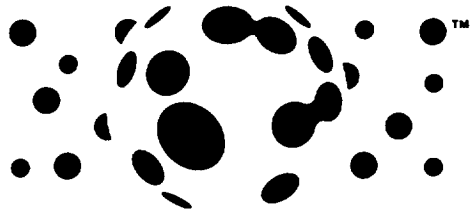

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FINAL TECHNICAL REPORT

Cooperative Agreement NCC 2-1088 Entitled:
"PLANETARY EVOLUTION AND MICROBE-ENVIRONMENT INTERACTIONS"

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Period of Performance: January 1, 1999 to June 30, 2002

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NASA

During the three-year period of this Cooperative Agreement my laboratory conducted physiological and ecological studies regarding the abiotic/biotic relationship in microbial ecosystems. The overall objective of the research performed in the three years of this cooperative agreement is to determine how living systems respond to environmental stress. To accomplish this objective microbes were subjected to a variety of different environmental stresses including decreases in water activity, changes in radiation flux, and nutrient limitation. This research addressees two subsets of the astrobiology questions including: If life arose on early Mars what would have become of it once Mars lost most of its atmosphere and water? and if Earth organisms traveled to Mars what would become of them. To help answer this question we used microbes as model systems not only because they were the first living systems to arise, but also because they are the most likely to be able to withstand a broad range of environmental factors. To meet this objective we conducted laboratory and field work.

Planetary Protection

Microbial Enumeration: The primary objective of this work was to develop and test an accurate bioassay system for determining the number of live and dead microbes as well as their reduction after cleaning and sterilization on spacecraft landers, equipment and instruments that are landed on the surface of Mars. The specific technology preliminarily developed employs differential fluorescent staining, visualization through fiber optic digital scanning probe microscopy and computer assisted image processing.

During the course of landed missions to Mars measures must be taken to ensure that the planet be protected from possible terrestrial contamination by these missions. The new planetary protection cleaning requirement for the removal of dead microbes, in addition to live microbes, from spacecraft to be landed on Mars requires the development of a new technology for determining the dead and live bioload on spacecraft surfaces. This investigation was initiated to assess and develop such a technique. Aliquots of washed live and dead spores of Bacillus subtilis strain HA 101, and live and dead cells of Pseudomonas fluorescens (ATCC # 17400) in different ratios were placed onto the surface of sterile aluminum coupons and dried. The surface was then sprayed with Molecular Probes Live/Dead stain and dried. The numbers of live and dead microbial cells per unit area on the surfaces were then quantified using digital fiber optic microscopy and computer assisted image processing. The data indicate that this technique can be used to assess the total bioload on environmental surfaces, but can give a false live positive result for 20% of spores tested (Figure 1).

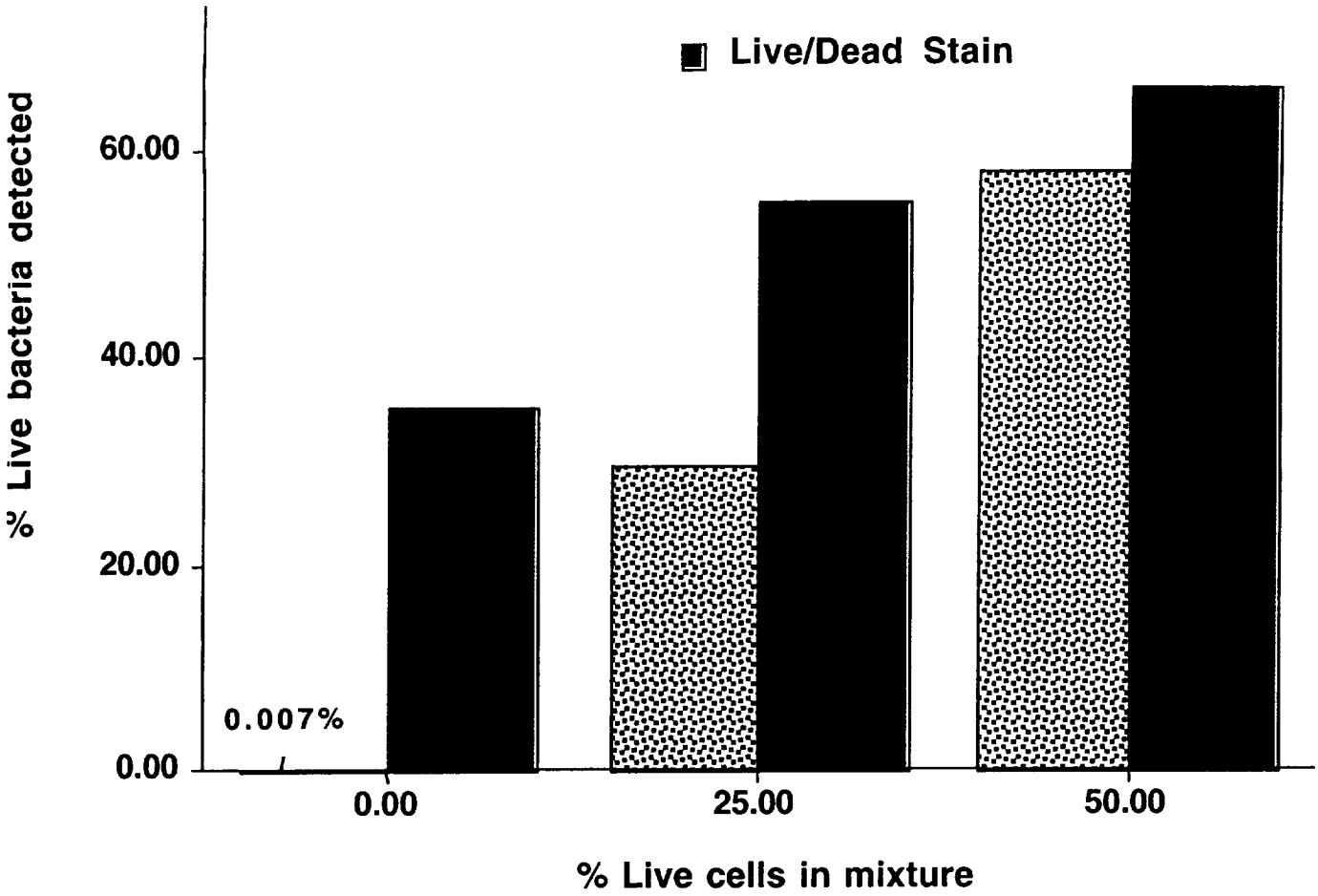

Figure 1. Comparison of MPN and Live/dead stain results from known mixtures of live and dead Bacillus subtilis spores.

Survival of Deinococcus radiodurans vs. Haloarcula-G. This task began during the third year of this Agreement. The hypothesis is that because Deinococcus radiodurans is among the most resistant organisms to gamma radiation that it will also be extremely resistant to UV radiation, and therefore may survive a journey to Mars aboard a spacecraft better tat most other organisms. The primary objective of this work was to compare the survival rates of *Deinococcus radiodurans* (an organism well known for its radiation resistance) to that of *Haloarcula*-G (an organism that has successfully survived exposure to UV radiation in space) when both are exposed to ultraviolet radiation and or desiccation. The overall approach was to expose dried monolayers of the test organisms to increasing doses of UV radiation in the laboratory using a deuterium lamp (Oriel model 6316) as the UV radiation source. The number of live and dead organisms was determined using Molecular Probes Live-Dead stain and the most probable number method.

Survival of Terrestrial Microorganisms on Spacecraft Components and Analog Mars Soils under Simulated Martian Conditions. During the next 10 years, several robotic missions to Mars will send at least four orbiters and four landers to the Martian surface culminating in two missions to collect rock and soil samples and return them safely to Earth by 2008. The objectives of the sample-return missions to Mars are to acquire rock and soil

samples in order to study the geochemistry, mineralogy, and petrology of Martian rocks. In addition, there will be a significant effort made to study the rock and soil samples for the presence of an extant Mars microbiology. Critical to the success of the sample-return missions will be modeling the microbial ecology of outbound spacecraft, and predicting whether terrestrial microorganisms might survive, grow, and replicate on Mars. If terrestrial microorganisms present on spacecraft surfaces survive and replicate on Mars, then their presence might impact the success of the sample-return missions. The past years' efforts on this task were primarily geared toward the design and construction of a Mars simulation chamber. This work was done at Kennedy Space Center. Our effort for the past year was to help Andrew Schuerger develop the requirements and design the simulation chamber.

Microbial monolayers were deposited onto aluminum coupons. Simulated Mars Dust was deposited onto 1/3 of the treated coupons. One-third of the treated coupons will serve as non-dust/+UV controls, and 1/3 of the treated coupons will serve as non-dust/non-UV controls. The non-dust/non-UV control coupons were prepared as described but then wrapped in aluminum foil to prevent UV irradiation. The thickness of SMD to be used in this experiment represents the minimum dust thickness capable of reducing UV inactivation of *B. subtilis* by 50%. One treated coupon of each microorganism was placed into individual wells of a 6-well tissue culture plate (one coupon per well). Twenty-four tissue culture plates will be prepared in which 1/3 of the plates will contain coupons without dust, 1/3 of the plates will contain coupons treated with SMD, and 1/3 of the plates will contain coupons wrapped in aluminum. Two plates per treatment will be harvested on 0, 1, 7, & 14 days after the start of the experiment. After tissue culture plates are collected at each sample time, the coupons will be aseptically removed and processed as described above for Experiment I. Petri-plate cultures will be incubated at 30 C for 96 hrs, and the numbers of surviving microorganisms will be estimated for each coupon. The experiment will be conducted three times (n = 6 per treatment).

Populations of *B. subtilis* were reduced by over 99.9% after 30 sec UV irradiation when maintained at Earth-normal temperature (23 C), pressure (1060 mb), gas mixture (normal N_2/O_2 ratio), and illuminated with a Mars-normal UV fluence rate. 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 *B. subtilis* followed a biphasic response 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 $kJ \cdot m^{-2}$, respectively (Figure 6). The LD_{100} rates are defined as those treatments in which no viable bacteria were recovered.

The biocidal effects of Mars-normal UV irradiation on *B. subtilis* spores were similar at Mars-normal conditions of temperature (–10 C), pressure (8.5 mb), and gas mix (CO_2 >99.9% purity) compared to the results obtained at Earth-normal environments. 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 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 ($P \leq 0.01$) as the UV exposure times of bacterial spores 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. Although the UV exposure times between the Earth-normal (Figure 6) and the Mars-normal 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 a significant added effect of simulated Martian environmental conditions on the survival of bacterial spores.

Three different dust coatings were used to simulate aeolian deposition of dust onto spacecraft surfaces in order to determine the effects that dust particles 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 (Figure 5). In the 25 to 50 μm dust fraction, particles were estimated to cover between 30 and 50 endospores (Figure 5A), but in the smallest fraction (2 to 8 μm), dust particles often adhered to the aluminum coupons between individual bacterial endospores (Figure 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 hr 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 (Figure 10). When the four dust coatings were exposed to 8 hrs 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.

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 $kJ \cdot m^{-2}$ for *B. subtilis* (called lethal dose multiples) might be achieved under various optical depths of the Martian atmosphere. 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 ($W \cdot m^{-2}$) to accumulated UVC + UVB dose rates per minute ($kJ \cdot m^{-2} \cdot min^{-1}$) under each *tau* simulation, and then dividing 11.9 $kJ \cdot m^{-2}$ by the accumulated UVC + UVB dose rate per minute (Table 3). Daily UVC + UVB fluence rates (Table 3) were estimated based on 1 $W \cdot m^{-2}$ = 3.6 $kJ \cdot m^{-2} \cdot h^{-1}$; and the results multiplied by a conversion factor of 8. The conversion factor was based on integrating the daily diffuse and direct beam solar spectrum models of Applebaum and Flood (1990), Applebaum et al. (1996), and Haberle et al. (1993) and converting the total daily fluence rates on Mars to the number of hours at full solar intensity that would fall on Mars at a solar zenith angle of 0 degrees. Thus, 8 hours at maximum solar irradiation equals the integrated solar flux for a 12-h daylight portion of one Sol on Mars. The lethal dose multiples (Table 3) were calculated by dividing the daily UV dose rates by the lethal UV dose rate of 11.9 $kJ \cdot m^{-2}$ of *B. subtilis* (Figure 6). Results indicated that for optical depths of the Martian atmosphere from *tau* = 0.10 (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.

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

Spectral ranges (nm)	Earth solar constant ($W \cdot m^{-2}$) [x]	Mars solar constant ($W \cdot m^{-2}$) [y]	Mars chamber simulation ($W \cdot m^{-2}$) [z]
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

[x] Based on Arvesen et al., 1969, Applied Optics 8(11):2215-2232.
[y] Estimated as 43% of Earth's solar constant.
[z] Based on direct measurements of the Mars Simulation Chamber's UV-VIS-NIR fluence rates using an Optronic Laboratories OL-754 UV-VIS spectroradiometer. [*] denotes data estimated with a LiCOR 1800 VIS-NIR spectroradiometer.

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

Neutral density filters [x]	UV Transmission (200-400 nm) [y]	VIS-NIR Transmission (400-1100 nm) [y]	Optical depth (*tau*)	Predicted transmissivity of the Martian atmosphere [z]
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

[x] 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.
[y] Actual UV and VIS transmissivities of the ND filters as determined using a Beckman DU-640 spectrometer from Beckman Instruments, Inc., Fullerton, CA USA.
[z] 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. (3795).

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 *Bacillus subtilis* on horizontal exposed spacecraft surfaces on Mars.

Optical depth of atmosphere (*tau*)	Instantaneous UVC + UVB fluence rate on equatorial Mars ($W \cdot m^{-2}$) [w]	Time required to accumulate a lethal dose rate of 11.9 $kJ \cdot m^{-2}$ for *Bacillus subtilis* (min) [x]	Daily UVC + UVB fluence rates on Mars ($kJ \cdot m^{-2}$) [y]	Lethal dose multiples for *Bacillus subtilis* for one Sol [z]
Mars solar constant	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

[w] Based on the Mars solar constant derived from Arveson 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.

[x] Estimated by converting the instantaneous UVC + UVB fluence rates ($W \cdot m^{-2}$) to accumulated UVC + UVB dose rates per minute ($kJ \cdot m^{-2} \cdot min^{-1}$), and then dividing 11.9 $kJ \cdot m^{-2}$ (i.e., lethal dose rate for *B. subtilis*) by the accumulated UVC + UVB dose rate per minute.

[y] Based on 1 $W \cdot m^{-2} = 3.6\ kJ \cdot m^{-2} \cdot h^{-1}$; 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 Applebaum and Flood (1990), Applebaum et al. (1996), and Haberle et al. (1993).

[z] Lethal dose multiples represent the number of times a lethal dose of 11.9 $kJ \cdot m^{-2}$ for *B. subtilis* would be accumulated in one Sol under specific levels of *tau*.

EXPOSURE OF MICROBES TO SPACE ENVIRONMENT.

Past work has shown that certain microbes can survive short duration exposure to the space environment (Mancinelli et al., 1998). The results of the first year series of ground experiments suggested that the DPA grown cells survive better than the pigmented cells. This may be due to the fact that DPA blocks the synthesis of carotene at the point of phytofluene production creating a pool of intracellular phytofluene. We have further shown that some of the carotenoid precursors absorb UV radiation more efficiently than carotene. To further test this hypothesis, we exposed pigmented and nonpigmented *Haloarcula* G to the space environment. We also included a unicellular cyanobacteria (*Synechococcus*)isolated from the evaporite that was flown in the first space exposure experiment.

Washed mid-log phase cultures of *Synechococcus*, *Haloarcula-G* , and *Haloarcula-G* grown in the presence of diphenylamine (DPA) (to determine the potential role, if any, of the carotenoid pigment in survival by blocking the synthesis of _-carotene causing a build-up of phytofluene in the cell, were suspended in 25% NaCl, placed onto 7 mm quartz discs and dried overnight, creating a mono-layer of cells. The dried preparations were placed into the European Space Agency's Biopan exposure facility, launched into earth orbit and exposed to the space environment during two missions. Ten of each of the types of samples were exposed to solar UV radiation and space vacuum, and ten of each were in the dark exposed to space vacuum only. Ground controls were prepared similarly. The second flight exposed the organisms to the space environment for 12.5 days. The organisms received a dose of ~5,000 kJm^{-2} of UVA and ~3,333 Kjm^{-2} of UVB and C. Due to their short time in space the probability is negligible that they were hit by a cosmic ray particle[3]. The temperature on each flight fluctuated between −15 °C and + 20 °C. All samples were compared to ground controls. Survivability was determined by the most probable number technique and with Molecular Probe's Bac-Lite Live/Dead stain.

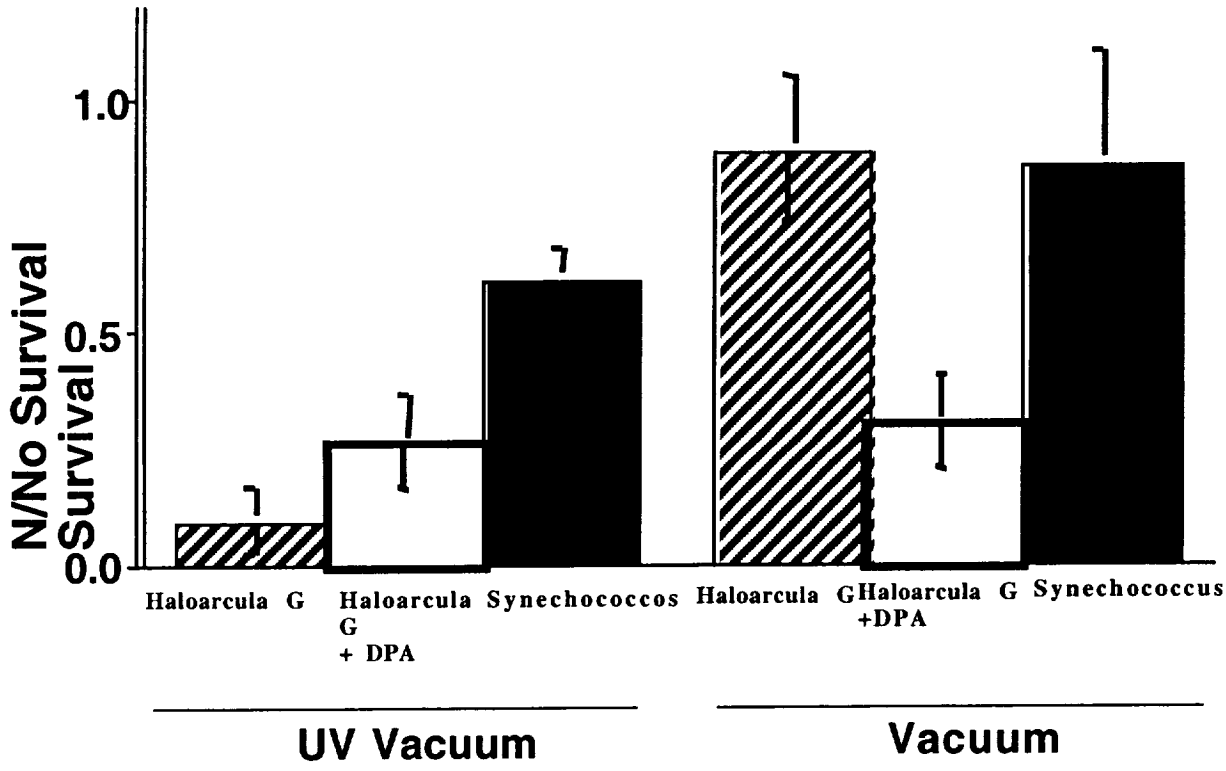

Figure 2. Survival of Haloarcula G pigmented and non-pigmented (grown in the presence of DPA) and Synechococcus after exposure to UV radiation. No equals the original number of organisms not exposed to UV radiation or vacuum.

These halophilic microbes survived solar UV radiation and desiccation during both flights, with the non-pigmented DPA grown *Haloarcula*-G surviving better than the pigmented (Figure 2) and Synechococcus surviving better that both Haloarcula-G and Haloarcula-G + DPA. This result may be due to the ability of carotene precursors in the Haloarcula-G +DPA cells to protect the cells from UV radiation better than carotene (unpublished data). The better survival of the *Synechococcus* may also be due to pigments.

We surveyed microbial isolates used in previous space flight experiments (BIOPAN listed above) and organisms isolated from natural communities that inhabit extreme environments such as salt evaporation ponds (Cargill, Co.) and thermophilic environments of Yellowstone National Park. We tested candidate organisms in ground-based simulators at the DLR in Köln, Germany. We have begun to determine genetic and physiological mechanisms underlying resistance. These results will be used to predict which terrestrial organisms are most likely to survive interplanetary travel. Note that because during evolution on Earth desiccation and ultraviolet radiation were major issues facing organisms in their transition to land, the results obtained here will have significance for evolutionary biology.

Protection of microbes by Mars soil. Results from our first had year shown that if organisms are protected from UV radiation by a 2_m layer of soil or cell debris they will survive (Mancinelli and Klovstad, 2000). To further assess the survivability of microbes in a Martian environments a Mars dust storm was simulated with respect to UV flux, pressure and suspended fine regolith. Bacillus subtilis strain HA 101 obtained from G. Horneck (DLR Köln, Germany) was used as the test organism. This strain of the organism was used because it is known to survive exposure to the space environment for months to years (Horneck, 1994). Additionally, this strain carries the auxotrophic marker his HA101 making it easy to distinguish from other microbes that will allow us to easily check for possible contamination by other microbes(Horneck, 1993).

Bacillus subtilis strain HA 101 was grown to mid log phase until the sporulation rate was greater than 70% as determined microscopically. The spores were concentrated by centrifugation at 10,000 X g. for 20 minutes and resuspended in a 0.01M Tris buffer solution. The cells were then incubated with DNase and lysozyme to rid the suspension of any vegetative cells. The suspension was centrifuged again at 10,000 X g. for 20 minutes and washed three times with sterile deionized water and dried overnight at 30°C.

The organism was mixed with the sterile Martian soil analog (obtained from Johnson Space Center, the Mars soil standard) such that the number of microbes equals ~ 5 x 10^9 g^{-1} dry wt soil. The microbe/Mars soil analog sample is placed in the spherical chamber equipped with a variable speed rotor (to create the simulated dust storm), a light/UV illumination port equipped with a deuterium lamp as a UV source, and gas ports. The chamber was then sealed,

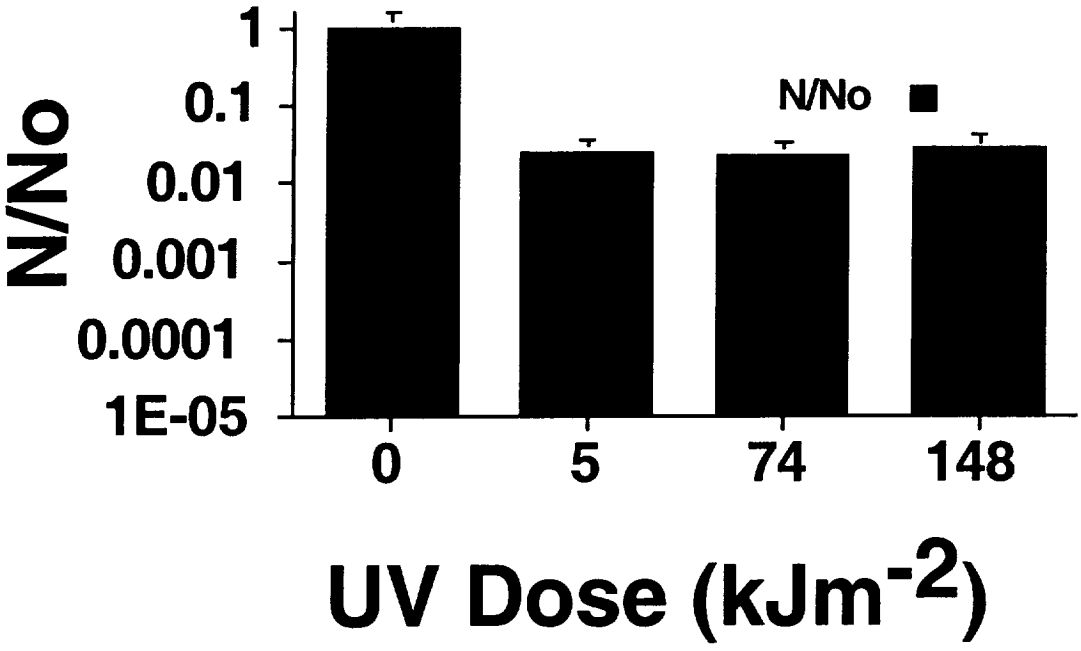

Figure 2. Bacillus subtilis spores exposed to increasing doses of UV radiation. Where No equals the original number of bacteria in the soil/bacteria mix not exposed to UV radiation.

flushed with a simulated Martian atmosphere (96.9% CO_2, 3% O_2, 0.1% H_2O), at a pressure of 10 torr then the sample was aseptically injected into the chamber. The microbe-dust mixture was exposed for 90 minutes, 24 hours and 48 hours. At the end of a test the dust was aseptically removed from the chamber by flushing N_2 gas through a port into a Whatman #1 filter assembly. The number of surviving microbes g^{-1} soil determined using the most probable number method (MPN) (Koch, 1994).

The data indicates that at least 10% of the microbes survive the exposure. The results of theses experiments suggests that microbes are protected from solar UV radiation by dust in a simulated Martian atmosphere. The results of the present study clearly indicate that if these organisms were to be picked up with the dust in a Martian dust storm they will survive travel into the Martian atmosphere. The implications of this are that once transported to Mars certain common Earth organisms can be distributed globally over the Martian surface and

survive. These data further suggest the potential for the global contamination of Mars by Earth organisms as a result.

MARS SAMPLE HANDLING FACILITY TESTBED DEVELOPMENT (MRSH)

We ascertained, by means of laboratory testing, the information needed to design a Test Bed for the biohazard assessment element of the MRSH. That element had to be capable of:

- Near-absolute containment of the sample
- Protection of the samples from contamination to a level consistent with the cleanliness of the MSR spacecraft.

The sample protection is needed both to avoid confusion in the experimental assessment of biohazard and also to ensure that the integrity of the samples is maintained for subsequent science analysis. To this end we designed and fabricated double walled isolation chambers with appropriate pressure differentials that allowed us to compare the applicability of this technology to that of a BSL-4 facility for biohazard analyses of samples returned from Mars. We expected to use remotely controlled manipulators rather than gloves.

Our rationale for proceeding with a double walled isolation chamber first is that more data exist regarding the use of BSL-4 facilities than on isolation chambers. Further, the primary goal of most BSL-4 type facilities is to protect the outside environment from the samples rather than the reverse. We believe that the challenge of protecting the samples from contamination (especially by live microbes, dead microbes & organic molecules) is in fact the greater challenge. We believe also that remotely-controlled sample manipulators will better serve the required two-way protection than the use of gloves would.

Principal potential contamination sources are 1) the isolation chamber itself, 2) the pass-throughs needed for cabling etc. and 3) the analytical instrumentation and equipment for sample manipulation, viewing etc.

We intended to systematically demonstrate that we can meet quantitative goals of cleanliness (derived from the goals established for MSR spacecraft cleanliness) and that we can achieve near-absolute containment (as measured by the helium detectors within the chamber walls where the minimum pressure is to be maintained). The chambers' design will be consistent with the analytical capability identified by the PP WG's. To that end we worked on developing a protocol to determine cleanliness and bioload of the chamber. Initially, this protocol will be based on the swipe protocol outlined in NPG 5340.1C and the newer witness plate concept being developed by JPL/Roger Kern and ARC/Rocco Mancinelli .

A secondary priority, not initially addressed, was to demonstrate that the instrumentation can achieve the needed levels of sensitivity and precision under the isolation conditions in question (note that the extreme cleanliness of the chambers should make it easier to achieve the required analytical capability). We anticipated that 3 different kinds of isolation chambers would be needed for the assessment of bio-safety and we planned to systematically

demonstrate required capability for all of these, beginning with the simplest. The 3 kinds of chambers will provide for:

1) Non-destructive analyses using optical microscopes, spectrometers and x-ray tomography
2) Destructive analyses requiring sample heating and analysis of the effluents
3) Analyses involving cell cultures

There was also the possibility that we would need to create a chamber in which testing using plants and rodents would take place. We postponed consideration of such a need until the PP WG had definitively identified this as a requirement. We began testing with a prototype chamber for non-destructive analyses. The planned steps were as follows:

- Survey analytical instrumentation consistent with PP WG recommendations
- Select affordable, representative instrument
- Outline initial concepts for sample analysis and handling
- Place instruments in the chamber to determine the feasibility of using the chamber, performing analyses and assessing instrument usage on cleanliness and bioload of the chamber.
- Determine feasibility of using the instruments in the chamber, first without and then with, a robotic arm (data and specifications to be provided by JSC).

We selected a digital light microscope equipped with a halogen lamp, and a remote lens system as the first instrument in developing our protocol for non-destructive analyses. This selection was based on:

- Biohazard analysis for examination of the sample for potential biohazards (e.g., organisms and fluorescing pigments) using light microscopy as well as fluorescence microscopy.
- Budget—the instrument selected must fit our FY '01 budget constraints
- Instrument availability
- Define preliminary protocols for analysis
- Define functional requirements for chamber
- Define preliminary test plan
- Design chamber including pass-throughs
- Procure instrumentation and associated equipment (a long-lead item)
- Procure chamber
- Finalize Test Plan

The heart of the matter lies in carrying out the test plan systematically and quantitatively:

- Implement Test Plan beginning with bare chamber and introducing the elements of equipment and instrumentation one by one
- Iterative evaluation of testing results and as necessary modification of cleaning procedures, out-gassing countermeasures etc. to meet quantitative goals
- Document protocols and procedures

We believe that this testing for the first chamber will establish whether our approach is fundamentally sound. Testing will be extended to a double-walled chamber with helium detectors in the inter-wall space (helium leakage is a far more sensitive measure than trying to detect the leakage of bio-molecules). Subsequently we will test each of the 3 different kinds of chamber.

We recognize that there are other aspects to consider such as 1) the ability of affordable remotely-controlled manipulators to meet our sample manipulation needs, 2) the ability to provide the chambers with air-locks that have the capability to facilitate the transfer of samples between chambers, 3) the ability to achieve the needed reliability of operation, 4) the ability to make repairs as necessary without jeopardizing the containment of the samples or contaminating them, 5) the integration of the isolation chambers and equipment into a total system, 6) the ability to carry out all the TBD protocols that will be identified by the PP WG, and 7) operations at low temperatures.

Thus there will be substantial effort required to work out bugs after the fundamentals of the approach have been demonstrated. This will require more time and resources than are available in FY '01 and '02. This work will be continued under Cooperative Agreement NCC 2–1359.

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Appendix A

Following is a list of publications resulting from this project:

Publications

Bishop J.L., Banin A., Mancinelli R.L., Klovstad M.R. (2001) Detection of soluble and fixed NH4+ in clay minerals by DTA and IR reflectance spectroscopy: A potential tool for planetary surface exploration. *Planetary Space Science*,50:11-19..

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