LOW BIOTOXICITIES OF ANALOG SOILS SUGGEST THAT THE SURFACE OF MARS MAY BE HABITABLE FOR TERRESTRIAL MICROORGANISMS. A. C. Schuerger[1], D. W. Ming[2], and D. C. Golden[3]. [1]University of Florida, Bldg. M6-1025, Space Life Sciences Lab, Kennedy Space Center, FL 32899; schuerg@ufl.edu; [2]Astromaterials Res. and Exploration Science Office, Mail Code KX, ESCG, Mail Code JE23, NASA, Houston TX 77058; douglas.w.ming@nasa.gov; [3]ESCG, Mail Code JE23, Houston TX 77058; d.c.golden1@nasa.gov.

Introduction: Recent studies [1,2] on the interactive effects of hypobaria, low temperatures, and CO_2-enriched anoxic atmospheres on the growth of 37 species of mesophilic bacteria identified 14 potential biocidal agents that might affect microbial survival and growth on the martian surface. Biocidal or inhibitory factors include (not in priority): (1) solar UV irradiation, (2) low pressure, (3) extreme desiccating conditions, (4) extreme diurnal temperature fluctuations, (5) solar particle events, (6) galactic cosmic rays, (7) UV-glow discharge from blowing dust, (8) solar UV-induced volatile oxidants [e.g., O_2^-, O^-, H_2O_2, O_3], (9) globally distributed oxidizing soils, (10) extremely high salts levels [e.g., $MgCl_2$, NaCl, $FeSO_4$, and $MgSO_4$] in surficial soils at some sites on Mars, (11) high concentrations of heavy metals in martian soils, (12) likely acidic conditions in martian fines, (13) high CO_2 concentrations in the global atmosphere, and (14) perchlorate-rich soils. Despite these extreme conditions several studies have demonstrated that dormant spores or vegetative cells of terrestrial microorganisms can survive simulated martian conditions as long as they are protected from UV irradiation [3,4,5,6]. What has not been explored in depth are the effects of potential biotoxic geochemical components of the martian regolith on the survival and growth of microorganisms.

The primary objectives of the research included: (1) prepare and characterize Mars analog soils amended with potential biotoxic levels of sulfates, salts, acidifying minerals, etc.; and (2) use the simulants to conduct biotoxicity assays to determine if terrestrial microorganisms from spacecraft can survive direct exposure to the analog soils.

Materials and Methods: A Mars Simulation Chamber (MSC) was used to create conditions similar to equatorial Mars (described in full by Schuerger et al. [7]. The biotoxicity assays described below were conducted at martian conditions of: (a) 6.9 mbar, (b) -10 ^oC, (c) Mars normal equatorial UVC, UVB, and UVA irradiation from 200 to 400 nm, (d) optical depth of 0.1, and (e) atmospheric mix composed of the top five gases in the martian atmosphere [CO_2 (95.53%), N_2 (2.7%), Ar (1.6%), O_2 (0.13%) and H_2O (0.03%)].

Six Mars analog soils were prepared to simulate a range of potentially biotoxic soils and are derived from Mars Pathfinder, MER, and Phoenix data [8,9,10,11, 12,13]. The soils include simulants for (a) a non-toxic basalt, (b) acidic soil, (c) high-salt soil, (d) alkaline soil, (e) perchlorate soil, and (f) aeolian dust that combined materials from all soils. All simulants were crushed and sieved to pass a 500 µm screen.

In preliminary experiments, we determined the minimum depth of crushed basalt required to attenuate the biocidal effects of solar UV irradiation (200-400 nm) to be 1 mm. For added safety, we conducted all biotoxicity experiments with bacterial monolayers covered with 2 mm of the crushed and sieved Mars analog soils in order to factor-out UV irradiation from the biotoxicity assays.

Spores of *Bacillus subtilis* HA101 or vegetative cells of *Enterococcus faecalis* ATCC 292121 were applied to pre-sterilized aluminum coupons, dried, individually covered with 2 mm of one of the six Mars analog soils, and incubated for 7 d at Mars conditions similar to those experienced by the Viking, Pathfinder, Opportunity, and Spirit missions. Approximately 2 x 10^7 cfu's of spores or vegetative cells were applied to aluminum coupons prior to applying analog soils. One set of coupons for each soil was placed within the UV-VIS-NIR beam within the Mars chamber, and a 2nd set of coupons for each soil was shielded from UV irradiation, but exposed to martian conditions. The experiments were designed to simulate bioloads on spacecraft surfaces covered by desiccated Mars soils that might occur after rocket exhaust plumes loft surface fines during landing that then settle on to the upper decks of spacecraft. Following exposure to martian conditions, coupons were assayed for viable numbers of surviving spores or cells using a Most Probable Numbers (MPN) procedure originally described by Mancinelli and Klovstad [14] and modified by Schuerger et al., [5,6].

Controls for both species included the following: (1) sterile deionized water (SDIW) cell suspensions at T = 0; (2) desiccated cells on aluminum coupons assayed 24 h after preparing coupons; (3) Earth-control coupons without analog soils held 7 d at 1013 mbar, 24 ^oC, and in the dark; (4) Earth-control conditions without analog soils but maintained 7 d at 1013 mb, –15 ^oC, and in the dark (*E. faecalis* only); and (5) Mars coupons without analog soils held 7 d at 6.9 mbar, –10 ^oC, and shielded from UV-VIS-NIR irradiation in the Mars chamber.

Results: For *B. subtilis*, spores exposed 7 d under martian conditions, but not covered by any of the six analog soils, exhibited a modest 40% reduction in viability suggesting that there was a minor effect of hypobaria on the survival of spores. In contrast, hypobaria appeared to reduce the viability of *E. faecalis* cells by 2-3 logs over the same time period. Furthermore, of the six analog soils tested, only spores of *B. subtilis* covered by the high-salt soil (with and without UV irradiation), and the acidic soil (+UV only) exhibited a 70-80% decrease in viable spores after 7 d under martian conditions. Spores of *B. subtilis* for all other soils exposed to UV, or not, were similar to the non-soil and (-)UV Mars chamber controls. Thus, spores of *B. subtilis* exposed to all six analog soils and maintained for 7 d under simulated martian conditions were either unaffected by contact with the soils, or exhibited only modest < 1 log losses of viability.

For *E. faecalis*, vegetative cells were inactivated at rates much higher than those observed for *B. subtilis*. In general, cells of *E. faecalis* were reduced 2-3 logs for the Mars (-)UV/non-soil control, basalt, acidic, alkaline, aeolian, and perchlorate soils. For the soils listed above, both the (-) and (+) UV treatments responded in similar manners for specific soils, suggesting that hypobaria and desiccation were more important factors than the potential biotoxicity of the analog soils. In contrast, only cells of *E. faecalis* covered by high-salt soils exhibited dramatically lower levels of survival than the other treatments. In fact, no viable cells were recovered from the (+)UV/high-salt soil treatment; a 7.5 log decrease from the SDIW controls. In contrast, cells of *E. faecalis* covered by the high-salt soil and shielded from UV irradiation exhibited a 6 log reduction compared to SDIW controls, and did retain some survivors.

Ultraviolet photons appeared to interact with some soils in such a way as to lower the numbers of survivors for *E. faecalis* (high-salt soil) or *B. subtilis* (acidic soil). Although the direct UV photons were demonstrated to be attenuated 6 orders of magnitude by 2 mm of soils, the production of UV-induced volatile oxidants could not be ruled out. Thus, the differences observed between (-)UV versus (+)UV exposed samples with some soils may be the result of (1) long-term exposure to very low numbers of UV photons that are scattering between soil particles down and into the unconsolidated materials, or (2) the production of volatile biotoxic oxidants from the UV irradiation of the upper mineral surfaces. Further studies are required to identify the processes responsible for UV photons contributing to reductions in spore and cell viabilities on spacecraft materials buried by depths of soil (i.e., ≥ 2 mm depth) that presumably attenuate at least 6 orders of magnitude of UV photons between 200-400 nm.

Discussion: Results from both species suggests that hypobaria and desiccation were the primary two factors reducing the numbers of recovered spores or cells on coupons. The Mars analog soils appeared in general to be either non-toxic, or at most, capable of inducing only a modest biocidal effect. The only exception was the high-salt soil which significantly reduced the numbers of viable spores (*B. subtilis*) or cells (*E. faecalis*) recovered from coupons.

Recent papers by Stoker et al. [16] and Beaty et al. [17] have examined the habitability of the martian surface. The ecological models of Stoker et al. used 19 environmental factors to characterize the habitability of the Viking, Pathfinder, Spirit, Opportunity, and Phoenix landing sites. However, Stoker et al. did not include potential biocidal factors in their models. In addition, although Beaty et al. listed 20 environmental factors (with 7 factors potentially biocidal in nature), they concentrated their efforts on modeling the low limits of life relative to temperature (-20 ^{o}C) and water activity (a_w < 0.62). Based on the current results, it appears that the geochemistries of martian surface fines will not act to overtly inactivate terrestrial microorganisms on Mars, and suggest that the soils on Mars are not unusually or severely biotoxic. Thus, the habitability of the martian regolith/soil should be similar to terrestrial soils of similar chemistries. Since microbial life on Earth has been recovered from all surface soils, regardless of geochemistries, we conclude that soil geochemistries on Mars will not be growth limiting factors for terrestrial microorganisms (when transported) or extant Mars microbiota (if present).

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