Planetary and Space Science 72 (2012) 91–101

Contents lists available at SciVerse ScienceDirect

Planetary and Space Science

journal homepage: www.elsevier.com/locate/pss

Biotoxicity of Mars soils: 1. Dry deposition of analog soils on microbial colonies and survival under Martian conditions

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ARTICLE INFO

Article history:
Received 31 January 2012
Received in revised form
2 July 2012
Accepted 20 July 2012
Available online 31 July 2012

Keywords:
Biotoxic soils
Mars geochemistry
Biocidal soils
Regolith
Exobiology

ABSTRACT

Six Mars analog soils were created to simulate a range of potentially biotoxic geochemistries relevant to the survival of terrestrial microorganisms on Mars, and included basalt-only (non-toxic control), salt, acidic, alkaline, aeolian, and perchlorate rich geochemistries. Experiments were designed to simulate the dry-deposition of Mars soils onto spacecraft surfaces during an active descent landing scenario with propellant engines. Six eubacteria were initially tested for tolerance to desiccation, and the spore-former *Bacillus subtilis* HA101 and non-spore former *Enterococcus faecalis* ATCC 29212 were identified to be strongly resistant (HA101) and moderately resistant (29212) to desiccation at 24 °C. Furthermore, tests with *B. subtilis* and *E. faecalis* demonstrated that at least 1 mm of Mars analog soil was required to fully attenuate the biocidal effects of a simulated Mars-normal equatorial UV flux. Biotoxicity experiments were conducted under simulated Martian conditions of 6.9 mbar, −10 °C, CO_2-enriched anoxic atmosphere, and a simulated equatorial solar spectrum (200–1100 nm) with an optical depth of 0.1. For *B. subtilis*, the six analog soils were found, in general, to be of low biotoxicity with only the high salt and acidic soils exhibiting the capacity to inactivate a moderate number of spores (<1 log reductions) exposed 7 days to the soils under simulated Martian conditions. In contrast, the overall response of *E. faecalis* to the analog soils was more dramatic with between two and three orders of magnitude reductions in viable cells for most soils, and between six and seven orders of magnitude reductions observed for the high-salt soil. Results suggest that Mars soils are likely not to be overtly biotoxic to terrestrial microorganisms, and suggest that the soil geochemistries on Mars will not preclude the habitability of the Martian surface.

1. Introduction

The habitability of the Martian lithosphere will depend on a range of factors in which conducive conditions for microbial survival, metabolism, growth, and evolution exceed inhibitory or biocidal conditions. Furthermore, habitability of the Martian surface can be divided into two parts in which predicting conditions conducive for terrestrial life are compared to conditions that may support an extant Martian microbiota. The conditions for the persistence of terrestrial and Martian life on the surface of Mars are likely to overlap with some factors (yet to be determined), but also may be mutually exclusive. Recently, Stoker et al. (2010) proposed an ecological approach for predicting the potential habitability of the Martian surface using 19 environmental factors, with emphasis on water availability, temperature, presence of nutrients and an energy source, and protection from solar UV irradiation. However, Stoker et al. (2010) did not address potential biotoxic factors that might be present in habitable niches at the surface or near-subsurface of Mars. Beaty et al. (2006) in modeling the abundance of Special Regions on Mars listed 20 environmental factors that might affect the survival and reproduction of terrestrial microbes on Mars, with several factors representing inhibitory or biocidal soil conditions. But similar to Stoker et al. (2010), Beaty et al. (2006) did not extensively model soil biotoxic factors. Instead, Beaty et al. (2006) emphasized the roles of water activity (a_w), low-temperature thresholds for terrestrial life (−20 °C), and mapping Mars environments in thermodynamic disequilibrium (e.g., polar caps, subsurface brines, recent gullies, surficial ices in craters) relevant to the characterization of habitable Special Regions on Mars.

In contrast, Schuerger and coworkers (Berry et al., 2010; Schuerger and Nicholson, 2006) studying the growth of terrestrial microorganisms under simulated Martian conditions

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http://dx.doi.org/10.1016/j.pss.2012.07.026

identified 14 potential biocidal or inhibitory factors likely to be present on the surface of Mars. Of the 14 factors, five were environmental conditions considered ubiquitous on Mars and included solar UV irradiation, extreme desiccation, hypobaria (1–14 mbar), an anoxic CO_2-enriched atmosphere, and low surface temperatures. Solar particle events and galactic cosmic rays were considered external factors that occur infrequently or at low dosage, respectively. And seven factors involved potentially biotoxic edaphic factors widely distributed in localized sites on the surface. Based on the studies cited above, the edaphic factors considered most likely to be biotoxic or inhibitory to terrestrial life on Mars include (not in priority): (1) salinity, pH, and Eh of available liquid water; (2) oxidizing soils created by soil chemical reacitons and not by UV-induced processes (e.g., creation of oxidants by the anoxic hydration of pyrite; Davila et al., 2008); (3) high salt levels; (4) presence of heavy metals; (5) acidic conditions in some soils; (6) perchlorates; and (7) presence of UV-induced volatile oxidants (e.g., O_2^-, O^-, H_2O_2, NO_x, O_3).

The global fine-grained surficial dust and regolith on Mars is highly oxidized and contains basaltic materials, nanophase iron oxides (npO_x), and SO_4- and Cl-bearing salts (Morris et al., 2006). 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 are very similar, which is attributed to global mixing of fine-grained materials due to winds (Bell et al., 2000; Bruckner et al., 2003; Gellert et al., 2004; Klingelhofer et al., 2004; Morris et al., 2004). However, there are soils that have very high concentrations of sulfate salts (e.g., Fe-, Mg-, and Ca-sulfates in the Paso Robles soils on Husband Hill in Gusev crater; Ming et al., 2006, 2008), high silicate-rich soils near Home Plate in Gusev crater (Ming et al., 2008), soils at the Phoenix landing site that contain perchlorates (Hecht et al., 2009) and Ca-rich carbonates (Boynton et al., 2009), and high Cl and Br soil units in Gusev Carter (Haskin et al., 2005; Wang et al., 2006).

The objectives of the current work were to: (1) determine the depth of analog soils required to fully attenuate the biocidal effects of UV irradiation on microorganisms, (2) characterize the effects of desiccation on microbial survival, and (3) determine if Mars analog soils are overtly toxic to at least two terrestrial microorganisms typically recovered from spacecraft surfaces. The biotoxicity experiments were designed to simulate the dry deposition of Mars soils onto spacecraft surfaces during a powered descent landing scenario that lifts and scatters surface fines away from descent engines and onto the upper surfaces of landing pads, struts, instrument decks, solar panels, and radioisotope thermoelectric generators (RTG). Thus, the experiments were designed to test the overt biotoxic nature of a diversity of Mars analog soils placed in direct contact with microbial cells under simulated Martian conditions. Research on the biotoxic effects of hydrated analog soils and extracted soil solutions will be published elsewhere. Although significant organic and elemental contributions from accreting interplanetary dust particles (IDPs) and chondrites occur on Mars (Flynn, 1996; Stoker et al., 1993), inputs of chondritic materials to the Mars analog soils were ignored in the current work.

2. Materials and methods

2.1. Composition of Mars regolith

The chemical composition of surface soils has been largely determined by X-ray fluorescence (XRF) instruments onboard six landers/rovers that have landed at various locations on the surface. The term "soil" is used here to denote loose unconsolidated materials that can be distinguished from Martian rocks, bedrock, or strongly cohesive sediments. No implication of the presence or absence of organic materials or living matter is intended by this term in reference to Mars (e.g., Bell et al., 2000). Typical Martian soils have very similar compositions at the six landing sites on Mars. The uniform composition among the sites is thought to be in part due to homogenization of Martian fines by global dust storms (Clark et al., 1982; Wänke et al., 2001; Gellert et al., 2004).

Typical and atypical soils have been encountered by the Spirit rover in Gusev crater. There are a few subtle mineralogical and chemical differences in the typical Gusev soils or Laguna class materials (Ming et al., 2008; Morris et al., 2008). Laguna class materials have been separated into subclasses based upon subtle chemical differences and their Fe^{3+}/Fe_T ratios (Ming et al., 2008; Morris et al., 2008). Doubloon subclass soils appear to be a mixture of local rocks (Wishstone and Watchtower class rocks) and typical Gusev soils (Ming et al., 2008). Doubloon is enriched in Ti, Al, and P that are all geochemical indicators for Wishstone and Watchtower rocks. Boroughs subclass soils have a distinct enrichment in Cl, Br, and S compared to the other subclasses. Boroughs soils were exposed in trenches excavated by the rover's wheels out on the plains of Gusev crater. The higher S, Cl, and Br and the apparent correlation of Mg with S in the soil trenches suggest that these elements have been mobilized by water and transported downward in the soil profile (Haskin et al., 2005; Wang et al., 2006).

Atypical soils in Gusev crater contain very high S or very high Si. Paso Robles class soils at the Spirit landing site have very high S, contain ferric sulfates, and formed by aqueous alteration of basaltic materials under acid-sulfate conditions (Ming et al., 2006; Morris et al., 2006; Yen et al., 2008). Another unique soil class called Gertrude Weise was encountered by Spirit along the eastern side of a circular volcanic feature called Home Plate. Gertrude Weise soils are highly enriched in Si and Ti and depleted in all other elements except Cr compared to typical Gusev crater basalts (Ming et al., 2008). Silicon, Ti, and Cr are chemical indicators that this high Si soil is the residue that remained after acid–sulfate leaching (i.e., bleaching) of a host material (Morris et al., 2000; Squyres et al., 2008). The depletion of all the other elements suggests that these elements were removed by acidic solutions. A third unique soil type called Eileen Dean was also discovered on the eastern side of Home Plate and contains high Mg and magnetite (Ming et al., 2008).

Although the Mars Phoenix Scout Mission did not have a XRF instrument as part of the payload suite, the Wet Chemistry Laboratory (WCL) and Thermal Evolved Gas Analyzer (TEGA) identified perchlorate salts and carbonates in soils at the landing site (Hecht et al., 2009; Boynton et al., 2009; Sutter et al., 2012). The pH of Phoenix soils was alkaline ($pH = 7.7 \pm 0.5$) and contained 0.4% to 0.6% perchlorate (ClO_4) by mass. And a Viking class alkaline soil ($pH > 8$) was included in the assays based on Quinn and Orenberg (1993).

2.2. Mars analog soils

Based upon the soils that have been encountered during robotic missions to the surface, six soil types were created to simulate a range of potentially geochemical and mineralogical properties to test the survival of terrestrial microorganisms under Martian conditions. The six soil classes include a control (unaltered basalt only), high salt (Burroughs subclass), acidic (Paso Robles class), alkaline (Viking soils), weakly alkaline/perchlorate (Phoenix), and aeolian (Laguna class) soil types.

Mars analog soils were prepared by grinding mixtures of representative minerals (given in Table 1) to pass through a 200 μm stainless steel sieve five soils were prepared to respresent