EXTREMOPHILES FOR ECOPOIESIS: DESIRABLE TRAITS FOR AND SURVIVABILITY OF PIONEER MARTIAN ORGANISMS

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ABSTRACT

Humanity is on the verge of having the capability of constructively directing environmental changes on a planetary scale. Within the foreseeable future, we will have the technology to modify Mars' environment, and make it a habitable planet. However, we do not have enough information to determine the course of such an event. To our knowledge, no known terrestrial organism has the capability of living on Mars' surface under present conditions. However, with some modification, Mars' environment could be brought into the survival and growth range of currently known microorganisms. Using the SHOT Ecopoesis Testbed, we performed survival/growth experiments to determine the suitability of potential pioneering life forms for Mars. Included among the potential pioneers were five genera of cyanobacteria (*Anabaena*, *Chroococcidiopsis*, *Plectonema*, *Synechococcus* and *Synechocystis*), three partially-characterized Atacama Desert heterotrophic eubacterial strains, and several desert varnish isolates. Microorganisms were exposed to a present-day mix of martian atmospheric gases, but at a pressure of 100 mbar (10 times Mars' current atmospheric pressure). Cultures were inoculated into samples of JSC Mars-1 soil stimulant and exposed to full-spectrum simulated martian sunlight. Day/night temperature cycled from 26°C to -80°C and back. Preliminary results indicate that both autotrophic and heterotrophic bacteria can survive in the simulated engineered martian environment.

INTRODUCTION

In relation to terrestrial organisms, Mars' environment is harsher than any on Earth. The combination of low temperature, low atmospheric pressure, low moisture, high atmospheric CO_2 fraction and high UV flux is not experienced by any known Earth organism. However, Earth organisms may be found in environments that include at least one of the martian environmental factors (although Mars is still harsher in most respects). The dry valleys of Antarctica provide a low temperature, low moisture, high UV environment. Chile's Atacama Desert is arguably the driest place on Earth. Some hot springs and volcanic vents produce anoxic environments with very high CO_2 levels. Alpine and stratospheric environments possess low pressure, low temperature and high UV.

Each of these terrestrial environments harbors some form of life. Organisms living there have adapted to the extreme conditions, and those adaptations are genetically encoded. In theory, genes encoding favorable characteristics from one organism could be transferred to another organism, which already possesses some other characteristics for extreme environment survival. With enough genetic alterations, we could conceivably "build" a microorganism—a "marsbug"—that could survive, reproduce and grow in a somewhat modified martian environment.

Ten years ago, Hiscox and Thomas (Hiscox and Thomas, 1995) detailed some of the characteristics that an ideal marsbug might possess. Here, we elaborate upon and update these characteristics, and summarize some initial experiments in which organisms with some of these characteristics were tested under approximated martian conditions.

DESIRABLE TRAITS FOR PIONEER MICROORGANISMS

For the purposes of this paper, we assume that initial, non-biological, modification of Mars' environment would occur before pioneer microorganisms are introduced. At minimum, Mars would have an atmospheric pressure of at least 25 mbar, significant periods of above-freezing temperatures, and significant bodies of liquid water (Thomas, 1995). At 25 mbar, water boils at 9°C, providing a narrow temperature window at which water remains liquid. Also, recent studies suggest that Earth bacteria do not grow at pressures below 25 mbar (Schuerger et al., 2006a; Schuerger and Nicholson, 2005). Even with initial modification, the martian environment would be at least as harsh as the least hospitable environments on Earth. Accordingly, pioneer microorganisms would require many, if not all, of the following characteristics (summarized in Table I).

Autotrophy. Mars has no known reserves of organic materials. Pioneer organisms would need to make their

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Table I. Desirable characteristics for pioneer martian microorganisms. *The lists of microorganisms and references are examples only, and are not meant to be exhaustive.*

Characteristic	Example Microorganisms	References
Autotrophy: oxygenic photosynthesis	Cyanobacteria, algae	(Blankenship and Hartman, 1998; Burger-Wiersma and Matthijs, 1990; Dismukes et al., 2001; Fay, 1983; Goodwin, 1980; Ho and Krogman, 1982; Kaplan et al., 1988; Tabita, 1994; Xiong and Bauer, 2002)
Autotrophy: chemosynthesis	*Nitrosamonas*, *Nitrobacter*, *Thiobacillus*, methanogens	(Atlas, 1997; Boston et al., 1992; Chyba and Hand, 2001; Northup et al., 2003; Stevens and McKinley, 1995)
Psychrophily	*Pyramimonas*, *Fragilariopsis*, *Pseudo-nitzschia*, *Porosira*, *Entomoneis*, *Nitzschia*.	(Chen and Berns, 1978; Christner et al., 2003; Daugbjerg, 2000; Gaidos et al., 2004; Gilichinsky et al., 2003; McMinn et al., 2005; Morita, 1975)
CO_2 tolerance	*Anabaena*, *Plectonema*, *Cyanidium*, *Nannochloris*	(Negoro et al., 1991; Seckbach et al., 1970; Seckbach and Libby, 1970; Thomas et al., 2005)
Hypoxia tolerance	*Anabaena*, *Plectonema*, *Cyanidium*	(Seckbach et al., 1970; Thomas et al., 2005; Zehnder and Svensson, 1986)
Carbonate dissolution	*Matteia*, other cyanobacteria and algae	(Crispim et al., 2003; Friedmann et al., 1993)
Denitrification	*Pseudomonas*, *Paracoccus*, *Streptomyces*	(Baker et al., 1998; Hart et al., 2000; Kumon et al., 2002; Zumft, 1997)
Nitrogen fixation	*Anabaena*, *Chroococcidiopsis*, *Cyanothece*, *Synechococcus*, *Trichodesmium*	(Almon and Böger, 1988; Berman-Frank et al., 2001; Böhme, 1998; Fay, 1983; Friedmann and Kibler, 1980; Golden and Yoon, 1998; Klingler et al., 1989; Mitsui and Cao, 1988; Olson et al., 1998; Potts et al., 1983; Schneegurt et al., 2000; Wolk, 1988)
Osmotic tolerance, desiccation resisistance	*Chroococcidiopsis*, *Halomonas*, *Klebsormidium*, *Oscillatoria*, *Phormidium*, endospore-forming bacteria, extreme halophiles	(Abyzov et al., 1967; Billi et al., 2001; Billi et al., 2000; Cockell et al., 2005; de Winder et al., 1989; Frankenberg-Schwager et al., 1975; Imshenetsky and Lysenko, 1965; Kraegeloh and Kunte, 2002; Olson et al., 1998)
UV/ionizing radiation resistance	*Aspergillus*, *Bacillus*, *Chroococcidiopsis*, *Deinococcus*, *Mycobacterium*, *Rubrobacter*	(Battista et al., 1999; Bauche and Laval, 1999; Billi et al., 2000; Cockell et al., 2005; Ferreira et al., 1999; Imshenetsky et al., 1967; Imshenetsky et al., 1977)
Hypobaric tolerance	*Aspergillus*, *Mycobacterium*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*	(Frankenberg-Schwager et al., 1975; Hawrylewicz et al., 1967; Imshenetsky et al., 1977; Imshenetsky et al., 1970; Silverman and Beecher, 1967)
Switchable genes and pathways	Cyanobacteria, facultative autotrophs, genetically modified agricultural plants	(Garlick et al., 1977; Guay and Silver, 1975; Sarhan and Danyluk, 1998; Sorokin et al., 2000)

own biomolecules from inorganic constituents via photosynthesis or chemosynthesis. Photosynthesis would replace the CO_2 in Mars' atmosphere with O_2—a key process in planetary engineering (Graham, 2004; McKay, 1998; McKay et al., 1991; Thomas, 1995). Iron and manganese-oxidizing chemolithotrophs produce dark-colored byproducts (Dorn and Oberlander, 1981), which decrease the albedo of rock surfaces, and would aid in the heating of Mars (Boston et al., 2004).

Antioxidants. Oxygenic photosynthesis produces a variety of reactive oxygen species (ROS) as normal byproducts of electron transfer. In addition, Mars' surface is currently a highly oxidizing environment, presumably due to UV-produced ROS (Hunten, 1979; Plumb et al., 1989; Yen et al., 2000). Currently, we do not know whether initial engineering efforts would change the oxidizing nature of Mars' surface. However, other environmental stresses, such as chilling, increase the amount of ROS produced by photosynthesis (Clare et al., 1984; Hodgson and Raison, 1991a; Hodgson and Raison, 1991b; Thomas et al., 1999; Wise, 1995; Wise and Naylor, 1987). Pioneer microorganisms would require robust antioxidant systems in order to detoxify both internally- and externally-generated ROS.

Psychrophily. Even under engineered conditions, Mars will be cold. Truly psychrophilic organisms have optimum growth temperatures below 20°C (Atlas, 1997). The ability to grow at low temperature requires an entire suite of enzymes that operate well at low temperature as well as highly unsaturated membrane lipids (Chattopadhyay and Jagannadham, 2001; Gerday et al., 2000; Morita, 1975; Russell, 2000; Zecchinon et al., 2001). Thus, psychrophilic autotrophs may be good starting organisms to which other characteristics could be added.

CO_2 tolerance. Mars' atmosphere currently contains 95% CO_2, albeit at low pressure. We expect initial engineering

efforts to increase the total atmospheric pressure primarily through the release of additional CO_2 from the polar caps and from possible carbonate rocks, which would further increase the proportion of CO_2. At high CO_2 levels, decreased pH becomes a factor as well. When CO_2 dissolves in water, it forms carbonic acid with a pH of 5.5 - 6.0 at 1000 mbar. Fortunately, several cyanobacteria and at least one alga survive and grow in >20% CO_2 at 1000 mbar, and many microorganisms grow within the pH range of 5 - 6 (Negoro et al., 1991; Seckbach et al., 1970; Seckbach and Libby, 1970; Thomas et al., 2005).

Hypoxia tolerance. Mars' atmosphere contains very little O_2. Pioneer microorganisms will have to grow during extended periods of hypoxia or anoxia. Even though photosynthesis produces O_2, plants require at least 50 mbar pO_2 for proper development (due in part to the large proportion of non-photosynthetic tissues) (Alpi and Beevers, 1983; Atwell et al., 1982; Barclay and Crawford, 1982; Jackson and Drew, 1984). Most chemoautotrophs also require O_2 in order to oxidize inorganic molecules for energy production (Atlas, 1997), thus limiting their initial growth on Mars. Fortunately, most of the CO_2-tolerant photoautotrophic microbes, cited previously, also grow anaerobically.

Carbonate dissolution. As mentioned previously, carbonates are a potential source of atmospheric CO_2. Many cyanobacteria and algae cause damage to limestone-based structures due to carbonate dissolution—the conversion of carbonate ions into CO_2 (Crispim et al., 2003). If Mars has extensive carbonate deposits, such organisms could speed the process of thickening Mars' CO_2 atmosphere while also providing oxygen. The Antarctic cyanobacterium, *Matteia*, has been specifically suggested for this purpose (Friedmann et al., 1993).

Denitrification. Although Mars' atmosphere could be thickened by the addition of CO_2, photosynthesis will remove the CO_2 and replace it with O_2. An atmosphere of pure O_2 would result in catastrophic combustion. On Earth, the atmosphere is buffered with N_2, which is largely nonreactive. If Mars possesses significant nitrate deposits, these can be converted to N_2 via the process of denitrification (Thomas, 1995). Denitrifying bacteria use nitrate in place of O_2 for respiration, and thus are usually hypoxia tolerant as well. *Pseudomonas aeruginosa*, a heterotrophic facultative anaerobe, grows well and denitrifies under conditions similar to those expected during ecopoesis (Hart et al., 2000). Additionally, some chemoautotrophs utilize nitrate in place of oxygen (Oremland et al., 2002), thus avoiding the problem of hypoxia and chemosynthesis mentioned previously.

Nitrogen fixation. Later in the process of planetary engineering, biologically-usable nitrogen will need to be recycled from the atmosphere. Nitrogen fixation (Mancinelli, 1996)—the opposite of denitrification—would be detrimental during the initial stages, but would be required later in order to stabilize biological communities (Thomas, 1995). *Azotobacter vinelandii* and *Azomonas agilis* have the capability of fixing nitrogen at pN_2 as low as 5 mbar (Klingler et al., 1989). Many genera of cyanobacteria also fix nitrogen (Böhme, 1998; Fay, 1983; Friedmann and Kibler, 1980; Golden and Yoon, 1998; Mitsui and Cao, 1988; Mulholland and Capone, 2000; Olson et al., 1998; Potts et al., 1983; Schneegurt et al., 2000; Zehr et al., 2001).

Osmotic tolerance. The initial bodies of water that form on Mars probably will contain significant amounts of dissolved salts; they may also be transient. Pioneer microorganisms will need to tolerate osmotic stress, and survive periods of desiccation. A large number of Gram-positive bacteria form endospores that allow long periods of dormancy under adverse conditions. Many non-spore-forming bacteria also survive periods of desiccation. The period between dormancy and the resumption of metabolism should be as short as possible for pioneering marsbugs so that they can take full advantage of periods of favorable conditions. Some cyanobacteria undergo seasonal desiccation and quickly become active during wet periods (de Winder et al., 1989; Hershovitz et al., 1991a; Hershovitz et al., 1991b; Scherer et al., 1984).

Ultraviolet and ionizing radiation resistance. Mars has very little free O_2 and only a very thin ozone layer that changes with season and latitude (Barth et al., 1974; Lefèvre et al., 2004), which would offer little or no protection to potential marsbugs. Even though Mars receives about half as much total solar radiation of Earth, the amount of UV at the surface is much higher. Sun-exposed surfaces on Mars receive sterilizing doses of UV radiation. However, presumed planetary engineering processes would release additional CO_2 into the atmosphere, and reduce the UV flux at the surface. For example, Mars currently receives approximately 3.5 Watts m^{-2} UVC at the equator during the Vernal Equinox, but with an atmosphere of 500 mbar CO_2, the UVC flux drops to 0.9 Watts m^{-2} (Cockell et al., 2000). Also, Mars doesn't internally generate a magnetic field like Earth's, and possesses only patchy remnant crustal magnetic fields (Acuna et al., 1999; Acuna et al., 1998; Connerney et al., 2004; Connerney et al., 2001). Although some localized remnant magnetic fields are up to 30 times stronger than those of Earth (Connerney et al., 2001), the lack of a global magnetic field allows the surface of Mars to receives more cosmic radiation than Earth – 20-30 centiSieverts/year (Cucinotta et al., 2001). A marsbug would need mechanisms to resist radiation damage, and repair any damage that occurs. Endolithic cyanobacteria, which have been suggested as models for martian microorganisms (Friedmann and Ocampo-Friedman, 1994; Friedmann and Ocampo-Friedmann, 1984; Thomas and Schimel, 1991), protect themselves from UV by their habitats (porous rocks) and by the production of photoprotective pigments (Villar et al., 2005). The bacterium, *Deinococcus radiodurans*, survives very high doses of ionizing radiation due to its efficient DNA repair mechanisms (Battista et al., 1999; Bauche and Laval, 1999; Levin-Zaidman et al., 2003; Venkateswaran et al., 2000). Desiccation resistant strains of the

cyanobacterium *Chroococcidiopsis* also exhibit resistance to ionizing radiation, presumably due to efficient DNA repair as well (Billi et al., 2000). Protective pigments and repair enzymes potentially could be genetically added to other microorganisms, increasing their abilities to live in the martian environment.

Hypobaric tolerance. Even after initial engineering efforts, the atmospheric pressure of Mars would be far lower than that of Earth. While many bacteria can survive the desiccation that may occur at low pressure, a marsbug would have to actively metabolize and grow. As mentioned previously, bacteria may have a 25 mbar lower limit for active growth (Schuerger et al., 2006a; Schuerger and Nicholson, 2005). Bacteria have been isolated from the upper atmosphere; however, their metabolic state remains controversial (Imshenetsky et al., 1977; Wainwright et al., 2003; Wainwright et al., 2004). They may be actively growing without ever "touching ground," or they may be dormant while being transported through the air. If these bacteria do indeed remain active while in the upper atmosphere, they may have physiological characteristics that could be useful on Mars.

"Switchable" genes. While the introduction of new genes into organisms is relatively easy, their regulation is more problematic. Many of the characteristics described here are metabolic opposites (e.g., respiration and photosynthesis, denitrification and nitrogen fixation). The ability to control pioneer microorganisms over large areas with the application of a very dilute controlling agent would be highly desirable. Large numbers of genes are switched on and off in organisms that have multiple energetic pathways (Garlick et al., 1977; Guay and Silver, 1975; Sorokin et al., 2000). "Switchable" genes are also desirable in genetically engineered agricultural crops to make them resistant to environmental stresses (Sarhan and Danyluk, 1998); research in this area may also benefit planetary engineering.

A relatively small number of experiments have examined the effects of parts of Mars' environment on potential pioneer organisms, including UV radiation (Cockell et al., 2005; Hansen et al., 2005; Nicholson and Schuerger, 2005; Schuerger et al., 2003), high CO_2 (Hart et al., 2000; Kanervo et al., 2005; Thomas et al., 2005), low pressure and hypoxia (Boston, 1981; Kanervo et al., 2005; Paul et al., 2004). However, these experiments only integrated 2-5 of the variable conditions that would occur during ecopoeisis on Mars. To address this problem, SHOT, Inc. built a simulator capable of specifically replicating most of the environmental parameters (with the notable exceptions of gravity and cosmic radiation) on Mars during all phases of planetary engineering (Thomas et al., in review).

ECOPOESIS SIMULATIONS

The SHOT Martian Environment Simulator has been operational since May 2005. As of October 2005, six experiments have been performed with durations of 24 hours to six weeks (Thomas et al., accepted for publication). The initial experiments were primarily to determine logistical and analytical needs for longer-duration research, but we also determined short-term survival and growth characteristics for a variety of heterotrophic and autotrophic bacteria.

A 24-hour day was used in all experiments. Illumination was provided by a xenon arc lamp (Sylvania 69263-0 Short Arc Lamp, XBO, 1000 W/HS OFR) fitted with a solar filter that provided a close approximation of solar radiation. Photosynthetically active radiation at sample level ranged from 15 µmol photons m^{-2} s^{-1} in the shaded region to 1000 µmol photons m^{-2} s^{-1} in direct light. Total ultraviolet radiation (250-400 nm) was 1.7 µmol photons m^{-2} s^{-1} in the shaded region, and 50 µmol photons m^{-2} s^{-1} in direct light. The temperature regime cycled from a low of -80°C to a high of 26°C, diurnally. This approximated the lower latitudes of Mars during the vernal equinox (Carr, 1996), but probably is more extreme than what would occur after the initial stages of planetary engineering. As of October 2005, all of the experiments were performed at an atmospheric pressure of 100 mbar—10 times Mars' current highest pressure, but only 10% of Earth's atmospheric pressure. For experiments of 14 days or less, we used simulated atmosphere of 95% CO_2, 2.7% N_2, 1.6% Ar and 0.13% O_2 (Owen et al., 1977). For longer-duration experiments, we used an atmosphere of 100% CO_2. In most of the experiments, water was added (1 mL per day or less) in order to maintain atmospheric water saturation.

Cyanobacteria stock cultures were grown in liquid BG-11 medium, and diluted to A_{720} = 0.25 with fresh BG-11. Heterotrophic bacteria stock cultures were grown in trypticase soy broth (TSB), and diluted to A_{720} = 0.25 with fresh TSB. Several sample configurations were tested, including open trays, multi-well tissue culture plates and arrays of individual sample containers. Individual liquid cultures or mixtures of cultures were added to sterile JSC Mars-1 simulant (5-15 g, depending on the container) to the point of saturation. Desert varnish and cave microorganisms were grown on BG-11 agar. Agar cultures were than macerated and mixed with JSC Mars-1. While samples were in the simulator, parallel control samples were kept at 4°C in darkness. In cases where samples could not be analyzed at the SHOT facility, the samples were placed on ice and transported by car or by overnight courier.

Samples were analyzed for esterase activity via an assay of fluorescein diacetate (FDA) hydrolysis (Adam and Duncan, 2001; Schnürer and Rosswall, 1982) at the beginning and end of each experiment. The FDA hydrolysis assay indicates microbial metabolism across a wide variety of taxa, and correlates well with assays of respiration. Subsamples of 0.5 - 1.0 g were taken from each sample before and after each experiment and transferred into 15 mL centrifuge tubes. 5 mL of 60 mM K_2PO_4 buffer (pH 7.6) was added to each tube and briskly shaken for 10-20 seconds. Ten µL FDA in ethanol (5 mg

mL^{-1}) was added to each tube, and then all tubes were incubated for 3-5 hours at 25°C on a rocker table. Following incubation, the samples were extracted by adding 5 mL 2:1 chloroform:methanol. The samples were centrifuged for 10 minutes at 1000 x g, and the supernatant was measured spectrophotometrically at 490 nm.

Chlorophyll *a* extractions were also used to determine the relative abundance of photosynthetic organisms (Bowles et al., 1985; Myers et al., 1980). Subsamples of 0.5 - 1.0 g were taken from each sample before and after each experiment and transferred into 15 mL centrifuge tubes. 5 mL of 80% ethanol was added to each tube. Tubes were placed in a -20°C freezer overnight, and then were centrifuged for 10 minutes at 1000 x g. The supernatant was measured spectrophotometrically at 664 nm.

For the first two experiments (24 hours and 14 days), plate counts (Atlas, 1997) and trypan blue live-dead stains (Sigma-Aldrich Co., 2005) were also used to determine survival. However, the tests were very time-consuming with ambiguous results, and were discontinued in favor of the FDA and chlorophyll assays.

The full results of the initial experiments are reported elsewhere (Thomas et al., accepted for publication). Here, we summarize the most relevant results.

In experiments of seven days or longer duration, a "water cycle" became evident within the test chamber as water tended to condense at the ends as the chamber cooled at "night," and the samples at the ends of the chamber tended to be moister than samples in the middle. While this was disconcerting at first, it probably approximates the conditions on a planet-wide scale—some areas of Mars will be wetter than others, and life will become distributed according to moisture levels.

Live-dead microscopic assays and plate counts showed survival of all of the organisms tested in the overnight and 14-day trials. However, because of the small sizes of the cells tested, the live-dead assays were prone to large errors. Non-cellular material was sometimes counted as cells, and some cells were incorrectly counted as debris. Plate counts of cyanobacteria took 1-2 weeks in order to grow countable colonies. In addition, *Plectonema* and *Anabaena*, being filamentous, gave underestimated results from plate counts. Further use of these assays was discontinued.

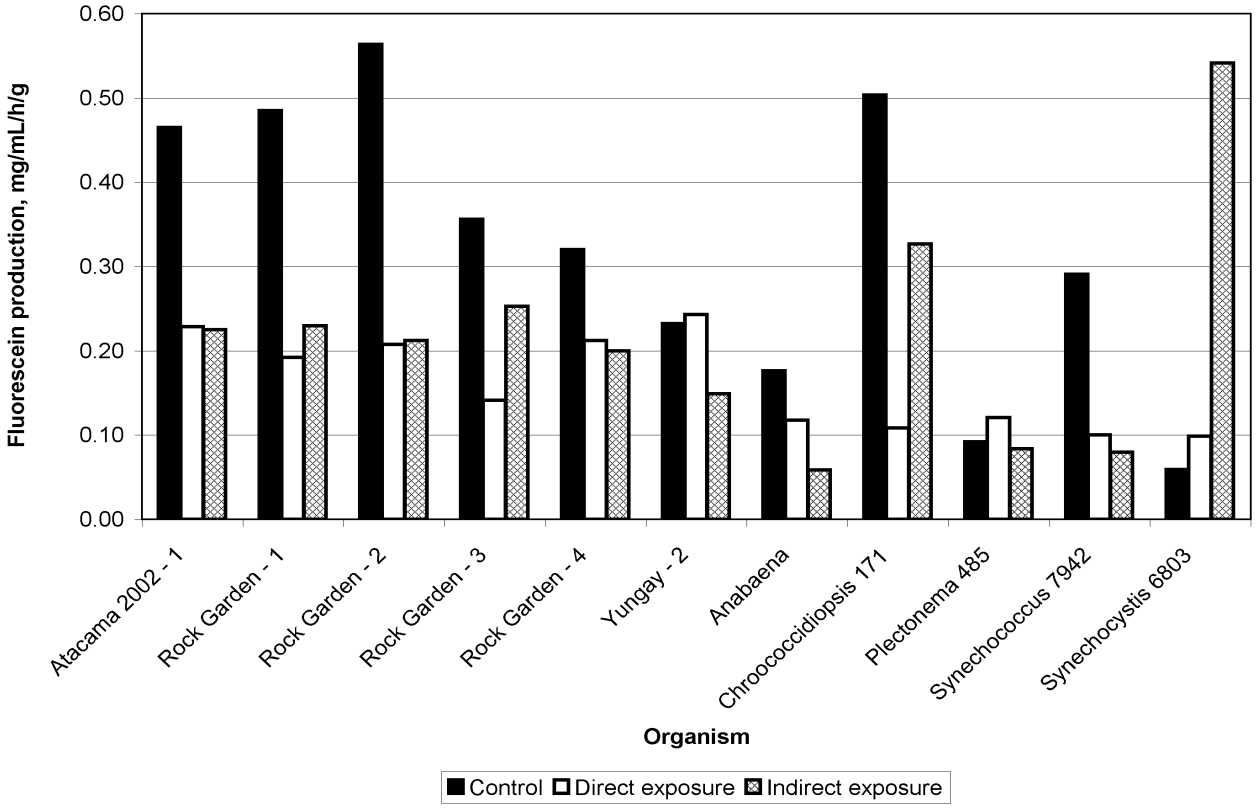

Figure 1. ***FDA hydrolysis by eubacteria and cyanobacteria after a 14-day ecopoesis trial.*** *Hydrolysis rates are per gram of total soil simulant. Samples were placed in two 24-well tissue culture plates—one in direct light, the other in shadow. "Atacama," "Rock Garden," and "Yungay" refer to partially-characterized, heterotrophic Atacama Desert isolates. "Atacama 2002" is a strain of Klebsiella oxytoca; "Rock Garden-2" is a strain of Bacillus licheniformis; and "Yungay-2" is another strain of Bacillus. The other "Rock Garden" strains appear to be species of Staphylococcus (but not S. aureus). All heterotrophic bacteria were exposed in JSC Mars-1 simulant amended with trypticase soy broth. The cyanobacteria (five organisms on the right side of the graph) were grown in JSC Mars-1 amended with BG-11 medium. Each bar represents a single sample.*

The heterotrophic bacteria, *Bacillus* and *Klebsiella*, grew very well during short-duration experiments in which they were supplied organic nutrients (Figure 1). These results indicate the hardiness of both sporogenic (*Bacillus*) and non-sporogenic (*Klebsiella*) bacteria. These organisms were discontinued in later experiments since organic nutrients are not expected to be available during the early ecopoeisis of Mars. However, since both genera are commonly associated with humans, these results have relevance for planetary protection issues associated with both robotic and human exploration of Mars. In our experiments, heterotrophic bacteria survived at least 14 days in the simulator. Although *Bacillus* spores are quickly inactivated in the presence of Mars levels of UV (Newcombe et al., 2005; Schuerger et al., 2003; Schuerger et al., 2006b), spores beneath the regolith would be protected. Similar experiments to ours (without UV) have shown even longer survival times of *Bacillus* under present day martian conditions (Nicholson and Schuerger, 2005). While these results are encouraging from a planetary engineering perspective, the presence of terrestrial "hitch-hikers" in the martian regolith could interfere with astrobiological research on Mars.

Several genera of cyanobacteria have been tested with a wide range of survival (Figures 1 and 3). Cyanobacterial survival in the simulator was somewhat correlated with CO_2 tolerance (Thomas et al., 2005), but desiccation resistance also had a role. *Anabaena*, *Plectonema* and *Chroococcidiopsis* appear to be very good candidates for further study. One strain of *Chroococcidiopsis* in particular (CCMEE 029), has been shown previously to have higher UV resistance than *Bacillus* (Cockell et al., 2005). UV resistance is especially important for cyanobacteria on Mars since they need exposure to light for photosynthesis, but that same exposure causes damage from UV radiation. A fine covering of regolith may provide UV shielding while still allow enough photosynthetically active radiation to reach the cells. Further research in this area will prove beneficial both for ecopoeisis research and in understanding niches for possible extant life on Mars.

Of the desert varnish and cave bacteria (Figure 2), *Pedomicrobium* had very high esterase activity after five weeks of exposure. Even though all of these strains have long generation times, *Pedomicrobium* may also be a good candidate for further study. However, at this time, most of these strains have only been partially characterized and more information is needed about their metabolism and environmental limits.

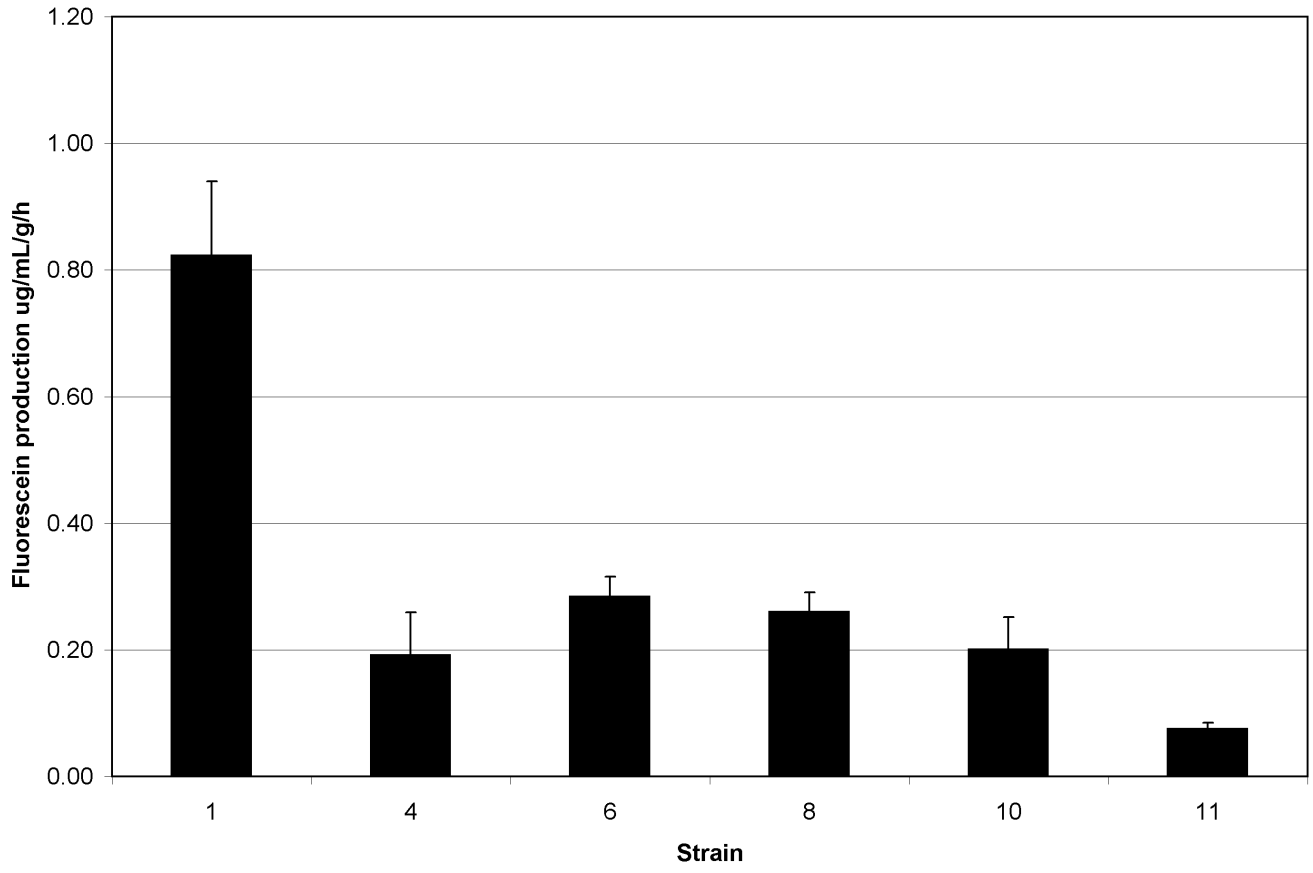

Figure 2. ***FDA hydrolysis by desert varnish and cave bacteria after a 5-week ecopoesis trial.*** *Pure cultures from agar plates were mixed with BG-11 amended JSC Mars-1 simulant and placed in individual polypropylene containers in direct light. Strain 1 has been identified as Pedomicrobium manganicum isolated from desert varnish. The other strains are partially-characterized isolates from cave and desert varnish environments. Each bar represents the mean of three samples; error bars = s.d.*

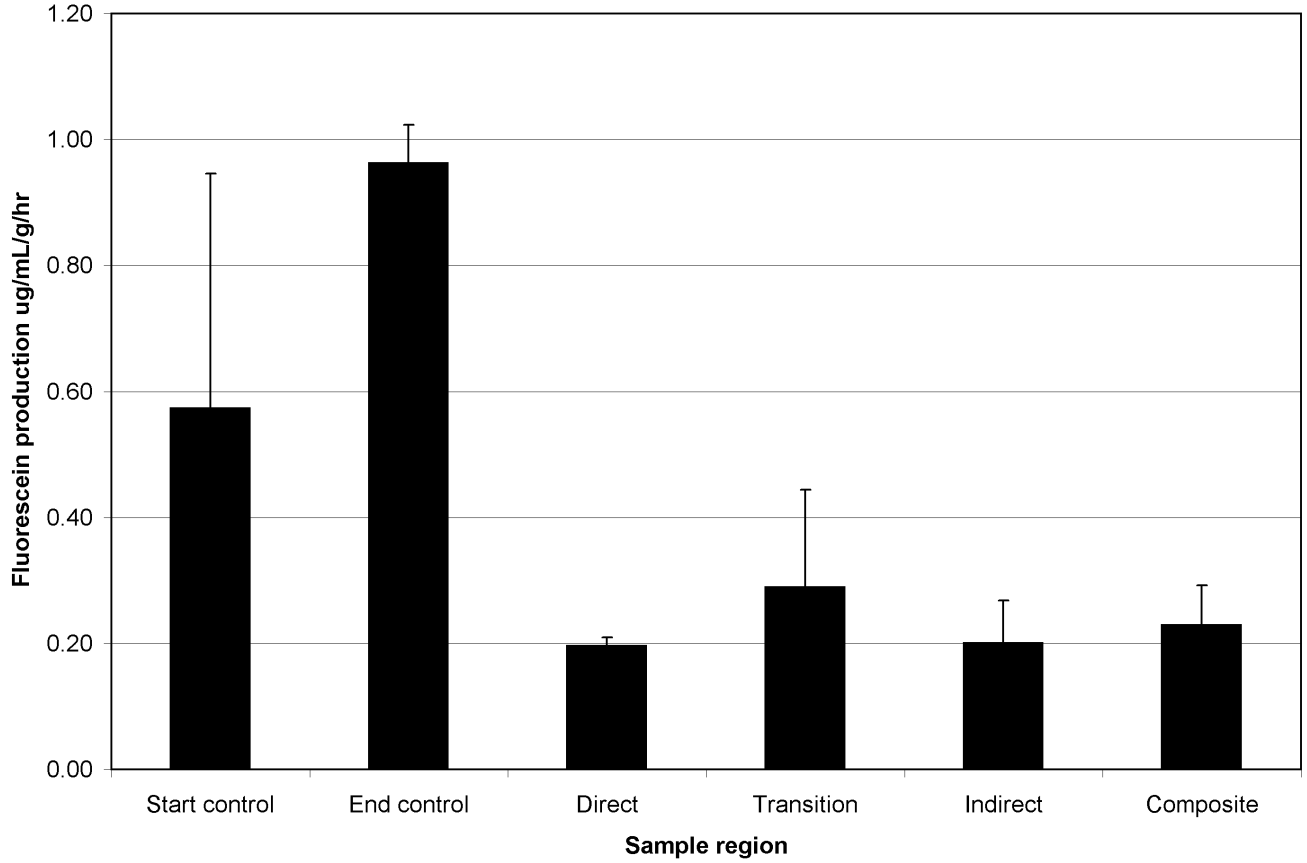

Figure 3. ***FDA hydrolysis within a simulated soil community after a 5-week ecopoesis trial.*** *A simulated soil community was formed by mixing cultures of Anabaena sp., Chroococcidiopisis CCMEE171 and CCMEE662, Plectonema boryanum, Klebsiella oxytoca, Bacillus licheniformis and Bacillus sp. in JSC Mars-1 simulant amended with BG-11 medium. The soil was spread over a polystyrene tray, which was placed half in light and half in shadow. After the experiment, the soil was divided into three regions: direct light, transition, and indirect light. Composite samples were mixtures of soil from all three regions. Three samples were obtained from each region and averaged; error bars = s.d.*

CONCLUSIONS

With the increasing availability of martian environment simulators, the science of planetary engineering is moving from the theoretical to the experimental realm. Although planetary-scale ecopoesis is still in the distant future, population and community level experiments are possible and are currently in progress. In addition to providing insight about possible future life on Mars, these experiments also tell us about the survivability of Earth organisms in the present martian environment.

As additional simulators become available, voluntary standardization of environmental parameters will be desirable. We envision a consortium that would set guidelines for planetary simulators so that experiments undertaken with different simulators would still be directly comparable. Efforts to form such a consortium are currently underway.

Ecopoeisis also provides an exciting opportunity to engage students in science. While many students have heard of terraforming through *Star Trek* and other science fiction stories, they usually do not realize that serious science underlies the fiction. Ecopoesis can be used as a focal point for discussions of geology, environmental science, microbiology, ecology and other disciplines to show the interdisciplinary nature of planetary science—whether that planet is Earth, Mars or one that has not been discovered yet.

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REFERENCES

Abyzov, S. S., Zavarzin, G. A., Ivanov, M. V., Seregin, V. I., and Shirokov, O. G. 1967. On the possibility of protracted anabiosis in micro-organisms. *Life Sciences and Space Research* 5: 261-267.

Acuna, M. H., Connerney, J. E. P., Ness, N. F., Lin, R. P., Mitchell, D., Carlson, C. W., McFadden, J., Anderson, K. A., Reme, H., Mazelle, C., Vignes, D., Wasilewski, P., and Cloutier, P. 1999. Global distribution of crustal

magnetization discovered by the Mars Global Surveyor MAG/ER experiment. *Science* 284: 790-793.

Acuna, M. H., Connerney, J. E. P., Wasilewski, P., Lin, R. P., Anderson, K. A., Carlson, C. W., McFadden, J., Curtis, D. W., Mitchell, D., Reme, H., Mazelle, C., Sauvaud, J. A., d'Uston, C., Cros, A., Medale, J. L., Bauer, S. J., Cloutier, P., Mayhew, M., Winterhalter, D., and Ness, N. F. 1998. Magnetic field and plasma observations at Mars: initial results of the Mars Global Surveyor mission. *Science* 279: 1676-1680.

Adam, G., and Duncan, H. 2001. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biology and Biochemistry* 33: 943-951.

Almon, H., and Böger, P. 1988. Hydrogen metabolism of the unicellular cyanobacterium *Chroococcidiopsis thermalis* ATCC29380. *FEBS Letters* 49: 445-449.

Alpi, A., and Beevers, H. 1983. Effects of O_2 concentration on rice seedlings. *Plant Physiology* 71: 30-34.

Atlas, R. M. 1997. *Principles of Microbiology*. Wm. C. Brown Publishers, Dubuque, IA.

Atwell, B. J., Waters, I., and Greenway, H. 1982. The effect of oxygen and turbulence on elongation of coleoptiles of submergence-tolerant and -intolerant rice cultivars. *Journal of Experimental Botany* 33: 1030-1044.

Baker, S. C., Ferguson, S. J., Ludwig, B., Page, M. D., Richter, O. M. H., and van Spanning, R. J. M. 1998. Molecular genetics of the genus *Paracoccus*: metabolically versatile bacteria with bioenergetic flexibility. *Microbiology and Molecular Biology Reviews* 62: 1046-1078.

Barclay, A. M., and Crawford, R. M. M. 1982. Plant growth and survival under strict anaerobiosis. *Journal of Experimental Botany* 33: 541-549.

Barth, C. A., Hord, C. W., Stewart, A. I., Lane, A. L., Dick, M. L., and Anderson, G. P. 1974. Mariner 9 Ultraviolet Spectrometer Experiment: seasonal variation of ozone on Mars. *Science* 179: 795-796.

Battista, J. R., Earl, A. M., and Park, M. J. 1999. Why is *Deinococcus radiodurans* so resistant to ionizing radiation? *Trends in Microbiology* 7: 362-365.

Bauche, C., and Laval, J. 1999. Repair of oxidized bases in the extremely radiation-resistant bacterium *Deinococcus radiodurans*. *Journal of Bacteriology* 181: 262-269.

Berman-Frank, I., Lundgren, P., Chen, Y. B., Kupper, H., Kolber, Z., Bergman, B., and Falkowski, P. 2001. Segregation of nitrogen fixation and oxygenic photosynthesis in the marine cyanobacterium *Trichodesmium*. *Science* 294: 1534-1537.

Billi, D., Friedmann, E. I., Helm, R. F., and Potts, M. 2001. Gene transfer to the desiccation-tolerant cyanobacterium *Chroococcidiopsis*. *Journal of Bacteriology* 183: 2298-2305.

Billi, D., Friedmann, E. I., Hofer, K. G., Caiola, M. G., and Ocampo-Friedmann, R. 2000. Ionizing-radiation resistance in the desiccation-tolerant cyanobacterium *Chroococcidiopsis*. *Applied and Environmental Microbiology* 66: 1489-1492.

Blankenship, R. E., and Hartman, H. 1998. The origin and evolution of oxygenic photosynthesis. *Trends in the Biochemical Sciences* 23: 94-97.

Böhme, H. 1998. Regulation of nitrogen fixation in heterocyst-forming cyanobacteria. *Trends in Plant Science* 3: 346-351.

Boston, P. J. 1981. Low-pressure greenhouses and plants for a manned research station on Mars. *Journal of the British Interplanetary Society* 34: 189-192.

Boston, P. J., Ivanov, M. V., and McKay, C. P. 1992. On the possibility of chemosynthetic ecosystems in subsurface habitats on Mars. *Icarus* 95: 300-8.

Boston, P. J., Todd, P., and McMillen, K. 2004. Robotic lunar ecopoiesis test bed: bringing the experimental method to terraforming, AIP #654. *Space Technology and Applications Forum 2003 Proceedings*. American Institute of Physics, College Park, MD.

Bowles, N. D., Paerl, H. W., and Tucker, J. 1985. Effective solvents and extraction periods employed in phytoplankton carotenoid and chlorophyll determinations. *Canadian Journal of Fishery and Aquatic Science* 42: 1127-1131.

Burger-Wiersma, T., and Matthijs, H. C. P. 1990. The biology of the Prochlorales. Pages 1-24 in Codd, G. A., Dijkhuizen, L., and Tabita, F. R., eds. *Autotrophic Microbiology and One-Carbon Metabolism*. Kluwer Academic Publishers, Dordrecht.

Carr, M. H. 1996. *Water on Mars*. Oxford University Press, New York.

Chattopadhyay, M., and Jagannadham, M. 2001. Maintenance of membrane fluidity in Antarctic bacteria. *Polar Biology* 24: 386-388.

Chen, C. H., and Berns, D. S. 1978. Comparison of the stability of phycocyanins from thermophilic, mesophilic, psychrophilic and halophilic algae. *Biophysical Chemistry* 8: 203-213.

Christner, B. C., Kvitko, B. H., 2nd, and Reeve, J. N. 2003. Molecular identification of Bacteria and Eukarya inhabiting an Antarctic cryoconite hole. *Extremophiles* 7: 177-83.

Chyba, C. F., and Hand, K. P. 2001. Life without photosynthesis. *Science* 292: 2026-2027.

Clare, D. A., Rabinowitch, H. D., and Fridovich, I. 1984. Superoxide dismutase and chilling injury in *Chlorella ellipsoidea*. *Archives of Biochemistry and Biophysics* 231: 158-163.

Cockell, C. S., Catling, D. C., Davis, W. L., Snook, K., Kepner, R. L., Lee, P., and McKay, C. P. 2000. The ultraviolet environment of Mars: biological implications past, present, and future. *Icarus* 146: 343-359.

Cockell, C. S., Schuerger, A. C., Billi, D., Friedmann, E. I., and Panitz, C. 2005. Effects of a simulated martian UV flux on the cyanobacterium, *Chroococcidiopsis* sp. 029. *Astrobiology* 5: 127-140.

Connerney, J. E. P., Acuña, M. H., Ness, N. F., Spohn, T., and Schubert, G. 2004. Mars crustal magnetism. *Space Science Reviews* 111: 1-32.

Connerney, J. E. P., Acuña, M. H., Wasilewski, P. J., Kletetschka, G., Ness, N. F., Rème, H., Lin, R. P., and Mitchell, D. L. 2001. The global magnetic field of Mars and implications for crustal evolution. *Geophysical Research Letters* 28: 4015-4018.

Crispim, C. A., Gaylarde, P. M., and Gaylarde, C. C. 2003. Algal and cyanobacterial biofilms on calcareous historic buildings. *Current Microbiology* 46: 79-82.

Daugbjerg, N. 2000. *Pyramimonas tychotreta*, sp. nov. (Prasinophyceae), a new marine species from Antarctica: light and electron microscopy of the motile state and notes on growth rates. *Journal of Phycology* 36: 160-171.

de Winder, B., Matthijs, H. C. P., and Mur, L. R. 1989. The role of water-retaining substrata on the photosynthetic response of three drought tolerant phototrophic micro-organisms isolated from a terrestrial habitat. *Archives of Microbiology* 152: 458-462.

Dismukes, G. C., Klimov, V. V., Baranov, S. V., Kozlov, Y. N., DasGupta, J., and Tyryshkin, A. 2001. The origin of atmospheric oxygen on Earth: the innovation of oxygenic photosynthesis. *Proceedings of the National Academy of Sciences USA* 98: 2170-2175.

Dorn, R. I., and Oberlander, T. M. 1981. Microbial origin of desert varnish. *Science* 213: 1245-1247.

Fay, P. 1983. *The Blue-Greens*. Edward Arnold (Publishers) Ltd., London.

Ferreira, A. C., Nobre, M. F., Moore, E., Rainey, F. A., Battista, J. R., and da Costa, M. S. 1999. Characterization and radiation resistance of new isolates of *Rubrobacter radiotolerans* and *Rubrobacter xylanophilus*. *Extremophiles* 3: 235-8.

Frankenberg-Schwager, M., Turcu, G., Thomas, C., Wollenhaupt, H., and Bucker, H. 1975. Membrane damage in dehydrated bacteria and its repair. *Life Sciences and Space Research* 13: 83-88.

Friedmann, E. I., Hua, M., and Ocampo-Friedmann, R. 1993. Terraforming Mars: dissolution of carbonate rocks by cyanobacteria. *Journal of the British Interplanetary Society* 46: 291-292.

Friedmann, E. I., and Kibler, A. P. 1980. Nitrogen economy of endolithic microbial communities in hot and cold deserts. *Microbial Ecology* 6: 95-108.

Friedmann, E. I., and Ocampo-Friedman, R. 1994. A primitive cyanobacterium as pioneer organism for terraforming Mars. *Advances in Space Research*.

Friedmann, E. I., and Ocampo-Friedmann, R. 1984. The antarctic cryptoendolithic ecosystem: relevance to exobiology. *Origins of Life* 14: 771-776.

Gaidos, E., Lanoil, B., Thorsteinsson, T., Graham, A., Skidmore, M., Han, S. K., Rust, T., and Popp, B. 2004. A viable microbial community in a subglacial volcanic crater lake, iceland. *Astrobiology* 4: 327-44.

Garlick, S., Oren, A., and Padan, E. 1977. Occurrence of facultative anoxygenic photosynthesis among filamentous and unicellular cyanobacteria. *Journal of Bacteriology* 129: 623-629.

Gerday, C., Aittaleb, M., Bentahir, M., Chessa, J. P., Claverie, P., Collins, T., D'Amico, S., Dumont, J., Garsoux, G., Georlette, D., Hoyoux, A., Lonhienne, T., Meuwis, M. A., and Feller, G. 2000. Cold-adapted enzymes: from fundamentals to biotechnology. *Trends in Biotechnology* 18: 103-107.

Gilichinsky, D., Rivkina, E., Shcherbakova, V., Laurinavichuis, K., and Tiedje, J. 2003. Supercooled water brines within permafrost-an unknown ecological niche for microorganisms: a model for astrobiology. *Astrobiology* 3: 331-41.

Golden, J. W., and Yoon, H. S. 1998. Heterocyst formation in *Anabaena*. *Current Opinion in Microbiology* 1: 623-629.

Goodwin, T. W. 1980. Algae. Pages 207-256. *The Biochemistry of the Carotenoids: Plants*. Richard Clay (The Chaucer Press), Ltd., Suffolk.

Graham, J. M. 2004. The biological terraforming of Mars: planetary ecosynthesis as ecological succession on a global scale. *Astrobiology* 4: 168-95.

Guay, R., and Silver, M. 1975. *Thiobacillus acidophilus* sp. nov.; isolation and some physiological characteristics. *Canadian Journal of Microbiology* 21: 281-288.

Hansen, A. A., Merrison, J., Nørnberg, P., Lomstein, B. A., and Finster, K. 2005. Activity and stability of a complex bacterial soil community under simulated Martian conditions. *International Journal of Astrobiology* 4: 135-144.

Hart, S. D., Currier, P. A., and Thomas, D. J. 2000. Denitrification by *Pseudomonas aeruginosa* under simulated engineered martian conditions. *Journal of the British Interplanetary Society* 53: 357-359.

Hawrylewicz, E. J., Hagen, C., Tolkacz, V., and Ehrlich, R. 1967. Effect of reduced barometric pressure on water availability related to microbial growth. *Life Sciences and Space Research* 5: 174-186.

Hershovitz, N., Oren, A., and Cohen, Y. 1991a. Accumulation of trehalose and sucrose in cyanobacteria exposed to matric water stress. *Applied and Environmental Microbiology* 57: 645-648.

Hershovitz, N., Oren, A., Post, A., and Cohen, Y. 1991b. Induction of water-stress proteins in cyanobacteria exposed to matric- or osmotic-water stress. *FEMS Microbiology Letters* 83: 169-172.

Hiscox, J. A., and Thomas, D. J. 1995. Genetic modification and selection of microorganisms for growth on Mars. *Journal of the British Interplanetary Society* 48: 419-426.

Ho, K. K., and Krogman, D. W. 1982. Photosynthesis. Pages 191-214 in Carr, N. G. and Whitton, B. A., eds. *The Biology of Cyanobacteria*. University of California Press, Berkeley.

Hodgson, R. A. J., and Raison, J. K. 1991a. Lipid peroxidation and superoxide dismutase activity in relation to photoinhibition induced by chilling in moderate light. *Planta* 185: 215-219.

Hodgson, R. A. J., and Raison, J. K. 1991b. Superoxide production by thylakoids during chilling and its implication in the susceptibility of plants to chilling-induced photoinhibition. *Planta* 183: 222-228.

Hunten, D. M. 1979. Possible oxidant sources in the atmosphere and surface of Mars. *Journal of Molecular Evolution* 14: 71-8.

Imshenetsky, A. A., Abyzov, S. S., Voronov, G. T., Kuzjurina, L. A., Lysenko, S. V., Sotnikov, G. G., and Fedorova, R. I. 1967. Exobiology and the effect of physical factors on micro-organisms. *Life Sciences and Space Research* 5: 250-260.

Imshenetsky, A. A., and Lysenko, S. V. 1965. Ultra-high vacuum and microorganisms. *Life Sciences and Space Research* 3: 142-148.

Imshenetsky, A. A., Lysenko, S. V., Kasakov, G. A., and Ramkova, N. V. 1977. Resistance of stratospheric and mesospheric micro-organisms to extreme factors. *Life Sciences and Space Research* 15: 37-39.

Imshenetsky, A. A., Lysenko, S. V., and Komolova, G. S. 1970. The effect of high vacuum on oxidative reactions in bacteria and the activity of certain enzymes. *Life Sciences and Space Research* 8: 77-81.

Jackson, M. B., and Drew, M. C. 1984. Effects of flooding on growth and metabolism of herbaceous plants. Pages 47-128 in Kozlowski, T. T., ed. *Flooding and Plant Growth*. Academic Press, Orlando.

Kanervo, E., Lehto, K., Ståhle, K., Lehto, H., and Mäenpää, P. 2005. Characterization of growth and photosynthesis of *Synechocystis* sp. PCC 6803 cultures under reduced atmospheric pressures and enhanced CO_2 levels. *International Journal of Astrobiology* 4: 97-100.

Kaplan, A., Marcus, Y., and Reinhold, L. 1988. Inorganic carbon uptake by cyanobacteria. Pages 534-539. *Methods in Enzymology*. Academic Press, New York.

Klingler, J. M., Mancinelli, R. L., and White, M. R. 1989. Biological nitrogen fixation under primordial Martian partial pressures of dinitrogen. *Advances in Space Research* 9: 173-6.

Kraegeloh, A., and Kunte, H. J. 2002. Novel insights into the role of potassium for osmoregulation in *Halomonas elongata*. *Extremophiles* 6: 453-62.

Kumon, Y., Sasaki, Y., Kato, I., Takaya, N., Shoun, H., and Beppu, T. 2002. Codenitrification and denitrification are dual metabolic pathways through which dinitrogen evolves from nitrate in *Streptomyces antibioticus*. *Journal of Bacteriology* 184: 2963-2968.

Lefèvre, F., Lebonnois, S., Montmessin, F., and Forget, F. 2004. Three-dimensional modeling of ozone on Mars. *Journal of Geophysical Research* 109: 1-20.

Levin-Zaidman, S., Englander, J., Shimoni, E., Sharma, A. K., Minton, K. W., and Minsky, A. 2003. Ringlike structure of the *Deinococcus radiodurans* genome: a key to radioresistance? *Science* 299: 254-256.

Mancinelli, R. L. 1996. The nature of nitrogen: an overview. *Life Support and Biosphere Science* 3: 17-24.

McKay, C. P. 1998. Past, present, and future life on Mars. *Gravitational and Space Biology Bulletin* 11: 41-50.

McKay, C. P., Toon, O. B., and Kasting, J. F. 1991. Making Mars habitable. *Nature* 352: 489-496.

McMinn, A., Pankowski, A., and Delfatti, T. 2005. Effect of hyperoxia on the growth and photosynthesis of polar sea ice algae. *Journal of Phycology* 41: 732-741.

Mitsui, A., and Cao, S. 1988. Isolation and culture of marine nitrogen-fixing unicellular cyanobacteria *Synechococcus*. Pages 105-113 in Packer, L. and Glazer, A. N., eds. *Cyanobacteria*. Academic Press, Inc., San Diego.

Morita, R. Y. 1975. Psychrophilic bacteria. *Bacteriological Reviews* 39: 144-67.

Mulholland, M. R., and Capone, D. G. 2000. The nitrogen physiology of the marine N_2-fixing cyanobacteria *Trichodesmium* spp. *Trends in Plant Science* 5: 148-153.

Myers, J., Graham, J.-R., and Wang, R. T. 1980. Light harvesting in *Anacystis nidulans* studied in pigment mutants. *Plant Physiology* 66: 1144-1149.

Negoro, M., Shioji, N., Miyamoto, K., and Miura, Y. 1991. Growth of microalgae in high CO_2 gas and effects of SO_X and NO_X. *Applied Biochemistry and Biotechnology* 28-29: 877-886.

Newcombe, D. A., Schuerger, A. C., Benardini, J. N., Dickinson, D., Tanner, R., and Venkateswaran, K. 2005. Survival of spacecraft-associated microorganisms under simulated martian UV irradiation. *Applied and Environmental Microbiology* 71: 8147-8156.

Nicholson, W. L., and Schuerger, A. C. 2005. *Bacillus subtilis* spore survival and expression of germination-induced bioluminescence after prolonged incubation under simulated Mars atmospheric pressure and composition: implications for planetary protection and lithopanspermia. *Astrobiology* 5: 536-44.

Northup, D. E., Barns, S. M., Yu, L. E., Spilde, M. N., Schelble, R. T., Dano, K. E., Crossey, L. J., Connolly, C. A., Boston, P. J., Natvig, D. O., and Dahm, C. N. 2003. Diverse microbial communities inhabiting ferromanganese deposits in Lechuguilla and Spider Caves. *Environmental Microbiology* 5: 1071-86.

Olson, J. B., Steppe, T. F., Litaker, R. W., and Paerl, H. W. 1998. N_2-fixing microbial consortia associated with the ice cover of Lake Bonney, Antarctica. *Microbial Ecology* 36: 231-238.

Oremland, R. S., Hoeft, S. E., Santini, J. M., Bano, N., Hollibaugh, R. A., and Hollibaugh, J. T. 2002. Anaerobic oxidation of arsenite in Mono Lake water and by a facultative, arsenite-oxidizing chemoautotroph, strain MLHE-1. *Applied and Environmental Microbiology* 68: 4795-802.

Owen, T., Biemann, K., Rushneck, D. R., Biller, J. E., Howarth, D. W., and Lafleur, A. L. 1977. The composition of the atmosphere at the surface of Mars. *Journal of Geophysical Research* 82: 4635-4639.

Paul, A. L., Schuerger, A. C., Popp, M. P., Richards, J. T., Manak, M. S., and Ferl, R. J. 2004. Hypobaric biology: Arabidopsis gene expression at low atmospheric pressure. *Plant Physiology* 134: 215-23.

Plumb, R. C., Tantayanon, R., Libby, M., and Xu, W. W. 1989. Chemical model for Viking biology experiments: implications for the composition of the martian regolith. *Nature* 338: 633-635.

Potts, M., Ocampo-Friedmann, R., Bowman, M. A., and Tözún, B. 1983. *Chroococcus* S24 and *Chroococcus* N41 (cyanobacteria): morphological, biochemical and genetic characterization and effects of water stress on ultrastructure. *Archives of Microbiology* 135: 81-90.

Russell, N. J. 2000. Toward a molecular understanding of cold activity of enzymes from psychrophiles. *Extremophiles* 4: 83-90.

Sarhan, F., and Danyluk, J. 1998. Engineering cold-tolerant crops--throwing the master switch. *Trends in Plant Science* 3: 289-290.

Scherer, S., Ernst, A., Chen, T.-W., and Böger, P. 1984. Rewetting of drought-resistant blue-green algae: time course of water uptake and reappearance of respiration, photosynthesis, and nitrogen fixation. *Oecologia* 62: 418-423.

Schneegurt, M. A., Tucker, D. L., Ondr, J. K., Sherman, D. M., and Sherman, L. A. 2000. Metabolic rhythms of a diazotrophic cyanobacterium, *Cyanothece* sp. strain ATCC 51142, heterotrophically grown in continuous dark. *Journal of Phycology* 36: 107-117.

Schnürer, J., and Rosswall, T. 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Applied and Environmental Microbiology* 43: 1256-1261.

Schuerger, A. C., Berry, B., and Nicholson, W. L. 2006a. Terrestrial bacteria typically recovered from Mars spacecraft do not appear able to grow under simulated martian conditions. Pages 1397. *37th Lunar and Planetary Science Conference*, Houston, TX.

Schuerger, A. C., Mancinelli, R. L., Kern, R. G., Rothschild, L. J., and McKay, C. P. 2003. Survival of endospores of Bacillus subtilis on spacecraft surfaces under simulated martian environments: implications for the forward contamination of Mars. *Icarus* 165: 253-276.

Schuerger, A. C., and Nicholson, W. L. 2005. Synergistic effects of low pressure, low temperature, and CO_2

atmospheres inhibit the growth of terrestrial bacteria under simulated martian conditions. Pages 1366. *36th Lunar and Planetary Science Conference*, Houston, TX.

Schuerger, A. C., Richards, J. T., Newcombe, D. A., and Venkateswaren, K. 2006b. Rapid inactivation of seven *Bacillus* spp. under simulated Mars UV irradiation. *Icarus* 181: 52-62.

Seckbach, J., Baker, F. A., and Shugarman, P. M. 1970. Algae thrive under pure CO_2. *Nature* 227: 744-745.

Seckbach, J., and Libby, W. F. 1970. Vegetative life on Venus? Or investigations with algae which grow under pure CO_2 in hot acid media at elevated pressures. *Space Life Sciences* 2: 121-143.

Sigma-Aldrich Co. 2005. Use of trypan blue stain and the hemocytometer to determine total cell counts and viable cell number. Sigma-Aldrich Co.

Silverman, G. J., and Beecher, N. 1967. Survival of cocci after exposure to ultrahigh vacuum at different temperatures. *Applied Microbiology* 15: 665-667.

Sorokin, D. Y., Tourova, T. P., and Kuenen, J. G. 2000. A new facultatively autotrophic hydrogen- and sulfur-oxidizing bacterium from an alkaline environment. *Extremophiles* 4: 237-45.

Stevens, T. O., and McKinley, J. P. 1995. Lithoautotrophic microbial ecosystems in deep basalt aquifers. *Science* 270: 450-454.

Tabita, F. R. 1994. The biochemistry and molecular regulation of carbon dioxide metabolism in cyanobacteria. Pages 437-467 in Bryant, D. A., ed. *The Molecular Biology of Cyanobacteria*. Kluwer Academic Publishers, Dordrecht.

Thomas, D. J. 1995. Biological aspects of the ecopoeisis and terraformation of Mars: current perspectives and research. *Journal of the British Interplanetary Society* 48: 415-418.

Thomas, D. J., Boston, P. J., Todd, P., Boling, J., Campbell, K., Gregerson, R. G., Holt, A., III, McSpadden, T., and McWilliams, L. accepted for publication. Early results of ecopoeisis experiments in the SHOT martian environment simulator in Crossman, F., ed. *Proceedings of the 8th International Mars Society Convention*.

Thomas, D. J., and Schimel, J. P. 1991. Mars after the Viking missions: is life still possible? *Icarus* 91: 199-206.

Thomas, D. J., Sullivan, S. L., Price, A. L., and Zimmerman, S. M. 2005. Common freshwater cyanobacteria grow in 100% CO2. *Astrobiology* 5: 66-74.

Thomas, D. J., Thomas, J. B., Prier, S. D., Nasso, N. E., and Herbert, S. K. 1999. Iron superoxide dismutase protects against chilling damage in the cyanobacterium *Synechococcus* species PCC7942. *Plant Physiology* 120: 275-282.

Thomas, N. A., Todd, P., Metz, G. W., Platt, H., Kurk, A., and Thomas, D. J. in review. Performance evaluation of a laboratory test bed for planetary biology. *Gravitational and Space Biology*.

Venkateswaran, A., McFarlan, S. C., Ghosal, D., Minton, K. W., Vasilenko, A., Makarova, K., Wackett, L. P., and Daly, M. J. 2000. Physiologic determinants of radiation resistance in *Deinococcus radiodurans*. *Applied and Environmental Microbiology* 66: 2620-2626.

Villar, S. E., Edwards, H. G., and Cockell, C. S. 2005. Raman spectroscopy of endoliths from Antarctic cold desert environments. *Analyst* 130: 156-62.

Wainwright, M., Wickramasinghe, N. C., Narlikar, J. V., and Rajaratnam, P. 2003. Microorganisms cultured from stratospheric air samples obtained at 41 km. *FEMS Microbiology Letters* 218: 161-165.

Wainwright, M., Wickramasinghe, N. C., Narlikar, J. V., and Rajaratnam, P. 2004. Are these stratospheric nanoparticles bacteria? *Microbiology* 150: 756-8.

Wise, R. R. 1995. Chilling-enhanced photooxidation: the production, action and study of reactive oxygen species produced during chilling in the light. *Photosynthesis Research* 45: 79-97.

Wise, R. R., and Naylor, A. W. 1987. Chilling-enhanced photooxidation. Evidence for the role of singlet oxygen and superoxide in the breakdown of pigments and endogenous antioxidants. *Plant Physiology* 83: 278-282.

Wolk, C. P. 1988. Purification and storage of nitrogen-fixing filamentous cyanobacteria. Pages 93-95 in Packer, L. and Glazer, A. N., eds. *Cyanobacteria*. Academic Press, Inc., San Diego.

Xiong, J., and Bauer, C. E. 2002. Complex evolution of photosynthesis. *Annual Review of Plant Biology* 53: 503-521.

Yen, A. S., Kim, S. S., Hecht, M. H., Frant, M. S., and Murray, B. 2000. Evidence that the reactivity of the martian soil is due to superoxide ions. *Science* 289: 1909-1912.

Zecchinon, L., Claverie, P., Collins, T., D'Amico, S., Delille, D., Feller, G., Georlette, D., Gratia, E., Hoyoux, A., Meuwis, M. A., Sonan, G., and Gerday, C. 2001. Did psychrophilic enzymes really win the challenge? *Extremophiles* 5: 313-21.

Zehnder, A. J., and Svensson, B. H. 1986. Life without oxygen: what can and what cannot? *Experientia* 42: 1197-205.

Zehr, J. P., Waterbury, J. B., Turner, P. J., Montoya, J. P., Omoregie, E., Steward, G. F., Hansen, A., and Karl, D. M. 2001. Unicellular cyanobacteria fix N_2 in the subtropical North Pacific Ocean. *Nature* 412: 635-638.

Zumft, W. G. 1997. Cell biology and molecular basis of denitrification. *Microbiology and Molecular Biology Reviews* 61: 533-616.