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Survivability of *Psychrobacter cryohalolentis* K5 under Simulated Martian Surface Conditions

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ABSTRACT

Spacecraft launched to Mars can retain viable terrestrial microorganisms onboard that may survive the interplanetary transit. Such biota might compromise the search for life beyond Earth if capable of propagating on Mars. The current study explored the survivability of *Psychrobacter cryohalolentis* K5, a psychrotolerant microorganism obtained from a Siberian permafrost cryopeg, under simulated martian surface conditions of high ultraviolet (UV) irradiation, high desiccation, low temperature and low atmospheric pressure. First, a desiccation experiment compared the survival of *P. cryohalolentis* cells embedded, or not embedded, within a medium/salt matrix maintained at 25 °C for 24 hrs within a laminar flow hood. Results indicated that the presence of the medium/salt matrix enhanced survival of the bacterial cells by 1 to 3 orders of magnitude. Second, tests were conducted in a Mars Simulation Chamber to determine the UV tolerance of the microorganism. No viable vegetative cells of *P. cryohalolentis* were detected after 8 hrs of exposure to Mars-normal conditions of 4.55 W/m^2 UVC irradiation (200-280 nm), −12.5 °C, 7.1 mbars and a Mars gas mix composed of CO_2 (95.3%), N_2 (2.7%), Ar (1.6%), O_2 (0.2%) and H_2O (0.03%). Third, an experiment was conducted within the Mars chamber in which total atmospheric opacities were simulated at τ = 0.1 (dust free CO_2 atmosphere at 7.1 mbars), 0.5 (normal clear-sky with 0.4 = dust opacity and 0.1 = CO_2 only opacity) and 3.5 (global dust storm) to determine the survivability of *P. cryohalolentis* to partially shielded UVC radiation. The survivability of the bacterium increased with the level of UVC attenuation, although population levels

still declined several orders of magnitude compared to UVC absent controls over an 8-hr exposure period.

INTRODUCTION

Spacecraft that leave Earth inadvertently carry microscopic life forms onboard embedded within surface defects, wiring, electronic boards, and metal crevices (Schuerger, 2004). These microorganisms can endure the space environment, including conditions of high vacuum, extreme temperature fluctuations, high doses of solar UV irradiation, and other conditions hostile to living cells (Horneck *et al.* 2002; National Research Council 2005). Microbial contamination of spacecraft may pose a serious concern for space missions with life-detection payloads. A primary concern for near-term Mars surface missions is how to eliminate the risks of false-positives derived from the launched terrestrial bioloads (Rummel and Meyer, 1996; Schuerger, 2004). Thus, measurements of the survivability of bacteria under simulated martian surface conditions are essential to understanding which kinds of terrestrial microorganisms – if any – can potentially propagate on the surface of Mars (National Research Council 2005).

Recent studies have documented a high diversity of microorganisms in NASA spacecraft assembly facilities, which could readily attach to spacecraft (La Duc *et al.*, 2003; 2004; 2007; Link *et al.*, 2004; Moissl *et al.*, 2007; Venkateswaran *et al.*, 2001; 2003). Crawford (2005), Nicholson *et al.* (2005), and Tauscher *et al.* (2006) have hypothesized that maintaining a hygienic environment inadvertently selects for oligotrophic microbes. For example, spores of *Bacillus*

sp. and *Clostridium* sp. isolated from spacecraft clean rooms have indeed shown unusual resistance to UV irradiation (Link *et al.*, 2004; Newcombe *et al.*, 2005). These microorganisms and possibly others may be conditioned and pre-adapted to the harsh conditions of spaceflight (La Duc *et al.*, 2004). Survival of terrestrial microbes in interplanetary space requires resistance to extreme low pressures, extreme temperatures, (ranging from -171 °C to +111 °C), severe desiccation, solar UV irradiation, solar particle/radiation events, and cosmic rays (Schuerger, 2004). Despite these conditions, it has been estimated that a typical Mars spacecraft might retain $3x10^4$ – $2x10^7$ viable bacteria, located deep inside the vehicle, by the time it reaches the surface of the planet (Schuerger, 2004).

Schuerger *et al.* (2003; 2006) showed that after reaching the surface, 99.9% of *Bacillus subtilis* spores exposed to simulated martian conditions were killed within 30 sec, and greater than six orders of magnitude reductions were observed after 180 min. Results suggested that direct exposure to UV irradiation was the most limiting environmental factor for *B. subtilis* survival on the surface, whereas simulated Mars gas composition, pressure and temperature had no measurable effects (Schuerger *et al.*, 2003). Cockell *et al.* (2005) had similar UV-dependent results when studying the survivability of the cyanobacterium *Chroococcidiopsis* sp. 029 under simulated martian conditions. Together, these experiments predict a low probability of bacterial surface contamination on Mars, provided that terrestrial organisms are exposed to an ambient, daytime UV flux

(Schuerger *et al.*, 2003; 2006; Cockell *et al.*, 2005). The absence or attenuation of UV light, however, might create very different survivability scenarios. Both Schuerger *et al.* (2003) and Cockell *et al.* (2005) found that the survival of bacteria increased significantly when shielded from UV irradiation by thin layers of dust or rocks. In fact, *Chroococcidiopsis* sp. 029 retained viability after 8 hrs under rock coverage only 1 mm thick (Cockell *et al.*, 2005) and *B. subtilis* survived 8 hrs of UV irradiation when covered by only a 0.5 mm coating of dust (Schuerger *et al.*, 2003). In addition, Morozova *et al.* (2006) found that after a 22 d exposure to simulated thermo-physical conditions at martian low- and mid-latitudes, up to 90% of methanogenic archaea from Siberian permafrost survived in pure cultures as well as in environmental samples. It is conceivable that microbes on the external surfaces of Mars spacecraft will be protected from radiation by atmospheric dust events that deposit dust on the spacecraft surface, or by mission operations that involve drilling into rocks or driving through regolith.

Psychrophilic and psychrotolerant bacteria may possess unique capacities for dealing with the martian surface environment (Cockell *et al.*, 2005; National Research Council, 2005; Nicholson *et al.*, 2005). Throughout extremely cold climates on Earth, these specially-adapted microorganisms not only survive but thrive in conditions with low temperatures, oligotrophic nutrient regimes, and limited water resources (Cavicchioli, 2002). Recently cultured bacteria from

Siberian permafrost and Antarctic ice sheets can metabolize at temperatures down to –20 °C (Rivkina, 2000; Bakermans *et al.*, 2006).

The primary objective of the current study was to determine whether the psychrotolerant bacterium, *Psychrobacter cryohalolentis* K5, could survive under simulated Mars conditions if provided with adequate levels of nutrients, salts, and moisture. Survivability was defined by the viability of bacterial cells. The experiments herein did not measure growth or replication of *P. cryohalolentis*. The goal was to determine whether the survivability of *P. cryohalolentis* was greater than the mesophilic species tested previously in simulated martian conditions (Schuerger *et al.*, 2003; Cockell *et al.*, 2005; Newcombe *et al.*, 2005; Schuerger *et al.*, 2005; Schuerger and Nicholson, 2006; Tauscher *et al.*, 2006), particularly when protected from UV irradiation.

MATERIALS and METHODS

Strain isolation and physiology

Psychrobacter cryohalolentis K5 was isolated from a –12 °C subsurface hypersaline cryopeg in the Kolyma lowlands of Siberia, Russia. Bakermans *et al.* (2006) characterized the bacterium as a gram-negative, strictly aerobic, coccobacillus that was non-motile, non-pigmented, non-spore forming, and capable of growth in a pH range from 6.0 to 9.5 and a salinity range of 0 to 1.7 M NaCl. Cell dimensions were 0.9-1.3 μm long and 0.5-0.8 μm wide. The optimal

growth temperature for the species was 22 °C, but the growth range stretched from -10 to 30 °C (Bakermans *et al.*, 2006). At its optimal growth temperature, generation time was just under 5 hrs (Bakermans and Nealson, 2004).

Standard microbiological procedures

The *P. cryohalolentis* K5 strain was cultivated at room temperature (25 °C) in a liquid growth medium made from the following chemicals in 1 L of deionized water: 1 g of Bacto™ yeast extract, 5 g of Bacto™ peptone, and 17 g of sea salts. For solid media, 16.5 g of Bacto™ agar were added to 1 L of solution. All media ingredients were obtained from Becton Dickinson and Company (Sparks, MD, USA), except for the sea salts, which were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Inoculum of *P. cryohalolentis* was prepared 24 hrs prior to use by incubating vegetative cells in a test tube with 10 mL of liquid media at 25 °C. On solid media at 25 °C, colonies grew as circular, smooth, opaque and ~ 1 mm diameter after 36-48 hrs.

In order to simulate bacterial adhesion to, and survival on, spacecraft surfaces, vegetative cells of *P. cryohalolentis* were placed on aluminum coupons (2 cm x 1 cm x 1 mm) previously coated with a chromate conversion film (Iridite 14-2, MacDermid, Inc., Waterbury, CT, USA) (see Schuerger *et al.*, 2005; 2008). The Iridite surface treatment reduces corrosion on aluminum and is often used on spacecraft aluminum components (Schuerger *et al.*, 2005). The aluminum coupons were dry-heat sterilized at 130 °C for 24 hrs and allowed to cool to 25 °C

before bacteria were deposited onto the aluminum surfaces. Under aseptic conditions, vegetative cells of *P. cryohalolentis* from liquid cultures were applied to the center of coupons in 100-µL spots and allowed to dry at 25 °C in a laminar flow hood for 2 hrs (NuAire Inc., model NU-201-430, Plymouth, MN, USA). Once dried the media/salt residue would form a crystalline matrix that both encased bacteria and provided nutrients to sustain cells (Fig. 1). This coupon preparation procedure was standardized and used for all experiments. Media/salt residues on coupons were imaged using a high-resolution video microscope (model VH-7000, Keyence Corp. of America, Woodcliff Lake, NJ, USA).

Desiccation experiment for comparing coupon preparation techniques

A 24-hr desiccation experiment was conducted with *P. cryohalolentis* at room temperature (25 °C) and pressure (1013 mbar). The purpose of the experiment was twofold: (i) to evaluate the desiccation resistance of the bacterium on simulated spacecraft surfaces, and (ii) to determine if survivability was enhanced by encasement within the medium/salt matrix. Two sets of coupons were prepared for the experiment, hereafter referred to as 'media' and 'non-media' coupons. The media coupons contained *P. cryohalolentis* vegetative cells embedded in the medium/salt matrix and dried onto the surface of the coupons, as described in the previous section. For the non-media coupons, cells were grown on solid sea-salt media for 24 hrs at 25 °C, mechanically harvested and diluted in sterile deionized water (SDIW) to densities of 3.19 x 10^9 cells per mL as

quantified by a Spectronic-20 spectrometer (model 4001, Spectron Instruments, USA) set at 400 nm. Quantified cell suspensions were then spotted onto spacecraft coupons in 100-μL volumes per coupon. The T = 0 samples for both the media and non-media coupons were sampled, while the remaining coupons were placed in a Petri dish to desiccate in a dark laminar flow hood for 24 hours. The surviving bacteria from the media coupons for T = 0 and T = 24 hour samples were estimated by the Most Probable Number (MPN) method. The minimum detection limit of the MPN assay was 10 cells per coupon. Cells were re-suspended in 100 μL of liquid media, followed immediately by processing through a series of 10-fold liquid media dilutions, in which each diluted sample was plated onto solid media. For the non-media coupons, the method described by Schuerger *et al.* (2003; 2006) was followed, in which coupons were placed into plastic tubes with autoclaved deionized water and shaken with 1 g of heat-sterilized silica sand (24 h at 130 °C). Next, 10-fold serial dilutions with sterile water were performed and aliquots were dispensed onto solid media and the number of viable cells estimated by MPN. For both the media and non-media coupons, the numbers of viable bacteria were estimated after cultures had incubated at 25 °C for 42 hr.

Mars Simulation Chamber experiments

The Mars Simulation Chamber (MSC) is located in the Space Life Sciences Laboratory at Kennedy Space Center, FL, USA. Details of the MSC

construction and mechanical function have been published elsewhere (Schuerger *et al.*, 2008). The MSC system was capable of reproducing martian surface environmental conditions, including: pressure (down to 0.1 mbar), temperature (-100 to +200 °C), atmospheric gas composition (top five gases; see below), and atmospheric dust loads (optical depths [τ] from dust-free skies [0.1] to global dust storms [3.5]). Temperature for all experiments was maintained at –12.5 °C, based on average daytime highs recorded by the Viking 1 and 2 landers (Owen, 1992) and just below the minimum growth temperature reported for *P. cryohalolentis* (Bakermans *et al.*, 2006). Pressure was fixed at 7.1 mbar. A gas mixture (Boggs Gases, Titusville, FL) representing the martian atmosphere was proportioned into the chamber using a mass-flow controller and consisted of: CO_2 (95.3%), N_2 (2.7%), Ar (1.6%), O_2 (0.2%) and H_2O (0.03%). Ultraviolet irradiation was generated within the spectral range of 200-400 nm based on a Mars UV model described previously (Schuerger *et al.*, 2003; 2006). Fluence values for UVC (200-280 nm), UVB (280-320 nm), and UVA (320-400 nm) were 4.55, 8.82 and 36.95 W/m^2, respectively. Total UV flux from this calibration was 50.32 W/m^2.

For the first Mars simulation experiment, UV light was passed through neutral density filters (Maier Phototonics, Inc., Manchester Center, VT) to create an optical depth of $\tau = 0.1$, which simulated the surface of equatorial Mars at its mean orbital distance from the Sun, under dust-free skies (Schuerger *et al.*, 2003; 2006). Bacteria were prepared using the standard spotting technique on

aluminum coupons in which vegetative cells were embedded within a medium/salt matrix. The number of bacteria applied in 100-μL drops onto coupons was estimated to be 1.8×10^{10} from 9 replicates. Coupons were loaded into the MSC in pre-sterilized glass Petri dishes, which sat directly on the upper surface of the LN_2 cold-plate. The first experiment was designed to measure bacterial survival over increasing time exposures, where one sol was the equivalent of 8 hrs UV exposure and 16 hrs of darkness. Time-steps for the experiment were 0.5, 1, 3 and 9 sols. Each time-step had bacterial coupons divided into one of three groups (each with triplicate samples) designed to pinpoint the effect of UV irradiation on survivability: Mars (+UV), Mars (–UV), and Earth controls (-UV). The Mars (+UV) coupons were inside the MSC and exposed directly to UV irradiation; the Mars (–UV) coupons were also inside the MSC but completely protected from UV light; and the Earth control (–UV) coupons were placed outside the MSC, wrapped with aluminum foil, and left at room conditions (25 °C, 1013 mbar). Following the martian simulation, all coupons were assayed for viability using the MPN method described above. The experiment was repeated 1 week later under identical conditions, yielding a total of 6 replicates per treatment.

The second Mars simulation experiment was designed to determine how atmospheric dust loading might affect survivability of bacteria cells embedded within the dried media/salt matrix. Neutral density filters (Fig. 2) composed of

silica glass and a thin Ni-Cr-Fe alloy coating were used to establish optical depths (τ) of 0.1, 0.5 or 3.5 for UV irradiation, effectively simulating (i) a dust-free CO_2 atmosphere, (ii) a typical clear sky condition with low levels of dust, or (iii) a global dust storm condition (sensu Schuerger *et al.*, 2003; 2006). A UV spectrometer (model IL1400A, International Light, Newburyport, WA, USA), was used to measure UVC values of 4.55, 3.57 and 0.21 W/m^2 for optical depths of 0.1, 0.5, and 3.5, respectively. Alongside the three different simulated dust loading conditions, additional bacterial coupons were shielded from all UV irradiation within the MSC, placed in a sample holder fitted with an opaque aluminum plate instead of a neutral density filter (Fig. 2). A set of coupons were held as Earth controls outside of the MSC and maintained at room temperature conditions without UV exposures. Coupons were exposed to martian conditions of 7.1 mbar, −10 °C, and Mars gas composition for 24 hrs with a 1-sol UVC simulation (i.e., 8 hrs of UV irradiation), and immediately assayed for survival. The experiment was repeated under identical conditions, for a total of 6 replicates per treatment.

Statistical treatment of data

Analysis of survivability values (log-transformed means) was performed using the statistical program R version 2.3.1 (The R Foundation for Statistical Computing, Vienna, Austria, 2006). Data were subjected to one-way permutation tests, both rank and pairwise, to compare mean population differences across

groups and controls at a 95% confidence level ($P < 0.05$). For the Mars simulations, the Wilcox test was used to examine population values across time between different treatment groups. The Kruskal-Wallis rank sum test was used to analyze population values across time within individual treatment groups.

RESULTS

Survivability from desiccation experiment

The desiccation experiment sought to determine the effects of drying vegetative cells of *P. cryohalolentis* onto aluminum coupons either alone (i.e., no buffers or medium/salt matrix) or embedded in a medium/salt matrix. Cells of *P. cryohalolentis* in both the media and non-media treatments were desiccated under identical environmental conditions for 24 hrs. Survivability assays showed a decline of several orders of magnitude for both experimental groups over the desiccation interval (Fig. 3). Although both groups declined, cells of *P. cryohalolentis* embedded within the media/salt matrix survived better than the non-media cells (permutation test: $P = 0.032$); a 4-order of magnitude reduction compared to a 6-order of magnitude reduction, respectively, was observed. The rate of decline for the bacteria in the media group was consistent with the results reported in the Mars simulations. Although a difference in initial population values for media and non-media coupons existed due to the disparate preparation techniques, this did not affect measuring survivability as a proportional value.

Survivability from Mars Simulation Chamber experiments

The survivability of *P. cryohalolentis* was negatively affected by exposures to UV irradiation under simulated Mars low-dust sky conditions (τ = 0.1) (Fig. 4). Survivability was dependent upon the treatment type and length of time exposure (Kruskal-Wallis rank sum test: $P < 0.05$). The number of viable cells after the Mars (+UV) treatment were significantly lower compared to the Mars (–UV) and Earth control (–UV) groups (Wilcox Test: $P < 0.001$), and fell below the detection limits of the MPN assay by the end of sol 1. The difference between the number of viable cells on the Mars (–UV) chamber coupons and Earth control (–UV) coupons was not statistically significant (Wilcox Test: P = 0.156). Although surviving populations of *P. cryohalolentis* for both (–UV) treatments persisted for the 9 sol simulation, the number of viable cells still declined by 100x over the course of the experiment (Kruskal-Wallis rank sum test: Mars (–UV) coupons $P = 0.0149$; Earth control (–UV) coupons $P = 0.0120$).

In the second MSC experiment the recovered numbers of vegetative cells in each dust treatment were significantly lower than control treatments (Kruskal-Wallis rank sum test: $P < 0.001$) (Fig. 5). No viable cells of *P. cryohalolentis* were recovered from coupons exposed to a dust simulation of τ = 0.1. Furthermore, the τ = 0.5 dust simulation experiment yielded approximately 1 x 10^3 viable cells per coupon, a 4-order of magnitude decline from the (–UV) Mars controls. The greatest numbers of viable bacteria were recovered from the τ = 3.5

coupons even though it was several orders of magnitude lower than that of the initial time-step.

DISCUSSION

The desiccation experiment demonstrated that *P. cryohalolentis* was especially sensitive to drying, by exhibiting a > 6 order of magnitude population reduction in recovered cells desiccated for 24 hrs. In order for a terrestrial microbe to survive and proliferate on Mars, it would need to tolerate, at minimum, desiccating conditions during the six-month long interplanetary transit, and also on the dry planet surface for periods long enough to become dispersed into niches conducive for growth. Hence, the importance of the *P. cryohalolentis* population decline in the desiccation experiment was threefold: (1) some of the Mars Simulation Chamber viability reductions could be attributed to desiccation alone, (2) *P. cryohalolentis* did not appear tolerant to the kinds of desiccating conditions that would arise in a forward contamination context during a Mars robotic mission, and (3) even the presence of a media/salt matrix (i.e., biofilm) did not provide sufficient long-term protection from water stress to dramatically enhance survival over non-media treatments.

The inability of *P. cryohalolentis* to maintain its population in the 24-hr desiccation period was unexpected, considering that this bacterium was recovered from salty, oligotrophic permafrost (Bakermans *et al.*, 2006). When bacteria inoculated into liquid media were applied onto coupons, the dried residues were

composed of a crystalline matrix that (1) encased bacteria with an environmental barrier, and (2) provided nutrients to cells. The desiccation experiment revealed the extent of enhanced survivability was minimal, by showing in parallel, the declining population of the same *P. cryohalolentis* cells without the media/salt matrix. It is also worth noting that the desiccation experiment was conducted outside the MSC in a laminar flow hood at room-normal humidity [40-50% at 23 °C)], not the 0.03% H_2O present in the martian atmosphere. True desiccation would have required a more sophisticated drying device, such as the instrument described by Kendrick and Kral (2006), which would likely have further reduced the survivability of *P. cryohalolentis*.

Even assuming that *P. cryohalolentis* could tolerate interplanetary desiccation and reach the surface of Mars in viable form, the current study indicates that UV irradiation would kill any sun-exposed cells within 8 hrs or less. This death rate is slightly slower than previously reported values for the bacterium *Bacillus subtilis* HA101 (Schuerger *et al.*, 2003; 2006) and *Chroococcidiopsis* sp. 029 (Cockell *et al.*, 2005). The slightly increased length of time (up to 8 hrs) observed for survival with *P. cryohalolentis* might be related to the protective medium/salt matrix that surrounded the vegetative cells in the current study. For example, with *B. subtilis* HA101 (Schuerger *et al.*, 2003), no microbial biofilm was associated with endospores exposed to martian conditions, but some extracellular material was observed associated with cells of *Chroococcidiopsis* sp.

029 in other similar Mars simulations (Cockell *et al.*, 2005). Comparing these two papers with the results from the current study, the numbers of recovered viable cells may be correlated with an increase of extracellular material (*B. subtilis* < *Chroococcidiopsis* < *P. cryohalolentis*).

The first Mars simulation experiment isolated UV irradiation as the primary biocidal factor by showing significantly different survivability across Earth control (–UV), MSC (–UV), and MSC (+UV) treatments. Bacteria exposed to direct UV irradiation died within 1 sol, whereas cells exposed to identical conditions but shielded from UV light survived beyond that exposure length; yet did fall by 2 orders of magnitude by 9 sols. It is worth noting that the dramatic reductions observed for (+UV) exposed Mars samples occurred while the vegetative cells were embedded in a medium/salt matrix that would have been expected to partially or fully attenuate the UV irradiation. Previous studies (Mancinelli and Klovstad, 2000; Schuerger *et al.*, 2005) have suggested that biofilms might significantly enhance the survival of microorganisms under martian conditions by attenuating solar UV irradiation. However, the results of the current study do not support this conclusion.

Since Earth (–UV) and Mars (–UV) populations of *P. cryohalolentis* changed similarly across time and were sustained through the final time-step (sol 9), it was concluded that martian temperature, pressure, and gas composition had little effect on *P. cryohalolentis* survival. This result is consistent with previous

reports using a variety of different microorganisms (Schuerger *et al.*, 2003; Cockell *et al.*, 2005; Nicholson *et al.*, 2005; Schuerger *et al.*, 2006) and may be attributed primarily to water stress.

Increasing simulated dust levels enhanced the survivability of vegetative cells of *P. cryohalolentis* over 1 sol. The low-dust sky treatment ($\tau = 0.1$) killed all bacteria by 1 sol; reproducing results from the first MSC experiment Mars (+UV) coupons. Only one-sixth of the coupons under the normal clear-sky dust scenario ($\tau = 0.5$) retained viable bacteria after 1 sol simulations. The global dust storm scenario ($\tau = 3.5$) yielded higher survivability, but with only moderate significance, suggesting that even trace amounts of UV irradiation on Mars may dramatically reduce bacterial survival.

CONCLUSIONS

Psychrophilic and psychrotolerant microorganisms have been regarded as serious contamination threats to Mars (National Research Council, 2005). *P. cryohalolentis*, however, had virtually no tolerance to the martian environment, even during scenarios where UV irradiation was partially or fully attenuated. Since survival of *P. cryohalolentis* was strongly inhibited by martian surface conditions, subsequent growth, replication, and proliferation seem unlikely. Desiccation and UV irradiation were identified as the primary lethal factors in these simulations; consistent with results reported for mesophilic bacteria tested

under similar martian conditions (Cockell *et al.*, 2005; Newcombe *et al.*, 2005; Schuerger *et al.*, 2003; 2005; 2006; Tauscher *et al.*, 2006).

While simulated atmospheric dust conditions in the current study permitted small amounts of biocidal UV light to reach cells of *P. cryohalolentis*, natural UV-shields that more thoroughly block light on the martian surface are imaginable. Future research should continue to explore how the survivability of psychrophilic and psychrotolerant bacteria on the surface of Mars changes with UV-shielding. Covering bacteria with a thin layer of dust, for example, should increase survivability (Mancinelli and Klovstad, 2000; Schuerger *et al.*, 2003), as would embedding bacteria into rocks or crevices within spacecraft material (Schuerger *et al.*, 2005). If UV irradiation was totally eliminated as an environmental factor, desiccation could become the major constraint on survivability of *P. cryohalolentis*. In the results presented here, viable *P. cryohalolentis* cells at the final MSC experimental time-step (sol 9) should not necessarily be considered Mars-adapted, since our results, and those of (Beaty *et al.*, 2006) indicate that water stress might continue to decrease bacterial survivability over time. More research, therefore, is needed to understand the survivability of psychrophilic and psychrotolerant microbes over time in cold, extremely desiccating conditions. Identification of purported biogenic trace gases and microbial cells in ice cores suggest that certain bacteria can survive hundreds of thousands of years at –40 °C, maintaining DNA and protein integrity so that

when conditions improve cells can reactivate and grow (Price and Sowers, 2004; Napolitano and Shain, 2004). This might mean that contaminating bacteria cells which do not grow or proliferate on Mars during the period of robotic or human exploration could eventually be revitalized on a geological timescale (Willerslev *et al.*, 2004). Therefore, until the survivability of additional extremophile life forms has been evaluated under simulated martian conditions, the effort to classify microorganisms found in spacecraft assembly facilities and to develop economical methods to sanitize spacecraft seems to be an appropriate conservative measure (Schuerger *et al.*, 2003; La Duc *et al.*, 2004; Tauscher *et al.*, 2006; Moissl *et al.*, 2007).

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FIGURE LEGENDS

Fig. 1. Coupon preparation method for *Psychrobacter cryohalolentis* K5. (A) The standard aluminum coupon preparation procedure used for all experiments, with the 100-µL liquid media/salt matrix spotted on each coupon and containing *P. cryohalolentis* vegetative cells. (**B**) Microscopic view of a coupon surface after the liquid media spot had dried; i.e., media technique. (**C**) Microscopic view of a coupon surface prepared with liquid water; i.e., non-media technique.

Fig. 2. Experimental setup for Mars Simulation Chamber (MSC) atmospheric dust experiment. Treatments are shown resting on the liquid-nitrogen (LN_2) cold plate within the MSC system with neutral density filters calibrated to simulate different atmospheric dust loads. The Mars (–UV) control is located at the far back, while the three dust scenarios are in the foreground: Mars dust load simulations are shown for $\tau = 0.1$ (upper left); $\tau = 0.5$ (upper right); and $\tau = 3.5$ (bottom right). The coupons within the glass Petri dish (lower left) can be disregarded. The experiment delivered 8 hrs of UV irradiation during a total Mars simulation time of 24 hrs.

Fig. 3. Effect of preparation technique on *P. cryohalolentis* K5 desiccation over a 24-hr period. The standard liquid media preparation technique (used throughout this study) was compared to a water-diluted, non-media procedure which left bacterial cells unprotected on coupon surfaces. Both coupon preparation treatments were desiccated at 25 °C for 24 hrs and were then assayed for survival

by the MPN assay. Values are means of multiple replicates; *bars* represent standard errors of the means.

Fig. 4. Mars Simulation Chamber experiment under simulated low-dust sky (τ = 0.1) conditions. *Psychrobacter cryohalolentis* K5 doped coupons (i.e., embedded in the medium/salt matrix) were exposed to simulated martian conditions for five distinct time intervals. Values are means of six replicates; *bars* represent standard errors of the means.

Fig. 5. Atmospheric dust experiment varying irradiation exposure in MSC. *Psychrobacter cryohalolentis* K5 doped coupons were exposed to martian conditions under different simulated atmospheric conditions for 1 sol (i.e., 24-hr in MSC with 8-hr UV irradiation). For τ = 0.1, the asterisk indicates that the number of bacteria recovered were below the MPN detection limit of 10 cells per coupon (dotted line) for all replicates (i.e., 100% of coupons were negative). Values are means of six replicates; *bars* represent standard errors of the means.

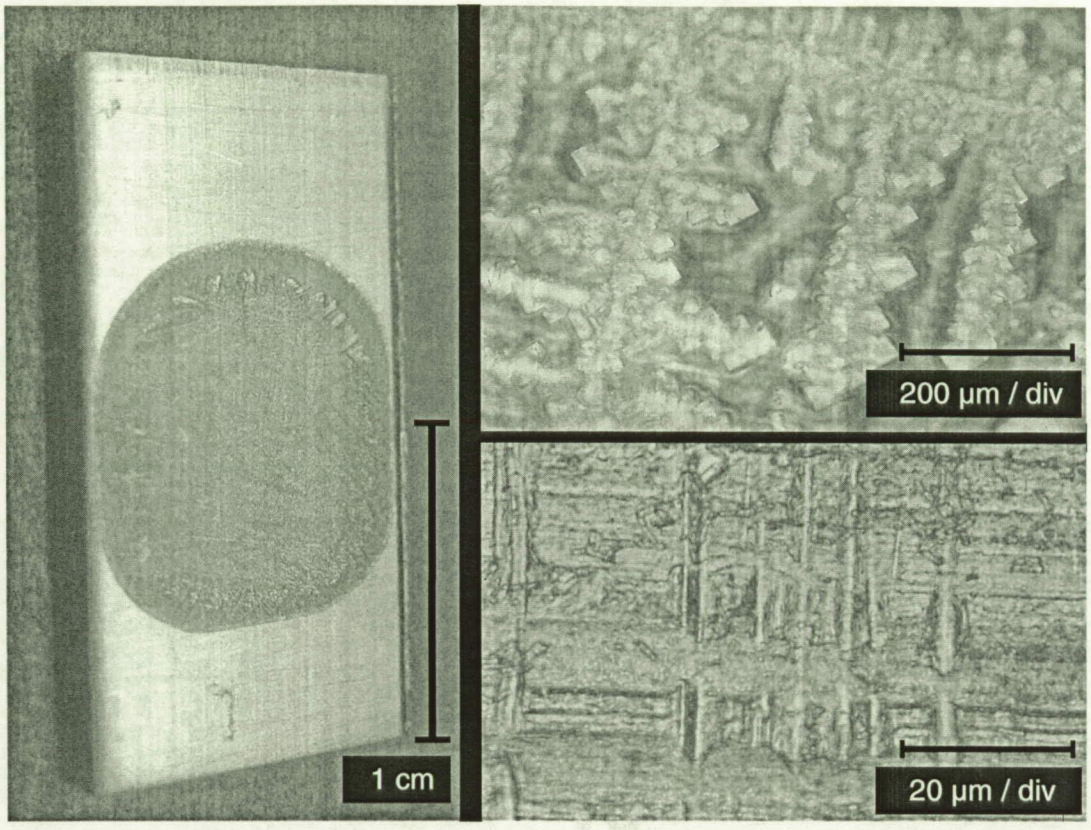
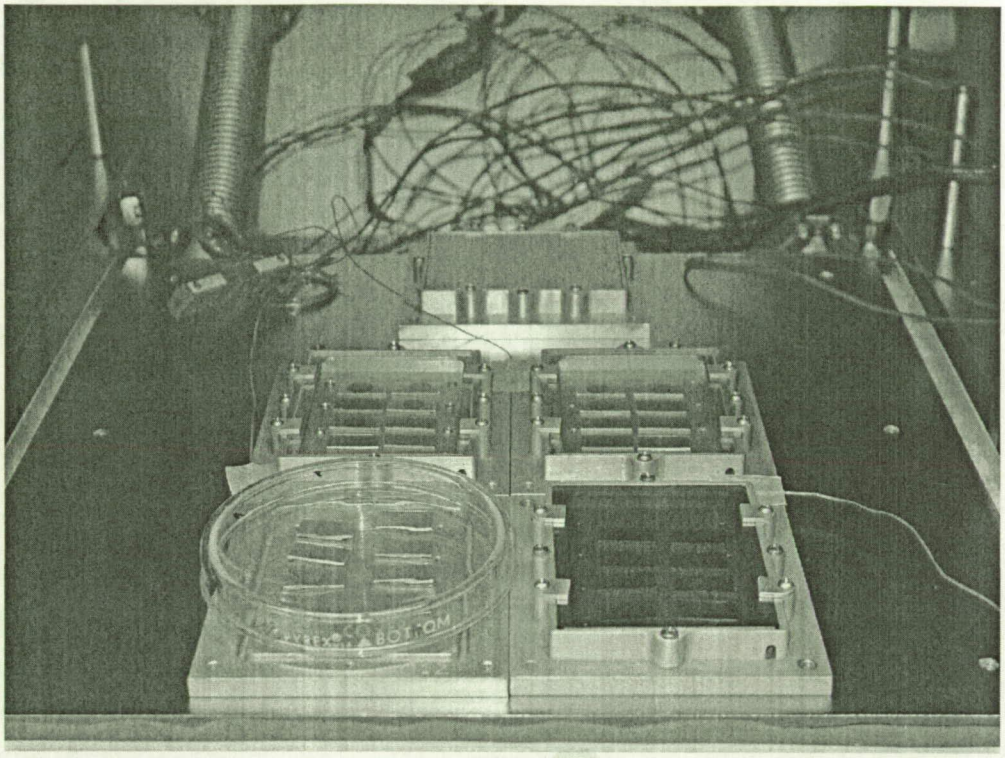


Fig. 1.



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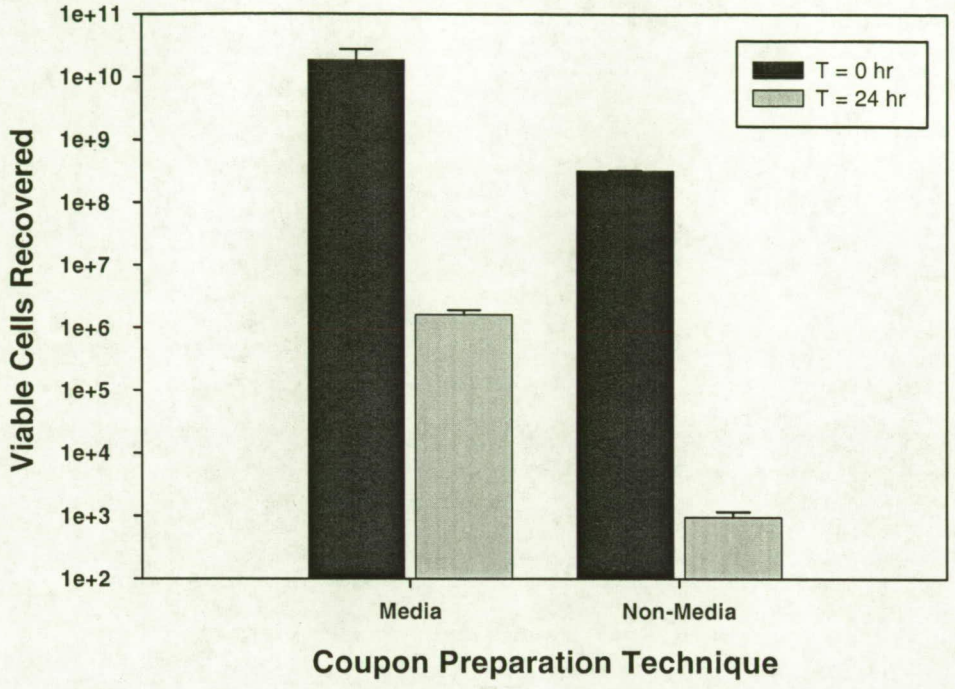
1e+11
1e+10
1e+9
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1e+5
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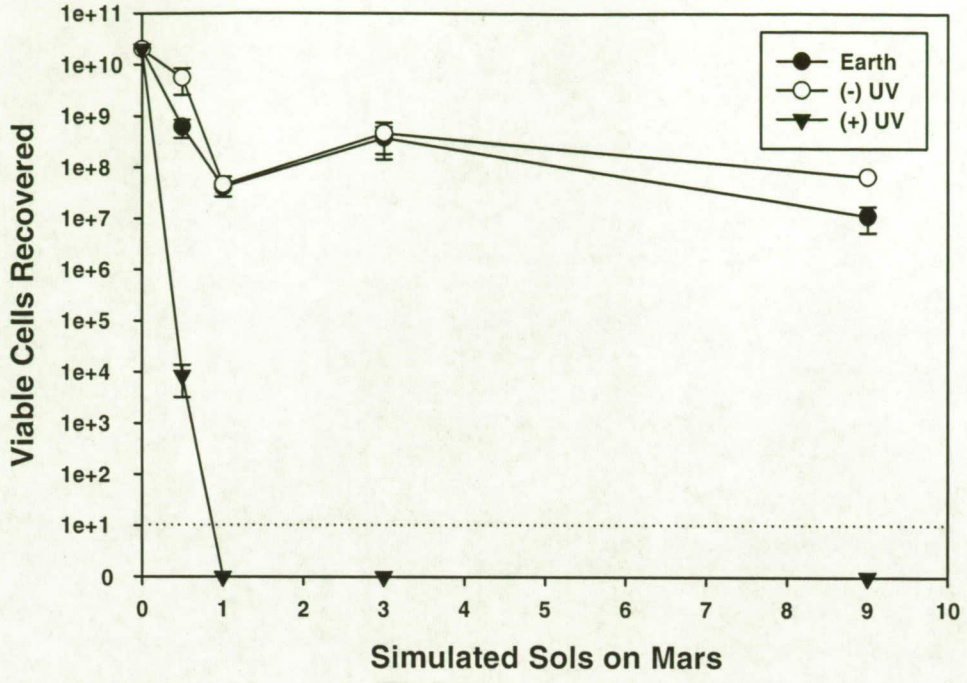
Viable Cells Recovered
T = 0 hr
T = 24 hr
Media
Non-Media
Coupon Preparation Technique

Fig. 3.


Viable Cells Recovered
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1e+10
1e+9
1e+8
1e+7
1e+6
1e+5
1e+4
1e+3
1e+2
1e+1
0
0
1
2
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4
5
6
7
8
9
10
Simulated Sols on Mars
Earth
(-) UV
(+) UV

Fig. 4.

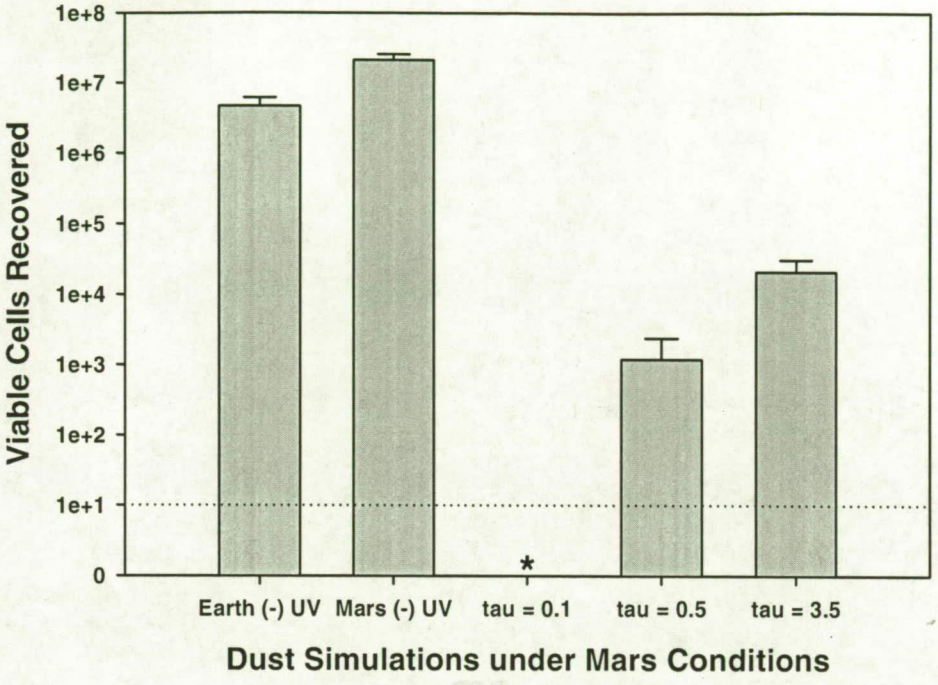
1e+8
1e+7
1e+6
1e+5
1e+4
1e+3
1e+2
1e+1
0
Viable Cells Recovered
*
Earth (-) UV
Mars (-) UV
tau = 0.1
tau = 0.5
tau = 3.5
Dust Simulations under Mars Conditions

Fig. 5.

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