BIOTOXICITY OF MARS ANALOG SOILS: MICROBIAL DISPERSAL INTO DESICCATED SOILS VERSUS EMPLACEMENT IN SALT OR ICE INCLUSION FLUIDS. A. C. Schuerger[1], D. W. Ming[2], and D. C. Golden[2]. [1]University of Florida, Bldg. M6-1025, Space Life Sciences Lab, Kennedy Space Center, FL 32899; email: schuerg@ufl.edu; [2]Astromaterials Research and Exploration Science Office, Mail Code JE23, NASA JSC, Houston, TX 77058; emails douglas.w.ming@nasa.gov and d.c.golden1@nasa.gov, respectively.

Introduction: Recent evidence from the Opportunity and Spirit rovers and the Mars Express mission suggests that the soils on Mars might be very high in biotoxic materials including sulfate salts, chlorides, and acidifying agents [1,2]. Yet, very little is known about how the chemistries of Mars soils might affect the survival and growth of terrestrial microorganisms.

In a recent paper on the interactive effects of hypobaria, low temperatures, and CO_2-enriched atmospheres on the growth of seven *Bacillus* spp., Schuerger and Nicholson [3] identified 13 potential biocidal agents that might affect microbial survival and growth on the martian surface. These factors include the following (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, and (13) high CO_2 concentrations in the global atmosphere. The Phoenix mission's discovery of perchlorates in the polar regolith [4] adds a 14th factor to this list. Despite these extreme conditions many studies have demonstrated that spores or dormant cells of terrestrial microorganisms can survive simulated conditions on Mars as long as they are protected from solar UV irradiation [5,6,7,8]. What has not been explored in depth has been the effects of potential biotoxic components in the martian regolith on the survival, growth, and adaptation of terrestrial microorganisms on Mars.

Nature of the martian regolith. The fine-grained surficial dust or regolith on Mars is often referred to in the literature as the "martian soil" [9]. The "global" soil is highly oxidized and contains basaltic materials (possibly from local basaltic rocks), nano-phase iron oxides, and $SO_4^{\square}$ and $Cl^{\square}$ bearing salts [1]. The chemistry of the fine-grained regolith or "soil" at the Pathfinder site, the two Viking landing sites, and the MER Spirit and Opportunity sites site are very similar, which is attributed to global mixing of fine-grained materials due to winds [9,10,11]. Minor variations in regolith compositions, especially for K_2O and Na_2O, have been observed in soil compositions at Pathfinder, Viking, and MER sites. Orbital gamma ray data also suggest that regional variations in K^+ and $Cl^{\square}$ are present [12]. The new MER data confirms that the chemically uniform fine-grained regolith probably represents a major surface component on Mars [10]. However, there are soils that have very high concentrations of sulfate salts (e.g., Fe-, Mg-, and Ca-sulfates in the soil called "Paso Robles" in Gusev crater) [1].

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

Materials and Methods: A Mars Simulation Chamber (MSC) was used to recreate conditions similar to equatorial Mars (described in full by Schuerger et al. [13]. The MSC system can accurately simulate five key components of the surface environment of Mars including: (a) pressures down to 0.1 mb, (b) UVC, UVB, and UVA irradiation from 190 to 400 nm, (c) dust loading in the atmosphere from optical depths of 0.1 to 3.5, (d) temperatures from –100 to +30 C, 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%)].

Two toxicological assays have been conducted with culturable mesophilic microbial species typically recovered from spacecraft surfaces. First, spores or vegetative cells of individual culturable species were applied to pre-sterilized iridited aluminum coupons, dried, covered with Mars analog soils to depths of up to 1 cm, placed in the Mars chamber, and incubated for 7 d at Mars conditions similar to those experienced by the Viking, Pathfinder, Opportunity and Spirit missions. Second, spores or vegetative cells of culturable species were added to water extracts of six Mars analog soils to determine if terrestrial microorganisms are capable of survival in hypersaline soil solutions. The soil solutions were obtained by vigorously mixing 50 g of each soil in 100 ml of 18 ohm deionized water for 2 hrs, filtered sequentially three times through cellulosic filters of 30, 0.45, and 0.22 µm nominal pore sizes, and dispensed into separate glass test tubes. Approximately 2 x 10^7 cfu's of spores or vegetative cells were added to each tube of soil solutions and then incubated at 24, 0, or □70 C for 7 d.

The "dry-assay" was designed to simulate bioloads on spacecraft surfaces covered by desiccated Mars soils during active descent onto the martian surface (i.e., rocket exhaust kicking up dusts that settle onto the upper decks of landers). The "wet-assay" was designed to simulate microbial species being emplaced into ice or salt inclusions during drilling operations by a rover.

Mesophilic species used in the dry-assays included *Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Pseu-*

domonas aeruginosa, Serratia liquifaciens, and Staphylococcus aureus. Species used in the wet-assay included *B. subtilis* and *E. faecalis*. Following exposure to experimental conditions, all materials doped with endospores or vegetative cells of microorganisms 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., [7,8].

Six Mars analog soils were prepared to simulate a range of potentially biotoxic soils and are derived from Mars Pathfinder, MER, and Phoenix data [1,2,9,10,11,12]. The soils include simulants for (a) a ground basalt that served as a non-toxic control (soil solutions = pH 8.62; EC 59 µS/cm), (b) acidic soil (pH 2.0; EC 38.8 mS/cm), (c) high salt soil (pH 3.14; EC 18.4 mS/cm), (d) alkaline soil (pH 10.18; EC 12.5), (e) perchlorate soil (pH 7.73; EC 5.56 mS/cm), and (f) aeolian soil that combined materials from all soils (pH 7.03; EC 6.71 mS/cm).

Results for the Dry-Assay: A desiccation experiment indicated that endospores of *B. subtilis* were very resistant to drying onto iridited aluminum coupons (used to simulate spacecraft surfaces), but that desiccation of all non-spore forming species yielded losses of viable cells between one to six orders of magnitude. Vegetative cells of *Serratia liquefaciens* were the most sensitive and cells of *Enterococcus faecalis* were the most resistant to desiccation of the non-spore forming species tested. A 7-d Mars simulation was conducted with endospores of *B. subtilis* and vegetative cells of *E. faecalis* to measure survival rates of both species when covered by 2 mm of either the basalt "control" soil or 2 mm of the high-salt soil and exposed to 6.9 mb total pressure, □10 C, continuous UVC irradiation at 4 W/m^2, and maintained within a Mars gas mix [7,13]. Cells of *E. faecalis* covered by the high-salt soil and exposed to martian conditions, with or without UV irradiation, were killed off to below detection limits of the assay within 7 d. Endospores of *B. subtilis* were reduced 1-2 orders of magnitude. All controls of both species exhibited high survival rates, and the interactive effects of (in order of biocidal activity) high-salt Mars analog soil, desiccation, and low pressure were concluded to be responsible for the significant decreases in viable numbers by both species.

Results for the Wet-Assay: In contrast, a wet-assay was conducted with *B. subtilis* and *E. faecalis* (as of this writing the *E. faecalis* experiments were still underway) in which endospores or vegetative cells, respectively, were exposed to aqueous soil solutions derived from all 6 soils listed above. Results for *B. subtilis* indicated no significant differences between controls and spores exposed to alkaline, perchlorate, or aeolian soil solutions. A very slight decrease in surviving spores was noted for the soil solutions from the high-salt and acid soil solutions. The decrease was only ½ order of magnitude below the controls and was close to the accuracy of the assay. Thus, for at least endospores of *B. subtilis*, the aqueous soil extractions did not appear to be biotoxic. Data for *E. faecalis* will be presented at the conference.

Discussion: Similar to several other microbial survival studies under martian conditions [5,6,7,8,15], interactive effects appear to impart significantly more stress on both endospores and vegetative cells than individual factors tested alone. The interactive effects of high-salt soil, desiccation, and low-pressure significantly decreased the viable cells recovered for both *B. subtilis* and *E. faecalis*. In contrast, endospores of *B. subtilis* exhibited only a slight decrease of no more than ½ an order of magnitude when exposed for 7 d in soil solutions from high salt and acid soils. No differences were observed for the soil solutions derived from the other Mars analog soils.

Experiments with multiple factors present on Mars that are likely to yield strong interactive effects are difficult to conduct due to requirements for large sample sizes and complex Mars simulators. For example, to conduct all possible interactions for the 14 biocidal factors (listed above) present on Mars would require 8.72 x 10^{10} possible combinations of experimental factors (i.e., 14 factorial). Something that is clearly outside the scope of human scientific study. However, because multiple biocidal factors are certainly present on Mars, combinations of stressing agents need to be tested if the astrobiology community is to gain an accurate assessment of how terrestrial microorganisms may, or may not, be able to survive, metabolize, replicate, and evolve on the surface of Mars. We recommend the following factors as the top priorities for interactive microbiology studies under simulated martian conditions: (1) anaerobic atmospheres, (2) high UVC fluence rates, (3) pressures near 6.9 mb, (4) desiccation, (5) biotoxicity of martian fines, and (6) soil oxidants.

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