Growth And Survival Of Bacteria In Simulated Martian Conditions

2008

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by

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B.S. University of Central Florida, 2005

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science in Biology
in the Department of Biology
in the College of Science
at the University of Central Florida
Orlando, Florida

Summer Term
2008

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ABSTRACT

Escherichia coli and *Serratia liquefaciens*, two common microbial spacecraft contaminants known to replicate under low atmospheric pressures of 25 mb, were tested for growth and survival in simulated martian conditions. Stressors of high salinity, low temperature, and low pressure were screened alone and in combination to determine how they might affect microbial activity. Growth and survival of *E. coli* and *S. liquefaciens* under low temperatures (30, 20, 10, or 5 °C) with increasing concentrations (0, 5, 10, or 20 %) of three salts believed to be present on the surface of Mars ($MgCl_2$, $MgSO_4$, NaCl) were monitored over 7 d. Results indicated higher growth rates for *E. coli* and *S. liquefaciens* at 30 and 20 °C and in solutions without salt or in 5 % concentrations. No increase in cell density occurred under the highest salt concentrations at any temperatures tested; however, survival rates were high, especially at 10 and 5 °C. Growth rates of *E. coli* and *S. liquefaciens* with and without salts at 1013, 100, or 25 mb of total atmospheric pressure were robust under all pressures. In a final experiment, *E. coli* was maintained in Mars-simulant soils in a Mars Simulation Chamber. Temperatures within the chamber were changed diurnally from -50 °C to 20 °C; UV light was present during daytime operation (8 hrs), and pressure was held at a constant 7.1 mb in a Mars atmosphere for 7 d. Results from the full-scale Mars simulation indicated that *E. coli* failed to increase its populations under simulated Mars conditions, but was not killed off by the low pressure, low temperature, or high salinity conditions. *Escherichia coli*, and potentially other bacteria from Earth, may be able to survive on Mars. Surviving bacteria may interfere with scientific studies

or, if future conditions become more favorable for microbial growth, modify the martian atmosphere and biogeochemistry.

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1. INTRODUCTION

Since the beginning of the Space Age, and in particular the age of robotic landers, the survivability of terrestrial microorganisms on planetary bodies like Mars has become an international concern. For this reason, the National Aeronautics and Space Administration (NASA) Office of Planetary Protection and the Committee on Space Research (COSPAR) have set guidelines for spacecraft sterilization and cleanliness. For space exploration missions in which robotic spacecraft are intended to land on a planetary body which has the potential to support life, the bioload of microbial contaminants must be reduced to concentrations within set guidelines (Chyba *et al*., 2006). These guidelines are designed to prevent the forward contamination of planetary surfaces and to assure the scientific integrity of future life detection experiments. It is currently unknown if terrestrial microorganisms typically found on spacecraft surfaces can grow and replicate under conditions encountered on the surface of Mars. To date, twelve spacecraft have landed or crashed onto the Mars surface as a result of United States, Russian, and European space program missions (*http://history.nasa.gov*).

Despite measures taken to significantly reduce microbial bioloads, diverse communities of terrestrial microorganisms remain on spacecraft interiors and exteriors at the time of launch (Chyba *et al*., 2006; La Duc *et al*., 2003, 2004; Schuerger *et al*., 2004). The diversity of microorganisms found on spacecraft surfaces are generally characteristic of the clean rooms within which the spacecraft are processed. Spacecraft assembly facilities are oligotrophic extreme environments in which only the most resilient species survive the high desiccation, low-nutrient conditions, controlled air circulation, and the rigors of bioburden reduction

(Venkateswaren *et al*., 2001). Characterization of the biological inventory of microorganisms on spacecraft has mostly been limited to isolation and identification using standard culture-based microbiological assays. These techniques, with their inherent biases, have limited the inventory to cultivable microbial species. Culture-based microbiological assays significantly underestimate the biological diversity present on spacecraft as traditional culture techniques fail to capture more than 99.9 % of present phylotypes (Chyba *et al*., 2006). Recently, the simultaneous use of culture-dependent *and* culture-independent techniques such as limulus amoebocyte lysate assay (LAL), adenosine triphosphate (ATP) bioluminescence assay, lipopolysaccharide-based microbial detection, DNA based PCR and clone library analyses has begun to reveal the breadth of microbial diversity present even on pre-sterilized spacecraft (La Duc *et al*., 2003, 2004; Venkateswaren *et al*., 2001). Known cultivable biological contaminants include, but are certainly not limited to species of *Bacillus*, *Escherichia*, *Staphylococcus*, *Pseudomonas*, *Acinetobacter*, *Streptococcus*, *Micrococcus*, and *Flavobacterium* (Schuerger *et al*., 2004).

The Martian landscape can be described as a cold and desolate desert of extremes. Martian surface temperatures can range from -140 °C to +20 °C, atmospheric water vapor content is about 1/10,000 of that on Earth, atmospheric gas composition is highly anoxic (~95% CO_2 and only 0.13% O_2), and the average pressure on Mars (7.1 mb) is far below the lowest pressure recorded on Earth at the summit of Mount Everest (330 mb) (Schuerger *et al*., 2004). The thin atmosphere and absent ozone layer allow a greater influx of solar ultraviolet light (UV) than Earth's atmosphere. Ultraviolet light may be the most biocidal of all factors to microbial survival on the martian surface because it damages DNA quicker than most vegetative

microorganisms can repair, resulting in cell death. Microorganisms found on exposed surfaces of spacecraft are killed within minutes of exposure; however, if covered by as little as a few hundred micrometers of martian soil, significant protection is provided (Schuerger *et al*., 2003; Cockell *et al*., 2000).

Martian soil may not be a hospitable medium for microbial growth. The Spirit and Opportunity rover missions have significantly contributed to the understanding of the chemical composition of the Martian regolith in recent years. Sulfate salts seem to dominate, but chloride, bromide, and iron salts appear to be present as well (Clark *et al*., 2005). Salts are in such abundance that any liquid water potentially present on or near the surface may be saturated by the salt ions, and, thus, may have extremely low water activities (Tosca *et al*., 2008). Toxic levels of Cr, Ni, and Zn may be present if mobilized in liquid water. Although precise rock classification is ongoing, minerals including jarosite and hematite appear to dominate outcrops at the Meridiani Planum exploration site (Clark *et al*., 2005). The presence of these rock types implicates water in the formation process, building a case for the presence of water in martian history.

Most of the water on Mars appears to be frozen in the polar caps or at the subsurface level (Feldman *et al*., 2003). Since the average pressure on Mars stays just above the triple point of water (0.1 ^{o}C at 6.9 mb), the existence of stable liquid water is unlikely; however, there is a narrow range of temperatures in which liquid water can exist on the surface of Mars (Haberle *et al*. 2001). It is hypothesized that as much as 29% of the surface can sustain conditions for liquid water to be present for up to ~37 sols per martian year (Haberle *et al*., 2001). This hypothesis underestimates spatial extent because these estimates are for pure liquid water whereas the high

salinity of martian soil should depress the freezing point of water (Brass, 1980). Geologic evidence indicates major events of liquid water upwelling from the subsurface, but it is unclear if these events occur today (Malin *et al*., 2000). There is, however, evidence for ephemeral frost events and frozen water on Mars. At the Viking 2 lander site, thin layers of frost were observed on the ground during winter mornings estimated to be a few thousandths of a centimeter thick (Svitek *et al*., 1990; Figure 1). Figure 2 shows frost around the rim of an impact crater on Vastitas Borealis filled with a lake of water ice. Thin films of water may also be available for biological use at the boundaries between ice grains or ice and dirt grains (Jakosky *et al*., 2003). Thus, although there may not be a significant source of liquid water available for long durations, there may be enough to support microbial growth for sporadic periods.

If the forward contamination of terrestrial microorganisms occurs, there would be both short and long-term consequences. In the short term, the forward contamination of Mars may complicate our ability to detect extant life on Mars, if it exists, and may result in a false positive reading for the first sign of extraterrestrial life. The orbit of Mars is chaotic, varying in both eccentricity and obliquity. In 100,000 to 200,000 years, changes in the eccentricity and obliquity of the planet will result in increased periods of direct Sun exposure, and, thus, higher sublimation rates of the polar caps. These changes in orbital characteristics will increase the amount of atmospheric CO_2 and water vapor leading to an increase in global pressure and temperature, dramatically affecting the habitability of Mars (Jakosky *et al*., 1995). Long-term survival of terrestrial microorganisms could mean the evolution and the distribution of life on Mars if future conditions become more favorable for growth.

Despite the harshness of the martian environment, this bleak planet has the potential to harbor life. Terrestrial microorganisms have been found in all ecological niches on Earth in which liquid water is present: thus the focus on water in studies of Mars. Extremophiles have been described in high altitude alpine deserts, in permafrost cores, and in hypersaline seas (Rothschild and Mancinelli, 2001); all of which are environments analogous to Mars. It is known that terrestrial microbes can thrive in these harsh conditions when tested one at a time, but it is unknown if microorganisms commonly found on spacecraft could metabolize and replicate in an environment like Mars where these harsh conditions persist in interactive stasis.

The primary objective of the current study was to expose common spacecraft contaminants to environmental stressors present on Mars to better understand the potential response of terrestrial microorganisms to the martian environment and to characterize the survival and growth processes of two species of mesophilic bacteria, *E. coli* and *S. liquefaciens,* to simulated martian temperatures, salinities, and pressures. It was predicted that low temperatures would dramatically retard cell proliferation, high salinities of any salt type would have biocidal effects on cell suspensions, and low pressure would have a weak inhibitory effect on cell growth of both *E. coli* and *S. liquefaciens*. It was expected that cells would not grow or survive in robust Mars simulations which test all these stressors in addition to ultraviolet irradiation.

2. MATERIALS AND METHODS

2.1 Microorganisms

Selections of microorganisms for the current study were based on results from a prior investigation of 22 species of mesophilic eubacteria, including multiple strains of common spacecraft contaminants, in simulated martian conditions (Berry *et al*. 2006; Schuerger *et al*. 2006). Results indicated *Escherichia coli* (Migula) 35218 and *Serratia liquefaciens* (Grimes and Hennerty) 27592 grew robustly at 25 mb pressure, in CO_2-enriched anaerobic atmospheres, at 30 °C, and on solid trypticase soy agar (Schuerger *et al*. 2006).

2.2 Growth Medium Preparation

Luria Bertani (LB) broth (10 g tryptone peptone and 5 g yeast extract per liter) was used for all liquid assays. Media were maintained in anaerobic atmospheres beginning immediately after autoclaving. Sterilized media were removed from the autoclave and immediately set in 7 L polystyrene containers along with two AnaeroPack sachets and a CO_2 indicator tablet (Mitsubishi Gas Chemical America, Inc., New York, NY). Van Horn *et al*. (1997) reported that AnaeroPack sachets remove oxygen to a concentration of less than 0.1 % within an hour. The vessels were flushed with a stream of carbon dioxide (CO_2) gas for approximately 25 seconds to reduce the time-lag required to reach anaerobic conditions. Thus, the LB liquid media was

allowed to cool under conditions that would minimize the dissolution of oxygen (O_2) back into the media.

2.4 Interactive Effects of Temperature and Salt on Bacterial Growth

Fresh cultures of *E. coli* and *S. liquefaciens* were prepared by growing bacteria in LB broth at 30 °C and 150 rpm for 24 hrs within a shaking microbial incubator (model Innova 4230, New Brunswick Scientific, Edison, NJ, USA). Starting suspensions of vegetative cells were then diluted to densities just below the detection limit of the spectrophotometer, corresponding to approximately 8×10^5 or 1×10^5 cells per inoculated tube for *E. coli* and *S. liquefaciens*, respectively. To confirm the desaturation of O_2 within the LB broth during the 7-d assays, the obligate aerobe, *Deinococcus radiodurans* R1, was inoculated into negative control tubes and placed within the vessels. An uninoculated tube of LB broth was also present as a second negative control to check for the continuation of sterility of the media during the 7-d assay.

Bacteria were grown in all possible combinations of three salts ($MgSO_4$, $MgCl_2$, and NaCl), four salt concentrations (0, 5, 10, or 20 %) and four temperatures (30, 20, 10, or 5 °C) to study the interactive effects of salt and temperature. Salts were selected based on published literature identifying $MgSO_4$, $MgCl_2$, and NaCl on Mars (Clark *et al*., 2005; Vaniman *et al*., 2005). Cultures were prepared, as described above, in 5 ml sterile LB broth containing each of the salts at each of the concentrations and incubated at each temperature. Cultures were maintained in shaking microbial incubators at temperature setpoints, under anaerobic conditions and 1013 mb pressure. Cell density was measured on 0, 1, 2, 4, and 7 d by optical density (OD)

using an ultraviolet-visible (UV-VIS) spectrophotometer (Spectronic Unicam, Rochester, NY, USA). For each OD measurement, cultures were removed from the polystyrene containers long enough to agitate samples, record OD, replace anaerobic pouches and indicator tablets, and flush with CO_2; total time for reading a set of cultures was approximately 10-15 min. Each treatment combination was performed in triplicate and the experiment was repeated once (n = 6). Optical densities lower than 0.010 were considered below the detection limit of the spectrophotometer. Data were normalized to ODs at time zero to account for small differences in starting OD values. Data were log-transformed and analyzed by analysis of variance (ANOVA) for a balanced factorial repeated measures design using version 11.5 of SPSS Windows (SPSS Inc., Chicago, IL, USA). Statistical analyses were limited to lower salt concentrations because many cultures did not grow in salt concentrations above 5% for $MgCl_2$ and NaCl. Therefore, data were analyzed across all salt concentrations for $MgSO_4$ and between salts at 0 and 5% for $MgCl_2$ and NaCl. Significant differences within treatment groups were estimated using an overlap rule for calculated 95% confidence intervals (Cumming *et al*., 2007).

A number of salt treatments (see below) failed to exhibit growth as measured by an increase of OD. This response might have been caused by (a) no growth of the test microorganisms within the salt-temperature treatments, or (b) positive growth below the detection limit of the OD procedure. Thus, additional assays were conducted using serial dilutions and direct plating onto LB agar to obtain viable cell counts of colony forming units (CFUs). The experimental set-up (as described above) was limited to those treatments which did not give a growth response. After 7 d of incubation, cultures were serially diluted, plated onto LB agar, and incubated overnight at 30 °C. Colony forming units were counted to determine the

number of viable cells in the original suspension. Each treatment was performed in triplicate and the experiment was repeated once (n = 6). Data were log transformed and analyzed by ANOVA for a balanced two way factorial design; 95 % confidence intervals were used to test for significant differences among treatments as before.

2.5 Interactive Effects of Salt and Low Pressure on Bacterial Growth

In order to measure interactive effects of hypobaric and hypersaline environments on the growth of *E. coli* and *S. liquefaciens*, tests were conducted at combinations of salts and atmospheric pressures thought to be only moderately restrictive to microbial activity. Inoculum and LB liquid media were prepared, as described above, except salts were limited to 0 or 5 % concentrations due to weak or no-growth responses at higher concentrations in the interactive salt and temperature assays (see above). Cultures were incubated for 7 d under anaerobic conditions within polycarbonate vacuum desiccators (model 08-642-7, Fisher Scientific, Pittsburgh, PA) maintained at 1013, 100, or 25 mb (Figure 3). Four anaerobic pouches and one indicator tablet were placed inside each hypobaric chamber. Two hypobaric chambers were placed within separate shaking microbial incubators set at 20 °C. Chambers were outfitted with in-line sterile filters (0.2 µm Polyvent-16 filter, Whatman, Inc., Florham Park, NJ) to inhibit airborne contaminants flowing into the hypobaric compartments. At the start of each test, CO_2 was flushed through the chambers and thereafter anaerobic pouches were replaced every 3 or 4 days to compensate for small amounts of oxygen leaking into the chambers. For experiments maintained at 25 mb, it was necessary to add approximately 0.5-0.75 ml of sterile deionized

water (SDIW) to maintain a consistent volume of liquid after 3-4 days and on day 7 when cultures were harvested. This was done to maintain a consistent volume of growth medium as water evaporated under low atmospheric pressure. Evaporation under 100 and 1013 mb was negligible. After 7 d, cultures were serially diluted and each dilution was plated on LB agar to obtain viable cell counts. Each treatment was performed in triplicate and the experiment was repeated once (n = 6). Data were log-transformed and analyzed by ANOVA for a balanced two-way factorial design; 95 % confidence intervals were used to test for significant differences among treatments.

2.6. Growth of Bacteria in Mars-Analog Soils

Several bench-top experiments were conducted to develop a procedure for the Mars Simulation Chamber (described below) experiment, and to gather preliminary data on microbial growth in Mars-analog soils. Mars-analog soils were composed of fine-grained volcanic palagonite from Hawaii (previously described by Schuerger *et al.*, 2003). To remove any biological contaminants, soils were autoclaved at 1.1 kg cm^{-1} and 121 ^{o}C for 30 min, and then stored at 130 °C overnight. Five grams of sterilized Mars-analog soil and 3.5 ml LB broth were mixed in small (60 mm diameter) petri dishes under aseptic conditions. These soil/media samples were inoculated with 100 µl of 24-hr-old cultures of *E. coli* or *S. liquefaciens*. Treatments included: (i) soils inoculated with *E. coli* or *S. liquefaciens* and then immediately assayed, (ii) soils desiccated for 24 hrs or 7 d by placing dishes in a laminar flow hood with lids open at 24 ^{o}C, (iii) soils incubated at 20, 4, or -20 °C for 24 hrs or 7 d, and (iv) blank samples

without soil (i.e., inoculum added to SDIW and immediately assayed). Negative controls were composed of three uninoculated petri dishes with soil/media mixtures desiccated in the laminar flow hood and assayed after 7 d. Incubated soils were transferred to 25 cc polystyrene tubes, and sampled using the MPN assay (described below). Each treatment was performed in triplicate and the experiment was repeated twice (n = 9). Data were log-transformed and analyzed by ANOVA for a balanced two-way factorial design; Tamhane's multiple comparisons test (SPSS, version 11.5) was used to determine differences between treatments. Ninety-five percent confidence intervals were used to test for significant differences among treatments.

2.7. Survival and Growth of *E. coli* in Mars Simulations

A Mars Simulation Chamber (MSC) (described by Schuerger *et al*., 2008) was used to determine if *E. coli* could survive and possibly grow under simulated martian conditions. Only *E. coli* was used for the MSC tests because vegetative cells of *E. coli* exhibited a stronger resistance to desiccation than vegetative cells of *S. liquefaciens* (based on results from the experiment described in Section 2.6). The MSC (Figure 4) is a stainless steel low-pressure cylindrical chamber with internal dimensions measuring 70 cm long by 50 cm in diameter. The MSC system can accurately simulate five key components of the surface environment of Mars including: (a) pressures down to 0.1 mb, (b) UV irradiation from 190 to 400 nm, (c) dust loading in the atmosphere from optical depths of 0.1 (dust-free sky) to 3.5 (global dust storm), (d) temperatures from -100 to +30 °C (based on Viking and Mars Pathfinder data [Golombek *et al*., 1999; Keiffer *et al*., 1976]), and (e) a Mars gas mix that included CO_2 (95.3 %), N_2 (2.7 %), Ar

(1.6 %), O_2 (0.13 %) and H_2O (0.03 %) (based on Viking data [Owen *et al*., 1992]). The Mars UV irradiation system has been described previously (Schuerger *et al*., 2003; 2006). The UVC (200-280 nm), UVB (280-320 nm), UVA (320-400 nm), and total UV fluence rates used in the current study were 4.0, 6.2, 25.9, and 35.6 W m^{-2}, respectively (Schuerger *et al*., 2006). The visible (VIS; 400-700 nm) and near-infrared (NIR; 700-1200 nm) fluence rates were 240 and 245 W m^{-2}, respectively (Schuerger *et al*., 2006).

Soils were prepared as described above (5 g soil, 3.5 mL LB media, 100 µl inoculum). Luria Bertani broth mixed into soils contained no salt, 5 % $MgSO_4$, or a 15 % salt mix (5 % $MgCl_2$, 5 % $MgSO_4$, and 5 % NaCl). Inoculated soil/LB-media mixes were allowed to desiccate for 24 hrs in an operating laminar flow hood with lids open at room temperature prior to placement in the MSC chamber. Three sets of treatments were prepared; one set placed within an operating laminar flow hood at Earth-normal conditions of pressure and temperature, and two sets of samples placed inside the MSC. Of the samples maintained within the MSC, one set was exposed to UV irradiation during daytime hours and one set was shielded from UV irradiation by a sheet of aluminum foil placed between the UV and no-UV treatments. Conditions within the MSC were maintained at 7.1 mb in a Mars atmosphere. Temperature was adjusted manually to create a diurnal fluctuation between 20 °C for 8 hrs of day and -50 °C for 16 hrs of night conditions. Soils inoculated with *E. coli* were maintained under the simulated martian conditions for 7 d.

A most probable number (MPN) microbial assay (Schuerger *et al*., 2003; 2006) was used to quantify viable bacteria bound to soil particles. In brief, soil samples were suspended in 50 ml sterile plastic centrifuge tubes containing 20 ml SDIW, sonicated for 4 min, vortexed for 2 min,

serially diluted, and 20 µl of each dilution pipetted into each of 16 wells of a 96-well plate pre-loaded with 180 µl of LB broth. The fully loaded 96-well dishes were incubated at 30 °C for 24 hrs. The MPN numbers were estimated as described previously (Schuerger *et al*., 2003). Each treatment was performed in triplicate and the experiment was repeated once (n = 6). Data were transformed using a 0.25 power transformation and analyzed by ANOVA for a balanced two-way factorial design; 95 % confidence intervals were used to test for significant differences among treatments.

3. RESULTS

3.1 Interactive Effects of Bacterial Growth between Temperature and Salt

Both *E. coli* and *S. liquefaciens* grew faster at warmer temperatures and lower salt concentrations regardless of salt type, but all treatments (salt type, concentration, temperature, and interaction terms) had significant effects on bacterial growth (Tables 1 and 2 ; Figures 5, 6, and 7). No-salt controls for both *E. coli* and *S. liquefaciens* grew vigorously at 30 and 20°C but growth slowed dramatically at 10 °C (Figure 5).

Escherichia coli did not grow at 5 °C in the no-salt controls assays, but *S. liquefaciens* was able to grow weakly at 5°C after 4 d. In the presence of 5 % $MgSO_4$, *E. coli* grew vigorously at 30 or 20 °C but not at lower temperatures (Figure 6). The same general pattern occurred when *E. coli* was grown in 10 % $MgSO_4$ except growth curves for 30 and 20 °C were significantly lower ($P \leq 0.05$). No increase in cell density for *E. coli* was observed in 20 % $MgSO_4$ for any incubation temperature. *Escherichia coli* also grew in 5 % $MgCl_2$ at 30 °C but only weakly at 20 °C and did not grow at 10 or 5 °C in $MgCl_2$. No growth of *E. coli* was detected in $MgCl_2$ for concentrations greater than 5 %. *Escherichia coli* grew weakly in 5 % NaCl at 30 °C but not at any other temperature or at any other concentration of NaCl.

Serratia liquefaciens grew strongly at 5 % $MgSO_4$ at 30 and 20 °C but had much slower growth at 10 °C and did not grow at 5 °C (Figure 7). At 10 % $MgSO_4$, the same pattern was observed for *S. liquefaciens*, but with lower growth rates when compared to 5% $MgSO_4$. No increases in growth rates for *S. liquefaciens* were observed for all temperatures tested with 20 %

$MgSO_4$. The same pattern occurred again in 5% $MgCl_2$ but with even lower growth rates. No increase in bacterial growth was observed for higher concentrations of $MgCl_2$. *Serratia liquefaciens* grew weakly in 5 % NaCl grown at 30, 20, or 10 °C with slightly more growth at 30 °C, but no increases in cell densities were observed at 5 °C or in higher concentrations of NaCl.

To confirm no-growth responses for the interactive salt and temperature experiment, a follow-up experiment was designed using a direct plating method to estimate the number of viable cells present in culture tubes after 7 d of treatment. Only treatments which resulted in no increase in OD were repeated. Salt and temperature treatments (see Figure 8 for specific treatments) were prepared, as described above. After 7d, treatments were serially diluted and plated on LB agar for direct enumeration of viable cells present in the high salt solutions. In all cases, the numbers of recovered cells of *E. coli* and *S. liquefaciens* were 1-5 orders of magnitude lower than the initial levels of inoculum (Figure 8). Thus, the primary effect of higher concentrations of $MgCl_2$, $MgSO_4$, and NaCl salts on *E. coli* and *S. liquefaciens* was to kill a percentage of vegetative cells when stored for 7 d in LB broth. Notably, no viable *S. liquefaciens* cells were recovered from 20% NaCl cultures maintained at 20 or 30 °C, but were recoverable at 10 °C and even higher numbers were recovered at 5 °C.

3.2 Interactive Effects of Salt and Low Pressure on Bacterial Growth

Escherichia coli generally grew robustly at low pressures (Figure 9), exhibiting approximately 2.5 orders of magnitude increases over the initial levels of inoculum. Surprisingly, *E. coli* grew significantly better in 100 and 25 mb atmospheres than in an Earth-

normal pressure of 1013 mb when incubated in 5 % $MgCl_2$ and NaCl solutions ($P \leq 0.05$). There was not a clear difference in bacterial growth among salts at 100 mb but a small decrease in growth occurred with $MgCl_2$ and NaCl treatments under 25 mb pressure. *Serratia liquefaciens* grew robustly under all pressures and in all salt concentrations; there were no significant differences in cell numbers among all three pressures tested ($P \geq 0.05$).

3.3 Growth of Bacteria in Mars-Analog Soils

Incubation of *Escherichia coli* at 20 °C for 1 and 7 d resulted in robust growth with significantly more growth in soils incubated for 7 d, as compared to other treatments ($P \geq 0.05$; Figure 10). The number of *E. coli* cells recovered from soils decreased about two orders of magnitude from initial inoculum in both the 1 and 7 d desiccation assays with no significant difference between 1- and 7-d treatments ($P \geq 0.05$). Survival was maintained at levels comparable to initial inocula when cells were incubated at 4 and -20 °C. There were no significant differences in *E. coli* cell densities between 4 and -20 °C ($P \geq 0.05$). *Escherichia coli* cells survived better at 4 and -20 °C than those desiccated at room temperature (24 °C). *Serratia liquefaciens* mirrored the results of *E. coli* with one important exception: survival greatly decreased in 7 d desiccation assays from initial inoculum levels by about 4 orders of magnitude compared to only 2 orders of magnitude for *E. coli*. Based on this result, *S. liquefaciens* was eliminated from the Mars simulations conducted within the MSC because *E. coli* appeared to have greater potential to withstand desiccation.

3.4 Survival and Growth of *E. coli* in Mars Simulations

Escherichia coli did not grow in Mars simulation assays, or in Earth controls (Figure 11). However, *E. coli* survived in simulated martian conditions, both with and without UV irradiation, at densities above or comparable to Earth controls (Figure 11). Results were similar to bench-top desiccation experiments in which recoverable cells decreased about 2 orders of magnitude after 7 d of desiccation (Figure 10). Recoverable cells from assays without UV irradiation were greater than assays with UV irradiation by less than half an order of magnitude. Highest rates of survival for all treatments were observed in 5% $MgSO_4$, and the no-salt and the 15% salt mixtures exhibited lower levels of survival (Figure 11). However, there was no significant difference in survival between soils without salt and with 15 % salt mix ($P \geq 0.05$). In general, the results between two repetitions of the experiment were very similar except in Earth controls for the 15% salt mixture in repetition #1, in which survival was unusually low. This difference is attributed to experimental error. The first and second repetitions of the experiment were graphed separately due to significant differences between runs coupled with a low treatment effect to run error ratio (see Table 11).

4. DISCUSSION

Escherichia coli was not able to grow and replicate under robust Mars simulations but most *E. coli* cells survived 7 d in simulated martian conditions, even under intense UV irradiation at the surface of the Mars analog soils. Survival of *E. coli* was actually higher on average under Mars simulations than Earth conditions, which was likely due to the preserving effects of low temperatures on cell viability. This possibility is supported by results of the experiment on survival of *E. coli* and *S. liquefaciens* in saline solutions at low temperatures: survival rates were highest at lower temperatures [5 and 10 °C, respectively (Figure 8)]. On average, the presence of intense UV irradiation decreased recoverable cells by less than half an order of magnitude when compared to soils not irradiated by UV. Intense UV does not penetrate the top layer of soil, so only microorganisms within the first few hundred micrometers were likely killed (Schuerger *et al*., 2003; Cockell *et al*., 2000). Previous experiments which tested soil samples from sites near spacecraft assembly facilities also report no growth in dried soils in simulated martian conditions (Foster *et al*. 1978). While the current study reported high survival rates extended over 7 d, Foster *et al*. (1978) reported similar results for simulations lasting up to 21 d.

Low pressure treatments had either no effect or a positive effect on growth in assays testing the replication of *E. coli* and *S. liquefaciens* under 1013, 100, and 25 mb pressures. Growth of *E. coli* and *S. liquefaciens* were not inhibited by low pressure in 5% $MgCl_2$, $MgSO_4$, or NaCl; both species increased viable cells by 2-4 orders of magnitude after initial inoculation at

100 or 25 mb. In contrast, *E. coli* in 5 % NaCl and 5 % $MgCl_2$ were inhibited at 1013 mb compared to 100 or 25 mb. Growth response of *E. coli* in 1013 mb was consistent with the results of experiments testing the effects of salt and low temperature on *E. coli*. A direct pressure effect on growth of bacteria within a liquid medium was also reported by Schuerger *et al*. (2006), except in that case, growth was inhibited under low pressures. The correlation between the low atmospheric pressures of 100 and 25 mb and the increased growth of *E. coli* cells suspended within a liquid medium was unclear. It is unknown what positive effect pressure has on bacterial growth on cells suspended in a liquid medium in which hydrostatic pressure is exerted on cells regardless of external pressure. It is hypothesized that the hypobaric conditions caused cells to expand in size, possibly causing alterations to membrane and protein structure and changes in osmotic stress. If these alterations occur under hypobaric conditions, the functionality of cell membranes may change, offering a possible explanation for the increase in growth of *E. coli* in solutions containing NaCl and $MgCl_2$ under low pressures. There may be a further connection between salt type and pressure effect. Magnesium sulfate had no effect on cell growth under low pressure, but the inhibitory effects of both chloride salts disappeared under 100 and 25 mb for *E. coli*. It is possible the chloride ion transport mechanism is affected by low pressure which resulted in the relief of osmotic stress.

While growth of *E. coli* or *S. liquefaciens* were not inhibited by hypobaric conditions (Figure 9), the desiccating effect of low pressure on hydrated soil had a significant biocidal effect on both bacteria (Figure 10). Desiccation will be a major obstacle for long-term survival of vegetative cells unless some mechanism of desiccation resistance is present on Mars.

Salts may serve two purposes in aiding the survival of microorganisms on the surface of Mars. First, salts depress the freezing point of water, making liquid water available over a broader range of temperatures. Clark and Van Hart (1981) report depressed freezing points for saturated salt solutions of about -5 °C, -30 °C, and -35 °C for $MgSO_4$, NaCl, and $MgCl_2$, respectively. However, even if liquid water was available over a broader range of temperatures, it is unlikely to fall within the limits for active growth and metabolism of mesophilic species, such as *E. coli*, but liquid water at lower temperatures may stretch the window for potential periods of growth of cold-tolerant microorganisms if they were delivered to Mars. *Escherichia coli* and *S. liquefaciens* are likely not to benefit from an increase in available liquid water at lower temperatures as *E. coli* was unable to grow below 10 °C and *S. liquefaciens* failed to grow at 5 °C (Figure 5). While the extended availability of liquid water may not aid the growth and metabolism of bacteria, due to other inhibitory factors like low temperature, it may aid in the survival of bacteria by hydrating cells more often, mitigating the effects of desiccation. Secondly, salts may provide protection from desiccation by encasing microbes in fluid inclusions within salt crusts or crystals (Adamski *et al*. 2006). In the case of the Mars simulations in the current study, the formation of salt crusts may have had a role in the survival of *E. coli* over the 7-d simulations. Inclusions can form within salt crusts as a result of the evaporation of highly saline solutions. Formation of inclusions may trap microorganisms once suspended in evaporated fluid, allowing microorganisms to potentially survive within these inclusions. Although controversial, a halotolerant bacterium has reportedly been isolated from a brine inclusion within a 250 million-year-old salt crystal (Vreeland *et al*., 2000). While this is an extreme example of bacterial survival within an inclusion, microbes may commonly become

trapped within salt crystals (Adamski *et al*. 2006). Though salts may aid cells in survival against desiccation, there is a balance between this benefit and the cost of high osmolarity on cell survival. Still, there is evidence that non-halotolerant microbes may find refuge within fluid inclusions; *Pseudomonas aeruginosa*, trapped in laboratory-grown halite crystals, survived up to 13 months after entrapment within halite crystals (Adamski *et al*. 2006).

Findings of the Mars Exploration Rovers, Spirit and Opportunity, include a diverse mineralogy of species which may implicate water in the formation process. At the Meridiani Planum landing site, Opportunity found sedimentary material containing sulfate-rich minerals such as jarosite, hematite and possibly gypsum (Squyres *et al*., 2004). It was hypothesized the formation of the minerals identified within the sedimentary rock at Meridiani Planum were formed by the evaporation and subsequent desiccation of shallow, acidic water, leaving aqueous-altered minerals behind. The Spirit Rover also found sulfate rich mineralogy in the Columbia Hills of Gusev Crater implying aqueous-alteration processes (Ming *et al*. 2006). Therefore, it may be possible there was a significant amount of water available to support life in Mars' past. It has even been suggested that sites where these minerals are found may be candidates for a sample-return mission to Mars (Squyres *et al*., 2004).

More benign conditions may have existed on Mars in the past (Clark *et al*., 2005; Squyres *et al*., 2004; Boynton *et al*., 2002) and could exist again with shifts in the obliquity and eccentricity of the planet (Jakosky *et al*. 1995). If such a shift were to occur, where terrestrial microorganisms from potentially-contaminated landing sites were able to proliferate, the global ecology and biogeochemistry of Mars could be affected. Earth's atmosphere has been altered in the past by microorganisms (Kasting and Seifert, 2002). Nearly all the O_2 in Earth's atmosphere

came from biological sources, mainly as a metabolic byproduct of photosynthetic cyanobacteria. Methanogens had an impact on the early Earth as producers of organic material and greenhouse gas. Earth's atmosphere and biogeochemistry is affected globally by microorganisms, so could the atmosphere and biogeochemistry of Mars. This demonstrates the importance and magnitude of the forward contamination of Mars by terrestrial bacteria.

While it is highly unlikely that terrestrial contaminants like *E. coli* and *S. liquefaciens* could grow on the surface of Mars in the current martian climate, it may be that vegetative cells, like *E. coli*, could remain in stasis for an indefinite period awaiting more favorable conditions. More research is required to determine how long *E. coli* cells can survive robust simulated martian conditions. The results of this study are based on the premise that nutrients and organic material may be available on Mars. No evidence to date has been found of organics on the martian surface but it is theorized as much as 2.4×10^8 g of reduced carbon may come from meteoritic sources alone each year (Benner *et al*., 2000). The on-going Phoenix mission should detect organics if they are present in martian soils at the north pole landing site (*http://www.nasa.gov*). If no organics are readily available in habitable environments on Mars, any surviving microorganisms would die off even in favorable conditions for microbial growth.

While it is not likely that *E. coli* or *S. liquefaciens* could survive to cause a global contamination event on Mars, there are numerous unknown species present on spacecraft that may pose a threat to planetary protection (Chyba *et al*., 2006; La Duc *et al*., 2003, 2004; Venkateswaren *et al*. 2001). Surprisingly, *E. coli*, a non-halotolerant, mesophilic bacterium survived robust simulated Mars conditions. Thus, it is feasible other species, better suited for the extreme conditions present on Mars, could survive as well as, if not better than, *E. coli*.

Future missions to Mars will likely become more complex and expand to unexplored areas of Mars. The Mars Exploration Program has planned missions to regions which may be habitable environments, including those indicating the existence of water in the past or present (Chyba *et al*., 2006). There is great interest in the research and development of robotic drills to bore beneath the martian surface in search of current or past habitable environments (Miller *et al*., 2004). These drills could potentially access water reservoirs which may be present underground. Conditions for survival of forward contaminants may be more favorable in these target regions if liquid water is available, indicating a need for the continuation of strict cleanliness protocols for spacecraft with life-detection objectives.

6. APPENDIX A – FIGURES

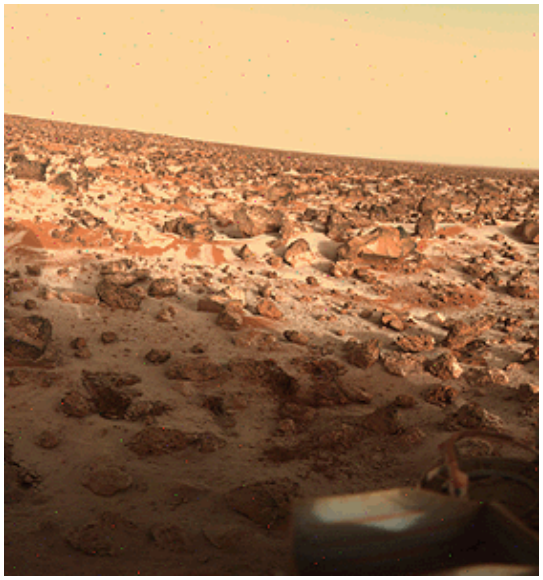

Figure 1. Viking 2 Lander Site. Frost layer around Viking 2 lander site is estimated to be a few thousandths of a centimeter thick (Viking Lander image 21I093; *http://nssdc.gsfc.nasa.gov).*

Figure 2. Impact Crater with Ice Lake. Image of impact crater on Vastitas Borealis filled with a lake of water ice and frost deposited around crater rim. Image taken by the Mars Polar Express February, 2005 (*http://www.esa.int*).

Figure 3. Low-pressure *polycarbonate* desiccator system used to reduce total atmospheric pressure down to 25 mb. Sterile in-line filters (0.2 µm pore-size) were added to the vacuum (right side) and venting (left side) lines. Four AnaeroPack pouches were placed within the desiccator to help scrub O_2 from the system.

Figure 4. Mars Simulation Chamber. The MSC system can accurately simulate five key components of the surface environment of Mars including: (a) pressures down to 0.1 mb, (b) UV irradiation, (c) dust loading in the atmosphere, (d) temperature, and (e) a Mars atmosphere composed of CO_2 (95.3 %), N_2 (2.7 %), Ar (1.6 %), O_2 (0.13 %) and H_2O (0.03 %).

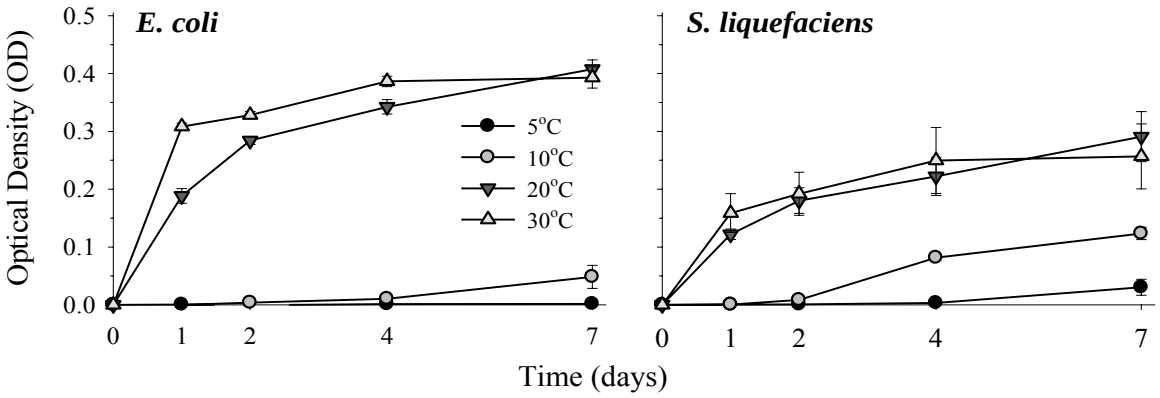

Figure 5. Growth of *Escherichia coli* and *Serratia liquefaciens* at 30, 20, 10 or 5 °C over 7 d in CO_2 atmospheres. Error bars denote 95% confidence intervals (n = 6).

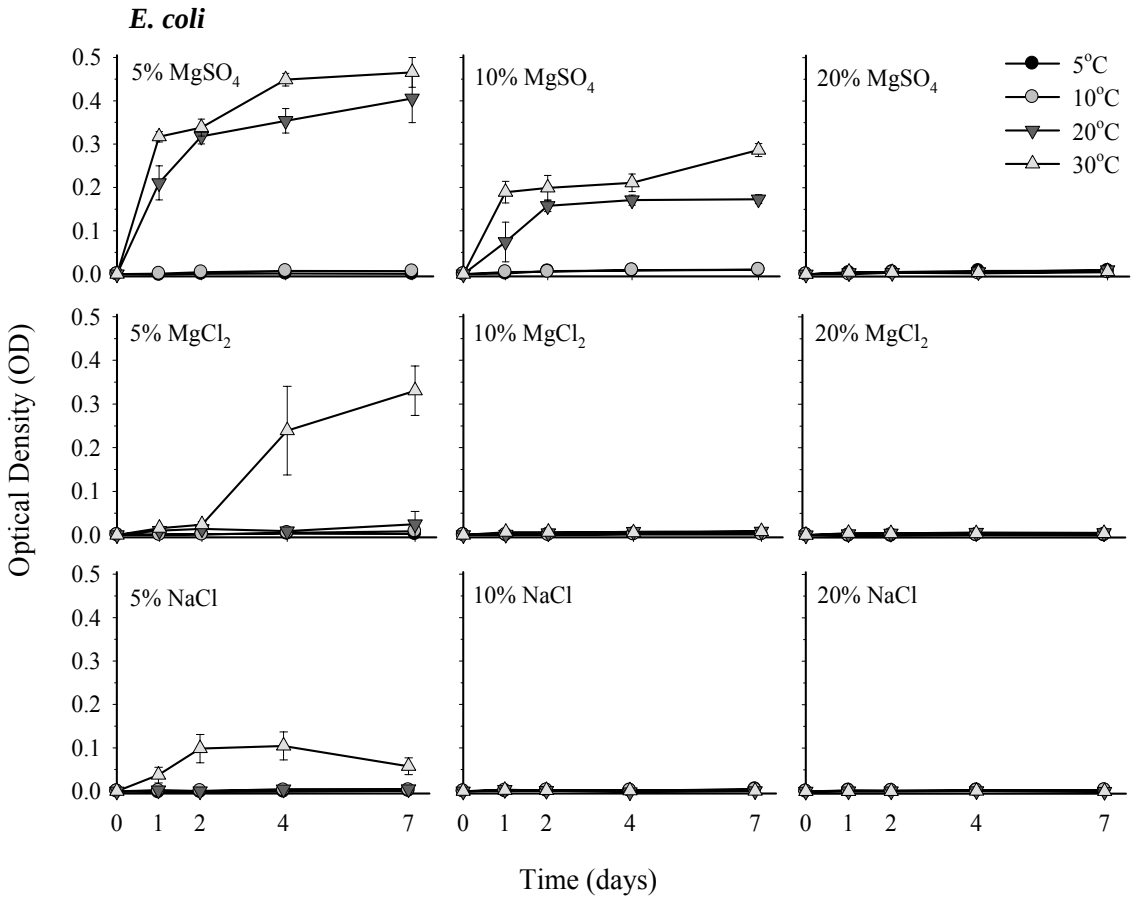

Figure 6. Growth of *Escherichia coli* in 5, 10, or 20 % $MgSO_4$, $MgCl_2$, and NaCl over 7 d in CO_2 atmospheres incubated at 30, 20, 10 or 5 °C. Error bars denote 95 % confidence intervals (n = 6).

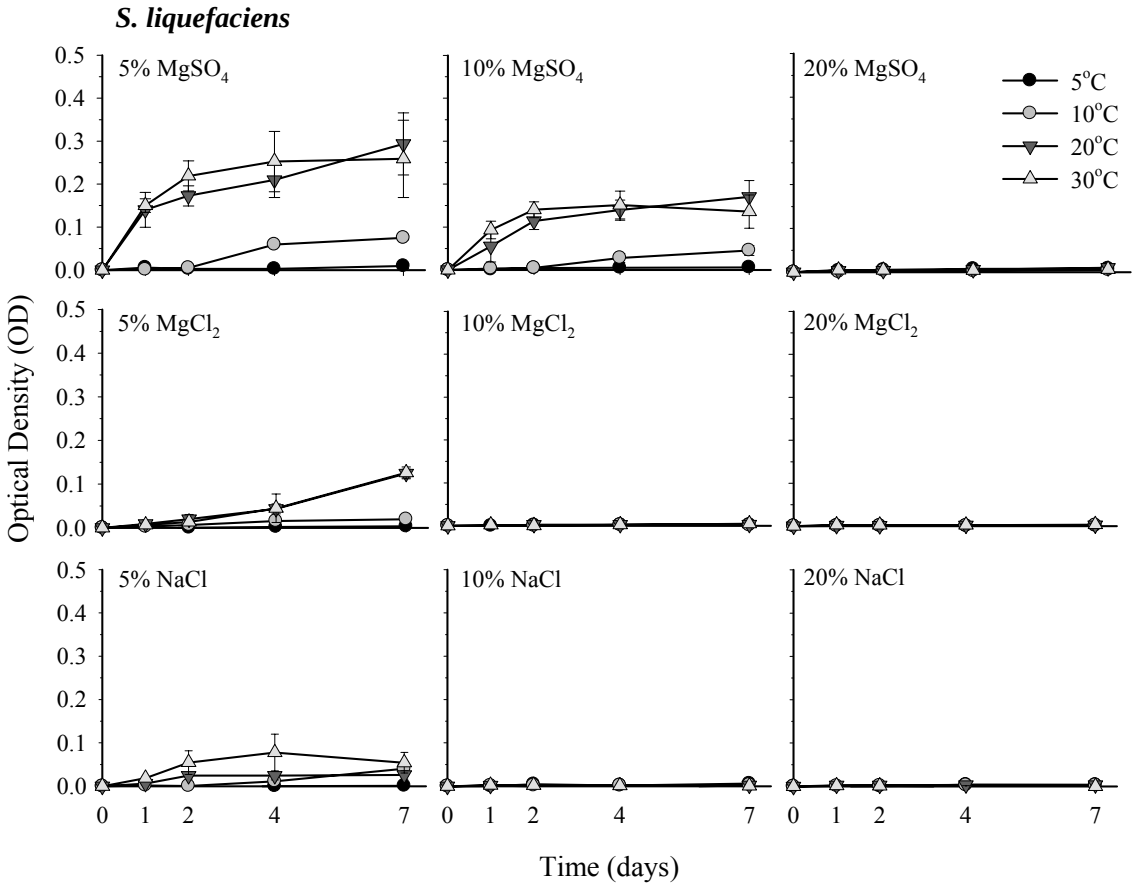

Figure 7. Growth of *Serratia liquefaciens* in 5, 10, and 20 % $MgSO_4$, $MgCl_2$, and NaCl over 7 d in CO_2 atmospheres incubated at 30, 20, 10 or 5 °C. Error bars denote 95 % confidence intervals (n = 6).

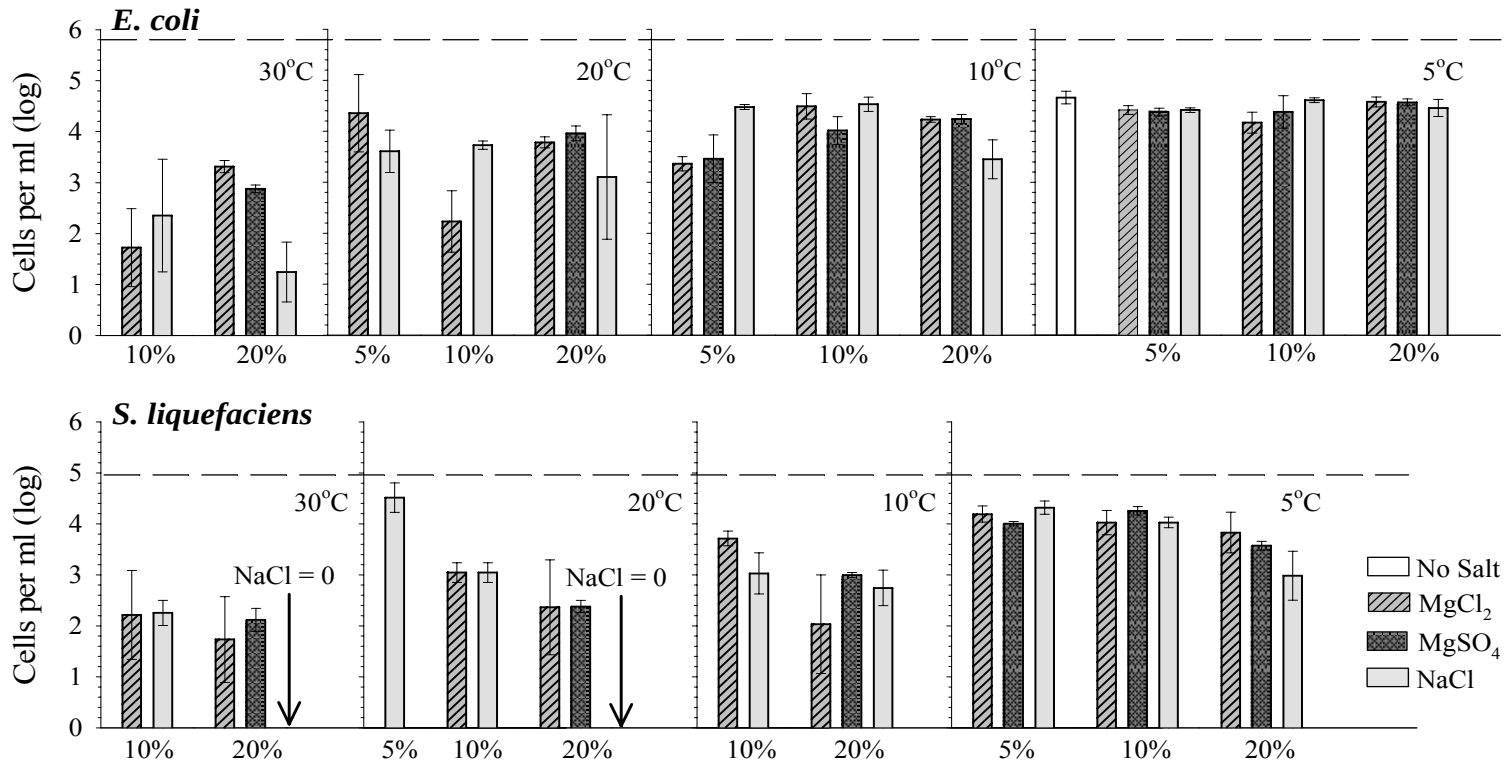

Figure 8. Survival of *Escherichia coli* and *Serratia liquefaciens* after 7 d under low temperatures and high salinity in CO_2 atmospheres. Cultures were harvested and counted after 7 d incubation at 30, 20, 10 or 5 °C. Dashed lines indicate starting levels of inoculum. Error bars denote 95 % confidence intervals (n = 6).

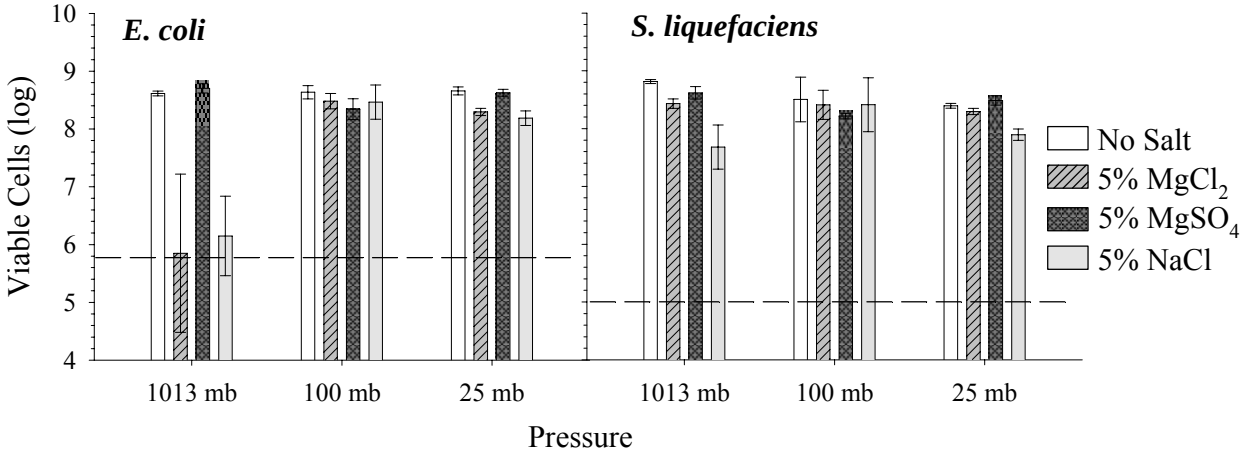

Figure 9. Mean cell densities of *Escherichia coli* and *Serratia liquefaciens* with and without salts under low pressure in CO_2 atmospheres. Cultures were incubated at 20 °C for 7 d under 1013, 100, or 25 mb. Dashed lines indicate starting levels of inoculum. Error bars denote 95% confidence intervals (n = 6).

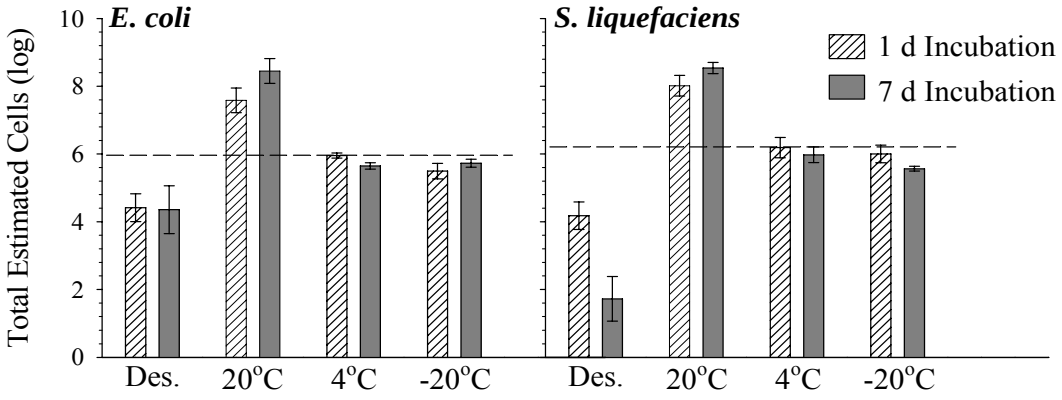

Figure 10. Mean cell densities of *Escherichia coli* and *Serratia liquefaciens* recovered from Mars analog soils after desiccation or incubation at 20, 4, or -20 °C for 1 or 7 d. Dashed lines indicate starting levels of inoculum. Error bars denote 95 % confidence intervals (n = 9).

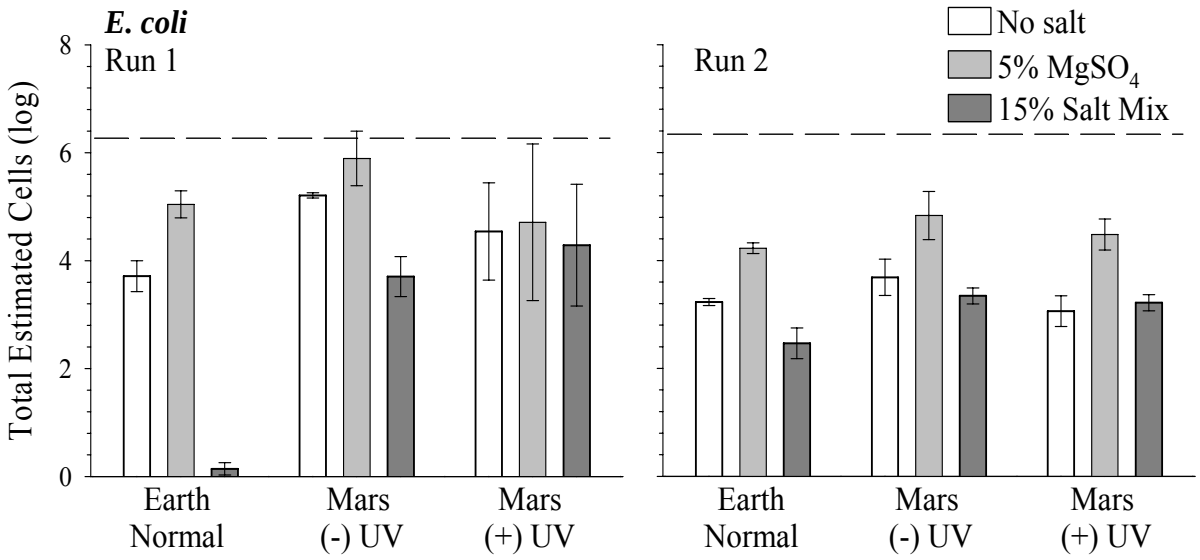

Figure 11. Recovery of viable cells of *Escherichia coli* in full-scale Mars simulations and Earth-controls. *Escherichia coli* cells were incubated for 7 d in Mars analog soils in Earth-normal conditions and in the MSC with and without UV exposure. Diurnal conditions within the MSC were changed from 20 °C and UV irradiation (daytime) to -50 °C without UV (nighttime). A Mars atmosphere was maintained at 7.1 mb. Dotted lines represent average initial inoculum for each repetition of the experiment. Error bars denote 95% confidence intervals (n = 6).

7. APPENDIX B - TABLES

Table 1. Interactive Effects of Temperature and Salt on Bacterial Growth - ANOVA for *E. coli* (n = 6).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	4.713	1	4.713	21473.707	.000
RUN	.008	1	.008	38.320	.000
TEMP	4.405	3	1.468	6690.310	.000
SALT	.794	2	.397	1809.417	.000
CONC	3.631	3	1.210	5514.406	.000
RUN * TEMP	.014	3	.005	20.834	.000
RUN * SALT	.001	2	.001	2.730	.068
TEMP * SALT	.760	6	.127	577.380	.000
RUN * TEMP * SALT	.016	6	.003	12.395	.000
RUN * CONC	.009	3	.003	13.532	.000
TEMP * CONC	3.533	9	.393	1788.474	.000
RUN * TEMP * CONC	.015	9	.002	7.441	.000
SALT * CONC	.794	6	.132	602.641	.000
RUN * SALT * CONC	.005	6	.001	3.656	.002
TEMP * SALT * CONC	.827	18	.046	209.250	.000
RUN * TEMP * SALT * CONC	.030	18	.002	7.579	.000
Error	.042	192	.000		

Dependent Variable: CELLS

Table 2. Interactive Effects of Temperature and Salt on Bacterial Growth - ANOVA for *S. liquefaciens* (n = 6).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	2.328	1	2.328	6021.425	.000
RUN	.002	1	.002	4.294	.040
TEMP	1.357	3	.452	1170.419	.000
SALT	.246	2	.123	318.764	.000
CONC	1.816	3	.605	1566.212	.000
RUN * TEMP	.039	3	.013	33.219	.000
RUN * SALT	.047	2	.023	60.604	.000
TEMP * SALT	.184	6	.031	79.277	.000
RUN * TEMP * SALT	.135	6	.022	58.110	.000
RUN * CONC	.022	3	.007	18.885	.000
TEMP * CONC	1.067	9	.119	306.571	.000
RUN * TEMP * CONC	.038	9	.004	10.950	.000
SALT * CONC	.448	6	.075	193.008	.000
RUN * SALT * CONC	.139	6	.023	59.835	.000
TEMP * SALT * CONC	.338	18	.019	48.564	.000
RUN * TEMP * SALT * CONC	.217	18	.012	31.124	.000
Error	.074	192	.000		

Dependent Variable: CELLS

Table 3. Survival of Temperature and Salt on Bacterial Growth - ANOVA for *E. coli* (n = 6).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	167.503(a)	61	2.746	15.948	.000
Intercept	1693.296	1	1693.296	9834.385	.000
TEMP	79.571	3	26.524	154.045	.000
SALT	.373	2	.186	1.083	.342
CONC	.697	2	.348	2.024	.137
RUN	1.475	1	1.475	8.569	.004
TEMP * SALT	3.486	6	.581	3.374	.004
TEMP * CONC	8.833	5	1.767	10.261	.000
SALT * CONC	15.890	4	3.972	23.071	.000
TEMP * SALT * CONC	12.795	7	1.828	10.616	.000
TEMP * RUN	2.340	3	.780	4.531	.005
SALT * RUN	2.405	2	1.203	6.985	.001
TEMP * SALT * RUN	2.961	6	.494	2.867	.012
CONC * RUN	.035	2	.018	.102	.903
TEMP * CONC * RUN	1.508	5	.302	1.751	.128
SALT * CONC * RUN	1.459	4	.365	2.118	.082
TEMP * SALT * CONC * RUN	9.784	7	1.398	8.118	.000
Error	21.350	124	.172		
Total	2844.850	186			
Corrected Total	188.853	185			

a R Squared = .887 (Adjusted R Squared = .831)
Dependent Variable: LOGCELLS

Table 4. Survival of Temperature and Salt on Bacterial Growth - ANOVA for *S. liquefaciens* (n = 6).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	230.527(a)	49	4.705	31.300	.000
Intercept	1015.316	1	1015.316	6754.890	.000
TEMP	45.998	3	15.333	102.007	.000
SALT	9.441	2	4.720	31.405	.000
CONC	49.714	2	24.857	165.375	.000
RUN	.161	1	.161	1.071	.303
TEMP * SALT	6.295	6	1.049	6.980	.000
TEMP * CONC	12.548	4	3.137	20.871	.000
SALT * CONC	7.856	4	1.964	13.067	.000
TEMP * SALT * CONC	15.569	3	5.190	34.528	.000
TEMP * RUN	1.005	3	.335	2.228	.090
SALT * RUN	1.089	2	.545	3.623	.030
TEMP * SALT * RUN	3.899	6	.650	4.324	.001
CONC * RUN	1.285	2	.642	4.273	.017
TEMP * CONC * RUN	1.739	4	.435	2.892	.026
SALT * CONC * RUN	.952	4	.238	1.584	.184
TEMP * SALT * CONC * RUN	8.309	3	2.770	18.427	.000
Error	15.031	100	.150		
Total	1515.606	150			
Corrected Total	245.558	149			

a R Squared = .939 (Adjusted R Squared = .909)
Dependent Variable: LOGCELLS

Table 5. Interactive Effects of Salt and Low Pressure on Bacterial Growth - ANOVA for *E. coli* (n = 6).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	79.580(a)	23	3.460	35.521	.000
Intercept	4704.982	1	4704.982	48302.465	.000
PRESSURE	20.542	2	10.271	105.444	.000
SALT	19.015	3	6.338	65.072	.000
RUN	.741	1	.741	7.607	.008
PRESSURE * SALT	24.945	6	4.157	42.682	.000
PRESSURE * RUN	.176	2	.088	.904	.412
SALT * RUN	4.079	3	1.360	13.960	.000
PRESSURE * SALT * RUN	10.081	6	1.680	17.249	.000
Error	4.676	48	.097		
Total	4789.237	72			
Corrected Total	84.255	71			

a R Squared = .945 (Adjusted R Squared = .918)
Dependent Variable: LOGCELLS

Table 6. Interactive Effects of Salt and Low Pressure on Bacterial Growth - ANOVA for *S. liquefaciens* (n = 6).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	10.538(a)	23	.458	49.564	.000
Intercept	5016.188	1	5016.188	542632.201	.000
PRESSURE	.224	2	.112	12.111	.000
SALT	3.295	3	1.098	118.798	.000
RUN	.274	1	.274	29.679	.000
PRESSURE * SALT	2.617	6	.436	47.180	.000
PRESSURE * RUN	2.693	2	1.347	145.660	.000
SALT * RUN	.319	3	.106	11.498	.000
PRESSURE * SALT * RUN	1.117	6	.186	20.132	.000
Error	.444	48	.009		
Total	5027.169	72			
Corrected Total	10.982	71			

a R Squared = .960 (Adjusted R Squared = .940)
Dependent Variable: LOGCELLS

Table 7. Growth of Bacteria in Mars-Analog Soils - ANOVA for *E. coli* (n = 6).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	120.999(a)	7	17.286	58.838	.000
Intercept	2513.320	1	2513.320	8555.060	.000
TREATMENT	118.678	3	39.559	134.656	.000
DAYS	.587	1	.587	1.999	.162
TREATMENT * DAYS	3.342	3	1.114	3.792	.014
Error	18.508	63	.294		
Total	2627.024	71			
Corrected Total	139.508	70			

a R Squared = .867 (Adjusted R Squared = .853)
Dependent Variable: LOGCELLS

Table 8. Growth of Bacteria in Mars-Analog Soils - Tamhane's multiple comparisons test for *E. coli* (n = 9).

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
-20 °C	20 °C	-2.3780(*)	.18110	.000	-2.9025	-1.8534
	4 °C	-.1885	.08476	.186	-.4271	.0501
	Desiccation	1.2255(*)	.21445	.000	.6029	1.8480
	4 °C	2.1895(*)	.17388	.000	1.6781	2.7008
	Desiccation	3.6034(*)	.26275	.000	2.8670	4.3399
	20 °C	-2.1895(*)	.17388	.000	-2.7008	-1.6781
	Desiccation	1.4140(*)	.20838	.000	.8022	2.0258
	20 °C	-3.6034(*)	.26275	.000	-4.3399	-2.8670
	4 °C	-1.4140(*)	.20838	.000	-2.0258	-.8022
20 °C	-20 °C	2.3780(*)	.18110	.000	1.8534	2.9025
4 °C	-20 °C	.1885	.08476	.186	-.0501	.4271
Desiccation	-20 °C	-1.2255(*)	.21445	.000	-1.8480	-.6029

Based on observed means.
* The mean difference is significant at the .05 level.

Dependent Variable: LOGCELLS

Table 9. Growth of Bacteria in Mars-Analog Soils - ANOVA for *S. liquefaciens* (n = 9).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	287.128(a)	7	41.018	149.250	.000
Intercept	2400.641	1	2400.641	8735.013	.000
TREATMEN	257.752	3	85.917	312.620	.000
DAYS	7.497	1	7.497	27.278	.000
TREATMEN * DAYS	21.879	3	7.293	26.536	.000
Error	17.589	64	.275		
Total	2705.357	72			
Corrected Total	304.717	71			

a R Squared = .942 (Adjusted R Squared = .936)

Dependent Variable: LOGCELLS
Tamhane

Table 10. Growth of Bacteria in Mars-Analog Soils - Tamhane's multiple comparisons test for *S. liquefaciens* (n = 9).

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
-20C	20C	-2.4937(*)	.13659	.000	-2.8763	-2.1110
	4C	-.2981	.12949	.155	-.6602	.0639
	desiccation	2.8307(*)	.36418	.000	1.7619	3.8995
	4C	2.1955(*)	.14442	.000	1.7920	2.5991
	desiccation	5.3243(*)	.36975	.000	4.2459	6.4028
	20C	-2.1955(*)	.14442	.000	-2.5991	-1.7920
	desiccation	3.1288(*)	.36718	.000	2.0549	4.2027
	20C	-5.3243(*)	.36975	.000	-6.4028	-4.2459
	4C	-3.1288(*)	.36718	.000	-4.2027	-2.0549
20C	-20C	2.4937(*)	.13659	.000	2.1110	2.8763
4C	-20C	.2981	.12949	.155	-.0639	.6602
desiccation	-20C	-2.8307(*)	.36418	.000	-3.8995	-1.7619

Based on observed means.
* The mean difference is significant at the .05 level.

Dependent Variable: POWER.25

Table 11. Experiment 5. ANOVA for *E. coli* (n = 6).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2149.932(a)	9	238.881	10.935	.000
Intercept	7225.763	1	7225.763	330.780	.000
ATM	380.758	2	190.379	8.715	.001
SALT	1165.372	2	582.686	26.674	.000
ATM * SALT	162.089	4	40.522	1.855	.136
RUN	441.625	1	441.625	20.217	.000
Error	939.319	43	21.845		
Total	10229.287	53			
Corrected Total	3089.251	52			

a R Squared = .696 (Adjusted R Squared = .632)

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