim 40\%$ in the mean atmospheric pressure, between ~ 6 and ~ 10 mbar [36]. These pressure fluctuations influence the total amount of UV reaching the surface and depress the shorter UVC and UVB spectral regions during times of higher pressure [28,37]. Mars also experiences periods during which its axial tilt relative to the Sun (i.e., obliquity) can be much more severe than that of Earth, which serves to exacerbate seasonal variations in UV flux striking the Martian surface [37]. The obliquity of Mars can vary between a low of nearly 10° to a high of 60° over cycles generally averaging 10^5 years [38]. In addition, on both

Mars and Earth, atmospheric density and thus protection from solar UV is an inverse function of altitude.

The atmospheric gas composition also exerts a strong effect on UV attenuation. For example, Earth's atmosphere is composed mainly of diatomic nitrogen ($\sim 78\%$) and oxygen ($\sim 21\%$). A portion of the oxygen in Earth's stratosphere interacts with sunlight and is converted to ozone, which strongly absorbs UV wavelengths shorter than ~ 300 nm [39]. This layer of stratospheric ozone shields the Earth's surface from all vacuum UV, UVC, and most UVB wavelengths [40]. The atmosphere of Mars, in contrast, consists mainly of carbon dioxide ($\sim 95\%$) with extremely low levels of oxygen ($\sim 0.13\%$) and ozone ($\sim 0.000004\%$) [41]. The major UV-absorbing gas in the Martian atmosphere is CO_2 which efficiently absorbs solar UV radiation shorter than 190 nm [32], so the UV environment on the Martian surface is much richer in UVC and UVB than on Earth's surface. Despite its greater distance from the Sun and the resulting fact that the Martian solar constant is only 43% that of Earth's, under clear-sky conditions the DNA-weighted UV irradiance on the Martian surface is approximately 3 orders of magnitude higher than that on Earth [37].

Atmospheric aerosols such as vapor droplets, water ice particles, and dust further absorb, reflect, and scatter incident UV. Atmospheric liquid droplets range from clouds of water vapor or ice particles on Earth and Mars to methane clouds in the atmosphere of Saturn's Moon Titan [42]. Solid particulates in the Earth's atmosphere include smoke from combustion and volcanism and airborne dust from windstorms. The Martian surface has been pulverized by meteor impacts and erosion such that the planet is covered by a layer of extremely fine dust, of which the smallest fines ($1\text{--}2\text{ }\mu\text{m}$ diameter) can be easily lofted into the atmosphere in regional-to-global scale dust storms which can last for months [43]. Summation of all factors in a planetary atmosphere that contribute to the attenuation of light in the solar spectrum (i.e., gas composition, atmospheric pressure, suspended dust, water ices, etc.) can be represented by a unitless term called optical depth (τ) [44]. In general, clear-sky conditions on Mars yield optical depths of the atmosphere that range between 0.3 and 0.5, and global dust storm conditions can yield optical depths of 3.5–5.0 [44,45]. A completely dust-free atmosphere on Mars at the mean atmospheric pres-

sure of 7.1 mbar would yield an optical depth of 0.1, which would produce an approximate 10% attenuation of the down welling UV irradiation [34]. Optical depths on Mars of 0.3 (clear-sky) to 3.5 (global dust storm) would correspondingly produce approximately 25–97% attenuations in the down welling UV radiation [34]. Therefore, airborne dust loading is an important component that attenuates incoming solar UV in the Martian atmosphere [34,37], and as we shall see below, dust can affect the survival rates of bacterial spores both in space [46] and under simulated Martian environmental conditions [34].

3. Spore UV resistance mechanisms

Spores of various *Bacillus* species are generally 10- to 100-fold more resistant to UV than are the corresponding vegetative cells [5,47–51]. UV resistance varies significantly between spores of different species and strains, and some strains producing extremely UV resistant spores have been isolated from environments subjected to high UV fluxes [19,22,52–54]. However, the reasons for these strain/species-specific differences in spore UV resistance are not yet known. Sporulation conditions can also affect the UV resistance of the resultant spores [55] but the specific reasons for this are again not known.

The major lethal target for UV radiation in spores is almost certainly DNA, and this appears to be the case over the whole UV spectrum. While UV can generate reactive oxygen species (ROS) in addition to generating lesions directly in DNA, the ROS appear likely to kill spores by generating single and/or double strand breaks in DNA [56].

3.1. Cellular protective mechanisms

Bacterial spores appear to be monogenomic in terms of their chromosome; thus, there will be no UV protection provided by the duplication of genetic information. However, natural plasmids carried by various *Bacillus* species are often present in multiple copies. Unfortunately, most genes on such plasmids are of unknown function and in the laboratory there is often little if any change in phenotype upon plasmid loss. Consequently, the role of multiple copies in the UV resistance of plasmid DNA is unclear.

A second potential mechanism for protection against UV is the accumulation of absorbing pigments, generally in the spore's outer layers, in particular the coats and outer membrane [57–60]. There is certainly evidence with growing bacteria that pigments in outer layers can protect against UV [61,62], presumably by absorbing the radiation before it penetrates to the DNA in the spore core or protoplast. In *Bacillus subtilis* spores the formation of coat pigment is due to the *cotA* gene product, a copper-dependent laccase that generates a probable melanin-like pigment, and melanic pigments can shield microbial cells against UV radiation [57,61,62]. Deletion of the *cotA* gene results in albino spores, and these spores are significantly more sensitive to artificial UVB, UVA, and simulated solar radiation than are the spores of its pigmented parent [57]. Spores of a *Bacillus thuringiensis* strain that produces a melanin pigment are also significantly more resistant to UVC and 366 nm radiation than are spores of the non-pigmented parent strain [60]. Red-pigmented spores of *B. atrophaeus* are reported to exhibit higher resistance to a simulated Martian UV environment; these spores contain protective carotenoid pigments that are particularly effective at absorbing UVA wavelengths [63]. As a further indication of the role of molecules in the spore's outer layers in UV protection, spores of some *B. subtilis* strains with defective coats are more sensitive to UVB, UVA, and full spectrum solar radiation, but not to UVC irradiation [59]. Thus, pigments likely provide some UV protection to spores, and further analysis of the UV resistance of spores of isogenic strains with and without various pigments would certainly be of value.

3.2. DNA photochemistry

The most effective UV wavelength for killing spores is UVC, which is ≥ 300 -fold more effective than are UVB, UVA, or full spectrum sunlight, and the photochemistry of DNA in spores exposed to UVC radiation has been best studied [5,47–51,64]. Most work on spore DNA photochemistry has been carried out with spores of *B. subtilis*, although where studied the results with spores of *B. cereus* and *Bacillus megaterium* are similar. Exposure of growing bacteria to UVC generates primarily cyclobutane-type dimers between adjacent pyrimidines on the same DNA strand; these cyclobutane pyrimidine dimers (CPDs) include those between adjacent thymines (TT), thymine and cytosine (TC or

CT), and cytosines (CC). A significant amount of the various (6-4) photoproducts are also generated between adjacent pyrimidines, and all of these pyrimidine photoproducts are potentially lethal lesions. In contrast to results with growing cells, by far the predominant photoproduct generated by UVC exposure of spores in water is a 5-thymine-5,6-dihydrothymine adduct termed spore photoproduct (SP) that is formed between adjacent thymine residues on the same DNA strand [65,66]. SP formation by UVC exposure of spores actually proceeds with slightly higher efficiency than does CPD formation in growing cells, but although SP is a potentially lethal lesion, SP is repaired extremely efficiently in the first minutes of spore germination (see below). In addition to SP, UVC can also generate some single and double strand breaks in spore DNA as well as a very small amount of CPDs, but the doses required for generation of the latter photoproducts are far, far above those needed to obtain high-level killing of spores [67]. There is also one report that tentatively concluded that significant levels of at least one (6-4) photoproduct are generated by exposure of spores to UVC [68], but this was not confirmed in a recent analysis of photoproducts generated in spores using enzymatic digestion followed by HPLC and mass spectrometry for photoproduct identification [69].

In contrast to results with UVC radiation, UVB does generate CPDs in spore DNA at physiological doses as well as some SP, while UVA generates only single and double strand breaks, as well as much SP [67]. Presumably, the strand breaks are caused by ROS generated by the UVA radiation. Unfortunately, there have not been studies on levels of (6-4) photoproducts generated by UVA and UVB irradiation of spores.

The effects of UV on spores are also dependent to some degree on the hydration level at which spores are irradiated. While spores irradiated dry at ambient pressure exhibit UVC and UVB sensitivity and DNA photochemistry similar to that of spores irradiated in water, dry spores irradiated with UVC or UVB under ultrahigh vacuum ($\leq 10^{-3}$ Pa) exhibit 10-fold lower resistance [64,69–72]. In addition, TT isomers including *trans*, *syn*-TT are generated by UVC and UVB at these low pressures, and UVC irradiation of dry spores at moderate vacuum (1–2 Pa) also generates significant amounts of TT, including *trans*, *syn*-TT [48]. However, levels of TT are very low (*cis*, *syn*-TT) or undetectable (*trans*, *syn*-TT) in dry spores irradiated with UVC at

Earth ambient pressure (10^5 Pa) ((6-4) photoproducts are also undetectable) or from spores irradiated in water [69–71]. The reasons for the changes in DNA photochemistry upon irradiation at low pressures are not clear, but presumably the degree of DNA hydration in spores at low vacuum is significantly lower than in spores in water or in dry spores at ambient pressure and humidity. Indeed, exposing spores to ultrahigh vacuum alone is sufficient to cause some DNA damage [73,74].

Spores contain extremely high levels ($\sim 10\%$ of their dry weight) as pyridine-2,6-dicarboxylic acid (dipicolinic acid (DPA)) and this compound sensitizes spores irradiated in water to UVC [50]. In contrast, DPA provides some protection against UVC when spores are irradiated dry at ambient pressure, and is also protective (3- to 7-fold) against UVB, UVA, and full spectrum sunlight when spores are irradiated either dry at ambient pressure or in water [64].

There appear to be a number of possible reasons for the novel photochemistry of DNA in spores. One is the presence of high levels of DPA in spores noted above. While DPA certainly influences the efficiency of killing of spores irradiated in water with UVC, it is not yet clear how DPA causes this effect, and to date there have been no detailed studies on the photochemistry of DNA in purified DPA-less spores. A second is the low degree of DNA hydration in the spore's central region or core, the site of spore DNA, even with spores in water. The spore core has only 25–45% of its wet weight as water depending on the species, while growing cells have 75–80% of their wet weight as water [75]. Indeed, the water content of the spore core is so low that enzymes are inactive in this compartment and soluble proteins are immobile [76,77]. Work in vitro has shown that poorly hydrated DNA has a different UV photochemistry than DNA in solution, as UVC irradiation of DNA in solution gives CPDs and (6-4) photoproducts and little if any SP, while irradiation of poorly hydrated DNA gives less CPDs and (6-4) photoproducts and significant levels of SP [78,79]. There is, however, some water in the spore core, as noted above, and small changes in core water levels of *B. subtilis* spores have no effect on spore UV resistance [80]. However, the precise degree of hydration of DNA in spores suspended in water is not known.

The third factor involved in spore UV photochemistry is certainly the most important one, and is the saturation of spore DNA with a group of novel DNA bind-

ing proteins, the small, acid-soluble proteins (SASP) of the α/β -type [50,81]. These small (60–70 aa) proteins are synthesized in the developing forespore at about the time the spore acquires UV resistance and the DNA's UV photochemistry changes to that of spore DNA [5,49,50]. Sufficient amounts of α/β -type SASP are accumulated to saturate the spore chromosome, and the chromosome is converted to a ring-like or toroidal structure by the binding of these proteins [82,83]. There are multiple α/β -type SASP in all *Bacillus* species that have been examined and the amino acid sequences of these proteins are highly conserved, both within one *Bacillus* species as well as across *Bacillus* species, and in *Clostridium* and *Sporosarcina* species as well. However, genes encoding α/β -type SASP are not found in non-spore forming bacteria and the amino acid sequences of these proteins are not similar to those of any other proteins and exhibit no common motifs found in DNA binding proteins. The α/β -type SASP protect spore DNA not only against UV damage, but also against damage caused by heat (depurination) and genotoxic chemicals [5,84]. The major *B. subtilis* chromosomal protein of growing cells, Hbsu is also present on the spore chromosome, and this protein may modify the effects of α/β -type SASP on spore DNA structure and properties to some degree [85].

B. subtilis has four genes encoding α/β -type SASP, and all are expressed in parallel during sporulation, although two (*sspA* and *sspB*) are expressed at a much higher level than the other two (*sspC* and *sspD*). However, all α/β -type SASP appear to be largely interchangeable in their effects on DNA both in vitro and in vivo [5,49,50,86,87]. Deletion of *sspA* and *sspB* gives spores (termed $\alpha^- \beta^-$) that lack ~80% of the total α/β -type SASP pool and these $\alpha^- \beta^-$ spores exhibit resistance to UVC that is lower than that of growing cells. Compared to results with wild-type spores, UVC irradiation of $\alpha^- \beta^-$ spores in water generates ~50% less SP and also ~1/2 the amount of CPDs formed in growing cells, as well as significant levels of (6-4) photoproducts [48,69,88]. Irradiation of $\alpha^- \beta^-$ spores with UVC in the dry state at ambient pressure gives similar results, while irradiation at low vacuum gives more *cis*, *syn*-TT and *trans*, *syn*-TT than with wild-type spores [48].

The photochemistry of DNA in spores can be largely duplicated in vitro using complexes of purified α/β -type SASP with DNA, as UVC irradiation of these com-

plexes generates very little CPDs and (6-4) photoproducts and significant levels of SP. However, the quantum efficiency of SP formation is lower in α/β -type SASP-DNA complexes than it is in spores [48,87]. Some of this lower efficiency of SP formation in vitro may be due to the absence of DPA, although other factors may also be involved. The photoproducts generated in these complexes by UVC appear to be intrastrand photoproducts, despite the generation of significant amounts of interstrand photoproducts upon UVC irradiation of poorly hydrated DNA under some conditions [69,89].

3.3. DNA repair mechanisms in spores

Since SP is a potentially lethal lesion, it must be repaired efficiently. Spores have at least three systems—recombinational repair (RR), nucleotide excision repair (NER), and spore photoproduct lyase (SP lyase)—for repair of UV damage to DNA, and the importance of these three systems varies depending on the damage to be repaired [5,49–51,72]. Recombinational repair appears to play little or no role in repair of damage by UVC and UVB, but may play a significant role in repair of damage caused by UVA. NER and SP lyase appear sufficient to explain the great majority of repair of damage by UVC and UVB in spores. The dormant spore appears to contain the enzymes of NER, and the levels of the proteins involved in this process that are under RecA control are further increased in the first minutes of germination of UVC irradiated spores [90]. As expected, *recA* spores are slightly less resistant to UVC than are wild-type spores and the NER system can repair CPDs (6-4) photoproducts and SP. SP lyase is involved only in repair of SP, which it cleaves to two thymine residues without DNA strand cleavage in a light independent reaction; this enzyme does not even bind to TT containing DNA [91]. The gene encoding SP lyase (*spIB*) is transcribed in the developing spore at approximately the same time as the *ssp* genes [92], and SP lyase is present in dormant spores [93]. Where it has been studied, levels of SP lyase are not increased by UV irradiation either during sporulation or of the dormant spore [92]. SP lyase is a homodimer, and is a member of the “radical-SAM” family of 4Fe–4S enzymes and uses *S*-adenosylmethionine as an essential cosubstrate, with the latter being cleaved ultimately to 5'-adenosine [94]. The reaction likely proceeds by formation of a 5'-adenosyl radical that abstracts a proton

from SP generating an SP radical [93–96]. Formation of this radical then leads to β -scission of the thymine-thymine bond and finally completion of the reaction by transfer of a proton from 5'-adenosine back to thymine, thus recycling the 5'-adenosyl radical [94–96]. While this mechanism is certainly reasonable in light of similarities between SP lyase and other “radical SAM” enzymes, further studies are needed to prove the mechanism definitively.

4. General factors influencing spore survival in space and on Mars

The interplanetary environment between Earth and Mars is composed of a number of factors that will impact the survival of terrestrial microorganisms on interior and exterior surfaces of spacecraft. The key biocidal factors within the interplanetary environment are: (i) high vacuum; (ii) solar UV irradiation; (iii) severe desiccating conditions; (iv) extreme temperature fluctuations; (v) charged particles in the Earth's Van Allen radiation belts; (vi) solar particle events (SPE); (vii) galactic cosmic rays (GCR) [97,98]. All of these factors are likely to contribute to the loss of microbial diversity and viable biomass on spacecraft surfaces. Immediately after reaching low Earth orbit (LEO), most spacecraft surfaces that are vented to the external environment will experience high vacuum, severe desiccating conditions and possibly extreme fluctuations of temperature. High-vacuum and extreme desiccating environments can significantly reduce the viability of microorganisms over just a few hours [32,99], and in general non-spore forming microorganisms are affected to a much greater extent than spore-forming species [100,101]. Apollo data indicate that temperature extremes on external surfaces of spacecraft components in the interplanetary environment can swing between -171 and $+111$ °C [97]. Those components that are heated to the upper levels of this range are likely to experience significant reductions in viable bioloads due exclusively to high temperatures. Based on these factors alone, it is likely that during the cruise phase to Mars the viability of microorganisms on the UV-protected but internally vented surfaces of spacecraft will be reduced 50–70% for spore-forming bacteria and up to 2–3 decades for non-spore forming microorganisms [34,101,102].

Many of the cultivable microorganisms typically recovered from spacecraft surfaces [11–16] exhibit relatively low resistance to UV irradiation [20,103,104]. Although endospores of some *Bacillus* spp. have been shown to exhibit much higher UV resistance than non-spore forming bacteria [20,34,104], spores of *Bacillus* spp. are not immune to solar UV irradiation and would be expected to be inactivated by one to several decades within a few tens of seconds to a few minutes under UV irradiation in LEO [105] or on Mars [34]. In fact, the total UV fluence rates in the unattenuated solar spectrum (including vacuum UV, UVC, UVB, and UVA) are so high that it is reasonable to expect that all sun-exposed surfaces on the exterior of spacecraft will be exposed to sterilizing levels of UV irradiation between 1000s and tens of 1000s of times the *effective lethal dose rates* of most (if not all) terrestrial microorganisms during the cruise phase to Mars [34]. Much research has demonstrated that UV irradiation is a very strong biocidal factor against terrestrial microorganisms, but this research also demonstrates clearly that UV irradiation can be blocked by relatively thin layers of dusts, metal oxides, UV-absorbing pigments, and opaque materials [46,105,106]. Consequently, while UV radiation can be extremely biocidal to microorganisms on spacecraft and ejected planetary debris, the biocidal effects are only for surface or very shallow subsurface sites.

Also of significant importance to the survival of microorganisms during interplanetary travel are the effects of charged particles in the Earth's Van Allen radiation belts, SPE, and GCR [107,108]. These factors differ from solar UV in that they are in general of lower dosage per unit of time, and are not limited to affecting microbial survival to surface populations alone, as is solar UV irradiation. In contrast to the low penetrating power of UV radiation, Van Allen radiation, SPE, and GCR processes will affect deeper layers within spacecraft and meteorites, but longer exposure times are required to achieve significant reductions in microbial bioloads.

Spacecraft and ejected planetary meteoritic material passing through the Earth's Van Allen Belts will be exposed to relatively high levels of charged particles (mainly electrons and protons) that can interact with solid matter and cause Bremsstrahlung radiation (i.e., secondary cascades of particles) composed of X-rays and γ -rays [109]. However, the transit times through these radiation belts are generally of short duration,

and may not impart significant levels of biocidal radiation to either outgoing or incoming spacecraft or ejected interplanetary debris. Of significantly greater concern than Van Allen radiation are SPE and GCR processes. SPE are generated from solar flares from the Sun and are comprised mainly of protons with a small contribution from α -particles and heavier nuclei [107,108]. GCR originate outside the Solar System and are composed of about 98% nuclei and 2% electrons and positrons [107,108]. The nuclear component of GCR is composed of about 87% protons, 12% α -particles, and about 1% heavier nuclei [107]. The effects of GCR proton bombardment on the survival of terrestrial microorganisms have been studied for interplanetary environments, and the results generally support the conclusion that during a 3-year Mars mission, microbial populations might be reduced between 20 and 90% on exterior surfaces of spacecraft by GCR alone [99,106,107]. However, during severe SPE the reductions in microbial bioloads on and within spacecraft might be significantly higher. Good estimates of the biocidal effects of SPE and GCR exposures are difficult to calculate because the actual dose rates experienced by spacecraft components are a combination of a wide range of individual particles at widely varying energies that are difficult to accurately simulate [107]. However, accelerator and space-based experiments with *B. subtilis* spores using radiation transfer models have allowed estimates of the biocidal effect of GCR and SPE to be calculated [4].

In summary, solar UV radiation would be expected to sterilize most sun-exposed spacecraft surfaces within a few minutes after reaching LEO, with the total dosage rates reaching many 1000s of times the normal effective lethal dose rates published in the literature. On vented and UV-protected exterior surfaces of spacecraft, terrestrial microorganisms are not likely to undergo replicative growth during the cruise phase to Mars due to the extreme temperature swings, severe desiccating conditions, and high vacuum of the interplanetary environment. Thus, the launched bioload of exterior spacecraft surfaces would be highest just after the vehicle reached LEO, and then would be expected to significantly decline as the biocidal factors in the interplanetary environment continually reduced the viability of the launched bioload. Based on the above discussion, it seems reasonable to expect that the launched bioload on exterior spacecraft surfaces will be reduced

at least 1–2 decades during the outbound cruise phase of a mission to Mars [34,98].

On Mars, the UV irradiation, SPE and GCR will be lower than during the cruise phase between Earth and Mars, but extreme desiccating conditions, low atmospheric pressure, and wide temperature swings will persist [97,98]. As discussed above, UV irradiation will be reduced through attenuation by the Martian atmosphere. SPE and GCR will also be attenuated slightly by the Martian atmosphere (at a level of about 1% of the attenuation by Earth's atmosphere), and will be further reduced by half due to the shielding effects of the planet itself. However, both SPE and GCR will remain significant factors on Mars, which has led many to suggest that long-term human settlements on Mars will require significant levels of shielding accomplished perhaps by burying habitats under several meters of Martian regolith [97,102,109]. In addition, oxidants in the Martian regolith [110,111], UV emissions from corona-discharges of electrostatic energy in blowing dust [112,113], and toxic heavy metals in the regolith or atmospheric dust [114] may also contribute to the biocidal effects of the Martian surface.

5. Simulations and flight experiments

As mentioned above, one of the strongest sterilizing factors in the interplanetary environment between Earth and Mars is solar UV irradiation, which has been demonstrated in space to kill unprotected spores of *B. subtilis* within seconds [115]. The reason for this is the highly energetic UVC and vacuum UV radiation that is directly absorbed by DNA, as demonstrated by action spectroscopy in space [116]. In addition, space vacuum and UV appear to act synergistically. Spores of *B. subtilis* simultaneously exposed to solar UV radiation and space vacuum exhibited a 10-fold increase in UV-sensitivity as compared to spores irradiated at atmospheric pressure [117]. Photoproducts generated within the DNA of *B. subtilis* spores exposed to UV radiation in ultrahigh vacuum ($\sim 10^{-6}$ Pa) (characteristic of the space environment in LEO) consisted of *cis*, *syn*-TT and *trans*, *syn*-TT in addition to SP [70,117]. Recent work has shown that *B. subtilis* spores treated with UV under more moderate low pressures (1–2 Pa) characteristic of planetary bodies with thin atmospheres are also more UV sensitive and demonstrate the same

alterations in DNA photochemistry seen under ultra-high vacuum [48].

During the past 50 years, many studies have been published on the survival of spore-forming bacterial species (generally in the genus *Bacillus*) under simulated Martian conditions [34,99,100,102,106,118–127]. Although these studies examined a diversity of effects of simulated Martian conditions on spore survival, a few general conclusions may be drawn from this body of work. First, UV radiation was the key parameter that determined survivability of spores under simulated Martian conditions; direct exposure to UV radiation resulted in rapid and nearly complete inactivation of microbial cultures [34,99,102,118,125,126]. These results are consistent with other literature from microgravity experiments during Gemini, Apollo, Space lab, and LDEF missions in which spore survival was directly related to the duration and intensity of exposure to solar UV radiation [115,117,128–130]. Second, thin (tens of micrometers) to thick (centimeters) contiguous layers of Mars analog soils were generally adequate for protecting a significant proportion of test populations of spores from the lethal effects of UV irradiation [34,125]. In contrast, individual particles of Mars analog dust measuring up to 50 μm in diameter deposited over bacterial monolayers (a condition that more accurately simulates aeolian dust settling onto a spacecraft surface than thick contiguous layers) were found not to protect endospores of *B. subtilis* from the UV flux of a simulated Martian spectrum [34]. Third, spores survived well under low temperature, low pressure, and N_2 or CO_2 atmospheres, exhibiting reductions in microbial populations of only one to several orders of magnitude [34,119–123]. Fourth, although survival of spores at reduced pressures has been reported (see above), there is still a direct effect of low pressure on the recovery of viable spores from spacecraft materials. For example, spores of *Bacillus* spp. exposed to low pressures similar to the surface of Mars or interplanetary space for several hours to several months exhibited between 20 and 70% reductions in spore survival compared to Earth controls at normal pressures [34,100,128–130]. Fifth, freeze-thaw cycles in the presence or absence of UV radiation generally did not reduce microbial survival rates under simulated Martian conditions [126]. Sixth, proton irradiation that has been used to simulate both SPE and GCR events may be a factor in reducing survival of terrestrial microorganisms on Mars

[99,106,124], but the effects on spore survival were generally not dramatic, supporting the conclusion that SPE and GCR events may not be that important to microbial survival over short durations of exposure time.

6. Concluding remarks

Both interplanetary space and the Martian surface environment are harsh and inhospitable environments for the survival, growth, and adaptation of terrestrial life. The primary biocidal factors that render these environments so inhospitable are high vacuum-UV and UVC fluence rates at the energetically more active short-wavelength portion of the spectrum, low atmospheric pressure, extreme desiccating conditions, low temperatures, oxidizing conditions in atmospheric dust and regolith, and the presence of SPE and GCR. Ultra-violet radiation in the interplanetary and Martian environments can deliver high lethal dose rates to sun-exposed surfaces yielding significant reductions in the launched bioloads. In contrast, due to the rather low penetrating power of UV radiation, its lethal effects can be effectively mitigated or prevented by a minimal amount of shielding. Dormant bacterial endospores are among the most UV-resistant cell types known, due to intrinsic mechanisms, which either prevent DNA damage in the spore or efficiently repair damage during subsequent spore germination. Thus, if protected from direct UV exposure, it is likely that a diversity of spore-forming species will have significantly longer periods of viability under conditions in interplanetary space or on Mars than has been discussed above for sun-exposed spores. However, a complete model on how spore-forming and non-spore forming terrestrial microorganisms might survive transport to other planetary bodies has not yet been fully developed. Thus, the potential for the movement of microorganisms among the various planetary bodies within our Solar System remains poorly constrained.

The implications of these issues for planetary protection are also quite important for the success of future robotic and human exploration missions in the Solar System. A clearly stated goal of both the Mars Exploration Program and NASA's Astrobiology Roadmap is the search for life elsewhere in the Solar System [131,132]. Mars and Europa are currently two key

targets for this search, and of critical importance to the success of these missions will be the understanding of how efficiently terrestrial microorganisms will survive the transit to these planetary bodies, and then how well they will survive, grow, and replicate under the actual conditions on or within Mars and Europa. Indeed, human directed panspermia via launched microbial bioloads on current spacecraft may be as likely or unlikely as natural undirected panspermia via launched geological materials from impact events. Both scenarios currently require additional modeling, and require new empirically derived data to verify the veracity of the tenets of the models before final conclusions can be drawn.

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References

- [1] S. Arrhenius, Die Verbreitung des Lebens im Weltenraum, Die Umschau 7 (1903) 481–485.
- [2] H. Richter, Zur Darwinschen Lehre, Schmidts Jahrbuch Ges. Med. 126 (1865) 243–249.
- [3] W. Thomson (Lord Kelvin), 1871 Presidential address to the British Association, in: Popular Lectures and Addresses, MacMillan and Company, England, 1894, pp. 132–205.
- [4] C. Mileikowsky, F.A. Cucinotta, J.W. Wilson, B. Gladman, G. Horneck, L. Lindgren, J. Melosh, H. Rickman, M. Valtonen, J.Q. Zheng, Natural transfer of viable microbes in space. I. From Mars to Earth and Earth to Mars, *Icarus* 145 (2000) 391–427.
- [5] W.L. Nicholson, N. Munakata, G. Horneck, H.J. Melosh, P. Setlow, Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments, *Microbiol. Mol. Biol. Rev.* 64 (2000) 548–572.
- [6] B.C. Clark, A.L. Baker, A.F. Cheng, S.J. Clemett, D. McKay, H.Y. McSween, C.M. Pieters, P. Thomas, M. Zolensky, Survival of life on asteroids, comets and other small bodies, *Origins Life Evol. Biosphere* 29 (1999) 521–545.
- [7] B.J. Gladman, J.A. Burns, M. Duncan, P. Lee, H.F. Levinson, The exchange of impact ejecta between the terrestrial planets, *Science* 271 (1996) 1387–1392.
- [8] B. Gladman, Destination Earth: Martian meteorite delivery, *Icarus* 130 (1997) 228–246.
- [9] B.J. Gladman, J.A. Burns, Mars meteorite transfer: simulation, *Science* 274 (1996) 161–162.
- [10] R.M. Brockett, J.K. Ferguson, M.R. Henney, Prevalence of fungi during Skylab missions, *Appl. Environ. Microbiol.* 36 (1978) 243–246.
- [11] M.S. Favero, Microbiologic assay of space hardware, *Environ. Biol. Med.* 1 (1971) 27–36.
- [12] M.S. Favero, J.R. Puleo, J.H. Marshall, G.S. Oxborrow, Comparative levels and types of microbial contamination detected in industrial clean rooms, *Appl. Microbiol.* 14 (1966) 539–551.
- [13] T.L. Foster, L. Winans, Psychrophilic microorganisms from areas associated with the Viking spacecraft, *Appl. Microbiol.* 30 (1975) 546–550.
- [14] C.M. Herring, J.W. Brandsberg, G.S. Oxborrow, J.R. Puleo, Comparison of media for detection of fungi on spacecraft, *Appl. Microbiol.* 27 (1974) 566–569.
- [15] J.R. Puleo, N.D. Fields, B. Moore, R.C. Graves, Microbial contamination associated with the Apollo 6 spacecraft during final assembly and testing, *Space Life Sci.* 2 (1970) 48–56.
- [16] J.R. Puleo, N.D. Fields, S.L. Bergstrom, G.S. Oxborrow, P.D. Stabekis, R.C. Koukol, Microbiological profiles of the Viking Spacecraft, *Appl. Environ. Microbiol.* 33 (1977) 379–384.
- [17] A.C. Schuerger, Microbial contamination of advanced life support (ALS) systems poses a moderate threat to the long-term stability of space-based bioregenerative systems, *Life Support Biosphere Sci.* 5 (1998) 325–337.
- [18] G.R. Taylor, Space microbiology, *Ann. Rev. Microbiol.* 28 (1974) 121–137.
- [19] R.T. Dillon, W.R. Gavin, A.L. Roark, C.A. Trauth Jr., Estimating the number of terrestrial organisms on the moon, *Space Life Sci.* 4 (1973) 180–199.
- [20] M.T. La Duc, W. Nicholson, R. Kern, K. Venkateswaran, Microbial characterization of the Mars Odyssey spacecraft and its encapsulation facility, *Environ. Microbiol.* 5 (2003) 977–985.
- [21] K. Venkateswaran, M. Satomi, S. Chung, R. Kern, R. Koukol, C. Basic, D. White, Molecular diversity of a spacecraft assembly facility, *Syst. Appl. Microbiol.* 24 (2001) 311–320.
- [22] K. Venkateswaran, N. Hattori, M.T. La Duc, R. Kern, ATP as a biomarker of viable microorganisms in clean-room facilities, *J. Microbiol. Meth.* 52 (2003) 367–377.
- [23] K. Venkateswaran, M. Kempf, F. Chen, M. Satomi, W. Nicholson, R. Kern, *Bacillus nealsonii* sp. nov., isolated from a spacecraft-assembly facility, whose spores are gamma-radiation resistant, *Int. J. Syst. Evol. Microbiol.* 53 (2003) 165–172.
- [24] D.L. DeVincenzi, H.P. Klein, Planetary protection, sample return missions and Mars exploration: history, status, and future needs, *J. Geophys. Res.* 103 (E12) (1998) 28,577–28,585.
- [25] J.D. Rummel, Planetary exploration in the time of astrobiology: protecting against biological contamination, *Proc. Natl. Acad. Sci. U.S.A.* 98 (2001) 2128–2131.
- [26] J.B. Barengoltz, Microbiological cleanliness of the Mars Pathfinder spacecraft, in: Proceedings of the 43rd Annual

- Technical Meeting “Contamination Control”, Institute of Environmental Science, 1997, pp. 242–248.
- [27] J.R. Puleo, S.L. Bergstrom, J.T. Peeler, G.S. Oxborrow, Thermal resistance of naturally occurring airborne bacterial spores, *Appl. Environ. Microbiol.* 36 (1978) 473–479.
- [28] J. Applebaum, D.J. Flood, Solar radiation on Mars, *Solar Energy* 45 (1990) 353–363.
- [29] Commission Internationale de l’Eclairage, International Lighting Vocabulary, 3rd ed., Commission Internationale de l’Eclairage, Paris, 1991, Publication CIE 17 nr (E-1.1).
- [30] World Health Organization, Global Solar UV Index: A Practical Guide. A Joint Recommendation of the World Health Organization, World Meteorological Organization, United Nations Environment Programme, and the International Commission on Non-Ionizing Radiation Protection, World Health Organization, 2002.
- [31] C.S. Cockell, A.L. Andrady, The Martian and extraterrestrial UV radiation environment-1. Biological and closed-loop ecosystem considerations, *Acta Astronaut.* 44 (1999) 53–62.
- [32] W.R. Kuhn, S.K. Atreya, Solar radiation incident on the Martian surface, *J. Mol. Evol.* 14 (1979) 57–64.
- [33] M.R. Patel, J.C. Zarnecki, D.C. Cutling, Ultraviolet radiation on the surface of Mars and the Beagle 2 UV sensor, *Planet. Space Sci.* 50 (2002) 915–927.
- [34] A.C. Schuerger, R.L. Mancinelli, R.G. Kern, L.J. Rothschild, C.P. McKay, Survival of endospores of *Bacillus subtilis* on spacecraft surfaces under simulated Martian environments: implications for the forward contamination of Mars, *Icarus* 165 (2003) 253–276.
- [35] L.E. Floyd, J.W. Cook, L.C. Herring, P.C. Crane, SUSIM’S 11-year observational record of the solar UV irradiance, *Adv. Space Res.* 31 (2003) 2111–2120.
- [36] R.W. Zurek, J.R. Barnes, R.M. Haberle, J.B. Pollack, J.E. Tillman, C.B. Leovy, Dynamics of the atmosphere of Mars, in: H.H. Kieffer, B.M. Jakosky, C.W. Snyder, M.S. Matthews (Eds.), *Mars*, University of Arizona Press, Tucson, AZ, 1992, pp. 835–933.
- [37] C.S. Cockell, D.C. Catling, W.L. Davis, K. Snook, R.L. Kepner, P. Lee, C.P. McKay, The ultraviolet environment of Mars: biological implications past, present, and future, *Icarus* 146 (2000) 343–359.
- [38] J. Laskar, P. Robutel, The chaotic obliquity of the planets, *Nature* 361 (1993) 608–612.
- [39] P. Warneck, *Chemistry of the Natural Atmosphere*, Academic Press, New York, NY, 1988.
- [40] F. Urbach, R.W. Gange (Eds.), *The Biological Effects of UVA Radiation*, Praeger Publishers, New York, NY, 1986.
- [41] T. Owen, K. Biemann, D.R. Rushneck, J.E. Biller, D.W. Howarth, A.L. Lafleur, The composition of the atmosphere at the surface of Mars, *J. Geophys. Res.* 82 (1977) 4635–4639.
- [42] M.E. Brown, A.H. Bouchez, C.A. Griffith, Direct detection of variable tropospheric clouds near Titan’s south pole, *Nature* 420 (2002) 795–797.
- [43] M.G. Tomasko, L.R. Dose, M. Lemmon, P.H. Smith, E. Wegryn, Properties of dust in the Martian atmosphere from the imager on Mars Pathfinder, *J. Geophys. Res.* 104 (1999) 8987–9007.
- [44] R.A. Kahn, T.Z. Martin, R.W. Zurek, The Martian dust cycle, in: H.H. Kieffer, B.M. Jakosky, C.W. Snyder, M.S. Matthews (Eds.), *Mars*, University of Arizona Press, Tucson, AZ, 1992, pp. 1017–1053.
- [45] D.S. Colburn, J.B. Pollack, R.M. Haberle, Diurnal variations in optical depth at Mars, *Icarus* 79 (1989) 159–189.
- [46] G. Horneck, P. Rettberg, G. Reitz, J. Wehner, K. Strauch, C. Panitz, V. Starke, C. Baumstark-Khan, Protection of bacterial spores in space, a contribution to the discussion on panspermia, *Origins Life Evol. Biosphere* 31 (2001) 527–547.
- [47] W.L. Nicholson, P. Fajardo-Cavazos, DNA repair and the ultraviolet radiation resistance of bacterial spores: from the laboratory to the environment, *Recent Res. Devel. Microbiol.* 1 (1997) 125–140.
- [48] W.L. Nicholson, B. Setlow, P. Setlow, UV photochemistry of DNA *in vitro* and in *Bacillus subtilis* spores at earth-ambient and low atmospheric pressure: implications for spore survival on other planets or moons in the solar system, *Astrobiology* 2 (2002) 417–425.
- [49] P. Setlow, Resistance of bacterial spores to ultraviolet light, *Comments Mol. Cell. Biol. Biophys.* 5 (1988) 253–264.
- [50] P. Setlow, Resistance of bacterial spores to ultraviolet light, *Environ. Mol. Mutagen.* 38 (2001) 97–104.
- [51] W.L. Nicholson, P. Fajardo-Cavazos, R. Rebeil, T.A. Slieman, P.J. Riesenman, J.F. Law, Y. 50e, Bacterial endospores and their significance in stress resistance, *Antonie van Leeuwenhoek* 81 (2002) 27–32.
- [52] J.N. Bernardini, J. Sawyer, K. Venkateswaran, W.L. Nicholson, Spore UV and acceleration resistance of endolithic *Bacillus pumilus* and *Bacillus subtilis* isolates obtained from Sonoran desert basalt: implications for lithospermia, *Astrobiology* 3 (2003) 709–717.
- [53] M.T. La Duc, M. Satoni, K. Venkateswaran, *Bacillus odyseeyi* sp. Nov., a round-spore-forming bacillus isolated from the Mars Odyssey spacecraft, *Int. J. Syst. Evol. Microbiol.* 54 (2004) 195–201.
- [54] L. Link, J. Sawyer, K. Venkateswaran, W.L. Nicholson, Extreme spore UV resistance of *Bacillus pumilus* isolates obtained from an ultra-clean spacecraft assembly facility, *Microbial Ecol.* 47 (2004) 159–163.
- [55] W.L. Nicholson, J.F. Law, Method for the purification of bacterial endospores from soils: UV resistance of natural Sonoran desert soil populations of *Bacillus* spp. with reference to *Bacillus subtilis* strain 168, *J. Microbiol. Meth.* 35 (1999) 13–21.
- [56] R.M. Tyrell, Inducible responses to UV-A exposure, in: F. Urbach (Ed.), *Biological Responses to Ultraviolet-A Radiation*, Valdemar Publishing, Overland Park, KN, 1992, pp. 59–64.
- [57] M.F. Hullo, I. Moszer, A. Danchin, I. Martin-Verstraete, CotA of *Bacillus subtilis* is a copper-dependent laccase, *J. Bacteriol.* 183 (2001) 5426–5430.
- [58] C. Mitchell, S. Iyer, J.F. Skomurski, J.C. Vary, Red pigment in *Bacillus megaterium* spores, *Appl. Environ. Microbiol.* 52 (1986) 64–67.
- [59] P.J. Riesenman, W.L. Nicholson, Role of the spore coat layers in *Bacillus subtilis* spore resistance to hydrogen peroxide, artificial UV-C, UV-B, and solar UV radiation, *Appl. Environ. Microbiol.* 66 (2000) 620–626.

- [60] D. Saxene, E. Ben-Dov, R. Mansherob, Z. Barak, S. Boussiba, A. Zaritsky, A UV tolerant mutant of *Bacillus thuringiensis* spp. kurstaki producing melanin, *Curr. Microbiol.* 44 (2002) 25–30.
- [61] A.A. Imshenetsky, S.V. Lysenko, S.P. Lach, Microorganisms of the upper layer of the atmosphere and the protective role of their cell pigments, *Life Sci. Space Res.* 17 (1979) 105–110.
- [62] P.Z. Margalith, *Pigment Microbiology*, Chapman & Hall, London, UK, 1992, pp. 5–31.
- [63] R. Moeller, G. Horneck, *Bacillus* endospores: an ideal exobiological tool, Abstracts of the 35th Committee on Space Research (COSPAR) Scientific Assembly, 2004 (Abstract COSPAR04-A-02596).
- [64] T.A. Slieman, W.L. Nicholson, Role of dipicolinic acid in survival of *Bacillus subtilis* spores exposed to artificial and solar UV radiation, *Appl. Environ. Microbiol.* 67 (2001) 1274–1279.
- [65] J.E. Donnellan Jr., R.B. Setlow, Thymine photoproducts but not thymine dimers found in ultraviolet-irradiated bacterial spores, *Science* 149 (1965) 308–310.
- [66] H.A. Varghese, 5-Thyminyl-5,6-dihydrothymine from DNA irradiated with ultraviolet light, *Biochem. Biophys. Res. Commun.* 38 (1976) 484–490.
- [67] T.A. Slieman, W.L. Nicholson, Artificial and solar UV radiation induces strand breaks and cyclobutane pyrimidine dimers in *Bacillus subtilis* spore DNA, *Appl. Environ. Microbiol.* 66 (2000) 199–205.
- [68] J.A. Lindsay, W.G. Murrell, A comparison of UV induced DNA photoproducts from isolated and non-isolated developing bacterial forespores, *Biochem. Biophys. Res. Commun.* 113 (1983) 618–625.
- [69] T. Douki, B. Setlow, P. Setlow, Effects of the binding of-type small, acid-soluble spore proteins on the photochemistry of DNA in spores of *Bacillus subtilis* and in vitro. *Photochem. Photobiol.*, 2005, in press.
- [70] C. Lindberg, G. Horneck, Action spectra for survival and spore photoproduct formation from *Bacillus subtilis* irradiated with short wavelength (200–300 nm) UV at atmospheric pressure and in vacuo, *J. Photochem. Photobiol.* 11 (1991) 69–80.
- [71] C. Lindberg, G. Horneck, Thymine photoproduct formation and inactivation of intact spores of *Bacillus subtilis* irradiated with short wavelength UV (200–300 nm) at atmospheric pressure and in vacuo, *Adv. Space Res.* 12 (1992) 275–279.
- [72] Y. Xue, W.L. Nicholson, The two major spore DNA repair pathways, nucleotide excision repair and spore photoproduct lyase, are sufficient for the resistance of *Bacillus subtilis* spores to artificial UV-C and UV-B but not to solar radiation, *Appl. Environ. Microbiol.* 62 (1996) 2221–2227.
- [73] K. Dose, A. Bieger-Dose, R. Dillman, M. Gill, O. Kerz, A. Klein, H. Meinert, T. Nawroth, S. Risi, C. Stridde, ERA-experiment “Space Biochemistry”, *Adv. Space Res.* 18 (1995) 119–129.
- [74] N. Munakata, M. Saitou, N. Takahashi, K. Hieda, F. Morohoshi, Induction of unique tandem-base change mutations in bacterial spores exposed to extreme dryness, *Mutat. Res.* 390 (1997) 189–195.
- [75] P. Gerhardt, R.E. Marquis, Spore thermoresistance measurements, in: I. Smith, R.A. Slepecky, P. Setlow (Eds.), *Regulation of Prokaryotic Development*, American Society for Microbiology, Washington, DC, 1989, pp. 43–63.
- [76] A.E. Cowan, D.E. Koppel, B. Setlow, P. Setlow, A soluble protein is immobile in dormant spores of *Bacillus subtilis* but is mobile in germinated spores: implications for spore dormancy, *Proc. Natl. Acad. Sci. U.S.A.* 100 (2003) 4209–4214.
- [77] P. Setlow, Mechanisms which contribute to the long-term survival of spores of *Bacillus* species, *J. Appl. Bacteriol.* 76 (1994) 129S–134S.
- [78] M.H. Patrick, D.M. Gray, Independence of photoproduct formation on DNA conformation, *Photochem. Photobiol.* 24 (1976) 507–513.
- [79] R.O. Rahn, J.L. Hosszu, Influence of relative humidity on the photochemistry of DNA films, *Biochim. Biophys. Acta* 190 (1969) 126–131.
- [80] E. Melly, P.C. Genest, M.E. Gilmore, S. Little, D.L. Popham, A. Driks, P. Setlow, Analysis of the properties of spores of *Bacillus subtilis* prepared at different temperatures, *J. Appl. Microbiol.* 92 (2002) 1105–1115.
- [81] A. Driks, Proteins of the spore core and coat, in: A.L. Sonenshein, J.A. Hoch, R. Losick (Eds.), *Bacillus subtilis* and its Closest Relatives: from Genes to Cells, American Society for Microbiology, Washington, DC, 2002, pp. 527–536.
- [82] K. Pogliano, E. Harry, R. Losick, Visualization of the subcellular location of sporulation proteins in *Bacillus subtilis* using immunofluorescence microscopy, *Mol. Microbiol.* 18 (1995) 459–470.
- [83] K. Ragkousi, A.E. Cowan, M.A. Ross, P. Setlow, Analysis of nucleoid morphology during germination and outgrowth of spores of *Bacillus* species, *J. Bacteriol.* 182 (2000) 5556–5562.
- [84] A. Sohail, C.S. Hayes, P. Divvela, P. Setlow, A.S. Bhagwat, Protection of DNA by α/β -type small, acid-soluble proteins from *Bacillus subtilis* spores against cytosine deamination, *Biochemistry* 41 (2002) 11325–11330.
- [85] M.A. Ross, P. Setlow, The *Bacillus subtilis* Hbsu protein modifies the effects of α/β -type small, acid-soluble spore proteins on DNA, *J. Bacteriol.* 182 (2000) 1942–1948.
- [86] C.S. Hayes, Z.-Y. Peng, P. Setlow, Equilibrium and kinetic binding interactions between DNA and a group of novel, non-specific DNA binding proteins from spores of *Bacillus* and *Clostridium* species, *J. Biol. Chem.* 275 (2000) 35040–35050.
- [87] W.L. Nicholson, B. Setlow, P. Setlow, Ultraviolet irradiation of DNA complexed with α/β -type small, acid-soluble proteins from spores of *Bacillus* or *Clostridium* species makes spore photoproduct but not thymine dimers, *Proc. Natl. Acad. Sci. U.S.A.* 88 (1991) 8288–8392.
- [88] B. Setlow, P. Setlow, Thymine containing dimers as well as spore photoproduct are found in ultraviolet-irradiated *Bacillus subtilis* spores that lack small acid-soluble proteins, *Proc. Natl. Acad. Sci. U.S.A.* 84 (1987) 421–423.
- [89] T. Douki, G. Laporte, J. Cadet, Inter-strand photoproducts are produced in high yield within A-DNA exposed to UVC radiation, *Nucleic Acids Res.* 31 (2003) 3134–3142.

- [90] B. Setlow, P. Setlow, Role of DNA repair in *Bacillus subtilis* spore resistance, *J. Bacteriol.* 178 (1996) 3486–3495.
- [91] T.A. Slieman, R. Rebeil, W.L. Nicholson, Spore photoproduct (SP) lyase from *Bacillus subtilis* specifically binds to and cleaves SP (5-thymine-5,6-dihydrothymine) but not cyclobutane pyrimidine dimers in UV-irradiated DNA, *J. Bacteriol.* 182 (2000) 6412–6417.
- [92] M. Pedraza-Reyes, F. Gutiérrez-Corona, W.L. Nicholson, Temporal regulation and forespore-specific expression of the spore photoproduct lyase gene by sigma-G RNA polymerase during *Bacillus subtilis* sporulation, *J. Bacteriol.* 176 (1994) 3983–3991.
- [93] R. Rebeil, Y. Sun, L. Chooback, M. Pedraza-Reyes, C. Kinsland, T.P. Begley, W.L. Nicholson, Spore photoproduct lyase from *Bacillus subtilis* spores is a novel iron-sulfur DNA repair enzyme which shares features with proteins such as Class III anaerobic ribonucleotide reductase and pyruvate-formate lyases, *J. Bacteriol.* 180 (1998) 4879–4885.
- [94] R. Rebeil, W.L. Nicholson, The subunit structure and catalytic mechanism of the *Bacillus subtilis* DNA repair enzyme spore photoproduct lyase, *Proc. Natl. Acad. Sci. U.S.A.* 98 (2001) 9038–9043.
- [95] R.A. Mehl, T.P. Begley, Mechanistic studies on the repair of a novel photolesion: the spore photoproduct, *Org. Lett.* 1 (1999) 1065–1066.
- [96] J. Cheek, J.B. Broderick, Direct H atom abstraction from spore photoproduct C-6 initiates DNA repair in the reaction catalyzed by spore photoproduct lyase: evidence for a reversibly generated adenosyl radical intermediate, *J. Am. Chem. Soc.* 124 (2002) 2860–2861.
- [97] G. Horneck, R. Facius, G. Reitz, P. Rettberg, C. Baumstark-Khan, R. Gerzer, Critical issues in connection with human planetary missions: protection of and from the environment, *Acta Astronaut.* 49 (2001) 279–288.
- [98] A.C. Schuerger, Microbial ecology of the surface exploration of Mars with human-operated vehicles, in: C.S. Cockell (Ed.), *Martian Expedition Planning*, Univelt Publishers, Santa Barbara, CA, 2004, pp. 363–386 (American Astronautical Society publication AAS 03-322).
- [99] J. Koike, T. Hori, Y. Katahira, K.A. Koike, K.L. Tanaka, K. Kobayashi, Y. Kawasaki, Fundamental studies concerning planetary quarantine in space, *Adv. Space Res.* 18 (1996) 339–344.
- [100] C.A. Hagen, J.F. Godfrey, R.H. Green, The effect of temperature on the survival of microorganisms in a deep space vacuum, *Space Life Sci.* 3 (1971) 108–117.
- [101] G. Horneck, Astrobiology studies of microbes in simulated interplanetary space, in: P. Ehrenfreund (Ed.), *Laboratory Astrophysics and Space Research*, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1999, pp. 667–685.
- [102] A.C. Schuerger, J.T. Richards, D.A. Newcombe, K.J. Venkateswaran, Survival of seven *Bacillus* spp. under simulated Mars UV irradiation suggests minimum forward contamination around landing sites, *Int. J. Astrobiol.* 2004 (Suppl. 1) (2004) 77.
- [103] B. Keller, G. Horneck, Action spectra in the vacuum UV and far UV (122–300 nm) for inactivation of wet and vacuum-dry spores of *Streptomyces griseus* and photoreactivation, *J. Photochem. Photobiol. B: Biol.* 16 (1992) 61–72.
- [104] R.M. Tyrrell, A common pathway for protection of bacteria against damage by solar UVA (334, 365 nm) and an oxidizing agent (H₂O₂), *Mutat. Res.* 145 (1985) 129–136.
- [105] G. Horneck, Exobiological experiments in earth orbit, *Adv. Space Res.* 22 (1998) 317–326.
- [106] J. Koike, T. Oshima, K.A. Koike, H. Taguchi, K.L. Tanaka, K. Nishimura, M. Miyaji, Survival rates of some terrestrial microorganisms under simulated space conditions, *Adv. Space Res.* 12 (1992) 271–274.
- [107] G. Horneck, Radiobiological experiments in space: a review, *Int. J. Radiat. Appl. Instrum. Part D* 20 (1992) 185–205.
- [108] L.W. Townsend, J.W. Wilson, The interplanetary radiation environment and methods to shield from it, in: C.R. Stoker, C. Emmhart (Eds.), *Strategies for Mars: A Guide to Human Exploration*, American Astronautical Society, San Diego, CA, 1996, pp. 283–323.
- [109] O.W. Lazareth, M. Divadeenam, H. Ludewig, M.S. Spergel, S. Mughabghab, E.C. Selcow, T.E. Ward, J.R. Powell, Human radiation dose received during a manned Mars mission, in: T.R. Meyer (Ed.), *The Case for Mars IV: The International Exploration of Mars*, American Astronautical Society, San Diego, CA, 1997, pp. 139–146.
- [110] J.F. Bell III, et al., Mineralogic and compositional properties of Martian soil and dust: results from Mars Pathfinder, *J. Geophys. Res.* 105 (2000) 1721–1755.
- [111] R.V. Morris, D.C. Golden, J.F. Bell III, T.D. Shelfer, A.C. Scheinost, N.W. Hinman, G. Furniss, S.A. Mertzman, J.L. Bishop, D.W. Ming, C.C. Allen, D.T. Britt, Mineralogy, composition, and alteration of Mars Pathfinder rocks and soils: evidence from multispectral, elemental, and magnetic data on terrestrial analogue, SNC meteorite, and Pathfinder samples, *J. Geophys. Res.* 105 (2000) 1757–1817.
- [112] C.R. Buhler, C.I. Calle, Chemical implications due to the low electrical breakdown in the Martian atmosphere, in: *Proceedings Electrostatics Society of America, IEEE/IAS Joint Conference*, Laplainan Press, Morgan Hill, CA, 2003, pp. 565–579.
- [113] W.M. Farrell, M.L. Kaiser, M.D. Desch, J.G. Houser, S.A. Cummer, D.M. Wilt, G. Landis, Detecting electrical activity from Martian dust storms, *J. Geophys. Res.* 104 (2003) 3795–3801.
- [114] F.H. Hauck, *Safe on Mars: Precursor Measurements Necessary to Support Human Operations on the Martian Surface*, National Academy Press, Washington, DC, 2002.
- [115] G. Horneck, Response of *Bacillus subtilis* spores to space environment: results from experiments in space, *Origins Life Evol. Biosphere* 23 (1993) 37–52.
- [116] G. Horneck, U. Eschweiler, G. Reitz, J. Wehner, R. Willimek, K. Strauch, Biological responses to space: results of the experiment “exobiological unit” of ERA on EURECA 1, *Adv. Space Res.* 16 (1995) 105–111.
- [117] G. Horneck, H. Bucker, G. Reitz, H. Requardt, K. Dose, K.D. Martens, H.H. Mennigmann, P. Weber, Microorganisms in the space environment, *Science* 225 (1984) 226–228.

- [118] R.H. Green, D.M. Taylor, E.A. Gustan, S.J. Fraser, R.L. Olson, Survival of microorganisms in a simulated Martian environment, *Space Life Sci.* 3 (1971) 12–24.
- [119] C.A. Hagen, E.J. Hawrylecz, R. Ehrlich, Survival of microorganisms in a simulated Martian environment. I. *Bacillus subtilis* var. *globigii*, *Appl. Microbiol.* 12 (1964) 215–218.
- [120] C.A. Hagen, E.J. Hawrylecz, R. Ehrlich, Survival of microorganisms in a simulated Martian environment. II. Moisture and oxygen requirements for germination of *Bacillus cereus* and *Bacillus subtilis* var *niger* spores, *Appl. Microbiol.* 15 (1967) 285–291.
- [121] E.J. Hawrylecz, C. Hagen, V. Tolkacz, R. Ehrlich, Effect of reduced barometric pressure on water availability related to microbial growth, *Life Sci. Space Res.* 5 (1967) 174–186.
- [122] E.J. Hawrylecz, C.A. Hagen, R. Ehrlich, Response of microorganisms to a simulated Martian environment, *Life Sci. Space Res.* 3 (1964) 64–73.
- [123] A.A. Imshenetsky, L.A. Kouzyurina, V.M. Jakshina, On the multiplication of xerophilic micro-organisms under simulated Martian conditions, *Life Sci. Space Res.* 11 (1973) 63–66.
- [124] J. Koike, T. Oshina, Planetary quarantine in the solar system. Survival rates of some terrestrial microorganisms under simulated space conditions by proton irradiation, *Acta Astronaut.* 29 (1993) 629–632.
- [125] R.L. Mancinelli, M. Klovstad, Martian soil and UV radiation: microbial assessment on spacecraft surfaces, *Planet. Space Sci.* 48 (2000) 1093–1098.
- [126] E. Packer, S. Scher, C. Sagen, Biological contamination of Mars II. Cold and aridity as constraints on the survival of terrestrial microorganisms in simulated Martian environments, *Icarus* 2 (1963) 293–316.
- [127] C.A. Hagen, E.J. Hawrylecz, B.T. Anderson, M.L. Cephus, Effect of ultraviolet on the survival of bacteria airborne in simulated martian dust clouds, *Life Sci. Space Res.* 8 (1970) 53–58.
- [128] H. Bucker, G. Horneck, H. Wollenhaupt, M. Schwager, G.R. Taylor, Viability of *Bacillus subtilis* spores exposed to space environment in the M-191 experiment system aboard Apollo 16, *Life Sci. Space Res.* 12 (1974) 209–213.
- [129] G. Horneck, H. Bucker, G. Reitz, Long-term survival of bacterial spores in space, *Adv. Space Res.* 14 (1994) 41–45.
- [130] P.R. Lorenz, J. Hotchin, A.L. Markusen, G.B. Orlob, C.L. Hemenway, D.S. Hallgren, Survival of microorganisms in space, *Space Life Sci.* 1 (1968) 118–130.
- [131] D.J. Des Marais, L.J. Allamandola, S.A. Benner, A.P. Boss, D. Deamer, P.F. Falkowski, J.D. Farmer, S.B. Hedges, B.M. Jakosky, A.H. Knoll, D.R. Liskowsky, V.S. Meadows, M.A. Meyer, C.B. Pilcher, K.H. Nealson, A.M. Spormann, J.D. Trent, W.W. Turner, N.J. Woolf, H.W. Yorke, The NASA astrobiology roadmap, *Astrobiology* 3 (2003) 219–235.
- [132] J.B. Garvin, O. Figueroa, F.M. Naderi, NASA's new Mars exploration program: the trajectory of knowledge, *Astrobiology* 1 (2001) 439–446.