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Survival of endospores of *Bacillus subtilis* on spacecraft surfaces under simulated martian environments: implications for the forward contamination of Mars

Andrew C. Schuerger,[a,*] Rocco L. Mancinelli,[b] Roger G. Kern,[c] Lynn J. Rothschild,[d] and Christopher P. McKay[e]

[a] *Dynamac Corporation, Mail Code DYN-3, Kennedy Space Center, FL 32899, USA*
[b] *SETI Institute, NASA–Ames Research Center, Moffett Field, CA 94035, USA*
[c] *Jet Propulsion Lab, Calif. Institute of Technology, Mars Exploration Directorate, Pasadena, CA 91109, USA*
[d] *Ecosystem Science and Technology Branch, NASA–Ames Research Center, Moffett Field, CA 94035, USA*
[e] *Space Science Division, NASA–Ames Research Center, Moffett Field, CA 94035, USA*

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Abstract

Experiments were conducted in a Mars simulation chamber (MSC) to characterize the survival of endospores of *Bacillus subtilis* under high UV irradiation and simulated martian conditions. The MSC was used to create Mars surface environments in which pressure (8.5 mb), temperature (−80, −40, −10, or +23 °C), gas composition (Earth-normal N_2/O_2 mix, pure N_2, pure CO_2, or a Mars gas mix), and UV-VIS-NIR fluence rates (200–1200 nm) were maintained within tight limits. The Mars gas mix was composed of CO_2 (95.3%), N_2 (2.7%), Ar (1.7%), O_2 (0.2%), and water vapor (0.03%). Experiments were conducted to measure the effects of pressure, gas composition, and temperature alone or in combination with Mars-normal UV-VIS-NIR light environments. Endospores of *B. subtilis*, were deposited on aluminum coupons as monolayers in which the average density applied to coupons was 2.47×10^6 bacteria per sample. Populations of *B. subtilis* placed on aluminum coupons and subjected to an Earth-normal temperature (23 °C), pressure (1013 mb), and gas mix (normal N_2/O_2 ratio) but illuminated with a Mars-normal UV-VIS-NIR spectrum were reduced by over 99.9% after 30 sec exposure to Mars-normal UV fluence rates. However, it required at least 15 min of Mars-normal UV exposure to reduce bacterial populations on aluminum coupons to non-recoverable levels. These results were duplicated when bacteria were exposed to Mars-normal environments of temperature (−10 °C), pressure (8.5 mb), gas composition (pure CO_2), and UV fluence rates. In other experiments, results indicated that the gas composition of the atmosphere and the temperature of the bacterial monolayers at the time of Mars UV exposure had no effects on the survival of bacterial endospores. But Mars-normal pressures (8.5 mb) were found to reduce survival by approximately 20–35% compared to Earth-normal pressures (1013 mb). The primary implications of these results are (a) that greater than 99.9% of bacterial populations on sun-exposed surfaces of spacecraft are likely to be inactivated within a few tens of seconds to a few minutes on the surface of Mars, and (b) that within a single Mars day under clear-sky conditions bacterial populations on sun-exposed surfaces of spacecraft will be sterilized. Furthermore, these results suggest that the high UV fluence rates on the martian surface can be an important resource in minimizing the forward contamination of Mars.

Keywords: Mars; Exobiology; Spectroscopy; Regoliths; Ultraviolet observations

1. Introduction

Microbial contamination of robotic spacecraft could pose a significant risk to near-term Mars surface missions by increasing the forward contamination of scientific payloads, local landing sites, or the global martian environment. Species of cultivable microorganisms on unmanned spacecraft surfaces have been characterized and include species of bacteria, yeasts, actinomycetes, and fungi that are closely associated with human activities in vehicle assembly areas (Favero et al., 1966; Favero, 1971; Puleo et al., 1973, 1977; Venkateswaran et al., 2001). Environmental microorganisms from soils and airborne deposition also can contribute to the microbial bioloads on unmanned spacecraft (Foster and

* Corresponding author.
E-mail address: schueac@kscems.ksc.nasa.gov (A.C. Schuerger).

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Winans, 1975; Ruschmeyer and Pflug, 1977). The most common cultivable bacteria recovered from unmanned spacecraft include species of *Staphylococcus*, *Micrococcus*, *Streptococcus*, *Bacillus*, *Corynebacterium*, and *Flavobacterium* (Favero et al., 1966; Favero, 1971; Puleo et al., 1973, 1977; Taylor, 1974; Venkateswaran et al., 2001). Species diversity and biomass of cultivable microorganisms recovered from unmanned spacecraft surfaces (Favero, 1971; Puleo et al., 1973, 1977; Venkateswaran et al., 2001) are nearly identical to those recovered from airborne dust in cleanrooms (Favero et al., 1966). In contrast, species of non-cultivable microorganisms have not been adequately characterized on spacecraft surfaces (see Venkateswaran et al., 2001, 2003 for recent progress in this area).

Although the cultivable microflora of unmanned spacecraft was well documented in the 1960's, most pre-Viking robotic spacecraft did not receive heat-sterilization treatments prior to launch (Favero, 1971). The microbial bioloads on these early spacecraft were estimated to range between 1×10^4 to 2×10^8 cultivable microorganisms per vehicle (Favero, 1971). During the Viking missions, spacecraft sanitation and assembly protocols were enhanced in order to reduce the total pre-sterilization bioloads to approximately 6×10^3 viable microorganisms per vehicle (Puleo et al., 1977). To reduce the bioload further, the fully integrated Viking 1 and 2 landers were heat-sterilized for 30 or 23 h, respectively, at 112 °C after internal portions of the vehicles reached at least 110 °C (Puleo et al., 1977; DeVincenzi et al., 1998). The heat-sterilization procedures reduced the bioloads on both landers to less than 2×10^{-4} viable spore-forming bacteria per vehicle (Puleo et al., 1977). The pre-sterilization bioloads and surface cleaning procedures used with the Viking landers (VL) continue to be considered as the standards for Mars surface missions (Rummel, 2001). However, current mission protocols for Mars do not automatically require heat-sterilization of fully integrated vehicles (DeVincenzi et al., 1998), and, thus, spacecraft bioloads present at launch for more recent missions are significantly higher than those encountered during Viking.

In order to mitigate against the microbial contamination of life-detection experiments or samples slated for Earth-return missions, the ecologies of microorganisms on spacecraft must by understood and modeled from initial assembly of spacecraft components until the termination of each mission on Mars. Key factors to model include pre-launch microbial ecologies of spacecraft, effects of spacecraft sanitation procedures on microbial viability, microbial survival during the cruise phase of each mission, dispersal of viable spores away from landed vehicles, and survival of microorganisms in martian environments. Of these factors, the microbial ecologies of unmanned spacecraft, sanitation procedures of spacecraft surfaces, and survival of terrestrial microorganisms in simulated martian environments have been the most widely studied. As discussed above, the species of common cultivable microorganisms on unmanned spacecraft are reasonably well understood, and the microorganisms of general concern are those associated with human activities in vehicle assembly areas (Favero et al., 1966; Favero, 1971; Puleo et al., 1973, 1977; Venkateswaran et al., 2001). Spacecraft sterilization procedures (Crow and Smith, 1995; Pflug, 1971; Sagan and Coleman, 1965) are outside the scope of the current study but include heat-sterilization, surface cleaning with biocidal chemicals, gaseous sterilants, and gas-plasma sterilization. Long-term survival of up to 5.9 years has been demonstrated for *Bacillus subtilis* in space, as long as endospores were protected from exposure to direct solar UV irradiation (Horneck et al., 1994, 1995). But the literature on survival of microorganisms under martian conditions has often involved simulations that failed to create accurate surface conditions now known to exist on Mars.

Over the past 50 years, at least 33 papers have been published reporting various conditions of microbial survival under simulated martian conditions (Foster et al., 1978; Green et al., 1971; Hagen et al., 1964, 1967; Hawrylewicz et al., 1962, 1964; Imshenetsky et al., 1973; Koike and Oshima, 1993; Koike et al., 1993, 1996; Kooistra et al., 1957; Mancinelli and Klovstad, 2000; Packer et al., 1963; Young et al., 1964, and the citations within these papers). Although these studies examined different aspects concerning microbial survival under simulated martian conditions, a few general conclusions may be drawn from this body of work. First, terrestrial microorganisms survived well under low temperature, low pressure, and N_2 or CO_2 atmospheres; exhibiting reductions in microbial populations of one to several orders of magnitude (Foster et al., 1978; Hagen et al., 1964, 1967; Hawrylewicz et al., 1962, 1964; Imshenetsky et al., 1973). Several studies (Hagen et al., 1964, 1967; Hawrylewicz et al., 1964) reported the recovery of equal or greater numbers of microbial populations at the end of specific experiments compared to initial populations; but these studies utilized increased levels of partial pressures of O_2 and slightly higher total atmospheric pressures (50–100 mb) than would be found on the surface or Mars. No papers were found in the literature that demonstrated microbial replication under robustly simulated Mars surface conditions. In addition, Packer et al. (1963) cautioned that slight increases in populations of microbes under simulated martian conditions might be due to deaggregation factors (on the order of 2 to 20 times) which might break-up small groups of cells, and, thus, artificially inflate microbial populations at the ends of experiments. Second, UV irradiation was the key parameter that determined survivability of microorganisms under simulated martian conditions; direct exposure to UV irradiation resulted in rapid and nearly complete inactivation of microbial cultures (Green et al., 1971; Koike et al., 1996; Mancinelli and Klovstad, 2000; Packer et al., 1963). Third, thin layers of Mars analog soil were generally adequate for protecting microorganisms from the lethal effects of UV irradiation (Mancinelli and Klovstad, 2000; Packer et al., 1963). Fourth, freeze-thaw cycles generally did not reduce microbial survival rates under simulated martian conditions (Foster et al., 1978; Packer et al., 1963;

Young et al., 1964). Fifth, proton irradiation (used to simulate galactic cosmic rays) may be a factor in reducing the survival of terrestrial microorganisms on Mars (Koike et al. 1992, 1996; Koike and Oshima, 1993), but these studies used extremely high proton dosage rates equal to between 200 to 250 years on Mars delivered within a few days to a few weeks. New research should be conducted that simulates reasonable daily proton flux rates in order to accurately predict short-term effects of proton irradiation on survival rates of terrestrial microorganisms on Mars.

In contrast, there are several key questions that have not been addressed in this body of literature. First, none of the studies discussed above tested microbial survival on actual spacecraft materials or components. Most of the studies tested microbial survival of either microorganisms maintained as dried biofilms or as pure cultures added to terrestrial soils or low-fidelity Mars analog soils (e.g., use of terrestrial field soils by Packer et al. (1963)). Second, UV irradiation models generally were either not included in the Mars simulations (most of the literature) or were not matched to accurate simulations of Mars UV fluence rates (Mancinelli and Klovstad, 2000; Hagen et al., 1970; Koike et al., 1996; Packer et al., 1963). For example, Hagen et al. (1970) did not characterize the spectral quality of the UV flux in their tests, and, thus, it is difficult to evaluate their results. Koike et al. (1996) tested microbial survival under a much broader range of UV irradiation (115 to 400 nm) than has been predicted for the martian surface (190 to 400 nm; sensu Kuhn and Atreya, 1979). Packer et al. (1963) exposed microbial samples to monochromatic UV irradiation at 254 nm, and used a high N_2 and low CO_2 atmosphere. Mancinelli and Klovstad (2000) used a calibrated deuterium lamp for their UV irradiation source, which supplied a much higher fluence rate in the short-wavelength UVC region (200 to 280 nm) compared to the solar spectrum (Arvesen et al., 1969). Only the work by Green et al. (1971) used an accurate UV simulation (200 to 2500 nm) supplied by xenon-arc lamps to study microbial survival under simulated martian conditions. However, Green et al. (1971) studied microbial survival in a low-fidelity Mars analog soil (limonite). Third, few studies selected species of terrestrial microorganisms based on their occurrence as contaminants on unmanned spacecraft. Microorganisms were generally common soil microorganisms or microorganisms associated with terrestrial sites from extreme environments. And fourth, a diversity of temperatures, gas compositions, pressures, and analog soils were used in previous Mars simulations (Green et al., 1971; Hagen et al., 1970; Packer et al., 1963), some of which differed significantly from what is currently known about Mars (Kieffer et al., 1992; Rieder et al., 1997; Schofield et al., 1997). No studies were found in the literature in which temperature, gas composition, pressure, UV irradiation, and spacecraft materials were simultaneously simulated for microbial survival experiments pertaining to near-term robotic missions to Mars.

The objectives of the current studies were to

(i) develop a robust UV irradiation model for Mars,
(ii) conduct a series of experiments to investigate the survival of endospores of *Bacillus subtilis* under robustly simulated Mars-like conditions,
(iii) determine the minimum UV dosage rate required to inactivate bacterial populations on spacecraft materials,
(iv) characterize the effects of temperature, gas composition, and pressure at the time of UV-exposure on the survival of bacterial populations, and
(v) study the protective role of dust coatings on bacterial survival on spacecraft components.

The bacterium, *Bacillus subtilis*, was chosen for these experiments based on its use in a significant portion of the previous studies on microbial survival under simulated martian environments (see above), its occurrence as a common microbial contaminant of unmanned spacecraft surfaces (Favero et al., 1966; Favero, 1971; Puleo et al., 1973, 1977; Taylor, 1974), and its resistance to a wide range of harsh environmental factors including heat (Nicholson et al., 2000), UV irradiation (Lindberg and Horneck, 1991; Nicholson et al., 2000; Setlow, 1988; Slieman and Nicholson, 2001), desiccation (Dose et al., 1991; Dose and Gill, 1995), low temperature (Nicholson et al., 2000), and high vacuum (Nicholson et al., 2000; Horneck et al., 1994, 1995). The current study does not examine the effects of martian conditions on non-cultivable microorganisms present on spacecraft surfaces, nor does it attempt to model a diversity of cultivable microbial species that might exhibit greater resistance to UV irradiation, high vacuum, desiccation, and temperature extremes than is found in *B. subtilis*. But the use of *B. subtilis* as a model does provide a good first-order approximation into the effects of the martian environment on the survival of common microorganisms on spacecraft surfaces.

2. Materials and methods

2.1. Mars simulation chamber

Simulated Mars experiments were conducted within a Mars simulation chamber (MSC) operated by the Materials Sciences Laboratory, Physical Testing Group in the Operations and Checkout (O&C) Building at Kennedy Space Center (KSC), FL (Figs. 1 and 2). The MSC is a stainless steel low-pressure cylindrical chamber with internal dimensions measuring 1.5 m long by 0.8 m in diameter. Temperature control of biological specimens was achieved with a liquid-nitrogen (LN2) cold plate (model TP2555 Thermal Platform, Sigma Systems Corporation, San Diego, CA USA) placed within the MSC and adjusted with an external control unit (Fig. 2A). Bacterial monolayers were deposited onto aluminum coupons, mounted to base plates of microbial holders (described below), and then attached to the LN2 cold plate (Fig. 2). Temperatures within the MSC were recorded

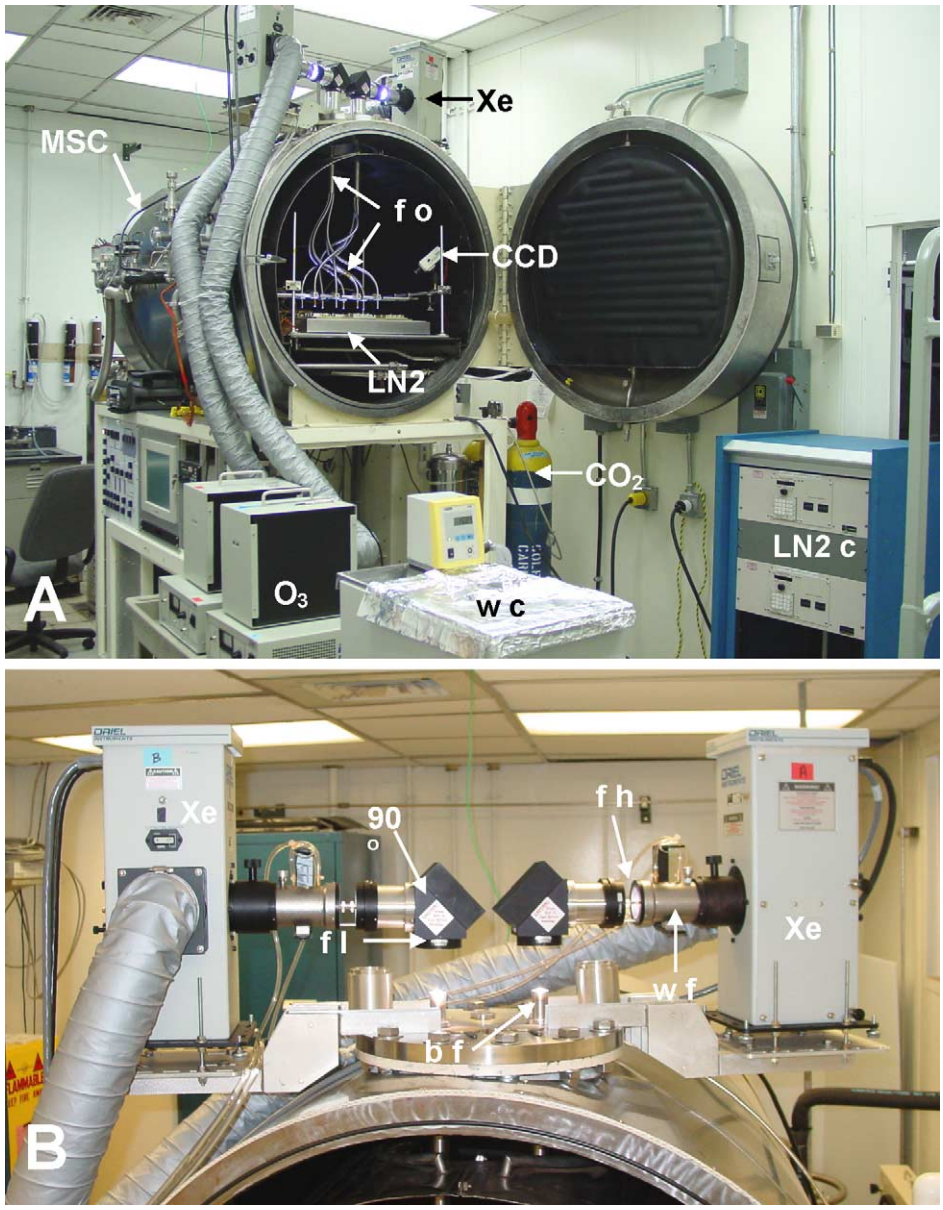

Fig. 1. (A) The Mars simulation chamber (MSC) was configured with a liquid-nitrogen cold-plate (LN2) programmed by an external controller (LN2 c). Carbon dioxide (CO_2), N_2, air, or Mars gas were supplied to the MSC via high-grade gas mixtures in standard K-bottles. Ozone produced by the xenon-arc (Xe) lamps was scrubbed by passing ozone-enriched cooling-air through charcoal filters (O_3). A charged-coupled device camera (CCD) was mounted within the MSC to view the UV-illuminated targets during each experiment. A water chiller (wc) supplied 15 °C water to water-filters mounted near Xe lamps. Various control panels can be seen on the left of the MSC. (B) The MSC was configured with two, 450 W Xe lamps that focused UV-VIS-NIR irradiation onto two bulkhead fittings (bf) and distributed the UV-enhanced light within the MSC via a series of fiber-optic bundles (fo). The UV-enhanced light from both Xe lamps passed through 6-cm water filters (wf) and 5-cm glass filter holders (fh), reflected off 90-degree beam turning mirrors (90°), and was focused by lenses (fl) onto the tops of 12.5 mm diameter fiber-optic bundles mounted within stainless steel bulkhead fittings (bf).

by a wireless datalogger (model 2625A Hydra Data Logger, Fluke Corp., Everett, WA, USA). Thermocouples were affixed to various surfaces and spacecraft materials within the MSC to accurately determine temperatures of bacterial monolayers placed upon the LN2 cold-plate. Control of atmospheric pressure was achieved to within ±0.1 mb (±10 Pa) of specific set-points. The pumping sub-system for the pressure control system within the MSC worked against a constant flow of gases supplied by bottled gas-mixes placed outside the MSC (Fig. 1A). Constant flow rates of the bottled gases were required to flush room-air from the MSC that leaked into the chamber during normal operations. Bottled gas-mixtures were purchased from a commercial vendor (Boggs Gases Co., Titusville, FL, USA) and contained either nitrogen (N_2 > 99.99% purity), carbon dioxide (CO_2 > 99.99% purity), air (N_2 at 78%, O_2 at 21%, and trace gases at approximately 1%), or Mars gas (composed of the following: CO_2 (95.3%), N_2 (2.7%), Ar (1.7%), O_2 (0.2%), and H_2O (0.03%)). The composition of the Mars gas was based on the results of the two Viking missions

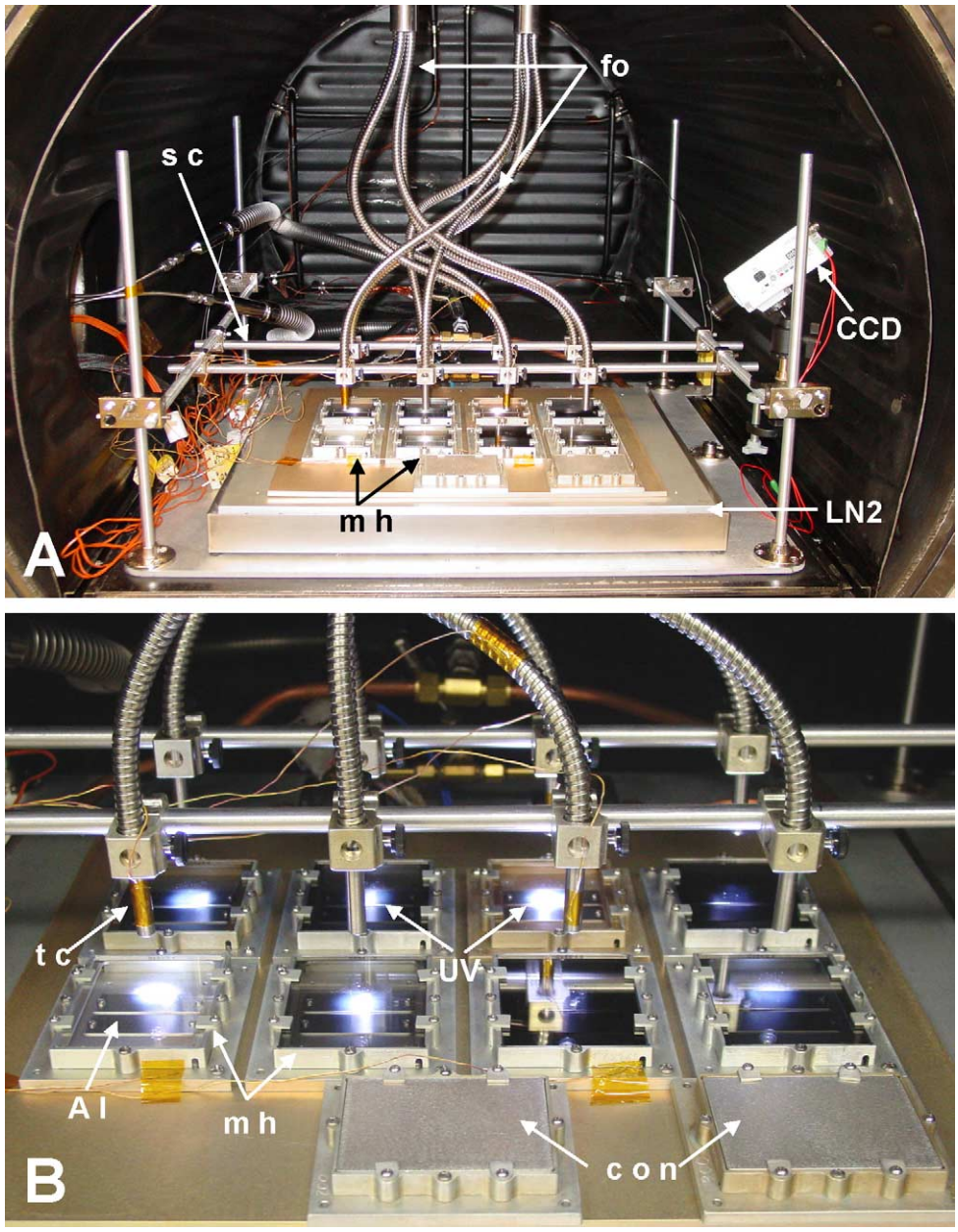

Fig. 2. (A) Eight, microbial holders (mh) were attached to the upper surface of the liquid-nitrogen (LN2) cold plate. The fiber-optic bundles (fo) that distributed the Mars-normal UV irradiation were held precisely in place by aluminum scaffolding (sc). A charged-coupled device (CCD) camera was used to monitor the glass plates on the microbial holders to confirm that no water ice or CO_2 frost formed during the course of each experiment. (B) The microbial holders usually contained one aluminum (Al) coupon per holder. Depicted here is a test in which the aluminum coupons illuminated with UV irradiation (UV) possessed bacterial monolayers, while the second aluminum coupon per holder was sterilized but without bacteria. The coupons without bacterial monolayers were then analyzed for cross-contamination. All tests were negative. Two microbial holders with aluminum covers were used as in-chamber controls (con). Thermocouple wires (tc) were attached to various surfaces with amber-colored kapton tape.

(reviewed by Owen (1992)). Gas compositions of all gases were monitored during experimental tests using a residual gas analyzer (RGA) (Transpector-2 Gas Analysis System, Leybold Inficon, East Syracuse, NY, USA). The RGA system was capable of partial pressure measurements of each gas from 1×10^{1} to 3×10^{-9} mb, depending on the mass-weight of the gas.

An ultraviolet (UV), visible (VIS), and near-infrared (NIR) illumination system was developed in order to deliver to the top surfaces of the bacterial monolayers a simulated Mars light environment. The UV-VIS-NIR system used two, 450 W xenon-arc lamps (model 6262, Oriel Instruments, Stratford, CA, USA) mounted on the outside of the MSC (Fig. 1). In Fig. 1B, the optical systems are shown in which the UV-enriched light first passed through 6-cm-long water filters (Oriel Instruments) to remove high-intensity mid-infrared (MIR) irradiation; light attenuation began at 1200 nm and was continuous out to 2500 nm (Fig. 3). The re-

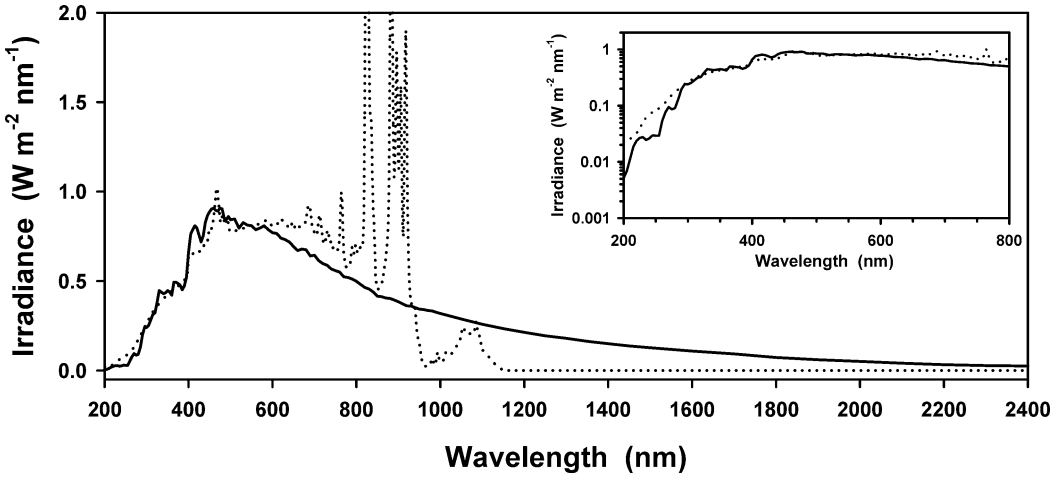

Fig. 3. The Mars solar constant (—) was based on the solar spectrum as reported by Arvesen et al. (1969) and modified by Kuhn and Atreya (1979) and Appelbaum and Flood (1990). The simulated Mars-normal UV-VIS-NIR irradiation (• • • • •) was measured after the MSC lighting system was fully calibrated to deliver a Mars solar constant flux to the upper surfaces of the aluminum coupons.

duction in MIR was required in order to prevent IR damage to the UV-transmitting fiber-optic bundles (described below). The UV-enhanced light was then reflected off of 90-degree beam-turning mirrors and focused onto the tops of two MSC bulkhead fittings. Each bulkhead fitting was configured with a 12.5 mm core of UV-transmitting fiber-optic bundles that transmitted the simulated Mars spectrum across the pressure differential of the MSC. All optical elements within the xenon-arc illumination systems were composed of highly purified fused-silica optics that transmitted UV irradiation down to 180 nm. Within the MSC (Fig. 2), fiber-optic arm assemblies (0.7 m long) were attached to the bottoms of the bulkhead fittings. The fiber-optic arm assemblies had common 12.5 mm thick fiber-optic bundles at the points of attachment to the bulkhead fittings, but were then split into four, 6.25 mm thick, fiber-optic arms at the distal ends of the fiber-optic assemblies. The ends of the fiber-optic arms were then held in-place by aluminum scaffolding assembled within the MSC (Fig. 2). Thus, eight separate fiber-optic arms could be precisely aligned to direct a simulated Mars-normal light spectrum onto biological specimens maintained within microbial holders (Fig. 2). The fiber-optic bundles were manufactured by CeramOptec (East Longmeadow, MA, USA) and were composed of individual fibers made of 200-µm diameter pure-silica cores of Optran UVNS non-solarizing fibers, 220 µm doped-silica cladding material, and wrapped in 245-µm polvimide coatings. The fiber-optic bundles were contained within flexible stainless steel sheaths that permitted a high-degree of movement within the MSC. The spectral range of the fiber bundles were rated by the manufacturer as 193–1200 nm. The transmissivity of the Optran non-solarizing fiber-optic bundles were ideal for these Mars simulations because the martian atmosphere is generally transparent to UV irradiation down to 190 nm (Kuhn and Atreya, 1979) and the in-line water filter (Fig. 1B) attenuated all MIR above 1200 nm.

2.2. Mars UV model

A Mars irradiance model was developed in order to match the UV-VIS-NIR output of the xenon-arc lamps to a reasonable simulation of the Mars solar constant. The Mars UV model was based on the work of Appelbaum and Flood (1990), Arvesen et al. (1969), and Kuhn and Atreya (1979); and was similar to the UV models developed by Cockell et al. (2000) and Patel et al. (2002). The Mars UV model was developed to represent the UV fluence rate on equatorial Mars without a significant contribution of ozone to UV absorption. Fluence rates from 200–2500 nm of Earth's solar constant (Arvesen et al., 1969) were scaled to the mean orbital distance to Mars producing a Mars solar constant that was 43% that of Earth's (Fig. 3). The mean integrated beam irradiance at the top of the atmosphere for Mars was modeled by Appelbaum and Flood (1990) to be 590 W m^{-2} from 200–40,000 nm. This was constrained slightly by limiting the UV-VIS-NIR fluence rate to 578 W m^{-2} based on a spectral range of 200–2500 nm, which was the range measurable with the existing spectrometers on-hand at KSC. The MSC UV-enhanced illumination system was precisely aligned and calibrated to deliver a simulated Mars solar constant to the surfaces of the bacterial monolayers placed on aluminum coupons and held within the microbial holders (Fig. 2B).

The data in Fig. 3 and Table 1 were collected using three different spectrometers to optimize the sensitivities of each instrument to specific spectral ranges. The UV region from 200 to 400 nm was measured with a model OL 754 high-resolution UV spectrometer from Optronic Laboratories, Inc. (Orlando, FL, USA). The VIS and NIR regions from 350 to 1100 nm were measured with a model LI-1800 field spectrometer from Li-COR, Ltd. (Lincoln, NB, USA). And the MIR region from 1000 to 2500 nm was measured with an ASD Field Spec Pro from Analytical Spectral Devices, Inc. (Boulder, CO, USA). The spectral data were convolved into a single spectrum (Fig. 3) and fluence rates for specific

Table 1
Simulated Mars irradiance levels for ultraviolet (UV), visible (VIS), near-infrared (NIR), and mid-infrared (MIR)

Spectral ranges (nm)	Earth solar constant ($W\,m^{-2}$)[a]	Mars solar constant ($W\,m^{-2}$)[b]	Mars chamber simulation ($W\,m^{-2}$)[c]
UVC (200–280)	7.39	3.18	5.86
UVB (280–315)	19.49	8.38	8.49
UVA (315–400)	89.28	38.39	36.56
Total UV (200–400)	116.16	49.95	50.91
VIS (400–700)	520.28	223.73	240.5
NIR (700–1100)	448.74	141.90	245.0
MIR (1100–2500)	259.05	162.48	0
Total IR (700–2500)	707.79	304.38	245.0
Total irradiance (200–2500)	1344.23	578.06	536.41

[a] Based on Arvesen et al. (1969).
[b] Estimated as 43% of Earth's solar constant from Kuhn and Atreya (1979).
[c] Based on direct measurements of the Mars Simulation Chamber's UV-VIS-NIR fluence rates using an Optronic Laboratories OL-754 UV-VIS spectroradiometer (200–400 nm), a LiCOR 1800 VIS-NIR spectroradiometer (400–1100 nm), and an Analytical Spectral Devices Field Spec (1100–2500 nm).

spectral ranges were estimated (Table 1). Furthermore, the spectral data in Fig. 3 and Table 1 were measured after the UV-VIS-NIR light had passed through all optical elements, including the fully assembled fiber-optic bundles, and was adjusted to equal the total UV fluence rate of 50 $W\,m^{-2}$ (from 200 to 400 nm) as predicted by the Mars solar constant model (Table 1). The primary objective in designing the MSC illumination system was to accurately simulate the total UV spectrum in the Mars solar constant, and then to measure the fluence rates of the VIS, NIR, and MIR spectral regions. The emphasis was placed on accurately simulating the UV region because it was assumed that UV light would be the primary biocidal factor in the Mars simulations. Thus, the simulated Mars-normal spectrum used here accurately reproduced the UV spectral region from 200–400 nm, was slightly higher in the VIS region from 400–700 nm, was significantly higher in the NIR (700–1100 nm), and did not reproduce the MIR region (1200–2500 nm) (Table 1). The higher NIR fluence rate here was not believed to be a problem because this region does not contribute to biocidal effects of light on bacteria. Furthermore, the irradiance level of the full spectrum from 200–2500 nm was only slightly lower than the predicted Mars solar constant (Table 1).

A series of neutral density filters were used to simulate the attenuation of the Mars solar constant by Rayleigh scattering and atmospheric dust. The ND filters were made of 3-mm thick fused-silica glass plates coated with increasing amounts of a nickel-chromium-iron alloy (manufactured by Maier Photonics, Inc., Manchester Center, VT, USA). The transmissivity characteristics of the ND filters are given in Fig. 4 and Table 2. All ND filters were spectrally flat in their transmission of light from 400–1100 nm (Fig. 4) but exhibited slight to moderate increases in attenuation of light at shorter wavelengths of UV (< 350 nm). The level of UV attenuation increased with increasing optical densities of the ND filters, and, thus, approximated a first-order simulation of UV attenuation by atmospheric dust on Mars. The optical densities of the ND filters were selected to simulate optical depths (*tau*) of the martian atmosphere under different dust-loaded conditions (Table 2). The transmissivities

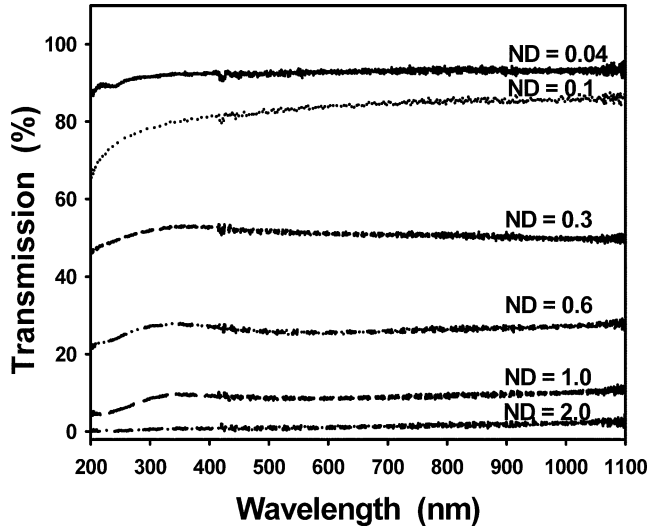

Fig. 4. Transmissivities of neutral density (ND) filters were measured with a Beckman DU-640 spectrometer (Beckman Instruments, Inc., Fullerton, CA, USA). All ND filters were spectrally flat from 400–1100 nm but exhibited slight to moderate increases in attenuation of light at shorter wavelengths of UV (< 350 nm). Scaling issues hide the approximately 50% reduction in UV irradiation observed below 350 nm for the ND = 2.0 filter.

of the ND filters were selected based on the models of Appelbaum and Flood (1990) for a solar zenith angle of zero and constrained to the net fluence rates for the total UV direct beam only. Diffuse irradiation could not be simulated in the configuration of the MSC lighting system used in the current study. Therefore, Beer's law (1) was used to match the transmissivity of the ND filters (directly measured with a Beckman DU-640 spectrometer from Beckman Instruments, Inc., Fullerton, CA, USA) to increasing levels of *tau* by the formula

$$T = e^{-tau/\mu} \tag{1}$$

where T equals the transmissivity of the atmosphere and μ equals the cosine of the solar zenith angle (from Haberle et al. (1993)). Furthermore, the UV transmissivity of the martian atmosphere down to 190 nm has not been adequately modeled, and, thus, no information was available on the ef-

Table 2
Simulated optical depths (*tau*) of the martian atmosphere using neutral density filters

Neutral density filters[a]	UV transmission (200–400 nm)[b]	VIS-NIR transmission (400–1100 nm)[b]	Optical depth (*tau*)	Predicted transmissivity of the martian atmosphere[c]
ND 0.04	91.1%	92.9%	0.1	90.5%
ND 0.1	77.1	84.5	0.3	74.1
ND 0.3	51.1	50.7	0.7	49.7
ND 0.6	26.0	26.4	1.4	24.7
ND 1.0	7.6	9.3	2.5	8.2
ND 2.0	0.5	1.5	3.5	3.0

[a] Ratings of neutral density filters were provided by the manufacturer (Maier Photonics, Inc., Manchester Center, VT, USA) and represented the optical densities of the filters.
[b] Actual UV and VIS transmissivities of the ND filters were determined with a Beckman DU-640 spectrometer from Beckman Instruments, Inc., Fullerton, CA, USA.
[c] Calculated transmissivities of the atmosphere on Mars of the direct beam at a solar zenith angle of zero. Based on Beer's law and described by Haberle et al. (1993).

fects that increasing dust-loads might have on changing the spectral quality of the down-welling or scattered UV irradiation. Based on the work of Appelbaum and Flood (1990) and Haberle et al. (1993), the global net irradiance levels on Mars (i.e., direct plus diffuse beams) in the visible and near-infrared should be higher than those simulated here for high optical depths, but the influence of increasing dust loads could not be adequately predicted for the UV spectral range, and, thus, are omitted in the current model. However, it is likely that the UV fluence rates will be higher than what is modeled here for high optical depths due to increased levels of diffuse UV irradiation, and, thus, the biocidal effects of UV irradiation reported here would be expected to proceed at a faster rate, particularly at high optical depths encountered under global dust storms.

2.3. Microbiological procedures

The bacterium, *Bacillus subtilis* strain HA 101, was obtained from G. Horneck (DLR, Koln, Germany) and served as the sole bacterial isolate for these experiments. The HA 101 strain possessed the auxotrophic marker gene, *his* HA 101, making it easy to differentiate from other bacteria that may have been present as environmental contaminants (Horneck, 1993). In addition, several internal controls were built into the microbiological procedures in order to confirm that only surviving populations of *B. subtilis* strain HA 101 were recovered from UV-exposed surfaces.

Endospores of *B. subtilis* were grown in a liquid sporulation medium, washed, and concentrated according to the procedures described by Mancinelli and Klovstad (2000). Concentrated suspensions of endospores were maintained at 4 °C for up to 8 weeks, after which, new populations of endospores were produced for ongoing experiments. The sporulation medium was also used in the bioassay procedure (described below) and was composed of the following in 1 liter of deionized water: 16 g Difco nutrient broth, 5 g KCl, 0.22 g $CaCl_2$, 1.6 mg $FeCl_3$, 3.4 mg $MnSO_4$, 12 mg $MgSO_4$, and 1 g D-glucose. All chemicals were obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA), except the nutrient broth medium, which was obtained from Fisher Scientific (Pittsburgh, PA, USA).

Bacterial monolayers of *B. subtilis* endospores were prepared on aluminum coupons and served as the primary experimental units for all experiments. Aluminum coupons (Seton, Inc. Brainford, CT, USA) measuring 5.3 cm × 1.7 cm × 1 mm were obtained from the manufacturer free of adhesive backings and drilled with two small 3-mm diameter holes centered at the ends of the longest dimension. The holes were used to screw the prepared coupons down to the base plates of the microbial holders (Fig. 2B). Aluminum coupons were dry-heat sterilized at 130 °C for 24 h and cooled to 24 °C prior to depositing bacterial monolayers onto the upper surfaces of the coupons. During preliminary tests, this sterilization procedure was found to inactivate large populations of *B. subtilis* strain HA 101 on a variety of metal surfaces (data not shown). Bacterial monolayers were prepared by diluting endospores in sterile deionized water (average OD = 0.034 at 400 nm) and pipetting 200 µl of the spore suspensions onto the centers of aluminum coupons. The average number of viable bacteria per 200-µl aliquot for these experiments was 2.45×10^6. Bacterial monolayers were then dried at 30 °C for 18 h and visually inspected for uniformity. Only aluminum coupons with smooth and uniform monolayers were chosen for experimental tests. Selected coupons were then mounted onto the base plates of the microbial holders (Fig. 2B) and transferred to the MSC for experiments under simulated martian conditions.

Under aseptic conditions, bacterial monolayers were deposited onto aluminum coupons and the coupons affixed to the base plates of pre-sterilized microbial holders. The microbial holders were then transported to the MSC system and attached to the LN2 cold-plate. The MSC door was closed and a Mars simulation test initiated. After concluding experimental tests (described below), the MSC was returned to an ambient Earth-normal environment, and the microbial holders removed. The bacterial monolayers were retuned to the lab where they were processed under aseptic conditions to estimate the numbers of surviving bacteria per coupon. The assay procedure was based on the work of Mancinelli and

Klovstad (2000) and is described elsewhere (Koch, 1994) as the Most Probable Numbers (MPN) procedure. In brief, UV-exposed aluminum coupons were transferred to sterile 50 cc plastic centrifuge tubes containing 20 ml of sterile deionized water (SDIW). The coupons were vigorously shaken for 10 sec and then vortexed for 2 min. For each coupon, a series of six, 10-fold dilutions were made in SDIW and then 20 µl of each dilution were transferred into each of 16 wells in a sterile 96-well micro-titre plate. All 96 wells of the micro-titre plates were previously filled with 180 µl of the sporulation medium described above. All micro-titre plates were incubated at 37 °C for 24 h, and the numbers of wells for each dilution exhibiting positive growth for HA 101 were counted. The numbers of positive wells per plate were converted to a three-digit code, and the code used to estimate the most probable number of surviving bacterial cells per coupon according to the procedures of Koch (1994). The numbers of surviving bacteria exposed to UV irradiation (N) were divided by the numbers of surviving bacteria in control treatments (No) placed within the MSC but not exposed to UV-irradiation. Thus, the values N/No represent the percentages of the original bacterial populations surviving after both UV-exposure and exposure to Mars-normal conditions of pressure, temperature, and gas composition.

2.4. Mars analog soil

Simulated martian dusts (SMD) were derived from a surficially altered ash of Pu'u Nene, a volcanic cone at 1850 m elevation on Mauna Kea's south flank, and described elsewhere (Allen et al., 1998). The palagonitic tephra was collected in 1985 by R. Singer and is very similar to the JSC Mars-1 soil simulant described by Allen et al. (1981, 1998). The volcanic palagonite closely matches the reflectance spectrum of Mars regolith (Morris and Gooding, 1990; Morris et al., 1993) and is similar in chemical composition, particle size, density, porosity, and magnetic properties of surface soils on Mars (Allen et al., 1981, 1998; Morris and Gooding, 1990). The palagonite is primarily composed of hawaiite, calcium feldspar, with traces of magnetite, hematite, pyroxene, olivine and glasses composed of SiO_2, Fe_2O_3, and CaO (Allen et al., 1998). The majority of the iron (64%) is present as nanophase (< 20 nm) ferric oxide particles (Allen et al., 1998). Three separate samples of SMD were prepared in a three-stage process. First, the Hawaiian palagonite was dry-sieved using a series of stainless steel screens with mesh openings from 125 to 45 µm. Second, the palagonite fraction that passed through the 45-µm sieve was suspended in SDIW, vigorously shaken for 1 min, and maintained for 2 h at 24 °C. Suspended soil particles that remained in the top 10 cm of the water column after 2 h were pipetted off and concentrated by centrifugation. And third, the SMD samples were dry-heat sterilized at 130 °C for 24 h prior to use. The SMD analogs were composed of the following: a fraction with large dust particles measuring 25–50 µm in diameter; a fraction with medium-sized dust particles measuring 10–25 µm in diameter; and a fraction with small dust particles measuring 2–8 µm in diameter. The sizes and cross-sectional areas of dust particles in SMD samples (Fig. 5) were measured with a high-resolution video imaging system (model VH-7000, Keyence Corporation of America, Woodcliff Lake, NJ, USA).

2.5. Operation of the Mars simulation chamber

A series of six experiments were conducted within the MSC system in which a basic operational procedure was used to cycle the MSC through various simulations of the martian surface. First, microbial holders with the appropriate treatments of bacterial monolayers, neutral density filters, and/or SMD coatings were attached to the LN2 cold-plate (Fig. 2). Two UV-blocking, 400 nm short-cutoff filters (model 57346, Oriel Instruments) were inserted into the light path of the xenon-arc lamps (Fig. 1B) and the alignment of the Mars-normal light emitted from the ends of the fiber-optic bundles was checked for each bacterial monolayer (Fig. 2B). Thermocouple wires were then attached to several microbial holders to monitor the temperatures of the bacterial monolayers. A CCD camera was installed within the MSC (Fig. 2A) so that live video images could be viewed during the course of each experiment in order to confirm that

(a) the alignment of the simulated Mars-normal light and the bacterial monolayers did not shift during the experiment, and
(b) that no water-ice or CO_2 frost formed on the neutral density filters.

Next, the door of the MSC was closed and the air evacuated. As the pressure within the MSC reached 50 mb, the flow of the atmospheric gas to be used during the experimental test was initiated. The MSC vacuum-control system generally required 15–20 min to stabilize at the simulated Mars-normal pressure of 8.5 mb. After 20 min, the LN2 cold-plate would be started and the MSC system allowed an additional 40–60 min to stabilize at specific set points. Only after the MSC systems stabilized at experimental set points for pressure, gas composition, and temperature were the bacterial monolayers exposed to the UV-enhanced simulated Mars solar spectrum. The UV exposures for various experiments (described below) were controlled precisely to the second. After UV-exposure, the bacterial monolayers were warmed slowly to room temperature (23 °C), the MSC was slowly vented and returned to Earth-normal pressures, and the microbial holders removed and returned to the lab for processing through the MPN procedure.

2.6. Experimental protocols

Six separate experiments were conducted under a diversity of simulated martian conditions. First, monolayers of *B. subtilis* were exposed to Mars-normal UV-VIS irradiation

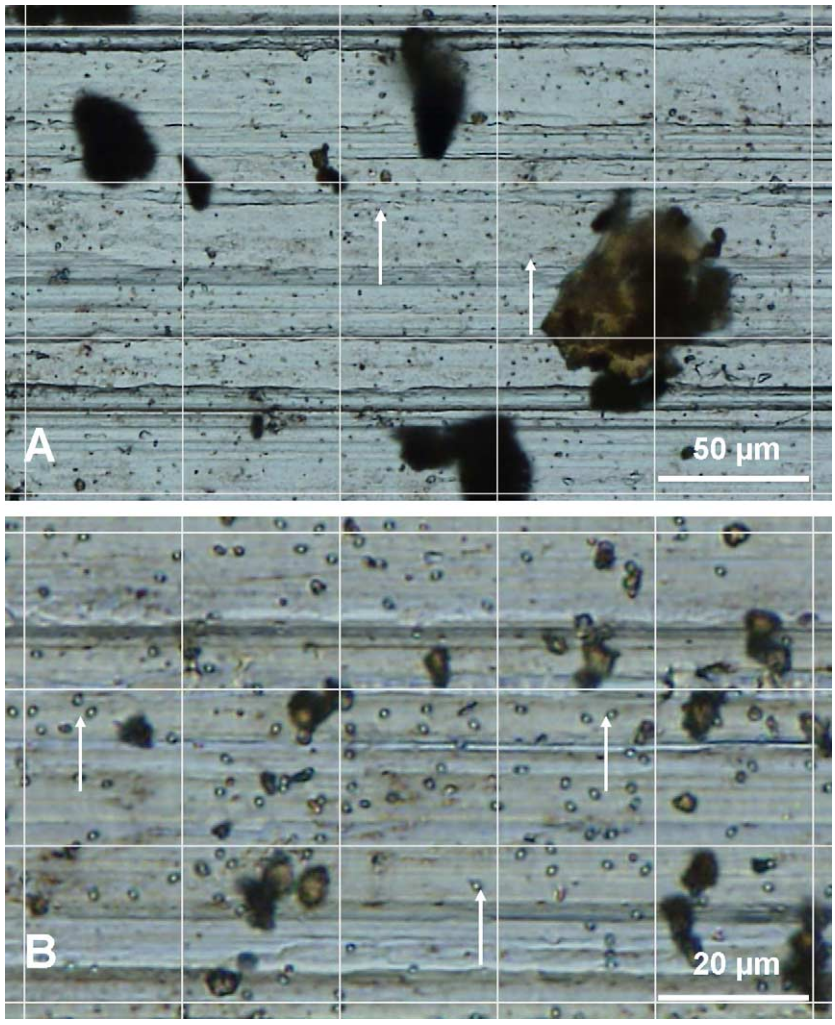

Fig. 5. (A) Large dust particles (25 to 50 µm in diameter) adhering to an aluminum coupon on which endospores (arrows) of *Bacillus subtilis* HA 101 were uniformly applied. The largest dust particles were estimated to cover between 30 and 50 endospores. (B) Small dust particles (2 to 8 µm in diameter) adhering to an aluminum coupon in which most of the smaller dust particles were observed to adhere to the aluminum surfaces between individual endospores.

for 0, 0.25, 0.5, 1, 1.5, 2, 5, 10, 15, 20, 25, or 30 min under Earth-normal environmental conditions of 1013 mb, 23 °C, and normal N_2/O_2 gas composition (78% and 21%, respectively). The experiment was used as a baseline study to determine how rapidly populations of *B. subtilis* were inactivated under Mars-normal UV light without other potential stressing agents involved in the simulation. The simulated optical depth (*tau*) of the atmosphere in this experiment was $tau = 0.1$ (Rayleigh scattering alone).

Second, bacterial monolayers were placed in the MSC and exposed to Mars-normal UV-VIS irradiation for 0, 1, 5, 10, 30, or 60 min under Mars-normal environmental conditions of 8.5 mb, −10 °C, and pure CO_2 atmosphere. In addition, this experiment simulated increased dust loading in the martian atmosphere using a series of neutral density filters. The simulated optical depths of the martian atmosphere were 0.1 (Rayleigh scattering alone), 0.3 (similar to clear-sky conditions on Mars during Viking), 0.7, 1.4, 2.5, and 3.5 (similar to global dust storm conditions on Mars during Viking) (Colburn et al., 1989; Kahn et al., 1992). The selected optical depths simulated only the attenuation of the direct beam UV and VIS fluence rates on Mars. Total MSC time at Mars-normal conditions for the neutral density filter experiment was 3 hours.

Third, bacterial monolayers were placed within the MSC at Mars-normal environmental conditions of 8.5 mb and −10 °C, exposed to 5 min of Mars-normal UV-VIS light, and then treated in separate simulations to different gas compositions of the atmosphere. Four gas mixtures were tested including N_2 (> 99.99% pure), CO_2 (> 99.99% pure), air (N_2 at 78%, O_2 at 21%, and trace gases at approximately 1%), or Mars gas (composed of the following: CO_2 (95.3%), N_2 (2.7%), Ar (1.7%), O_2 (0.2%), and H_2O (0.03%)). As described above, the purity of each gas mix was confirmed with an on-line residual gas analyzer. Gas mixtures were selected based on previous literature (Green et al., 1971; Hagen et al., 1970; Imshenetsky et al., 1967; Koike et al., 1995; Mancinelli and Klovstad, 2000; Packer et al., 1963) in which Mars simulations of microbial survival were carried out in a variety of gas mixes without pre-testing whether the

gas mixtures of the simulated atmospheres contributed to the survival or death of test organisms. Total MSC time at Mars-normal conditions for the gases experiment was 3 hours.

Fourth, monolayers of *B. subtilis* were placed within the MSC at Mars-normal environmental conditions of 8.5 mb and pure CO_2 atmosphere, exposed to 5 min of Mars-normal UV-VIS light, and then treated in separate simulations to -80, -40, -10, or $+23\,^{\circ}C$. Total MSC time at Mars-normal conditions for the temperature experiment was 4 hours. Temperatures were selected to represent the range of day-time high temperatures on Mars recorded by both Viking landers in the northern hemisphere ($-80\,^{\circ}C$ for winter months, $-40\,^{\circ}C$ for spring and fall months, and $-10\,^{\circ}C$ for summer months) (Kieffer et al., 1992) or predicted to occur for southern latitudes near perihelion ($+23\,^{\circ}C$) (Kieffer et al., 1977).

The fifth experiment involved the application of several different dust coatings to bacterial monolayers to determine whether dust particles could protect bacteria from the biocidal effects of UV irradiation under simulated martian conditions. Three separate dust coatings were applied as non-contiguous layers of individual particles of increasing size (Fig. 5). The non-contiguous dust coatings were created by first covering the bacterial monolayers with 0.5 mm thick layers of dust composed of particles measuring 2–8, 10–25, or 25–50 μm in diameter. Then, the aluminum coupons were rotated 90-degrees to a vertical position and most of the dust removed by gently tapping the aluminum coupons onto the bottoms of sterile petri dishes. A fourth treatment was composed of 0.5 mm thick contiguous layers of dust applied in such a way as to completely cover bacterial monolayers. All procedures were conducted under aseptic conditions, and the dust treatments were pre-sterilized at $130\,^{\circ}C$ for 24 h and cooled to $24\,^{\circ}C$. Controls were conducted to confirm that the dust treatments were sterile, and that the procedure could be carried out without contaminating either the dust or the bacterial monolayers. Separate chamber tests were conducted in which dust-coated bacterial monolayers were exposed to 1 or 8 h of simulated Mars-normal UV-VIS irradiation. An 8-h treatment delivered the equivalent UV-dosage of one martian Sol at the equator (based on the daily UV fluence models of (Appelbaum and Flood, 1990)). The dust-coated bacterial monolayers were maintained at Mars-normal conditions of 8.5 mb, $-10\,^{\circ}C$, pure CO_2, and exposed to simulated Mars-normal UV-VIS irradiation. The dust was not removed from the dust-coated coupons prior to assaying the coupons for viable endospores of *B. subtilis*. Total MSC time at Mars-normal conditions for the dust experiment was 12 hours.

The last experiment involved a procedure for evaluating the effects of pressure on bacterial survival. Operational runs of the MSC at Mars-normal pressures (8.5 mb) in the experiments described above contained a set of two controls placed inside the MSC and a second set of two controls placed outside the MSC. The controls were composed of bacterial monolayers on aluminum coupons mounted within separate microbial holders, and in which the microbial holders were fitted with aluminum plates on the covers to block all light from striking the bacterial monolayers. Numbers of surviving bacteria placed inside the MSC (N) were divided by the numbers of surviving bacteria placed outside the MSC (No). The values of N/No were tabulated for each experiment and represent the numbers of surviving bacteria placed within the MSC compared to bacteria maintained in Earth-normal environments.

2.7. Statistical procedures

Statistical analyses were conducted with version 8.0 of the PC-based Statistical Analysis System (SAS) (SAS Institute, Inc., Cary, NC, USA). For most experiments, 0.25 power transformations were used to induce homogeneity of variances of individual treatments; all data is presented as untransformed data. Transformed data were subjected to analysis of variance procedures (PROC GLM) followed by protected least-squares mean separation tests ($P \leqslant 0.05$). Linear regression models were generated with PROC REG ($P \leqslant 0.05$). Data in the experiment to measure the effects of pressure on bacterial survival were analyzed with student t-tests ($P \leqslant 0.05$) in which the experimental means of surviving populations inside the MSC were individually tested against the Earth-normal control constant of $N/No = 1$.

3. Results

Populations of *B. subtilis* endospores were reduced by over 99.9% after 30 s UV irradiation when maintained at Earth-normal temperature ($23\,^{\circ}C$), pressure (1013 mb), gas mixture (normal N_2/O_2 ratio), and illuminated with a Mars-normal UV fluence rate (Fig. 6). Bacterial populations decreased more slowly between 30 sec and 10 min, and it required at least 15 min of Mars-normal UV exposure to inactivate 100% of the bacterial populations. The biocidal effects of the simulated Mars-normal UV flux on endospores followed a biphasic response (Fig. 6, insert) in which bacterial survival was characterized by an initial rapid decrease ($y = -1.99x + 0.83$; $P < 0.0001$; $r^2 = 0.753$) followed by a slower second phase with a significantly smaller slope value ($y = -0.00006x + 0.00054$; $P = 0.028$; $r^2 = 0.134$). The effective lethal dosage (LD) rates for UVC + UVB (200 to 315 nm) for the 99.9% ($> LD_{99}$) and 100% (LD_{100}) kill levels were 0.39 and 11.9 $kJ\,m^{-2}$, respectively (Fig. 6). The LD_{100} rates are defined for the current study as those treatments in which viable bacteria were not recovered with the MPN assay.

The biocidal effects of Mars-normal UV irradiation on *B. subtilis* endospores were similar at Mars-normal conditions of temperature ($-10\,^{\circ}C$), pressure (8.5 mb), and CO_2 atmosphere (Fig. 7) compared to the results obtained at Earth-normal environments (Fig. 6). Bacterial populations were reduced greater than three decades ($> 99.9\%$) for *tau* values of 0.1, 0.3, and 0.7 at UV exposures of 1-min, but

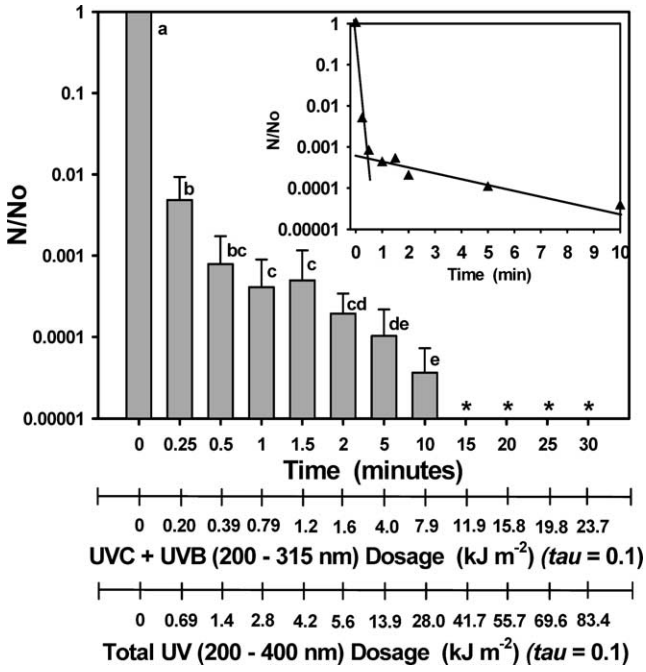

Fig. 6. Effects of Mars-normal UV irradiation on survival of endospores of *Bacillus subtilis* HA 101 on aluminum coupons maintained at Earth-normal pressure (1013 mb), temperature (+23 °C), and gas composition (normal N_2/O_2 ratio). Different letters indicate significant differences among treatments based on ANOVA and protected least-squares mean separation tests ($P \leqslant 0.05$; $n = 6$); bars represent standard errors of the means. Where *N* equals the number of survivors per treatment and *No* equals the number of viable endospores on the in-chamber controls. The [*] denotes treatments in which no viable endospores were recovered in the MPN assay.

were only moderately reduced (80 or 20%) for *tau* values of 2.5 or 3.5, respectively. As the time of UV exposure was increased to 10 min, the survival rates of *B. subtilis* were between 0.001 and 0.00001% for *tau* simulations of 0.1 to 2.5. Only the *tau* simulation of 3.5 (global dust storm levels) yielded significant survival rates of *B. subtilis* at 10 min. However, greater than 99% of bacterial populations were inactivated ($P \leqslant 0.01$) as the UV exposure times of bacterial endospores were extended to 60 min for $tau = 3.5$. One hundred percent of bacteria were inactivated at *tau* simulations of 0.1, 0.3, 0.7, and 1.4 in UV exposure times greater than 10 min (Fig. 7). Although the UV exposure times between the Earth-normal (Fig. 6) and the Mars-normal (Fig. 7) environmental simulations were slightly different, the overall effects of UV irradiation on the survival of *B. subtilis* were very similar between the two trials. Compared to the Earth-normal tests, there did not appear to be significant added effects of simulated martian environmental conditions on the survival of bacterial endospores.

Two related experiments were conducted to determine the influence of gas composition and temperature on the biocidal effects of Mars-normal UV irradiation on *B. subtilis* endospores. In the gas experiment, pressure and temperature were held at 8.5 mb and −10 °C, respectively, while the gas composition was changed. In the temperature experiment, pressure and gas composition were held at 8.5 mb and pure CO_2, respectively, while the temperature was altered. In both experiments *tau* simulations were set at 0.3, 1.4, 2.5, and 3.5. Bacterial monolayers were exposed to 5 min of Mars-normal UV irradiation after the MSC was equilibrated at the desired set points. Results indicated that gas composition (Fig. 8) and temperature (Fig. 9) had no effects on the survival rates of *B. subtilis* endospores under simulated Mars-normal conditions. In both experiments, only different *tau* simulations affected bacterial survival rates. In both experiments, en-

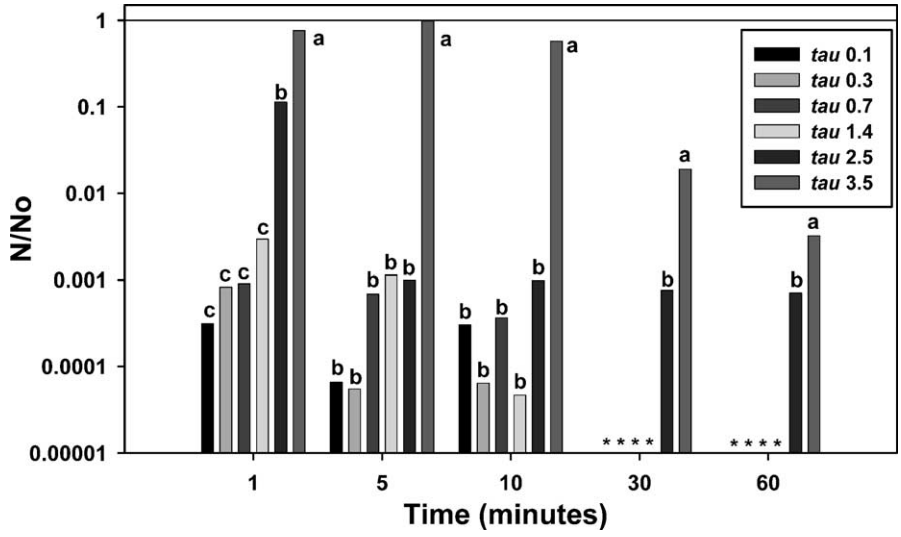

Fig. 7. Effects of Mars-normal UV irradiation on survival of endospores of *Bacillus subtilis* HA 101 on aluminum coupons exposed to Mars-normal pressure (8.5 mb), temperature (−10 °C), and gas composition (CO_2 at > 99.99% purity). Simulations of different optical depths (*tau*) of the martian atmosphere were achieved through the use of neutral density filters. Different letters indicate significant differences among *tau* treatments within each time category based on ANOVA and protected least-squares mean separation tests ($P \leqslant 0.05$; $n = 4$). Error bars were deleted for clarity, but were approximately 1/2 of a log for each treatment. Where *N* equals the number of survivors per treatment and *No* equals the number of viable endospores on the in-chamber controls. The [*] denotes treatments in which no viable endospores were recovered in the MPN assay.

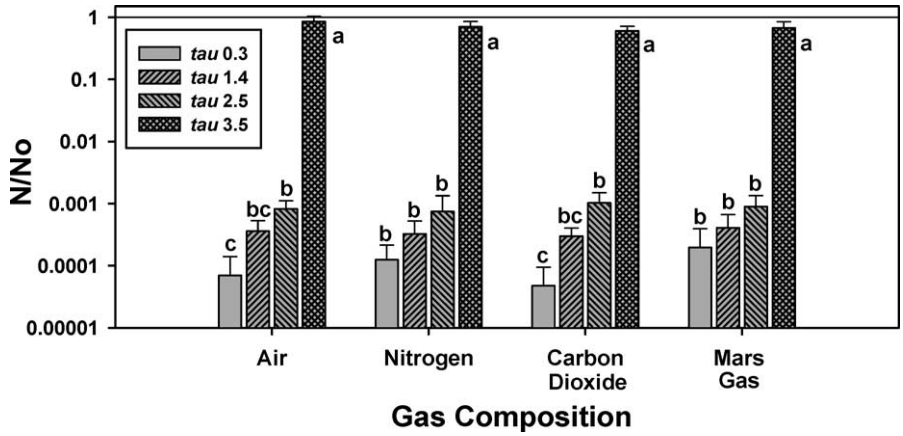

Fig. 8. Effects of gas composition and Mars-normal UV irradiation (5-min exposure) on survival of endospores of *Bacillus subtilis* HA 101 on aluminum coupons exposed to Mars-normal pressure (8.5 mb), and temperature (−10 °C). Different letters indicate significant differences among *tau* treatments within each gas category based on ANOVA and protected least-squares mean separation tests ($P \leqslant 0.05$; $n = 6$); bars represent standard errors of the means. Where *N* equals the number of survivors per treatment and *No* equals the number of viable endospores on the in-chamber controls.

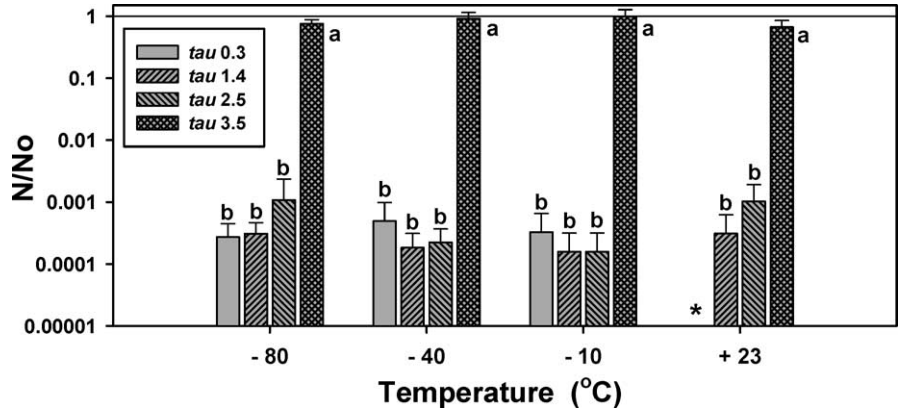

Fig. 9. Effects of temperature and Mars-normal UV irradiation (5-min exposure) on survival of endospores of *Bacillus subtilis* HA 101 on aluminum coupons exposed to Mars-normal pressure (8.5 mb), different temperatures, and gas composition (CO_2 at > 99.99% purity). Different letters indicate significant differences among *tau* treatments within each temperature category based on ANOVA and protected least-squares mean separation tests ($P \leqslant 0.05$; $n = 6$); bars represent standard errors of the means. Where *N* equals the number of survivors per treatment and *No* equals the number of viable endospores on the in-chamber controls. The [*] denotes treatments in which no viable endospores were recovered in the MPN assay.

dospores of *B. subtilis* were reduced between three (99.9%) and four decades (99.99%) for *tau* levels of 0.3, 1.4, and 2.5, but only between 2 and 18 % for *tau* levels of 3.5. The *P* values for gas composition and temperature terms in the ANOVA models were not significant at $P > 0.10$, while all *P* values for *tau* simulations were significant at $P \leqslant 0.01$.

Three different dust coatings were used to simulate aeolian deposition of dust onto spacecraft surfaces in order to determine the effects that dust particles might have on bacterial survival under simulated martian conditions. The particle sizes in the three dust coatings averaged 2 to 8, 10 to 25, and 25 to 50 μm in diameter. These three dust coatings were applied as non-contiguous layers in which individual dust particles were randomly distributed over the bacterial monolayers (Fig. 5). In the 25- to 50-μm dust fraction, particles were estimated to cover between 30 and 50 endospores (Fig. 5A), but in the smallest fraction (2 to 8 μm), dust particles often adhered to aluminum coupons at points between individual bacterial endospores (Fig. 5B). A fourth dust treatment created 0.5 mm thick contiguous layers of dust that extended beyond the boundaries of bacterial monolayers. All tests were conducted under *tau* simulations of 0.1. After 1 h of UV exposure, the smallest dust fraction (2 to 8 μm particles) failed to protect endospores of *B. subtilis* against Mars-normal UV irradiation, while the intermediate (10 to 25 μm) and large (25 to 50 μm) dust fractions offered a moderate amount of protection (Fig. 10). When the four dust coatings were exposed to 8 h of simulated Mars UV irradiation at Mars-normal environments, only the 0.5 mm thick contiguous layers of dust were found to protect bacteria from the biocidal effects of UV (Fig. 10).

In previous experiments (Figs. 6 thru 9), the number of viable spores of *B. subtilis* recovered for all *tau* simulations were observed to be less than the control treatments where N/No equaled 1.0. However, in the dust experiment the populations of *B. subtilis* recovered from the 0.5 mm thick contiguous layers were always higher than the controls ($N/No = 1.0$). It is likely that this response was due to a

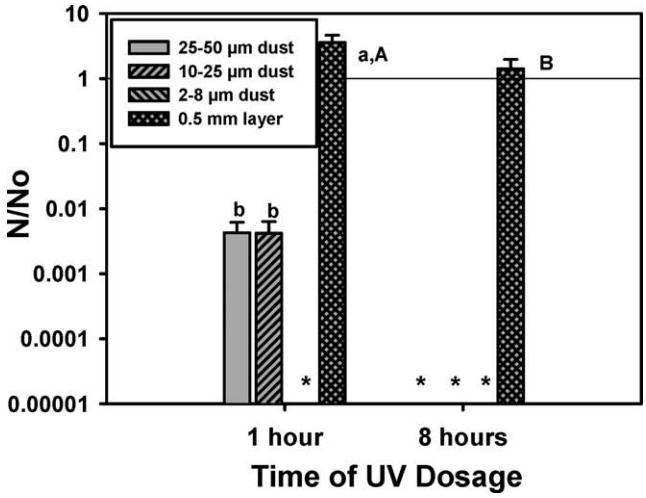

Fig. 10. Effects of dust coatings and Mars-normal UV irradiation (1-h and 8-h exposures) on survival of endospores of *Bacillus subtilis* HA 101 on aluminum coupons exposed to Mars-normal pressure (8.5 mb), temperature (−10 °C), and gas composition (CO_2 at > 99.99% purity). Different lower-case letters indicate significant differences among dust treatments within each time category based on ANOVA and protected least-squares mean separation tests ($P \leqslant 0.05$; $n = 6$); bars represent standard errors of the means. Different upper-case letters indicate significance between time treatments for the 0.5 mm contiguous layer. Where *N* equals the number of survivors per treatment and *No* equals the number of viable endospores on the in-chamber controls. The [*] denotes treatments in which no viable endospores were recovered in the MPN assay.

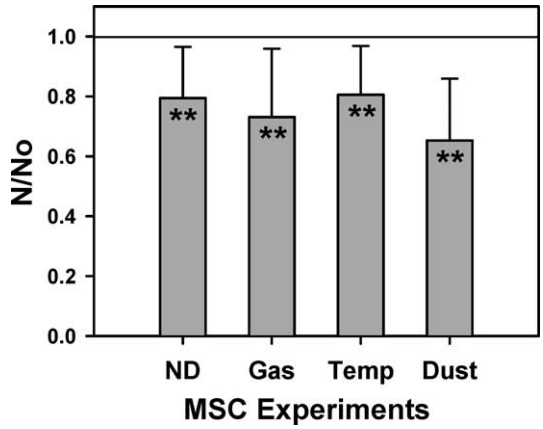

Fig. 11. Effects of pressure on survival of endospores of *Bacillus subtilis* HA 101 on aluminum coupons exposed to Mars-normal environments in experiments depicted in Figs. 7, 8, 9, and 10, and Earth-normal conditions of pressure (1013 mb), temperature (+23 °C), and gas composition (normal N_2/O_2 ratio). Each experiment in the Mars simulation chamber (MSC) was analyzed separately by standard paired *t*-tests between the Mars-normal controls (*N*) inside the MSC versus the Earth-normal controls (*No*) outside the MSC (** denotes significance at $P \leqslant 0.05$; where $n = 40$, 24, 24, and 12 for the ND filter, gas, temperature, and dust experiments, respectively). Error bars are 95% confidence levels for each comparison. Where *N* equals the number of survivors per treatment and *No* equals the number of viable endospores on the in-chamber controls.

higher recovery rate of bacterial endospores in the 0.5 mm dust layer treatment due to the presence of abrasive dust in the extraction medium. (Recall that the dust was not removed from the dust-coated coupons prior to assaying the coupons for viable endospores of *B. subtilis*.) The numbers of surviving bacteria recovered from the 0.5 mm thick treatments after 1 or 8 h of UV exposure were compared to determine if the populations of surviving bacteria were lower after 8 h than for 1 h of UV exposure. Results indicated that populations of surviving *B. subtilis* were significantly lower after 8 h of UV irradiation ($P = 0.001$). This suggests that although the 0.5 mm contiguous dust layer offered significant protection from UV irradiation, the protection was not 100%, and that sufficient levels of UV irradiation were able to penetrate the 0.5 mm dust layers to negatively impact the survival of bacterial spores adhered to aluminum coupons.

In experiments described in Figs. 7 thru 10, a set of internal and external controls were maintained in order to determine the effects of Mars-normal (inside the MSC controls) versus Earth-normal (outside the MSC controls) environments on the survival rates of *B. subtilis*. Procedures were different among the four experiments, so a direct statistical analysis was not possible. Instead, the numbers of surviving bacteria were compared using individual student *t*-tests for each external (*No*) versus internal control (*N*) treatments. Control treatments used microbial holders fitted with opaque aluminum plates such that no UV irradiation could contact the bacterial monolayers. Results indicated that in all four experiments ($P \leqslant 0.05$), the numbers of surviving bacteria under Mars-normal environments were between 20 to 35% less than the numbers of bacteria recovered in the Earth-normal controls (Fig. 11). Because the temperature and gas composition within the MSC during UV-exposure of bacteria were found not to affect the survival of bacteria, we conclude that this response is most consistent with the effects of pressure on bacterial survival than other experimental parameters.

Based on the results given above, calculations were conducted to estimate

(a) the number of minutes at various optical depths required to accumulate lethal UVC + UVB doses at solar zenith angles near 0 degrees,
(b) the daily UVC + UVB fluence rates on Mars, and
(c) numbers of times that lethal doses of 11.9 kJ m^{-2} for endospores of *B. subtilis* HA-101 (called lethal dose multiples) might be achieved under various optical depths of the martian atmosphere (Table 3).

The instantaneous UVC + UVB fluence rate for the Mars solar constant (Table 1) was used to estimate the instantaneous UV fluence rates at the surface of Mars under various optical depths of the atmosphere (based on the data in Table 2). The numbers of minutes required to accumulate lethal UVC + UVB dose rates for all *tau* simulations were estimated by converting the instantaneous UVC + UVB fluence rates (W m^{-2}) to accumulated UVC + UVB dose rates per minute (kJ m^{-2} min^{-1}) under each *tau* simulation, and then dividing 11.9 kJ m^{-2} by the accumulated UVC + UVB dose rate per minute (Table 3). Daily UVC + UVB flu-

Table 3
First-order approximations of the times required to accumulate lethal UVC + UVB doses, daily UVC + UVB fluence rates, and estimations of the lethal dose multiples for endospores of *Bacillus subtilis* on horizontal sun-exposed spacecraft surfaces on Mars

Optical depth of atmosphere (*tau*)	Instantaneous UVC + UVB fluence rate on equatorial Mars ($W\,m^{-2}$)[b]	Time required to accumulate a lethal dose rate of UVC + UVB ($11.9\,kJ\,m^{-2}$) for *Bacillus subtilis* (min)[c]	Daily UVC + UVB fluence rates on Mars ($kJ\,m^{-2}$)[d]	Lethal dose multiples for endospores of *Bacillus subtilis* for one Sol[e]
0.0[a]	11.56	17	332.9	27.9
0.1	10.46	20	301.3	25.3
0.3	8.57	23	246.7	20.7
0.7	5.75	35	165.6	13.9
1.4	2.86	69	82.4	6.9
2.5	0.95	208	27.4	2.3
3.5	0.37	536	10.2	0.9

[a] Top of the atmosphere levels for Mars solar constant.
[b] Based on the Mars solar constant derived from Arvesen et al. (1969) and Kuhn and Atreya (1979) in Table 1, and on data presented in Table 2. Values are for solar zenith angles near 0 degrees.
[c] Estimated by converting the instantaneous UVC + UVB fluence rates ($W\,m^{-2}$) to accumulated UVC + UVB dose rates per minute ($kJ\,m^{-2}\,min^{-1}$), and then dividing $11.9\,kJ\,m^{-2}$ (i.e., lethal dose rate for *B. subtilis*) by the accumulated UVC + UVB dose rate per minute.
[d] Based on $1\,W\,m^{-2} = 3.6\,kJ\,m^{-2}\,h^{-1}$; and then the resultant products multiplied by a conversion factor of 8 to represent the total integrated daily UV fluence rates on Mars. Based on the models of daily diffuse and direct beam solar irradiation of Appelbaum and Flood (1990) and Haberle et al. (1993).
[e] Lethal dose multiples represent the number of times a lethal dose of $11.9\,kJ\,m^{-2}$ for *B. subtilis* would be accumulated in one Sol under specific levels of *tau*.

ence rates (Table 3) were estimated based on $1\,W\,m^{-2} = 3.6\,kJ\,m^{-2}\,h^{-1}$; and the results multiplied by a conversion factor of 8. The conversion factor was determined by estimating the daily fluence rates on Mars for both the direct and diffuse beams from the solar spectrum models of Appelbaum and Flood (1990) and Haberle et al. (1993). Thus, 8 hours at maximum solar irradiation equals the daily integrated solar flux for a 12-h Sol on Mars. The lethal dose multiples (Table 3) were calculated by dividing the daily UVC + UVB dose rates by the lethal UVC + UVB dose rate of $11.9\,kJ\,m^{-2}$ of *B. subtilis* (Fig. 6). Results indicated that for optical depths of the martian atmosphere from *tau* = 0.1 (dust-free) to 3.5 (global dust storms), a lethal dose of UVC + UVB would be reached within approximately one Sol on horizontally flat spacecraft surfaces. However, for bacteria shielded from direct solar UV for all or part of each Sol, the lethal dose rates would be expected to increase. But until a robust model for diffuse UV irradiation on Mars is available, predictions on the survival of microorganisms on shaded portions of spacecraft cannot be completed.

4. Discussion

4.1. Biological responses to Mars-normal UV irradiation

The MSC lighting system was designed to deliver Mars-normal UV irradiation to bacterial monolayers deposited on aluminum coupons. Different ND filters were used to attenuate the Mars solar constant UV flux before the UV-enriched light contacted the bacterial monolayers. Together, the MSC lighting system and the ND filters provided a reasonable first-order simulation of the absorption, scattering, and reflectance back to space that dust particles in the martian atmosphere might impart on down-welling solar irradiation. For example, UV irradiation was partially attenuated below 350 nm for all ND filters and the levels of UV attenuation increased at higher simulations of *tau*. The amount of UV attenuation ranged from 10–15% for *tau* simulations of 0.3 and 0.7 and increased to 50% for a *tau* simulation of 3.5. The ND filters were selected to simulate a range of optical depths of the martian atmosphere between *tau* values of 0.1 (Rayleigh scattering only), 0.3 (clear-day conditions), and 3.5 (global dust storm conditions) based on Viking data from Colburn et al. (1989) and Kahn et al. (1992). The Mars UV model was consistent with other studies on solar irradiation on Mars (Appelbaum and Flood, 1990; Cockell et al., 2000; Haberle et al., 1993; Kuhn and Atreya, 1979; Patel et al., 2002) and was similar to the calculated UV fluence rates on Mars made by Cockell et al. (2000) and Patel et al. (2002). The Mars UV model did not simulate UV absorption by atmospheric ozone, and, thus, the UV model was developed to represent UV fluence rates on mid-latitude and equatorial Mars. Ozone on Mars has been reported to occur in the polar regions of the planet during fall and winter months for each hemisphere, but has not been observed in equatorial regions (Barth et al., 1973; James et al., 1994). Thus, the Mars UV model used here was developed to represent regions on Mars on which the Viking and Pathfinder missions were landed, and on which several future near-term Mars landers are likely to be placed.

Of particular importance in the development of the Mars UV model was the use of UV-enhanced xenon-arc lamps to provide broad-spectrum ultraviolet, visible, and near-infrared irradiation. Of the 33 papers found in the literature that conducted studies on microbial survival under simulated martian environments, only seven papers included UV irradiation as an experimental parameter (Green et al., 1971;

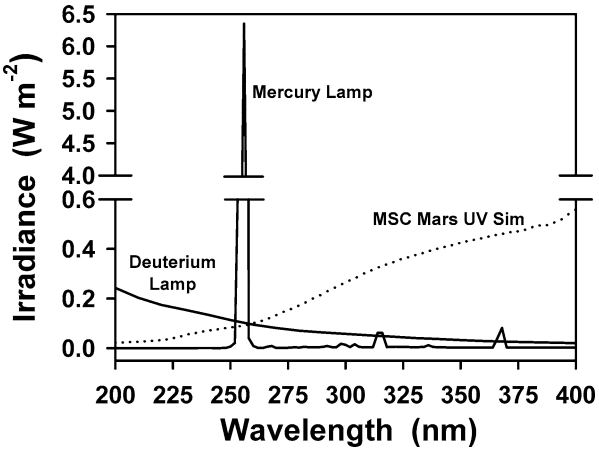

Fig. 12. Comparative spectra of a deuterium lamp (D2), a mercury-line lamp (Hg), and the Mars simulation chamber (MSC) UV simulation.

Hagen et al., 1970; Imshenetsky et al., 1967; Koike et al., 1995, 1996; Mancinelli and Klovstad, 2000; Packer et al., 1963). Of these, only one paper used xenon-arc lamps for creating Mars-normal UV fluence rates (Green et al., 1971). The other papers used either mercury-line (Hg) lamps with UV irradiation emitted at a peak wavelength of 254 nm (Hagen et al., 1970; Imshenetsky et al., 1967; Packer et al., 1963) or deuterium (D2) lamps with increasing UV fluence rates at lower wavelengths (Koike et al., 1995, 1996; Mancinelli and Klovstad, 2000). Comparative spectra of xenon-arc, Hg, and D2 lamps are given in Fig. 12; the fluence rates of each lamp are scaled to equal 50 $W\,m^{-2}\,s^{-1}$ for the UV range of 200–400 nm. Comparing the spectra of the Hg and D2 lamps to the Mars solar constant given in Fig. 3 clearly demonstrates that few studies on bacterial survival have accurately simulated the UV fluence rates on Mars in conjunction with simulated martian environmental conditions. Furthermore, the current study is the first report in which microbial survival on spacecraft surfaces is examined under accurately simulated Mars-normal pressure, temperature, gas composition, and UV fluence rates.

Ultraviolet irradiation of endospores of *B. subtilis* indicated that only 30 sec were required for the inactivation of 99.9% ($> LD_{99}$) of spore populations when bacterial monolayers were irradiated with Mars-normal UV fluence rates at $tau = 0.1$. The kill curve followed a bi-phasic response in which most of the population of *B. subtilis* was inactivated within 15–30 sec, followed by a slower reduction in the number of survivors that required up to 15 min of Mars-normal UV irradiation to achieve 100% kill (LD_{100}). The bi-phasic response curve might be explicable on the basis of

(i) a heterogeneous population of endospores in which a very small proportion of cells were highly resistant to UV irradiation,
(ii) small aggregates of endospores might have been present in which the underlying cells were protected from the biocidal effects of UV irradiation by the overlying endospores, or
(iii) both mechanisms were involved. Results were similar between *tau* simulations of 0.1 (Rayleigh scattering only) and 0.3 (clear-sky conditions), and, thus, indicated that bacterial inactivation on sun-exposed spacecraft surfaces will occur very rapidly under normal clear-sky conditions on Mars.

The lethal dose rate for endospores of *B. subtilis* (LD_{100}) for the UVC + UVB regions (200–315 nm) was 11.9 $kJ\,m^{-2}$, which was accumulated in 15 min under Mars-normal UV irradiation at a *tau* simulation of 0.1. This was consistent with the work of Mancinelli and Klovstad (2000) which reported a total UV (200–400 nm) lethal dose rate for *B. subtilis* of 12.3 $kJ\,m^{-2}$ for bacterial monolayers on aluminum coupons. Of special note is the fact that 450 W xenon-arc lamps were used in the current study and a 30 W deuterium lamp, was used by Mancinelli and Klovstad (2000). At first glance, the ultraviolet LD_{100} rates of the two studies appear to be derived from very different spectral ranges. But the apparent discrepancy is explicable on the basis that most of the UV flux in a D2 lamp is emitted below 325 nm (UVC + UVB) (see Fig. 12). Thus, the actual UVC + UVB fluence rates are similar in both studies. In general, UVC + UVB (200–315 nm) wavelengths contribute significantly more than UVA (315–400 nm) wavelengths to the biocidal effects of UV light on bacteria through the efficient absorption of short-wavelength UV irradiation by DNA (Jagger, 1985a). Although similar lethal dose rates were obtained here and by Mancinelli and Klovstad (2000), the times required to accumulate the lethal dose rates were decidedly different. In the current study, the LD_{100} rate of 11.9 $kJ\,m^{-2}$ for endospores of *B. subtilis* was accumulated in 15 min, while it required almost 3 h of irradiation by the D2 lamp to deliver the same lethal dose; the UV fluence rate was 3.076 $kJ\,m^{-2}\,h^{-1}$ in the Mancinelli and Klovstad study (2000). Thus, the current study gives a more accurate prediction of the actual time (in minutes) on Mars within which a lethal UV dose might be absorbed by microorganisms on sun-exposed spacecraft surfaces.

The total UV dose rates for the range between 200 and 400 nm reported here (41.7 $kJ\,m^{-2}$) and by Mancinelli and Klovstad (2000) (12.3 $kJ\,m^{-2}$) are significantly different because the xenon-arc lamps used in the current study emit much higher levels of UVA irradiation. Based on this difference, the use of Hg and D2 lamps may make it more difficult to accurately predict lethal dose rates for microorganisms on Mars, and, thus, we propose that xenon-arc lamps are the better choice for studying the effects of total UV irradiation on microbial survival on Mars, and should be used whenever possible for simulations of Mars-normal UV environments. Although UVC + UVB are generally viewed as contributing significantly greater proportions of biocidal activity compared to UVA (Jagger, 1985a; Nicholson et al., 2000), long-wavelength UVA photons can impact the sur-

vival of microorganisms (Jagger, 1985b, 1985c) and should not be ignored.

The use of ND filters to simulate UV attenuation by dust in the martian atmosphere demonstrated that populations of endospores of *B. subtilis* could be significantly reduced by accumulated UVC + UVB irradiation, even at high optical depths. For example, even at *tau* simulations of 3.5, greater than 99% of endospores were inactivated after only 60 min exposure to Mars-normal UV irradiation. In general, lethal UV dose rates for dormant bacteria are accumulative and are not necessarily correlated with instantaneous UV fluence rates (Nicholson et al., 2000). Thus, if the UVC + UVB lethal dose rate for *B. subtilis* (11.9 kJ m^{-2} at *tau* 0.1) is used as an upper limit for bacterial survival on spacecraft surfaces, then the times required to achieve full sterilization of spacecraft surfaces might be modeled for diverse landing sites, latitudinal positions, seasonal variations, and atmospheric opacities on Mars. Based on the current study with *tau* simulations of 0.1, 0.3, 0.7, 1.4, 2.5, or 3.5, the times required to achieve LD_{100} doses of UVC + UVB for endospores of *B. subtilis* on equatorial Mars with solar zenith angles near zero degrees are estimated as 20, 23, 35, 69, 208, and 536 min, respectively. These results are consistent with the survival rates of endospores of *B. subtilis* reported herein for the timed-exposures to Mars UV fluence rates under various simulations of *tau* and under Mars-normal environmental conditions (Fig. 7). Viewed another way, the LD_{100} doses for *tau* levels of 0.1, 0.3, 0.7, 1.4, 2.5, or 3.5 might be achieved 28, 25, 21, 14, 7, 2, and 1 times per Sol, respectively, on equatorial Mars. Lethal dose rates (LD_{100}) of UVC + UVB were also achieved for *tau* simulations of 0.1, 0.3, 0.7, and 1.4 of at least 30 min. In all experiments with unshielded bacterial monolayers in which the UV exposure times exceeded 15 min under *tau* simulations of 1.4 or lower, no viable endospores of *B. subtilis* were recovered. But even at high *tau* simulations of 2.5 or 3.5 that were similar to global dust storm conditions during both Viking missions (Colburn et al., 1989; Kahn et al., 1992), the results of the ND filter simulations suggest that bacterial monolayers can accumulate biocidal doses of UV irradiation under global dust storm conditions.

A key assumption of the current study was that the spore-forming bacterium, *Bacillus subtilis* HA 101, represented an approximate upper boundary of resistance to low temperature, low pressure, and high UV irradiation for cultivable microorganism commonly found on spacecraft surfaces (Horneck et al., 1994; Lindberg and Horneck, 1991; Nicholson et al., 2000; Morelli et al., 1962). Most other microbial species recovered from spacecraft surfaces are non-spore forming mesophilic species (Favero, 1971; Foster and Winans, 1975; Puleo et al., 1973, 1977) that are likely to be inactivated by UV irradiation in shorter periods of time than *B. subtilis* (Nicholson et al., 2000; Rambler and Margulis, 1980). Thus, the UVC + UVB LD_{100} rate of 11.9 kJ m^{-2} reported here for endospores of *B. subtilis* HA 101, might be considered a UV dose within which most spacecraft contaminants would be expected to be rendered inactive. Thus, actual times required for microbial inactivation on spacecraft surfaces on Mars might now be estimated by modeling how atmospheric dust loads, sun-elevation angles, latitudinal and seasonal variations, and orientation of spacecraft components affect the accumulated UV dose rates on spacecraft surfaces. Additional studies may push the upper-boundary for the lethal dose rate up if more UV-resistant bacterial species are recovered from spacecraft surfaces. However, the *B. subtilis* strain HA 101 was specifically selected for the current study based on its previously reported high levels of resistance to desiccation, low temperature, low pressure, and UV irradiation (Green et al., 1971; Horneck et al., 1994; Lindberg and Horneck, 1991; Mancinelli and Klovstad, 2000; Morelli et al., 1962; Nicholson et al., 2000), and, thus, the results reported here are likely to be good first-order approximations of microbial survival on spacecraft surfaces on Mars.

The quality of bacterial monolayers on spacecraft surfaces is another important factor in predicting actual UV lethal dose rates (LD_{99} or LD_{100}) for microbial species on Mars. Although great care was taken in the current study to select only bacterial monolayers that were smooth and uniformly distributed on aluminum coupons, the biphasic kill curve for *B. subtilis* suggests that a small amount of clumping may have occurred within a few monolayers. Direct observations of bacterial monolayers with high-resolution video microscopy demonstrated that > 99% of bacterial endospores were present as individual cells in monolayers. However, very low numbers of multi-celled clumps of between two and four endospores were periodically observed in bacterial monolayers. Thus, it seems reasonable that the second phase of the biphasic kill curve for *B. subtilis* may have been due, at least in part, to the partial protection of low numbers of endospores by over-lying cells. This is supported by the work of Mancinelli and Klovstad (2000) who reported that survival of *B. subtilis* endospores increased when multi-layered bacterial monolayers were exposed to high levels of UV fluence rates, and that multi-layered monolayers protected under-lying endospores of *B. subtilis* from UV irradiation. The key point is that the physical conditions of bacterial deposits on spacecraft surfaces have not been adequately studied, and, thus, actual LD_{100} rates for bacteria on spacecraft surfaces will depend on properly simulating the Mars-normal UV fluence rates, Mars environmental conditions, and the physical conditions of microbial deposits on spacecraft surfaces. The current study primarily addressed the effects of UV flux on bacterial survival, and we believe that the bacterial monolayers were a reasonable approximation of the conditions of most spore-forming bacteria on spacecraft surfaces. But a more complete picture of the microbial ecology of spacecraft, including the actual physical conditions of microbial deposits on spacecraft surfaces, is required in order to accurately predict the effects of UV irradiation on microbial survival on Mars. For example, rough surfaces on spacecraft components are more likely to possess

microsites that are conducive to the aggregation of microorganisms and may need to be treated differently than smooth surfaces during payload processing. In addition, endospores of *B. subtilis* HA 101 may have had some inherent heterogeneity of UV resistance that might have contributed to the bi-phasic kill-curve exhibited in Fig. 6. Although this possibility was not investigated during the current study, it should be considered in the overall modeling of microbial survival on spacecraft surfaces on Mars.

On Mars, aeolian deposits of dust on spacecraft surfaces could attenuate solar UV irradiation resulting in partial or complete shielding of viable bacteria, possibly increasing the times required for spacecraft sterilization. Several papers have characterized the effects of dust coatings on bacterial survival under UV irradiation, and in general they have reported that dust particles measuring 5–10 μm in diameter, or larger, can effectively block UV irradiation resulting in significant increases in bacterial survival (Green et al., 1971; Hagen et al., 1970; Horneck et al., 2001; Mancinelli and Klovstad, 2000; Packer et al., 1963). In these studies, simulated Mars dusts (Horneck et al., 2001; Mancinelli and Klovstad, 2000), Fe-montmorillonite (Mancinelli and Klovstad, 2000), limonite (Green et al., 1971; Hagen et al., 1970), crushed red sandstone (Horneck et al., 2001), and terrestrial field soils (Horneck et al., 2001; Packer et al., 1963) were used to shield bacteria suggesting that the UV-shielding effects were not limited to specific soil types or particle sizes. Thus, it was not anticipated that the Mars-normal UV simulations in the current study would have, in fact, inactivated populations of *B. subtilis* endospores (LD_{100}) covered with dust particles up to 50 μm in diameter. Endospores of *B. subtilis* were inactivated after 1-h UV exposures when covered with dust particles measuring 2–8 μm in diameter and after 8-h UV exposures when covered with dust particles measuring up to 50 μm in diameter. Only the 0.5-mm contiguous layer of simulated Mars dust was able to significantly protect *B. subtilis* from the Mars-normal UV flux. But even with the 0.5-mm contiguous layer, a significant reduction in surviving bacteria was noted between the 1-h and the 8-h UV exposures. The results reported here are not in agreement with previous studies (Green et al., 1971; Hagen et al., 1970; Horneck et al., 2001; Mancinelli and Klovstad, 2000; Packer et al., 1963), but are explicable if the thickness and quality of dust layers and the UV lamp-types are considered. First, the dust coatings used in the current study were composed of individual soil particles measuring 2–8, 10–25, or 25–50 μm in diameter in non-contiguous layers (Fig. 5). Most of the smaller dust particles (< 8 μm) were observed to adhere to aluminum coupons between individual bacterial endospores, while the largest dust particles were estimated to cover between 30–50 endospores. In most other studies, dust coatings were either composed of thick contiguous layers measuring from several millimeters to several centimeters (Green et al., 1971; Packer et al., 1963) or bacteria were mixed directly into aliquots of simulated Mars soils (Hagen et al., 1970; Horneck et al., 2001; Packer et al., 1963). Horneck et al. (2001) reported that mixing endospores of *B. subtilis* directly into fine-grained dusts or soils provided significantly higher protection against UV irradiation when compared to dust layers placed on tops of bacterial monolayers. Furthermore, aeolian dust on Mars measures 1–2 μm in diameter (Markiewicz et al., 1999; Pollack et al., 1995; Tomasko et al., 1999), and estimates during the Pathfinder mission indicated that surfaces were covered at the rate of only 0.28% per day (Landis and Jenkins, 2000). Thus, it appears that in previous studies the structure of dust coatings may not have accurately simulated the quality and quantity of dust falling on spacecraft surfaces on Mars.

Mancinelli and Klovstad (2000) did create dust coatings on bacterial monolayers in a similar manner as was used in the current study. They reported some protection of *B. subtilis* endospores by dust coatings, and, thus, are not in agreement with the results reported here. However, the effects of the spectral quality of UV lamps on UV penetration through simulated Mars dust have not been characterized, and it is possible that long-wavelength UVA irradiation present in the UV spectrum of the xenon lamps used here may be able to penetrate soil particles more efficiently than short-wavelength UVC or UVB. This possibility is supported by the work of Cockell et al. (2000) in which water suspensions of a Mars analog soil (JSC Mars-1 palagonite) were shown to attenuate UVC and UVB at much stronger rates than UVA. In addition, xenon lamps produce significant quantities of UVA photons while D2 and Hg lamps are generally deficient in UVA (Fig. 12); recall that the study by Mancinelli and Klovstad (2000) used a 30 W deuterium lamp to irradiate monolayers of endospores of *B. subtilis*. Thus, even though UVC and UVB photons generally exhibit greater biocidal effectiveness than UVA photons (Jagger, 1985a; Nicholson et al., 2000), the high UVA fluence rates of xenon lamps may have been enough to impart significant UV damage to endospores of *B. subtilis* covered by large dust particles leading to inactivation of endospores on aluminum coupons. This hypothesis is supported by two reports (Xue and Nicholson, 1996; Slieman and Nicholson, 2001) describing the effects of UVA irradiation on dormant endospores of *B. subtilis*. First, Xue and Nicholson (1996) reported that wild-type endospores of *B. subtilis* were 5-fold more resistant to UVC than to UVA irradiation when compared to mutants lacking DNA repair systems, suggesting that at increasing solar UV wavelengths, spores are inactivated by DNA damage not repairable by the common nucleotide excision repair or spore photoproduct-lyase repair systems, damage caused by UVA is conferred onto other photosensitive molecules other than DNA, or both. Second, Slieman and Nicholson (2001) reported that dried endospores of strains of *B. subtilis* rich in dipicolinic acid (DPA) were more sensitive to UVA than to either UVC or UVB irradiation when compared to mutants deficient in DPA. Dipicolinic acid is known to be an important factor in protecting endospores of *B. subtilis* against desiccation and heat, and is presumably involved in UV re-

sistance (reviewed by Nicholson et al., 2000 and Setlow, 1995). Thus, if UVA irradiation penetrates simulated Mars dust more efficiently than UVC or UVB irradiation, then the significantly higher fluence rates of UVA in the Mars-normal illumination produced by the xenon lamps used in the current study, compared to the D2 or Hg lamps used elsewhere (Hagen et al., 1970; Mancinelli and Klovstad, 2000; Packer et al., 1963), might have been responsible for the inactivation of endospores of *B. subtilis* reported here for monolayers covered by large individual dust particles. The larger-sized dust particles (10–25 and 25–50 μm treatments) did provide some protection from simulated Mars-normal UV fluence rates during 1-h UV exposures, but the protective effects were lost with the 8-h UV exposures. These results suggest that even if dust coatings on spacecraft surfaces do provide partial UV protection, longer exposures times of hours to several Sols might still inactivate surface contamination on spacecraft components. Based on these results, we conclude that aeolian dust deposited onto spacecraft surfaces may not protect surface populations of terrestrial contaminants on spacecraft. Current research activities are underway to characterize the effects of UV irradiation from xenon, D2, or Hg lamps on survival of endospores of *B. subtilis* on spacecraft surfaces covered by layers of simulated martian dusts of increasing thickness.

The effects of pressure alone on survival of *B. subtilis* were consistent with a number of studies at low pressures conducted for various lengths of time. In general, populations of *B. subtilis* are reduced 10 to 70% at pressures below 10 mb when exposed between a few hours (current study), several days (Green et al., 1971; Horneck et al., 1984), several weeks (Horneck et al., 2001; Morelli et al., 1962), or several months (Dose and Klein, 1996; Lorenz et al., 1968). Surviving populations of *B. subtilis* endospores generally decreased as the time of exposure to low pressures increased. The longest study of bacterial survival at low pressures in space was conducted on the Long Duration Exposure Facility (LDEF) placed in low-Earth-orbit in 1984 (Horneck, 1993; Horneck et al., 1994). Endospores of *B. subtilis*, strain HA 101, were exposed to high vacuum (10^{-6} mb) for 69 months (2107 days) and protected from direct solar UV irradiation. Bioassays of the bacterial monolayers after retrieval of the LDEF spacecraft indicated that 1–2% of *B. subtilis* endospores survived for 2107 days in space provided they were protected from UV irradiation (Horneck, 1993; Horneck et al., 1994). We conclude that pressure alone had only a minor effect on the results of studies reported here because the exposure times at Mars-normal conditions were less than 12 h. However, as the time of exposure to low pressure is increased, the survival of *B. subtilis* would be expected to decrease, and, thus, much longer exposures to pressures below 10 mb would cause more significant reductions in surviving bacterial populations than were described here.

Both temperature and gas composition were shown to have no effects on the biocidal role of UV irradiation on *B. subtilis*. These results were somewhat surprising because other studies had reported that temperatures below −10 °C could either enhance (Ashwood-Smith et al., 1968; Weber and Greenberg, 1985) or reduce (Ashwood-Smith et al., 1968) survival of *B. subtilis* during UV irradiation. We hypothesized that for low temperatures, the survival of *B. subtilis* under UV irradiation would be inversely correlated to temperature. The results from the current study did not support this hypothesis. The results of the gas composition experiment reported here suggest that differences in microbial survival in studies conducted under argon, carbon dioxide, nitrogen, air, or mixtures of these gases (Green et al., 1971; Hagen et al., 1970; Imshenetsky et al., 1967, Koike et al., 1995, 1996; Mancinelli and Klovstad, 2000; Packer et al., 1963) were due to factors other than gas composition.

4.2. Predictions for soft-landed and air-bag landed surface vehicles for Mars

Based on the results of the pressure, temperature, gas composition, dust coating, and UV irradiation experiments reported here, we conclude that of these five factors encountered during robotic operations on the surface of Mars, only solar UV irradiation will significantly impact microbial survival on sun-exposed spacecraft surfaces during the first Sol. The low-pressured environment on Mars may reduce populations slightly, but most of the reductions in microbial numbers due to low-pressure are likely to occur during the 6–8 month cruise phase between Earth and Mars. Thus, once a surface vehicle lands safely on Mars, the effects of low-pressure likely will be minor compared to the UV flux. The following are brief discussions of possible forward contamination issues for landing sites on Mars during air-bag and soft-landing surface missions.

The planetary protection (PP) program developed for the Mars Pathfinder mission (Barengoltz, 1997) identified the bioload limits at launch as not to exceed 300 aerobic spores m^{-2} and no more than a total of 3×10^5 aerobic spores for exposed surfaces of the entire lander. Using these numbers, results from the current study, and the literature discussed above, the probable microbial bioload surviving the Earth-Mars cruise phase and the first Sol on Mars may be predicted. First, we estimate that between 50 to 70% of the launched bioload on Pathfinder likely was inactivated during the 7-month cruise phase to Mars (based on the work of Dose and Klein, 1996; Green et al., 1971; Horneck et al., 1984, 2001; Lorenz et al., 1968; Morelli et al., 1962). Thus, prior to entry of Pathfinder into the martian atmosphere, the bioload on the vehicle likely had been reduced to approximately 150 aerobic spores m^{-2} and no more than a total of 1.5×10^5 aerobic spores for exposed surfaces of the entire lander. Pathfinder landed (L) safely on Mars at approximately 0400 local time (Golombek et al., 1997). The airbags were deflated and retracted by L + 74 min, and the petals opened by L + 87 min (Golombek et al., 1997).

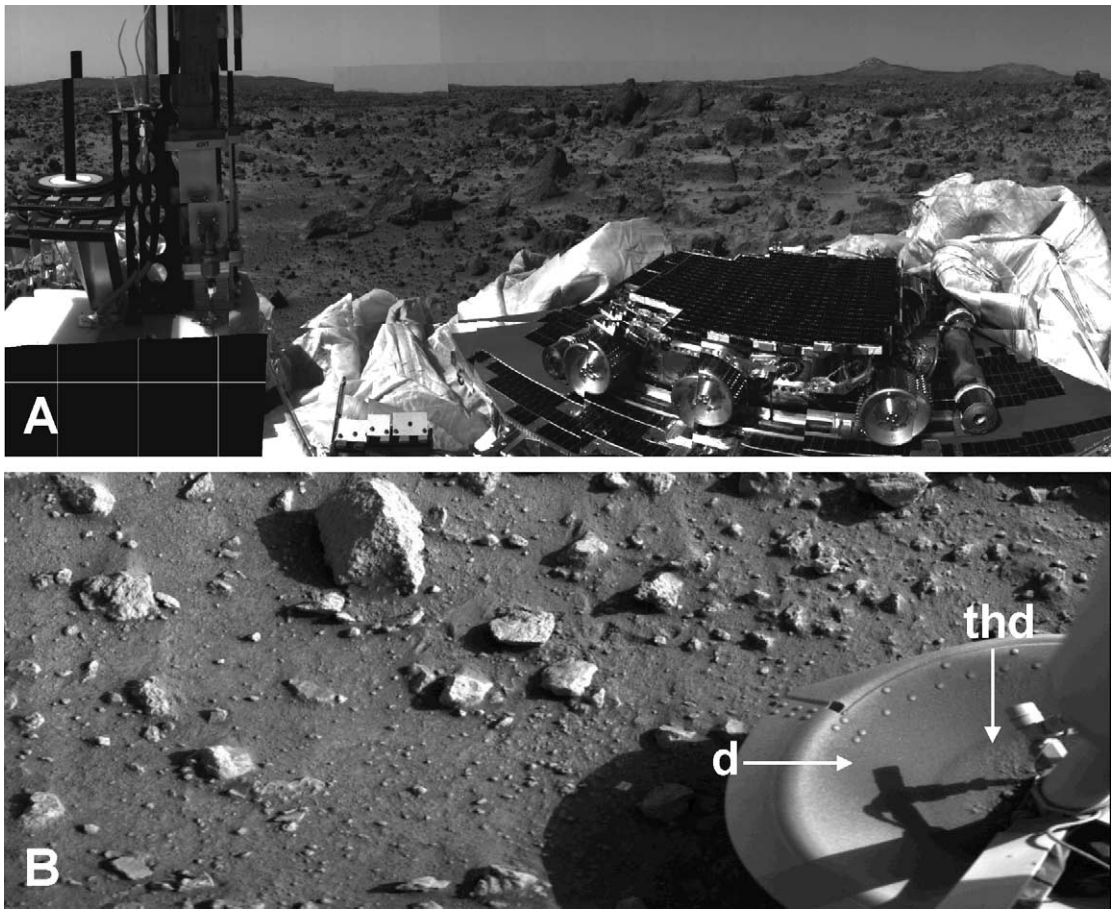

Fig. 13. (A) Pathfinder lander on Sol 2 with the non-deployed Sojourner rover on top of an extended petal. (B) Landing pad No. 3 on the Viking-1 lander depicting a thick layer of dust (thd) close to the landing-pad strut, and a thinner layer of dust (d) on the outer edge of the landing pad. The image of landing pad No. 3 was taken within 2–5 min after Viking-1 landed safely on Chryse Planitia (Mutch et al., 1976).

Thus, the upper surfaces of Pathfinder (Fig. 13A) were exposed to the martian atmosphere at approximately 0530 local time on Sol 1. If we assume that sunrise was at 0600 local time, then the upper surfaces of the Pathfinder vehicle were exposed to the martian environment for only 30 min before solar irradiation began illuminating spacecraft components. Second, aeolian dust on Mars measures 1–2 μm in diameter (Markiewicz et al., 1999; Pollack et al., 1995; Tomasko et al., 1999), and estimates during the Pathfinder mission indicated that surfaces were covered at a rate no greater than 0.28% per day (Landis and Jenkins, 2000). Thus, there was only about 30 min on Mars in which exposed surfaces of the vehicle could begin to accumulate aeolian dust before UV exposure. But the amount of dust accumulated on the exposed surfaces of the vehicle would have covered no more than approximately 0.006% of the spacecraft surface, and all of the aeolian deposited material would have been < 2 μm in diameter. Results reported here indicated that dust particles measuring 2–8 μm in diameter were unable to protect bacterial spores from the biocidal effects of Mars-normal UV irradiation, and, thus, it is reasonable to conclude that the smaller 1–2 μm sized dust particles that actually fell on Pathfinder surfaces during the first few hours on Sol 1 were inadequate in both size and abundance to protect bacteria from the biocidal effects of solar UV irradiation. Third, the optical depth of the martian atmosphere on Sol 1 during the Pathfinder mission was between $tau = 0.4$ and 0.5 (Smith et al., 1997; Smith and Lemmon, 1999). Based on the results presented here (and in particular on the lethal dose multiples in Table 3), it is likely that sun-exposed spacecraft surfaces on Pathfinder were sterilized several to many times during the first Sol on Mars. In contrast, spacecraft surfaces under the deflated air-bags and extended petals of Pathfinder (Fig. 13A) were likely to have received no lethal UV irradiation during Sol 1 on Mars.

In contrast to the air-bag landing system used by Pathfinder, soft-landed vehicles like Viking must use terminal descent engines that are likely to inject large quantities of dust into the air immediately around the landing vehicle at touchdown. Some of the suspended dust will be deposited onto spacecraft surfaces, and could offer significant UV protection for microorganisms if the dust layers accumulate to significant depths. For example, the first photograph returned from Viking 1 lander (Mutch et al., 1976) was taken within the first few minutes on the surface and clearly shows sig-

nificant deposits of dust on landing pad number 3 (Fig. 13B) (Seiff and Kirk, 1977). The deposit of Mars dust appears to be thick enough to have formed a contiguous layer on the upper surface of the landing pad. Based on the results presented here, one conclusion might be that this layer would indeed be enough to protect viable terrestrial microorganisms present on the upper surfaces of the Viking lander pads. However, the full picture of whether this could pose a forward contamination issue on a future soft-landed Mars mission must be based on modeling the entire process of microbial survival and UV exposure from payload processing, through the launch and cruise phases of the mission, and ending in the precise landing profile of the lander. Similar to Pathfinder, a soft-landed vehicle using descent engines would still be exposed to 6–8 months of high-vacuum during the cruise phase in which 50–70% of the viable launched bioload is likely to be lost before atmospheric reentry on Mars. Furthermore, the precise landing configuration and atmospheric reentry profile will have a significant impact on the survival of terrestrial microorganisms on exposed surfaces. Although the Viking landers were heat-sterilized at 112 °C for 30 h (VL1) and 23 h (VL2) (Puleo et al., 1977), future soft-landed Mars vehicles may not be. The following discussion uses the Viking landing profiles (Godwin, 2000; Seiff and Kirk, 1977; Soffen and Snyder, 1976; Soffen, 1977) as an example of how soft-landed vehicles might enter the martian environment.

Future soft-landed vehicles with descent engines may not pose a forward contamination risk to Mars because adequate UV exposure can be accumulated on spacecraft surfaces if the vehicles are soft-landed during daylight hours. Both Viking landers touched down at approximately 1600 local time on Mars (Soffen and Snyder, 1976). The optical depth of the martian atmosphere at landing was between 0.3 and 0.5 for both missions (Badescu, 1998), and the sun elevation angles above the horizons at the times of both landings were approximately 30 degrees. After the deorbit burns and reentries, both VL1 and VL2 supersonic parachutes were deployed at L − 85 s, and the aeroshells jettisoned at approximately L − 78 s. At L − 70 s, the lander legs were extended and began receiving solar UV exposures during the descent phases of the landings. The landing pads extended downward below the parachute shroud by approximately 0.2 m and were at least partially outside the shadows cast by the parachute shrouds (Godwin, 2000; Seiff and Kirk, 1977; Soffen and Snyder, 1976). At L − 40 s, the terminal descent engines were ignited and the parachutes were jettisoned. During the last 40 s of descent, the upper surfaces of both VL1 and VL2 landers, including all landing pads, were fully exposed to the UV environment in the martian atmosphere. The martian UV environment included both direct and diffuse UV irradiation, and it is likely that most surfaces on the landers were exposed to significant levels of solar UV irradiation as the descending vehicles changed their pitch, roll, and yaw characteristics. Furthermore, it is likely that the terminal descent engines did not inject dust into the local ambient environment at the landing sites until the vehicles were within 5–10 m of the surface, which would have occurred only for the last few seconds of descent. Thus, based on this landing profile and the results reported here, the landing pads of both Viking landers received solar UV for at least 40 s, and perhaps as long as 70 s prior to landing. And, the sun-exposed surfaces on the payload decks of both Viking landers received solar UV for approximately 40 s prior to landing. The kill curve for *B. subtilis* endospores reported here suggests that only 30 s may be required to achieve a three decade reduction in spore viability ($N/No \leqslant 0.001$), and, thus, most sun-exposed surfaces of the Viking Landers likely received enough UV dosage during descent to accumulate a lethal dosage in excess of the LD_{99} level for *B. subtilis*. Even if the Viking landers were not heat-sterilized prior to launch, it is likely that the upper sun-exposed surfaces of both vehicles would have experienced sufficient UV dosage to significantly reduce microbial populations before landing. Assuming that future near-term soft-landed vehicles will be launched with the same bioload as Pathfinder (i.e., 300 aerobic spores m^{-2} and no more than a total of 3×10^5 aerobic spores on exposed surfaces of the entire lander) and that approximately three decades reduction of viable bioload on sun-exposed surfaces is likely during the above modeled soft-landing scenario, then the vehicles might be predicted to retain no more than 0.3 viable aerobic spores m^{-2} on sun-exposed surfaces and perhaps as low as 1.5×10^2 viable aerobic spores for the entire lander at the time of touchdown. Thus, although dust deposition on spacecraft surfaces during a landing that utilizes terminal descent engines is an important issue for the forward contamination of Mars, the UV environment during daytime descents can be relied upon to achieve significant levels of sterilization prior to landing. However, if the landings occur at night, these conditions will not be present and the surviving bioload on spacecraft surfaces will not be impacted by solar UV irradiation until daybreak.

The models discussed above for air-bag and soft-landed vehicles are first-order approximations of likely landing scenarios on Mars. Several additional factors must be added to these models in order to accurately predict the total UV dosage received by Mars spacecraft. For example, landing at noon would increase the UV dosage and likely accomplish a greater level of sterilization than landing with lower sun elevation angles. Landing during periods of high optical depths in the atmosphere could significantly lower UV dosage rates such that spacecraft surfaces might receive only sub-lethal doses of UV irradiation on Sol 1. Seasonal and latitudinal considerations also will be important to accurately predict the total UV dosage a vehicle might receive during descent and landing on Mars. And lastly, the spatial orientation of spacecraft components relative to the sun at landing and during the first Sol on Mars will impact estimates of total UV effects on microbial survival of landed vehicles. However, given the rapid reductions in populations of *B. subtilis* endospores reported here, it seems reasonable to expect as

a first-order approximation that most sun-exposed surfaces of air-bag and soft-landed vehicles will be sterilized within one Sol on Mars even if the vehicles are landed during dust storms with optical depths > 3.5.

5. Conclusions

Mitigating the forward contamination of landing sites or life-detection experiments are important considerations for the design and implementation of surface missions to Mars. In order to correctly model this process, the microbial ecologies of spacecraft must be understood from initial assembly of spacecraft components through the operational termination of each mission. The primary factors to model include pre-launch microbial ecologies of spacecraft, effects of spacecraft sanitation procedures on microbial viability, microbial survival during the cruise phase of each mission, dispersal of viable spores away from landed vehicles, and survival of terrestrial microorganisms in the martian environment. The bacterium, *Bacillus subtilis*, was used in the current study as a model microorganism that is commonly recovered from spacecraft surfaces. However, most of the previously published literature on spacecraft contamination has not used modern molecular techniques to study the non-cultivable microbiota on spacecraft. Thus, the microbial survival model presented herein is only a first-order approximation of how terrestrial microorganisms might survive under martian conditions.

Microbial ecologies of cultivable microorganisms on robotic spacecraft have been relatively well characterized and are composed of microorganisms generally associated with human activity within spacecraft assembly facilities. Microorganisms shielded from direct exposure to solar UV irradiation during the 6–8 month cruise phase to Mars are likely to suffer reductions in their viability of perhaps 50–70% due to exposure to the high vacuum of interplanetary space. Once on the martian surface, microorganisms on sun-exposed spacecraft surfaces will experience temperature extremes of between -90 to $+20\,^{\circ}\mathrm{C}$, low atmospheric pressures between 6 to 10 mb, anaerobic conditions due to low pO_2 ($< 0.2\%$), high UV fluence rates, and galactic cosmic rays. Results and literature presented herein indicate that of these environmental conditions, only high UV fluence rates will significantly impact microbial survival on sun-exposed surfaces during the first few Sols on Mars. Results with *B. subtilis* endospores indicated that populations of viable bacteria were reduced three to four decades ($> 99.9\%$) when exposed to Mars-normal UV fluence rates for only 30 or 60 s under simulations of clear sky conditions ($tau = 0.3$). Furthermore, exposures to Mars-normal UV fluence rates lasting longer than 15 min inactivated endospores of *B. subtilis* to such an extent as to be non-recoverable from aluminum coupons. Although endospores of *B. subtilis* survived better when UV fluence rates were reduced in simulations of global dust storm conditions ($tau = 3.5$), results support the conclusion that terrestrial microorganisms will be inactivated very quickly on sun-exposed spacecraft surfaces under commonly encountered atmospheric conditions on Mars. In addition, most other cultivable microorganisms so far recovered from spacecraft surfaces are likely to exhibit even greater sensitivities to solar UV irradiation on Mars than the strain of *B. subtilis* used here. No information is presently available to suggest that non-cultivable microorganisms on spacecraft surfaces are more resistant than cultivable species to the harsh conditions found on Mars, and, thus, we believe that the current results are likely to be an effective model for the survival of most terrestrial microorganisms on Mars. But clearly there is a need for additional studies with other species of cultivable and non-cultivable microorganisms under simulated martian conditions.

Based on these results, the inactivation of microorganisms on sun-exposed spacecraft surfaces by UV irradiation on Mars likely will progress at a very rapid rate, taking only tens of seconds to a few minutes under clear sky conditions. In addition, microbial survival of microorganisms on spacecraft surfaces on Mars now may be predicted by modeling the accumulated UV dosage rates for different microbial species, orientations of spacecraft components, optical depths of the martian atmosphere, sun elevation angles, and latitudinal and seasonal variations on Mars.

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