SURVIVAL OF MICROORGANISMS ISOLATED FROM SPACECRAFT AND ASSEMBLY FACILITIES UNDER SIMULATED MARTIAN ENVIRONMENTS

Interim Report

JPL Task 1057

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A. OBJECTIVES

During the Viking missions, both landers were terminally heat-sterilized to assure that terrestrial microorganisms would not contaminate the life-detection experiments. However, the cost of designing and assembling the Viking landers was increased dramatically due to this requirement (NRC, 1997). The Planetary Protection protocols for the Mars Exploration Rovers (MER) did not require that the MER rovers be heat-sterilized prior to launch. Instead, NASA relied on a series of sequential sterilization steps to maintain the cleanliness of the MER vehicles (http://mars03-lib.jpl.nasa.gov/). *The results of this FY'03 DRDF study addresses the question of whether the forward contamination of Mars might be negligible due to sanitizing conditions experienced by the spacecraft after landing, and whether the methods used by NASA to sanitize the spacecraft are sufficient.*

The objectives of the proposed work are to examine the effects of direct and diffuse UV irradiation on the survival of terrestrial microorganisms typically recovered from spacecraft surfaces and assembly facilities. The UV experiments addressed four key components:

a. To develop a robust diffuse-UV-irradiation model for Mars in order to model microbial survival under clear sky conditions in which most of the UV reaching the surface is direct UV irradiation, and to model microbial survival under global dust storm conditions in which most of the UV is diffuse UV irradiation.
b. To study the effects of UVA, UVA+B, and total UV of the Mars simulated environment on the survival of microorganisms recovered from spacecraft surfaces.
c. To determine the minimum Mars-normal UV dosage rate required for inactivating 100% of microbial populations on spacecraft materials for each UV band.
d. To test the survival rates of UV-resistant microbial species recovered from spacecraft surfaces under robustly simulated Mars-like conditions in a Mars simulation chamber at KSC, FL.

B. PROGRESS AND RESULTS

Task#1. Develop a diffuse UV irradiation model for Mars under different optical depths, sun-elevation angles, latitudinal positions, and seasonal conditions on Mars

We developed a series of UV models that describe the diffuse, direct, and total UV environments on Mars for various atmospheric conditions. The direct UV beam describes UV

irradiation coming directly through the Martian atmosphere from the Sun. The diffuse UV beam describes UV irradiation that is forward-, side-, or back-scattered by various atmospheric components on Mars, producing a diffuse UV environment from the general sky. The total UV beam is the sum of these two factors. The models describe the effects of solar zenith angle, atmospheric dust, atmospheric pressure, atmospheric water ice, and polar ozone on the attenuation of UV irradiation at the Martian surface. Two examples of the UV models are given in Figure 1. Results of all of the models indicate that the strongest attenuating factors in the Martian atmosphere on solar UV irradiation are solar zenith angle and atmospheric dust. Water ice and atmospheric pressure had only minor effects on UV attenuation. Polar ozone occurs only periodically, and thus has a globally minor effect on UV irradiation reaching the surface, but could have a significant regional effect when ozone abundance is high at the Martian poles.

In addition, the UV reflectance of 15 spacecraft materials and 8 Mars-analog regolith samples were measured in order to model the UV environments on spacecraft components that are shielded from the direct UV beam (e.g., on the undersides of rovers and solar panels). The component of the UV environment on Mars that determines the rates of inactivation of terrestrial microorganisms on spacecraft surfaces is the "accumulated dose" of the "total UV beam" reaching the microbial cells. Results (Figure 2) indicated that most spacecraft materials were capable of reflecting UV irradiation down to 200 nm. Only two spacecraft materials (i.e., white epoxy paint 446-21-7925 and white paint NS43G) exhibited strong attenuation of UV light from 200 to 380 nm. Most other materials exhibited 1-decade reductions of UV irradiation down to 200 nm (compare the Spectralon reflectance target and the Chem Film-treated aluminum 6061 target in Figure 2), but these materials were generally spectrally flat to the absorption of UV photons. Thus, a generalized UV reflectance model for most spacecraft materials on Mars has been developed that suggests that most spacecraft materials will reflect approximately 10% of solar UV irradiation between 200 and 400 nm. Both the atmospheric UV models and the UV reflectance models developed here can now be used to accurately model whether significant dosages of UV irradiation will be accumulated by microbial cells on protected surfaces of spacecraft resulting in the inactivation of terrestrial microorganisms.

Task #2 and 3. UVA, UVB and UVC radiation resistance of spacecraft-associated microorganisms, and minimum UV dosage rate required for inactivating microbial populations (LD_{90} to LD_{100}).

Spacecraft isolates were identified (Table 1) using 16S rDNA sequence analysis. Spores of the isolates were added to sterile water at concentrations of 10^6 spores/ml and subjected to an accumulated exposure of 1 kJ (UVC) using a low-pressure mercury arc lamp. One-hundred-µl samples were removed and used to inoculate liquid medium (trypticase soy broth, TSB) or solid medium (trypticase soy agar, TSA). The results are shown in Table 1

Of the 45 organisms tested, 19 showed high resistance to UVC. Of these 19 organisms, 9 were selected and exposed to various forms of UV irradiation (Table 2). The full spectrum of UV irradiation (200 – 400 nm) was generated, using a UV-enhanced 450 W xenon-arc lamp (model 6262, Oriel Instruments, Stratford, CA, USA). Separate UV bands including UVA (315 – 400 nm), UVA+B (280 – 400 nm) irradiation was created with narrow-bandpass filters placed in the light path of the xenon-arc lamps. The UV fluence rates were adjusted to simulate a Mars-normal spectrum, determined by using an Optronics Laboratories OL754 UV-VIS spectroradiometer. The results of these carefully coordinated time-course studies are shown in

Table 2 as LD 50, LD 90 and LD 100 values in minutes. These 9 organisms were sent to Dr. Andrew Schuerger for completion of Task 4.

Dormant endospores of *B. pumilus*, SAFR-032, isolated from a spacecraft assembly facility building and exposed to a full-UV spectrum, exhibited a LD_{90} value of 4.5 minutes with a D value of ten minutes. In comparison, *B. subtilis 168*, a well-studied UV-sensitive species, had a LD_{90} value of 0.75 minutes and D value of 2 minutes under a full UV spectrum. Exposure to UV-A and UV A+B yielded LD_{90} values of >30 min and 28 min, respectively, for SAFR-32. The results show that UV-C is the main biocidal band and also demonstrate that organisms contaminating spacecraft assembly facilities can survive for short periods of time under a Mars-normal UV environment. It also raises real concerns about conditions that could enhance spore survival such as attenuation of UV irradiation by atmospheric dust, water ice, or ozone. Results of this work will help to refine the planetary protection programs for future near-term Mars lander, rover, and orbiter missions.

One organism, *Bacillus pumilus* SAFR-32, showed the greatest resistance to UV irradiation throughout the study (Table 2, Figure 3). The organism was much more resistant to UVA and UVA+B than the full UV spectrum. The results shown in Figure 3 illuminate the importance of biomass reduction with respect to the survivability of organisms to UV exposure. These results have direct implications for planetary protection.

Task #4. Determine the survival of UV-resistant microbial species recovered from spacecraft surfaces under robustly simulated Mars-like conditions (still in progress)

The effects of full Mars simulations on the survival of 7 *Bacillus* spp. under Mars-normal UV irradiation will be carried out in the remaining period of the project. The endospores of selected *Bacillus* species will be exposed to Mars-normal conditions of pressure (7 mb), temperature (-10 ^{o}C), atmospheric gas composition (95% CO_2), and UV irradiation (full UV spectrum at 50 W/m^2). Preliminary tests were conducted to determine the effects of Mars pressure (7 mb) alone on the survival of seven *Bacillus* spp. under Martian simulations. Results indicated that Mars pressure alone for up to 7 days had only a minor effect on the survival of dormant endospores of *B. pumilus* SAFR-32, *B. pumilus* FO-36B, *B. subtilis* HA-101, *B. subtilis* 42-HS1, *B. licheniformis* KL-196, *B. meagterium* KL-197, and *B. nealsonii* FO-092. Survival was reduced less than 20-30% compared to the Earth controls. The LD100 rates for dried endospores were estimated for the seven *Bacillus* spp. under robust simulations of the Mars UV irradiation environment. All species were significantly reduced within 1-15 min exposure to Mars UV irradiation. The most UV-sensitive species were *B. subtilis* 42-HS1, and *B. megaterium* KL-197. The most UV-resistant species were *B. pumilus* SAFR-32 and *B. licheniformis* KL-196.

C. SIGNIFICANCE OF RESULTS

This task tested the effects of UVA, UVA+B, and total UV environment on survival of microorganisms recovered from spacecraft surfaces. It was determined that UVC is the most potent band with respect to microbial survival. Studying these various wavelengths was critical to building a model for the survival of terrestrial microorganisms on Mars because the dust suspended in the Martian atmosphere can strongly attenuate UVC while allowing both UVA and

UVB to reach the surface. These results permit us to predict with great accuracy the likely time on the Martian surface required for the complete sterilization of both exposed and shaded spacecraft surfaces.

The results of this study clearly indicate that the UV environment on Mars is extremely harsh for the survival of microbes and that most microbial species on sun-exposed surfaces on spacecraft will be inactivated within a few tens of minutes to a few hours at equatorial latitudes. Even the highly UV-resistant endospores of *B. pumilus* SAFR-032 can still accumulate a lethal dose of solar UV irradiation on Mars within a single Sol if the atmospheric conditions are non-dusty. However, the diffusion of Mars solar radiation due to dust storms, negative influence of soil, and other factors should be carefully considered while implementing these results.

D. FINANCIAL STATUS

The total funding for this task was $200,000, of which $163,000 has been expended.

E. PERSONNEL

Dr. David Newcombe, a Caltech postdoc for JPL, was hired part-time for this project.

F. PUBLICATIONS

[1] David A. Newcombe, James N. Benardini, Andrew Schuerger, and Kasthuri Venkateswaran. "Survival of Spacecraft Associated Microbes Under Martian Simulated Solar Constant"/*104th General Meeting of American Society for Microbiology*, 2004 May, New Orleans.

G. REFERENCES

[1] National Research Council (NRC) (1997). *Mars Sample Return: Issues and Recommendations*. Washington D. C.: National Academy Press, 47 pp.

Table 1 List of Bacillus species tested for UV resistance at 1 kJ accumulated exposure

Species	Strain	Growth 24 48/24 48 hr	Species	Strain	Growth 24 48/24 48 hr
B. licheniformis	KL–196	++/++[1]	*B. pumulis*	SAFN–036	++/++
B. firmus	ATCC 14575	—/—	*B. pumulis*	SAFR–032	–+/–+
B. psychrodurans	VSE1–06	++/++	*B. pumulis*	ATCC 7061	–+/++
B. mojavensis	ATCC 51516	—/—	*B. pumulis*	KL–052	++/–+
B. fusiformis		—/—	*B. pumulis*	FO–033	++/++
B. thuringiensis	ATCC 10792	—/—	*B. pumulis*	84–1C	–+/++
B. firmus	10V2–2	++/++	*B. pumulis*	SAFN–029	–+/—
B. benzoevorans	ATCC 49005	—/—	*B. pumulis*	82–2C	++/++
B. gibsonii	ATCC 700164	++/++	*B. pumulis*	SAFN–037	—/—
B.nealsonii	FO–092	—/—	*B. pumulis*	ATCC 27142	—/—
B. mojavensis	KL–154	—/—	*B. pumulis*	SAFN–027	++/++
B. cereus	FO–11	++/++	*B. pumulis*	84–3C	—/—
B. neidei	NRRL BD–101	++/++	*B. pumulis*	015342–2 ISS	—/—
B. benzoevorans	SAFN–024	—/—	*B. mycodies*	FO–080	++/++
B. odyseensis	34hs–1	++/++	*B. subtilis*	ATCC 6051	++/++
B. niacini	51–8C	++/++	*B. thuringiensis*	SAFN–003	++/—
B. subtilis	168	++/++	*B. mycodies*	ATCC 6462	—/—
B. gibsonii	ATCC 700164	++/++	*B. subtilis*	42hs1	++/++
B. megaterium	ATCC 14581	++/—	*B. globgii*	RAVENS	–+/++
B. megaterium	KL–197	—/—	*B. sphaericus*	ATCC 14577	++/++
B. pumulis	84–4C	—/++[2]	*B. cereus*	ATCC 14579	++/++
B. pumulis	84–1C	—/++	*B. flexus*	ES–16	++/—
B. pumulis	51–3C	—/++			

1. Growth was categorized in liquid cultures by OD_{600} readings and on agar medium by colony counts. The + symbol indicates: OD_{600} reading >0.4 after 24 hr, 48 hr / >30 colonies after 24 hr, 48 hr.
2. These organisms showed growth on solid medium but not liquid and are still under investigation.

Table 2. Exposure of resistant strains to UV radiation at the Mars equatorial solar constant

Bacillus species		LD UVA (min)			LD UVA+B (min)			LD Full UV (min)		
		50	*90*	*100*	*50*	*90*	*100*	*50.0*	*90.0*	*100.0*
B. pumulis	*SAFR-32*	8.0	>30	>30*	10.5	27.0	>30	1.4	4.5	6.0
B. megaterium	*ATCC 14581*	21.5	>30	>30	9.2	25.1	>30	0.8	2.8	5.0
B. odysseyi	*34HS1*	1.5	>30	>30	3.5	17.5	>30	0.3	0.5	2.0
B. subtilis	*168.0*	6.9	>30	>30	1.8	18.0	>30	0.3	0.7	1.2
B. psychrodurans	*VSE1-06*	0.6	13.9	>30	2.0	>30	>30	0.4	0.9	1.1
B. pumulis	*SAFN-036*	0.5	1.0	20.0	1.5	2.0	>30	0.8	1.8	3.0
B. pumulis	*FO-33*	0.5	1.0	>30	4.6	18.0	>30	0.4	1.0	1.4
B. pumulis	*FO-36B*	1.2	13.4	>30	4.6	18.1	>30	0.7	1.9	3.0
B. subtilis	*42HS1*	2.0	>30	>30	10.0	26.0	>30	0.4	0.9	1.2

*Organisms that survived longer than the maximum exposure are listed with a > sign. These strains are still under investigation.

Figure 1. UV atmospheric models for mid-latitude ozone absorption (6%) and 7.12 mb of CO_2 at solar zenith angles (SZA) of 0 and 80 degrees. The models were developed during the FY03 DRDF-funded activities. Many additional models were developed to simulate UV attenuation in the Martian atmosphere due to CO_2 pressure, dust loading, water ice, ozone abundance, and solar zenith angles.

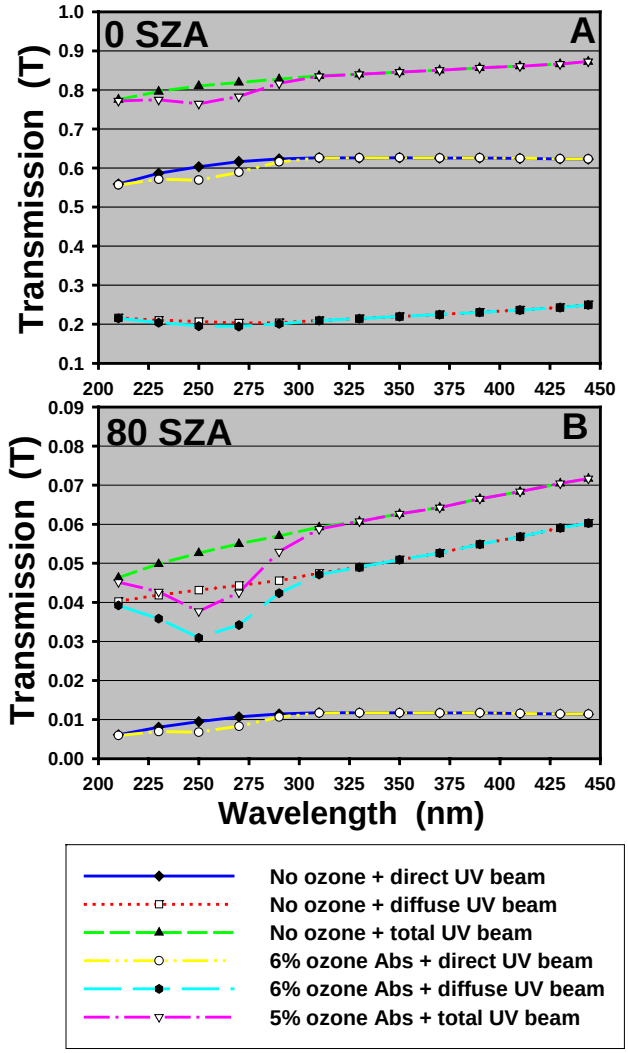

Figure 2. UV reflectance of spacecraft materials. Spectral scans of the standard reflectance target, Spectralon (99% reflectance), Chem Film-treated aluminum 6061, and white paint NS43G on aluminum. Note that the Chem Film-treated Al6061 is approximately 1-decade lower in UV reflectance (approx. 10%) than the Spectralon target, but that the white paint NS43G was at least 2-decades lower from 200 to 380 nm (approx. < 1%.

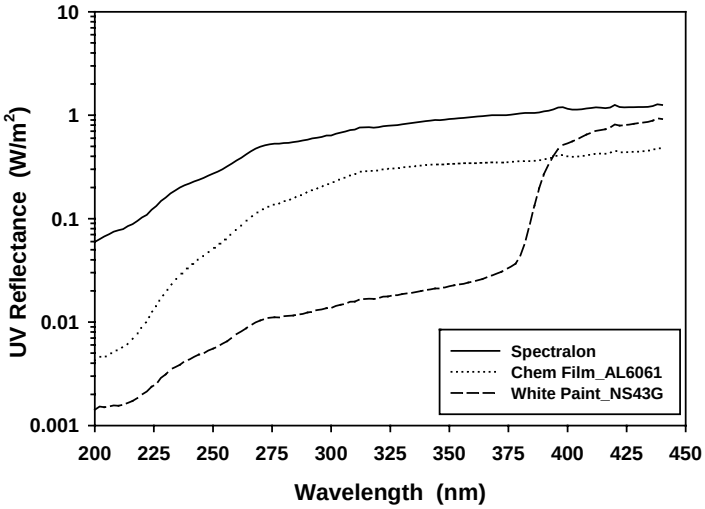
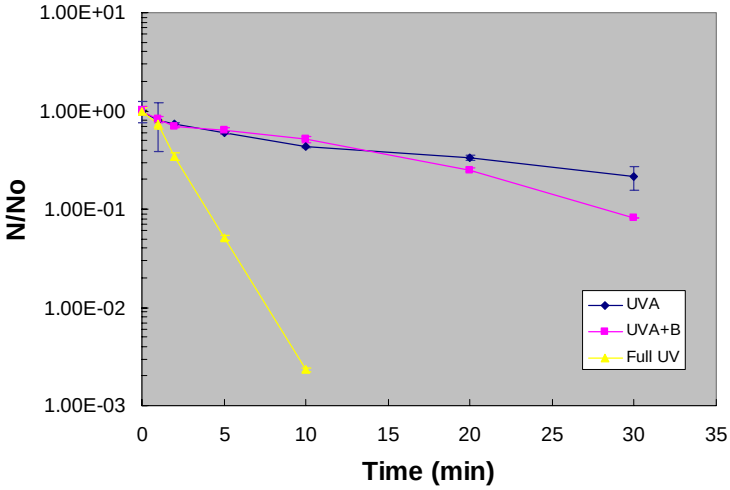
Figure 3. Exposure of *B. pumilus* SAFR-32 spores to various forms of UV irradiation. The data has been normalized by dividing by the starting concentration of spores. The error bars represent the standard deviation of 4 replicates normalized.