Terrestrial Microorganism and Biomarker Survival as a Function of Depth in a Mars Analog Regolith after Exposure to Mars Surface Conditions.
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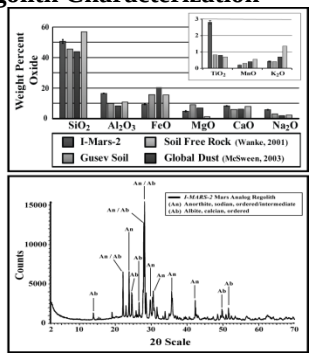
Introduction: Recent in-situ and observational data has provided significant evidence for past aqueous activity during the first billion years of Martian history in the form of geomorphological (*1*), mineralogical (*2, 3*) and geochemical (*4, 5*) analysis of the Martian surface. As a result, an active liquid water cycle would imply a period of Martian history characterized by an environment more tentatively more favorable to microbial life. Recent studies have focused on exposure of terrestrial microorganisms (*6-8*)and relevant biomolecules (*9-11*) to simulated Martian surface conditions in order to determine the feasibility of Earth-based organisms and their degradation products surviving long-term exposure to ambient Mars conditions. In order to ascertain the effective survival of terrestrial microorganisms and biologically relevant molecules at Mars surface conditions, we exposed several strains of microorganisms, DNA and amino acids to 41 days of simulated Martian UV, pressure and atmospheric conditions while embedded within a Mars regolith analog.

Experimental:

Regolith Preparation: Mars analog regolith was prepared from andesitic basalt composed of ordered and intermediately ordered sodian and calcian anorthite and albite. Sterile individual mineral constituents included magnesium sulfate, clinoptilolite, ferric oxide, Na-montmorillonite and anhydrous ferric sulfate and were added at approximately one weight percent of bulk composition, homogenized, and equilibrated with CO_2. The elemental composition of bulk basalt mimics the high-silica andesitic basalts thought to represent the low-latitude regolith compositions of the Martian surface as shown in Fig. 1.

Fig 1: Mars Analog Regolith Characterization

Sample	Mass (g)	Weight Percent
$MgSO_4$-x H_2O (Fisher ACS Certified)	30.1	0.95
Clinoptilolite (Fish Creek Mtns, NV)	28.4	0.9
Fe_2O_3 (Fisher #885359)	30.2	0.96
SWY-1 (Na-Montmorillonite)	30.2	0.96
$(Fe^{3+})_2(SO_4)_2$ (MP Biomed #5343J)	28.5	0.9
Collier Cone Andesitic Basalt	1046	33.1
Warner Valley Basalt	1964	62.2
TOTAL	3157.37	99.97



Sample Selection: Microorganism samples were selected based on selected desiccation resistance and organism-specific adaptations and included: *Hafnia, Marinobacter* and *Halomonas* strains, *Wangiella dermatiditis, Exiguobacterium sibircum, Methanobacterium veterum, Arthrobacter psychrolactophilus*, several nematode species and a species of tartagrade, as well as a DNA plasmid and a levorotary pool of amino acids. Samples were inoculated into Mars analog regolith at individual investigators' facilities prior to introduction into the TechShot Mars Ecopoiesis chamber.

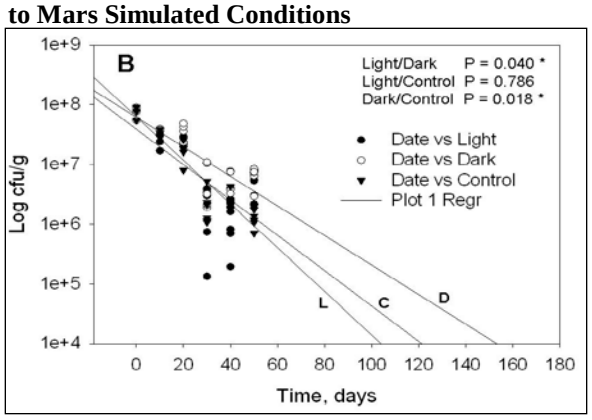
Experimental Chamber and Conditions: The TechShot Mars Ecopoiesis chamber mimics the diurnal cycle of a low-latitude, low-elevation region of present-day Mars during vernal equinox, including atmospheric composition and pressures, temperature, daily solar intensity cycle (up to 590 W m^{-2}), ultraviolet radiation flux (50 μmoles m^{-2} s^{-1}), and surface solar spectrum (down to 200 nm). Real-time water vapor concentration as a function of temperature and light/dark cycles was monitored using a chilled mirror hygrometer with low-pressure liquid nitrogen feed system. Diurnal temperature extremes ranged from -40 °C to 21 °C with 12 hours of daily UV exposure. Martian atmospheric water concentration was simulated using dual gas mixture yielding an atmospheric composition of approximately 1.35% N_2, 0.065% O_2, 0.035% CO, 0.005% H_2 and 200 ppm water vapor. The remaining balance was 50% Ar and 48.5% CO_2; atmospheric pressure was maintained at 15 mbar to provide the correct number of moles of each atmospheric component. Triplicate sampling of UV-exposed, shadowed and control samples occurred at 10 day intervals over the course of the 41 day experiment.

Results: Microbial and biomolecule samples were extracted from regolith analog at various depth intervals to ascertain the extent of burial depth on organism survival and organic carbon oxidation rates. Negative

results were obtained for *Halomona*, *Marinobacter*, and all nematode species even prior to exposure to Mars surface conditions and are inferred to be the result of loss due to desiccation. DNA-inoculated regolith also yielded negative results, indicating either complete oxidation of DNA as a result of interaction with the regolith or a complex cross-linking incorporation onto the regolith mineral grains.

Positive recoveries of microorganisms from exposed Mars analog regolith show a decrease in CFU with increasing simulation time and decreased survival rates in samples exposed to UV radiation. **Fig. 2** shows *Exiguobacterium* recovery with respect to time; esti-

Fig 2: *Exiguobacterium* Survival after Exposure to Mars Simulated Conditions

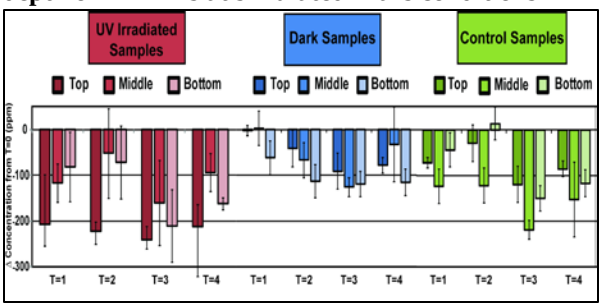


mated LD50 for UV exposed (L), control (C), and dark (D) samples were 105, 120 and 155 days, respectively; below 3 mm depth, the effects of UV are reduced and LD50 values increase from 105 to 120 days. Recoveries of *Hafnia* strains show similar UV dependence, with 7%, 17.9% and 54.9% survival rates in UV, dark and control samples, respectively. *M. veterum* samples showed no methane production after exposure to Mars conditions, although both live and dead cells were identified in samples. *A. psychrolactophilus* showed a five order of magnitude decrease in CFU/g regolith in UV, dark and control samples, indicating minimal effects from the presence of UV on organisms' survival rates.

Total hydrolysable amino acid (THAA) recoveries indicate secondary mechanisms of oxidation. In the upper centimeter of UV-exposed samples, approximately 40% of THAA's are lost in the first 10 days of the experiment; at 2 and 3 cm depth, significant losses in amino acids are not seen until after 20+ days of simulated Mars conditions. Dark samples show minimal changes in THAA concentration until after 10+ days at Mars conditions, and controls show a similar decrease in, with maximum loss occurring after day 20 (**Fig.3)**.

Discussion and Conclusions: Inferences of UV-C radiation as the primary limitation for both microor-

Fig 3: Concentration differences with respect to depth of THAA's at simulated Mars conditions



ganism survival and biomarker oxidation are investigated as a function of burial within the Mars analog regolith. Differences in survival rates from the uppermost regolith profiles and deeper layers would imply active photochemical processing of both microbial and biomarker moieties; however, calculations of UV-C exposed regolith surface area (<0.01% total area) (*9*), UV-C penetration depth (<1 mm) (*12*) and decreased survival in both dark and control samples imply alternate oxidation mechanisms. Studies have shown that the presence of water vapor yields no change in oxidation rates (*11*) due to the negligible water vapor absorption at wavelengths greater than 200 nm (*13*). Microorganism and biomarker survival is inferred to be controlled by the diffusion of water vapor, allowing microlayers of water to condense on mineral grains and yielding radical oxygen species; decreased survival in UV-exposed samples may represent a photochemical and/or kinetic effect on reactive species formation.

Terrestrial organisms and relevant biomarkers are shown to be able to survive extended periods of time on the Martian surface when embedded within subcentimeter scale Mars regolith analogs, and in some cases for periods of time extending beyond basic mission lifetimes. Missions designed for life detection must ensure proper planetary protection protocols are followed in order to minimize contamination risks of returned samples and in-situ life detection analyses.

References: [1] L. J. Wood, (2006) *GSA Bulletin* **118**, 557 [2] A. Gendrin *et al.*, (2005) *Science* **307**, 1587 [3] F. Poulet *et al.*, (2005) *Nature* **438**, 623 [4] L. A. Haskin *et al.*, (2005) *Nature* **436**, 66 [5] S. W. Squyres *et al.*, (2004) *Science* **306**, 1709 [6] S. Fendrihan *et al.*, (2009) *Astrobiology* **9**, 104 [7] A. A. Hansen *et al.*, (2009) *Astrobiology* **9**, 229 [8] D. J. Smith *et al.*, (2009) *Astrobiology* **9**, 221 [9] J. R. C. Garry, *et al.*, (2006) *Meteorit Planet Sci* **41**, 391 [10] C. R. Stoker, M. A. Bullock, (1997) *J Geophys Res-Planet* **102**, 10881 [11] I. L. ten Kate, *et al.*, (2006) *Planet Space Sci* **54**, 296 [12] R. L. Mancinelli, M. Klovstad, (2000) *Planet Space Sci* **48**, 1093 [13] C. Y. Chung, *et al.*, (2001) *Nucl Instrum Meth A* **467**, 1572