ASTROBIOLOGY
Volume 6, Number 2, 2006

Research Paper

Microbial Survival Rates of *Escherichia coli* and *Deinococcus radiodurans* Under Low Temperature, Low Pressure, and UV-Irradiation Conditions, and Their Relevance to Possible Martian Life

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ABSTRACT

Viability rates were determined for microbial populations of *Escherichia coli* and *Deinococcus radiodurans* under the environmental stresses of low temperature (−35°C), low-pressure conditions (83.3 kPa), and ultraviolet (UV) irradiation (37 W/m^2). During the stress tests the organisms were suspended in saltwater soil and freshwater soil media, at variable burial depths, and in seawater. Microbial populations of both organisms were most susceptible to dehydration stress associated with low-pressure conditions, and to UV irradiation. However, suspension in a liquid water medium and burial at larger depths (5 cm) improved survival rates markedly. Our results indicate that planetary surfaces that possess little to no atmosphere and have low water availability do not constitute a favorable environment for terrestrial microorganisms. Key Words: Extreme environments—Ultraviolet radiation—Mars—Environmental stress—Pressure—Temperature. Astrobiology 6, 332–347.

INTRODUCTION

Our understanding of the growth and limits for microbial life is evolving as science continues to explore and discover life in extreme environments that were once thought never to harbor life. Microorganisms are widely distributed on Earth and continually being discovered and identified in nearly every niche, including high pressure–high temperature, low temperature–high salinity, and high and low pH environments (Kushner, 1978; Cavicchioli, 2002). The ubiquity and dynamic nature of such a vast array of microorganisms demonstrate their ability to utilize a broad range of energy sources for metabolism (Madigan *et al*., 1997). Microorganisms have developed a variety of mechanisms in response to environmental stresses, which enable them to trap, conserve, and convert the energy required for their biosynthesis and growth (Hamilton, 1994). These mechanisms include phototrophy, lithotrophy, fermentation, and aerobic and anaerobic respiration.

Theoretically, life may appear in a particular environment if basic criteria are met (*e.g*., Irwin and Schulze-Makuch, 2001). These criteria in-

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clude a fluid medium, source of energy, and the constituents and conditions compatible with polymeric chemistry. There are many extreme environments on Earth that meet these criteria, but given the same opportunity on other planetary bodies, the question as to whether microorganisms remain viable or even exist has not yet been answered satisfactorily.

Microorganisms have been surveyed across a range of polar and other extreme cold environments on Earth. Deming (2002) described microbial diversity in polar environments of Antarctic and Arctic seawater, Antarctic and Arctic sea ice, and Arctic salt lakes. Microorganisms have been identified living in microscopic pockets of brine (Gilichinsky *et al*., 1993), encased in ice cores many thousands of years old (Siegert *et al*., 2000), and in porous sandstone and surface pavements in the Antarctic Dry Valleys (Doran *et al*., 1998; Wynn-Williams, 2000). Psychrophilic organisms have evolved and possess enzymes and cell membranes that allow them to function in extreme cold. Cronan (2002) described how bacteria modify their membranes in response to changing environmental conditions, and Nedwell (1999) showed that cold-adapted bacteria possess an increased proportion of unsaturated membrane lipids and a decreased proportion of branched-chain lipids to maintain membrane fluidity. Deming (2002) summarized findings with regard to the characterization of enzymes, proteins, membrane constituents, and genetic regulatory mechanisms that enable microorganisms to live in the cold (see also Georlette *et al*., 2004). Halophilic microorganisms are known to be well adapted to highly saline environments, including salt seas and lakes, the interstices of rock salt, and within saline soils (Kushner, 1978), and they can tolerate highly saturated NaCl concentrations. They are often highly pigmented and have a number of novel molecular characteristics. For example, specific proteins in the cell wall are negatively charged and stabilized by attracting the positively charged sodium ions (Na^+) abundant in the salty environment (Kates, 1986).

Many microbes have the potential to remain viable after exposure to extreme environmental conditions and stresses. This study builds upon the strategies utilized by microorganisms such as *Escherichia coli* and *Deinococcus radiodurans* to tolerate and adapt to changing conditions and stresses. *E. coli*, the world's most thoroughly studied life form and popular model organism, is known for its ability to respond and adapt to environmental stresses. Movement and transport into various environments has allowed *E. coli* to utilize RNA polymerase, which can respond to various intracellular and extracellular signals that regulate gene expression and produce responses to changing environmental stresses, which include osmotic pressures, mechanical stress, starvation, temperature extremes, and ultraviolet (UV) irradiation (*e.g*., EcoCyc., 2004).

D. radiodurans, known for its irradiation resistance, has a range of survival characteristics that allow it to survive in a variety of environments that are lethal to most other cellular species. For instance, *D. radiodurans* can withstand extreme radioactivity and UV irradiation, genotoxic chemicals, heat, and desiccation (Battista, 1997). The mechanisms by which *D. radiodurans* adapts to outside stresses are not clear. However, one adaptation technique recently identified in *D. radiodurans* is the accumulation of Mn(II) with low Fe(II) (Daly *et al*., 2004), which was suggested to facilitate resistance to irradiation. *D. radiodurans* also has an unusual tightly packed DNA with a laterally ordered toroid morphology that may contribute to its irradiation resistance and resistance to dehydration (Levin-Zaidman *et al*., 2003). The DNA of *D. radiodurans* is organized in a unique ring that prevents broken pieces of DNA generated by exposure to irradiation from floating freely within the cytoplasm, which prevents the loss of information. Any severed DNA fragments stay tightly locked in the ring and eventually come back together in the correct, original order, and the DNA strands are reconstructed.

It has been proposed, in recognition of the environmental flexibility of microorganisms on Earth, that some microorganisms with specific physiological capabilities could survive on Mars (Friedmann and Ocampo-Friedmann, 1984; Boston *et al*., 1992; McKay *et al*., 1992a,b; Stevens and McKinley, 1995). Recently, Cockell *et al*. (2005) exposed *Chroococcidiopsis* sp. 029 to a simulated martian UV flux and concluded that, even within an unattenuated UV flux, organisms could survive in lithic habitats if there was a source of liquid water and essential nutrients. Potential protected habitats have been postulated for Mars, such as sulfur-rich subsurface areas for chemoautotrophic communities, rocks for endolithic communities, and permafrost regions, hydrothermal vents, soil, and evaporate crystals (Horneck, 2000). However, the surface of Mars is cold, dry,

Table 1. Comparisons of Mars Environmental Conditions and Experimental Conditions

	Environmental conditions on Mars	*Experimental conditions*
Pressure	~600 Pa (6 mbar)	83.3 kPa (833 mbar)
Temperature	−123°C to 25°C	−35°C
UV irradiation (200 nm–400 nm)	8.4 to 67 W/m^2	37 W/m^2

subject to low atmospheric pressure, apparently chemically oxidizing, and exposed to a high flux of UV irradiation at short wavelengths. These stresses pose extreme challenges to the existence and growth of microbial organisms.

This study was designed to test microbial survival rates for some of these stresses, specifically low pressure, low temperature, and enhanced UV irradiation. As these are the same stresses that occur near the martian surface, this study has relevance to the survivability of microbial life on the surface or in the near surface of Mars. However, this study does not attempt to simulate actual martian environmental conditions. The tested pressure is much higher than the pressure that occurs on Mars, the strong diurnal fluctuations of martian temperature are not taken into account, and UV stresses do not reflect the UV radiation spectrum of Mars (Table 1). The objective, rather, is to test whether *E. coli* and *D. radiodurans* can remain viable in soil and water under environmental stress conditions common to planetary surfaces. The specific goals include: (1) determination of microbial viability rates of cells within and at different soil depths and in seawater and (2) comparison of microbial viability rates between *D. radiodurans* and *E. coli* populations under subzero temperature, low-pressure conditions, and extreme UV irradiation.

MATERIALS AND METHODS

Preparation of bacteria

D. radiodurans (ATCC strain 25073) and *E. coli* (ATCC strain 11229) were chosen as microbial representatives for this study. Each was grown and maintained on Trypticase soy broth at 37°C and stored at stationary phase under refrigeration until needed.

Prepared bacteria were transferred into conical tubes and centrifuged at 1,000 *g* for 10 min. The supernatant (Trypticase soy broth) was removed, and the pellet was washed once in 0.9% NaCl solution. Washed cells were added to Johnson Space Center (JSC) Mars-1 simulant soil, which was used because (1) it is nutrient poor, (2) it reflects soils present on a young Earth-type planet (weathered lava rock generated via hot-spot volcanism), and (3) the composition is well known (*e.g.*, Evans and Adams, 1979; Singer, 1982; Morris *et al.*, 1993; Allen *et al.*, 1998). The suspensions were transferred to sterilized glass beakers and immediately mixed with soil containing: (1) a high salt concentration (30 g of NaCl/L of distilled water), (2) a low salt concentration (0.5 g of NaCl/L of distilled water), or (3) seawater with a small amount of soil (Table 2). The exact procedure is shown in Fig. 1. Each mixture was then transferred aseptically to three individual glass beakers, which were subjected to their assigned environmental test conditions.

Environmental stresses and setup

Five experiments were conducted to measure the individual and combined effects of three different stresses: temperature, pressure, and UV irradiation. The effect on cell viability of the following five conditions were tested:

1. Subzero temperature (−35°C)
2. Low pressure (83.3 kPa)

Table 2. Prepared Media

Medium	*Soil*	*Water*
1. Saltwater soil	95% JSC Mars-1 simulant soil	5% saline water (30 g/L of NaCl/L)
2. Freshwater soil	95% JSC Mars-1 simulant soil	5% fresh water (0.5 g/L of NaCl/L)
3. Seawater	5% JSC Mars-1 simulant soil	95% sea salt water (30 g/L of sea salt/L)

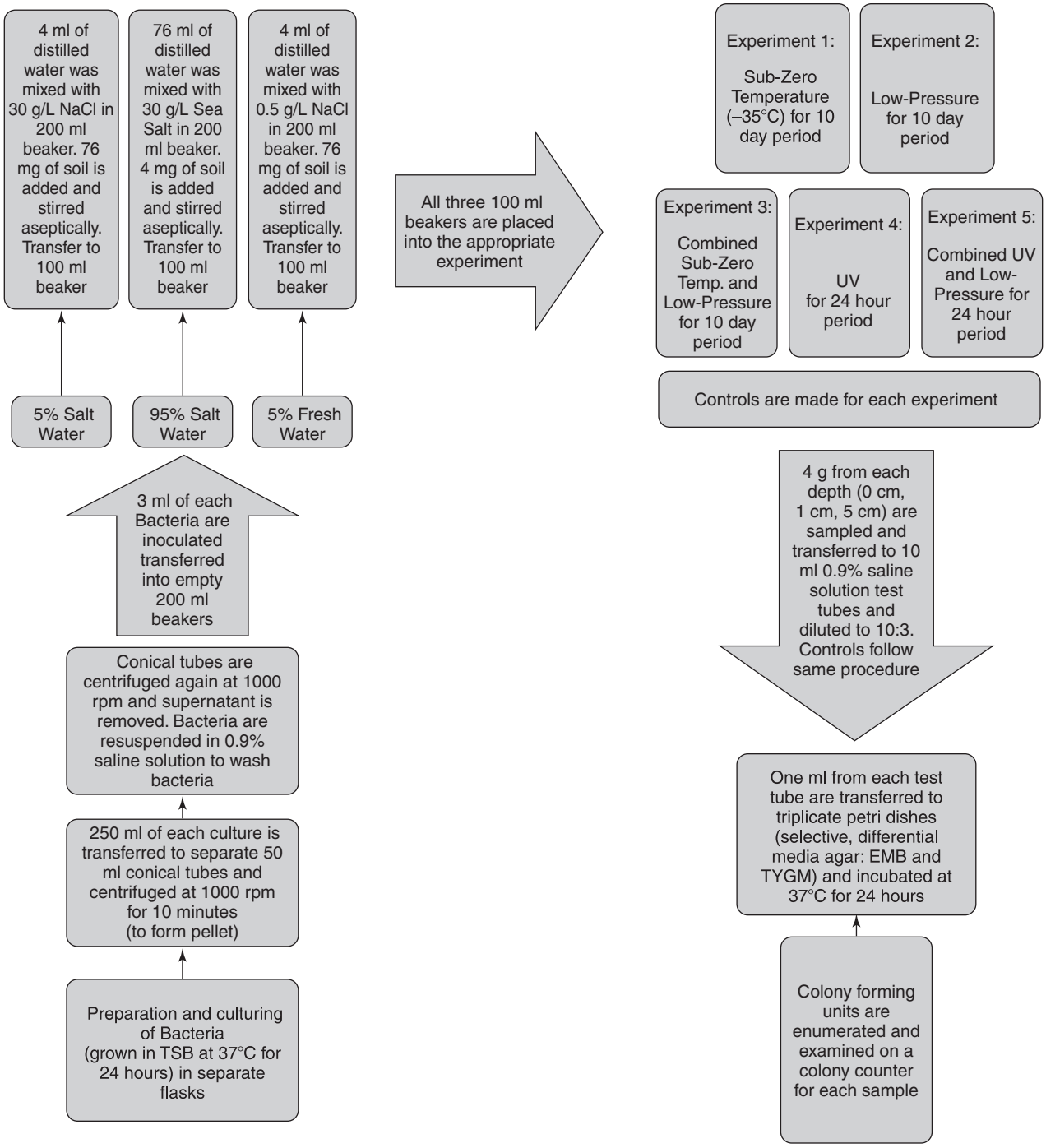

FIG. 1. Schematic diagram of the experimental setup and procedure for determination of the viability of bacteria. TSB, Trypticase soy broth.

3. Combination of subzero temperature (−35°C) and low-pressure conditions (83.3 kPa)
4. UV irradiation (37 W/m^2)
5. Combination of UV irradiation (37 W/m^2) and low-pressure conditions (83.3 kPa)

In addition to testing different types of media (freshwater soil, saltwater soil, and seawater), different depths were sampled to assess the magnitude of stress relevant to the depth within the soil column. Microbial viabilities

were tested in soils on the surface and at depths of 1 and 5 cm.

Experiment 1. The subzero temperature environmental setup involved placing triplicate samples of saltwater soil, freshwater soil, and seawater into a freezer with a temperature of −35°C. The samples were frozen for 10 days. The temperature was monitored with a subzero Celsius thermometer. A comparison of the experimental temperature conditions with those existing on Mars is provided in Table 1.

Experiment 2. A low-pressure atmosphere was created using a Gast (Benton Harbor, MI) model DAA oil-less diaphragm vacuum pump connected to a 3-quart vacuum jar and an industrial dry-ice cold trap to accelerate and enhance sublimation. A constant low pressure of 83.3 kPa was applied to the samples for 10 days. A comparison of the experimental pressure conditions with those existing on Mars is provided in Table 1.

Experiment 3. Frozen saltwater soil, freshwater soil, and seawater samples were placed into the vacuum chamber in the freezer for combined treatment of subzero temperature (−35°C) and low-pressure (83.3 kPa). These experimental conditions required the vacuum chamber to be connected by vacuum tubing to the vacuum pump through the freezer drain (for a 10-day period). Dry ice was added in the morning and afternoon of each day into the dry-ice cold trap until the end of the monitoring period.

Experiment 4. UV treatment required the placement of bacterial samples directly under a continuous UV output beam at room temperature. The high UV irradiation environment was simulated using a 30-W, ozone-free UV-deuterium lamp (model 63165, Oriel Instruments, Inc., Stratford, CT). The spectrum of the UV lamp compared with UV fluxes on Mars is provided in Fig. 2 and summarized in Table 1. The intensity of the deuterium lamp was 37 W/m^2 in the UV range from 200 to 400 nm at a sample distance of 25 mm. Samples were treated for 24 h continuously to represent days to weeks of clear-day exposure (Mancinelli and Klovstad, 2000). A condenser was connected to the lamp housing to allow the UV output beam to focus on the sample beakers that contained the medium with the bacteria.

Experiment 5. The glass beakers with the samples were placed into the vacuum chamber and exposed to the UV irradiation. The UV lamp was affixed at a distance of 25 mm above the vacuum chamber. The vacuum chamber consisted of polycarbonate designed to transmit more than 95% of the UV irradiation. Samples were exposed to low-pressure conditions (83.3 kPa) and UV irradiation (37 W/m^2) at room temperature for 24 h.

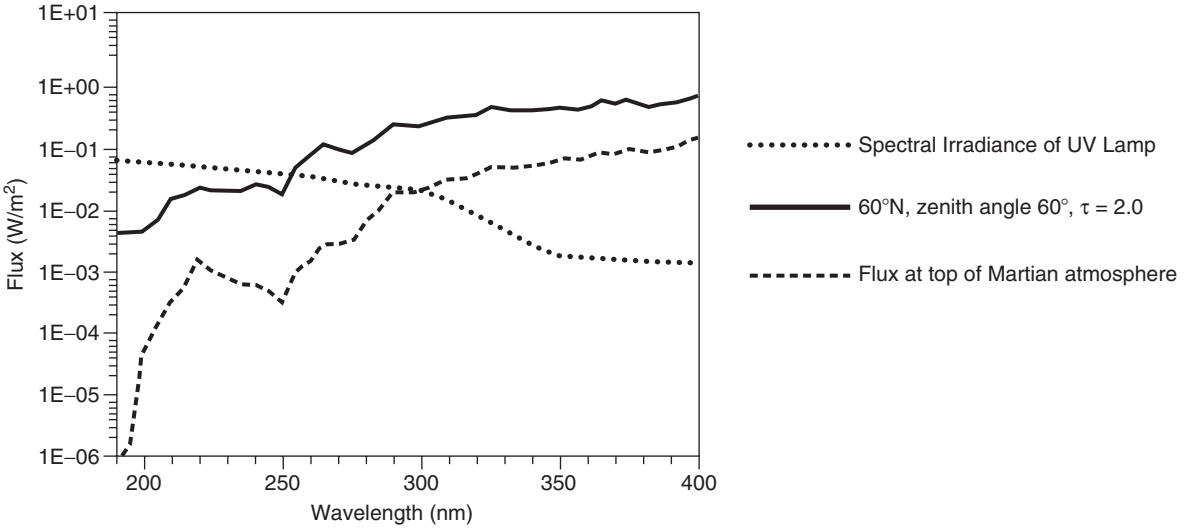

FIG. 2. Comparison among the spectral irradiance of the deuterium UV lamp, the UV flux at the top of the martian atmosphere, and at a low-intensity surface location on Mars. UV fluxes on the martian surface are typically between the lower two lines. The Mars fluxes shown are modified from Cockell *et al*. (2000).

Control groups. Controls were prepared and incubated following the procedures described in Fig. 1, except that the control samples were not placed into the different stress environments. Controls were placed in a cabinet at room temperature at 100 kPa pressure and zero UV flux. Controls were plated in triplicate and enumerated. Microbial survival rates were calculated for controls on two days—day 0 and day 10—to assess the change in microbial population growth over time in a non-stressed soil and water environment. *E. coli* and *D. radiodurans* populations in seawater essentially remained constant over the 10-day period [3,657 ± 148 colony-forming units (cfu)/ml for day 0 vs. 3,588 ± 181 cfu/ml for day 10]. In the soil samples, mean population totals for *E. coli* on day 0 and day 10 were 10,624 and 9,857 cfu/ml, respectively. Mean population totals for *D. radiodurans* for day 0 and day 10 were 14,259 and 17,464 cfu/ml, respectively. Day 0 represents the stationary growth phase of bacteria on a minimum nutrient basalt soil (JSC-1 stimulant soil). To be conservative, microbial viability counts for day 0 were used to represent the N_o for the control group.

Plating and enumeration of bacterial samples

Bacterial samples exposed to the five different environmental stresses were plated on EMB (*E. coli*) and TGY (*D. radiodurans*) media agar plates to evaluate microbial viability. Four grams of sample was gathered from the appropriate soil layers—surface, 1 cm in depth, and 5 cm in depth—and transferred to individual sterilized test tubes filled with 0.9% NaCl solution (Fig. 1). The suspension was further diluted in 0.9% NaCl, and various dilution solutions were prepared. The 10^{-3} dilution was found to be the optimum dilution for counting. Samples from the 10^{-3} dilution were plated, and colony numbers were determined (Fig. 1).

All tests and controls were done in triplicate. To determine viability or survival after treatment to various stresses, appropriate media were selected and prepared for *E. coli* and *D. radiodurans*. One milliliter of a 10^{-3} dilution sample was transferred to triplicate plates of EMB (*E. coli*) and TGY (*D. radiodurans*) media agar plates. Plates were incubated at 37°C for 24 h, after which bacterial colonies were enumerated to determine viability. In this study, viability or surviving microorganisms were determined as N/N_o, where N was the number of colony formers of the treated samples and N_o the number of colony formers of the untreated controls.

RESULTS

Experiment 1: subzero temperature (−35°C for 10 days)

Viability for *E. coli* populations was approximately 3% or less after exposure to low temperature (−35°C) for 10 days. Microbial populations from saltwater soil and freshwater soil samples displayed little to no resistance to subzero temperature at all three tested depths (surface, 1 cm, and 5 cm). In contrast, a survival rate of about 20% was observed in frozen seawater samples after being exposed to Experiment 1 (Table 3).

The *D. radiodurans* microbial populations in saltwater soil samples declined with increasing depth to survival rates from 47% to 27%, while microbial populations in freshwater soil exhibited little or no decline after treatment. In seawater samples, about 57% of the *D. radiodurans* cell populations survived exposure to −35°C temperature after 10 days (Table 3).

Experiment 2: low pressure (83.3 kPa for 10 days)

After treatment with low-pressure conditions (and associated desiccation stress), *E. coli* populations were completely eliminated after 10 days at all depths and in all soil samples. In contrast, seawater samples demonstrated a microbial survival rate of about 73% (Table 4). Because of evaporation and sublimation, the volume of seawater decreased by about 20% during the experiment, which increased salinity. In contrast, *D. radiodurans* had much higher microbial survival rates after exposure to low-pressure conditions. Survival rates for *D. radiodurans* were greater than 30% at all depths. In freshwater soil, the highest microbial viability rates were observed (63% to higher than 100%), while in saltwater soil the microbial viability rates declined from 52% to 31% with increasing depth after treatment. In contrast to the soils, microbial survival rates in seawater samples were at 100%, with no apparent effect on microbial viability (Table 4).

Experiment 3: combined subzero temperature and low pressure (−35°C and 83.3 kPa for 10 days)

Combined treatment of subzero temperature and low pressure for 10 days resulted in an *E. coli*

Table 3. Experimental Viability Results for *E. coli* and *D. radiodurans* After Subzero Temperature Treatment of −35°C for 10 Days

	E. coli					D. radiodurans				
Soil composition, depth	*Control (cfu/ml)*	*Mean (cfu/ml)*	*Viability*[a]	*SD*	*95% CI*	*Control (cfu/ml)*	*Mean (cfu/ml)*	*Viability*[a]	*SD*	*95% CI*
Saltwater soil										
Surface	1,441	39	2%	± 12 cfu/ml	33%	2,070	981	47%	± 106 cfu/ml	12%
1 cm	1,349	28	2%	± 13 cfu/ml	50%	2,867	1,211	42%	± 339 cfu/ml	32%
5 cm	2,146	10	0.4%	± 5 cfu/ml	50%	3,051	828	27%	± 287 cfu/ml	39%
Freshwater soil										
Surface	1,901	21	1%	± 2 cfu/ml	10%	1,518	981	65%	± 95 cfu/ml	11%
1 cm	782	31	3%	± 12 cfu/ml	45%	705	2,741	>100%	± 723 cfu/ml	26%
5 cm	1,012	32	3%	± 11 cfu/ml	41%	1,748	2,898	>100%	± 347 cfu/ml	16%
Seawater										
Surface	1,993	399	20%	± 53 cfu/ml	15%	2,300	1,318	57%	± 397 cfu/ml	34%

Saltwater soil contained 30 g/L of saline water [95% soil and 5% water (30,000 mg/L of NaCl)], freshwater soil contained 0.5 g/L of fresh-water [95% soil and 5% water (500 mg/L of NaCl)], and seawater contained 30 g/L of sea salt water [5% soil and 95% water (30,000 mg/L of sea salt)]. Numbers are given in cfu/ml. CI, confidence interval; SD, standard deviation.

[a]Viability or surviving microorganisms were determined as N/N_o, where N was the number of colony formers of the treated samples and N_o the number of colony formers of the untreated controls.

viability from 20% to larger than 100%. Microbial populations in freshwater soil samples had higher viability rates than saltwater soil samples. In both saltwater and freshwater soil samples, microbial viability was highest at a depth of 1 cm. Survival rates for seawater samples were reduced by 80% (Table 5). No measurable drop in water volume was noted during the experiment.

D. radiodurans exhibited higher viability rates compared with *E. coli* after treatment to combined subzero temperature and low pressure for 10 days. Freshwater soil samples again had higher viability rates compared with saltwater soil samples. In fact, no significant drop in viability was observed at any depth in freshwater samples. Saltwater soil samples decreased in microbial vi-

Table 4. Experimental Viability Results for *E. coli* and *D. radiodurans* After Low-Pressure Treatment for 10 Days (83.3 kPa)

	E. coli					D. radiodurans				
Soil composition, depth	*Control (cfu/ml)*	*Mean (cfu/ml)*	*Viability*[a]	*SD*	*95% CI*	*Control (cfu/ml)*	*Mean (cfu/ml)*	*Viability*[a]	*SD*	*95% CI*
Saltwater soil										
Surface	1,441	0	0%	± 0 cfu/ml	0%	2,070	1,088	52%	± 723 cfu/ml	57%
1 cm	1,349	0	0%	± 0 cfu/ml	0%	2,867	1,257	44%	± 257 cfu/ml	18%
5 cm	2,146	0	0%	± 0 cfu/ml	0%	3,051	935	31%	± 443 cfu/ml	42%
Freshwater soil										
Surface	1,901	0	0%	± 0 cfu/ml	0%	1,518	1,441	94%	± 262 cfu/ml	21%
1 cm	782	0	0%	± 0 cfu/ml	0%	705	1,288	>100%	± 736 cfu/ml	65%
5 cm	1,012	0	0%	± 0 cfu/ml	0%	1,748	1,104	63%	± 243 cfu/ml	25%
Seawater										
Surface	1,993	1,456	73%	± 646 cfu/ml	50%	2,300	2,300	100%	± 397 cfu/ml	17%

Saltwater soil contained 30 g/L of saline water [95% soil and 5% water (30,000 mg/L of NaCl)], freshwater soil contained 0.5 g/L of fresh-water [95% soil and 5% water (500 mg/L of NaCl)], and seawater contained 30 g/L of sea salt water [5% soil and 95% water (30,000 mg/L of sea salt)]. Numbers are given in cfu/ml. CI, confidence interval; SD, standard deviation.

[a]Viability or surviving microorganisms were determined as N/N_o, where N was the number of colony formers of the treated samples and N_o the number of colony formers of the untreated controls.

TABLE 5. EXPERIMENTAL VIABILITY RESULTS FOR *E. COLI* AND *D. RADIODURANS* AFTER COMBINED SUBZERO TEMPERATURE AND LOW-PRESSURE TREATMENT FOR 10 DAYS (−35°C, 83.3 kPa)

	E. coli					D. radiodurans				
Soil composition, depth	*Control (cfu/ml)*	*Mean (cfu/ml)*	*Viability*[a]	*SD*	*95% CI*	*Control (cfu/ml)*	*Mean (cfu/ml)*	*Viability*[a]	*SD*	*95% CI*
Saltwater soil										
Surface	1,441	721	50%	± 116 cfu/ml	18%	2,070	1,794	87%	± 239 cfu/ml	12%
1 cm	1,349	889	66%	± 162 cfu/ml	21%	2,867	2,760	96%	± 239 cfu/ml	24%
5 cm	2,146	591	28%	± 125 cfu/ml	21%	3,051	2,346	77%	± 239 cfu/ml	28%
Freshwater soil										
Surface	1,901	1,303	68%	± 266 cfu/ml	23%	1,518	1,472	96%	± 211 cfu/ml	16%
1 cm	782	1,042	>100%	± 252 cfu/ml	35%	705	2,392	>100%	± 652 cfu/ml	31%
5 cm	1,012	935	92%	± 323 cfu/ml	39%	1,748	5,602	>100%	± 664 cfu/ml	13%
Seawater										
Surface	1,993	399	20%	± 141 cfu/ml	40%	2,300	4,692	>100%	± 356 cfu/ml	23%

Saltwater soil contained 30 g/L of saline water [95% soil and 5% water (30,000 mg/L of NaCl)], freshwater soil contained 0.5 g/L of fresh-water [95% soil and 5% water (500 mg/L of NaCl)], and seawater contained 30 g/L of sea salt water [5% soil and 95% water (30,000 mg/L of sea salt)]. Numbers are given in cfu/ml. CI, confidence interval; SD, standard deviation.

[a]Viability or surviving microorganisms were determined as N/N_o, where N was the number of colony formers of the treated samples and N_o the number of colony formers of the untreated controls.

ability no more than 23%. No reduction of microbial viability was observed in seawater samples either (Table 5).

Experiment 4: UV irradiation (37 W/m² for 24 h)

Microbial populations in surface soil samples had the lowest viability rates following UV treatment. After exposure to UV irradiation for 24 h, *E. coli* microbial populations on the surface of saltwater soil samples had a viability of 3%, while *E. coli* populations on the surface of freshwater soils did not survive UV irradiation and had a minimal survival rate of only 0.7% at a depth of 1 cm. Increased salt concentrations in saltwater soil samples appeared to improve viability at a depth of 1 cm (30% vs. 0.7%). Survival rates for microbial populations in seawater samples were shown to have a 66% reduction in viability (Table 6).

TABLE 6. EXPERIMENTAL VIABILITY RESULTS FOR *E. COLI* AND *D. RADIODURANS* AFTER UV IRRADIATION TREATMENT FOR 24 h (37 W/m²)

	E. coli					D. radiodurans				
Depth	*Control (cfu/ml)*	*Mean (cfu/ml)*	*Viability*[a]	*SD*	*95% CI*	*Control (cfu/ml)*	*Mean (cfu/ml)*	*Viability*[a]	*SD*	*95% CI*
Saltwater soil										
Surface	2,162	63	3%	± 4 cfu/ml	8%	3,434	1,824	53%	± 162 cfu/ml	10%
1 cm	4,140	1,257	30%	± 386 cfu/ml	35%	3,680	1,257	34%	± 386 cfu/ml	31%
5 cm	4,830	2,775	57%	± 1,037 cfu/ml	42%	2,438	2,438	100%	± 347 cfu/ml	16%
Freshwater soil										
Surface	2,162	0	0%	± 0 cfu/ml	0%	2,484	4	0%	± 1 cfu/ml	25%
1 cm	4,140	32	0.7%	± 11 cfu/ml	41%	2,898	3,450	>100%	± 239 cfu/ml	8%
5 cm	4,830	2,039	42%	± 492 cfu/ml	27%	4,554	4,278	94%	± 293 cfu/ml	8%
Seawater										
Surface	4,830	1,656	34%	± 414 cfu/ml	28%	1,533	902	59%	± 74 cfu/ml	9%

Saltwater soil contained 30 g/L of saline water [95% soil and 5% water (30,000 mg/L of NaCl)], freshwater soil contained 0.5 g/L of fresh-water [95% soil and 5% water (500 mg/L of NaCl)], and seawater contained 30 g/L of sea salt water [5% soil and 95% water (30,000 mg/L of sea salt)]. Numbers are given in cfu/ml. CI, confidence interval; SD, standard deviation.

[a]Viability or surviving microorganisms were determined as N/N_o, where N was the number of colony formers of the treated samples and N_o the number of colony formers of the untreated controls.

Samples obtained from the surface for *D. radiodurans* microbial populations had a 0% viability in freshwater soil, but a 53% viability in saltwater soil. However, in freshwater soil samples the microbial survival rate at a depth of 1 cm was significantly enhanced compared with the saltwater soil samples. In contrast to other depths, both saltwater and freshwater microbial viability at a depth of 5 cm experienced almost no effect from UV treatment. UV irradiation did affect seawater samples with an observed microbial viability of 59% (Table 6).

Experiment 5: UV irradiation and low pressure (37 W/m^2 and 83.3 kPa for 24 h)

Microbial populations of *E. coli* and *D. radiodurans* were significantly reduced by UV irradiation and low-pressure conditions. UV and low-pressure treatment resulted in less than 1% viability for both surface and 1-cm-deep samples from both saltwater and freshwater soils for both *E. coli* and *D. radiodurans* populations. However, microbial populations at a depth of 5 cm experienced survival rates ranging between 32% and 71% for *E. coli*, and were about 56% for *D. radiodurans*. Microbial populations in seawater samples were affected less by this combined stress, with observed survival rates of 69% and 91% for *E. coli* and *D. radiodurans*, respectively (Table 7).

DISCUSSION

A subzero temperature of −35°C reduced microbial populations of *E. coli* dramatically. The most obvious reason for this observation is the formation of ice crystals that pierced cellular membranes. Other associated challenges to subzero temperatures include mRNA unfolding, decreased affinity of enzymes and proteins for substrates, inaccessibility of organic matter, membranes becoming too rigid and unable to transport solutes from the environment, and inability of cells to maintain membrane fluidity (Nedwell, 1999). Any cellular cold-shock responses may have been too slow to protect the cells.

Low-pressure stress was very efficient in reducing microbial populations. While *E. coli* populations ceased to exist, *D. radiodurans* populations showed remarkably high survival rates (Table 4). Low pressure has been noted to cause changes in the volume of microbial cells, which affect vital physiological and biochemical processes. However, little is known as to how pressure exactly affects microbial viability. Bartlett (2002) suggested that *E. coli* has no SOS response to pressure changes. However, since the pressure difference between the experimental conditions and Earth surface conditions was relatively minor, the largest effect of the reduced pressure might have been the removal of acces-

Table 7. Experimental Viability Results for *E. coli* and *D. radiodurans* After UV Irradiation and Low-Pressure Treatment for 24 h (37 W/m^2, 83.3 kPa)

	E. coli					D. radiodurans				
Soil composition, depth	*Control (cfu/ml)*	*Mean (cfu/ml)*	*Viability*[a]	*SD*	*95% CI*	*Control (cfu/ml)*	*Mean (cfu/ml)*	*Viability*[a]	*SD*	*95% CI*
Saltwater soil										
Surface	2,898	0	0%	± 0 cfu/ml	0%	2,484	2	0%	± 2 cfu/ml	50%
1 cm	3,450	0	0%	± 0 cfu/ml	0%	2,484	3	0%	± 2 cfu/ml	33%
5 cm	3,864	1,232	32%	± 186 cfu/ml	17%	2,898	1,656	57%	± 414 cfu/ml	28%
Freshwater soil										
Surface	3,450	0	0%	± 0 cfu/ml	0%	1,656	11	0.6%	± 5 cfu/ml	46%
1 cm	2,484	0	0%	± 0 cfu/ml	0%	2,484	7	0.2%	± 3 cfu/ml	43%
5 cm	2,897	2,070	71%	± 414 cfu/ml	23%	2,530	1,380	55%	± 239 cfu/ml	20%
Seawater										
Surface	3,419	2,346	69%	± 632 cfu/ml	31%	3,174	2,898	91%	± 414 cfu/ml	16%

Saltwater soil contained 30 g/L of saline water [95% soil and 5% water (30,000 mg/L of NaCl)], freshwater soil contained 0.5 g/L of fresh-water [95% soil and 5% water (500 mg/L of NaCl)], and seawater contained 30 g/L of sea salt water [5% soil and 95% water (30,000 mg/L of sea salt)]. Numbers are given in cfu/ml. CI, confidence interval; SD, standard deviation.

[a]Viability or surviving microorganisms were determined as N/N_o, where N was the number of colony formers of the treated samples and N_o the number of colony formers of the untreated controls.

sible water in the soil, thus decreasing the stability of macromolecules. Without water in the soil, microbial populations may not be able to sustain the stability of chemical bonds to maintain macromolecular structures necessary for vital microbial functions, which would have made *E. coli* more susceptible to low-pressure stress and caused the significant loss of cell viability. Desiccation stress was the likely cause of the decrease in microbial viability rather than the slight to moderate decrease of pressure itself. This is also supported by the observation that viability rates in *D. radiodurans* were much higher because of its known dehydration resistance, which some authors infer to be a by-product of its irradiation resistance (Mattimore and Battista, 1996; Battista *et al*., 1999; Levin-Zaidman *et al*., 2003).

Unexpectedly, the combination of temperature and pressure stress treatment had only a moderate to minimal affect on microbial viabilities for both *E. coli* and *D. radiodurans*. It would be expected that the stresses experienced for single treatment of subzero temperature and low pressure would act cumulatively to reduce microbial survival rates. However, we observed only an average reduction of 33% and 7% in *E. coli* and *D. radiodurans* microbial populations, respectively. One possible explanation for this observation is that both microbial populations responded synergistically to these combined stresses. In addition, the dehydration stress in a low-temperature environment may be less severe if liquid pockets of water are protected by overlying ice layers. It was also observed that microbial populations in freshwater soil samples had higher viability rates than populations in saltwater soil samples. It appears that under these conditions microbial populations may have induced cold-shock responses and were efficiently maintaining the stability of DNA, proteins, and membranes.

UV irradiation was very efficient in reducing microbial populations on the surface and at a depth of 1 cm. Microbial survival rates in saltwater soil were much higher than in freshwater soil, which suggests that the effects of UV irradiation were reduced when solutes were present in the soil water (Table 6). *D. radiodurans* was more resistant to UV irradiation than *E. coli*, which is likely due to its highly effective radiation repair mechanisms and extra copies of its chromosome. UV irradiation mostly affected microbial populations at the surface and to a lesser degree at a depth of 1 cm. The large decrease in microbial viabilities at a depth of 1 cm is somewhat puzzling, since a 1-cm-thick soil cover should have provided sufficient shielding. However, it was observed that the soil samples dried out over the test period, which indicates that the UV irradiation enhanced desiccation stress down to a depth of at least 1 cm, and affected microbial populations adversely. Cockell *et al*. (2000) reported that most cellular UV damage that results in loss of microbial viability is due to damage of DNA. Hence, the surviving microorganism may have utilized UV-resistant responses and water molecules, solutes, and soil as protection. Schulze-Makuch *et al*. (2004) proposed that adaptation to a UV-rich and water-poor environment through directional selection is possible. Common strategies used by terrestrial organisms to cope with UV irradiation include the retreat to a protected environment, such as subsurface habitats, where layers of soil or water for protection are available (Pierson *et al*., 1993; Wynn-Williams and Edwards, 2000). Another interesting strategy is the utilization of organic compounds derived from dead cells for protection from UV irradiation (Marchant *et al*., 1991). Microbial populations are even known to survive space conditions for a limited time when shielded by very thin material (a few micrometers), especially when they are in a dormant spore form (Horneck, 1981; Koike *et al*., 1991; Nicholson *et al*., 2000). However, survival rates, microbial strategies, and radiation doses are different when microbes are in an active state in a soil–water medium on a planetary surface.

UV irradiation and low-pressure stress had a devastating effect on microbial viability rates in soils for both *E. coli* and *D. radiodurans* microbial populations (Fig. 3), especially at the surface and a shallow depth of 1 cm where survival rates for both microbial species were less than 1% (Table 7). About half of the bacterial populations still survived at a depth of 5 cm, but this stress was only applied for 24 h during the combined stress experiment compared with 10 days when testing the low-pressure effect only (Experiment 2, Table 8). Thus, low-pressure conditions and UV irradiation combined took clearly the largest toll on the microbial populations. Even *D. radiodurans* could not withstand these stresses near the surface. Microbial survival rates were significantly higher in a seawater medium (Fig. 4). The combined stresses might have caused the instability of macromolecules such as DNA, proteins/enzymes, and membranes, and neither *E. coli* nor *D.*

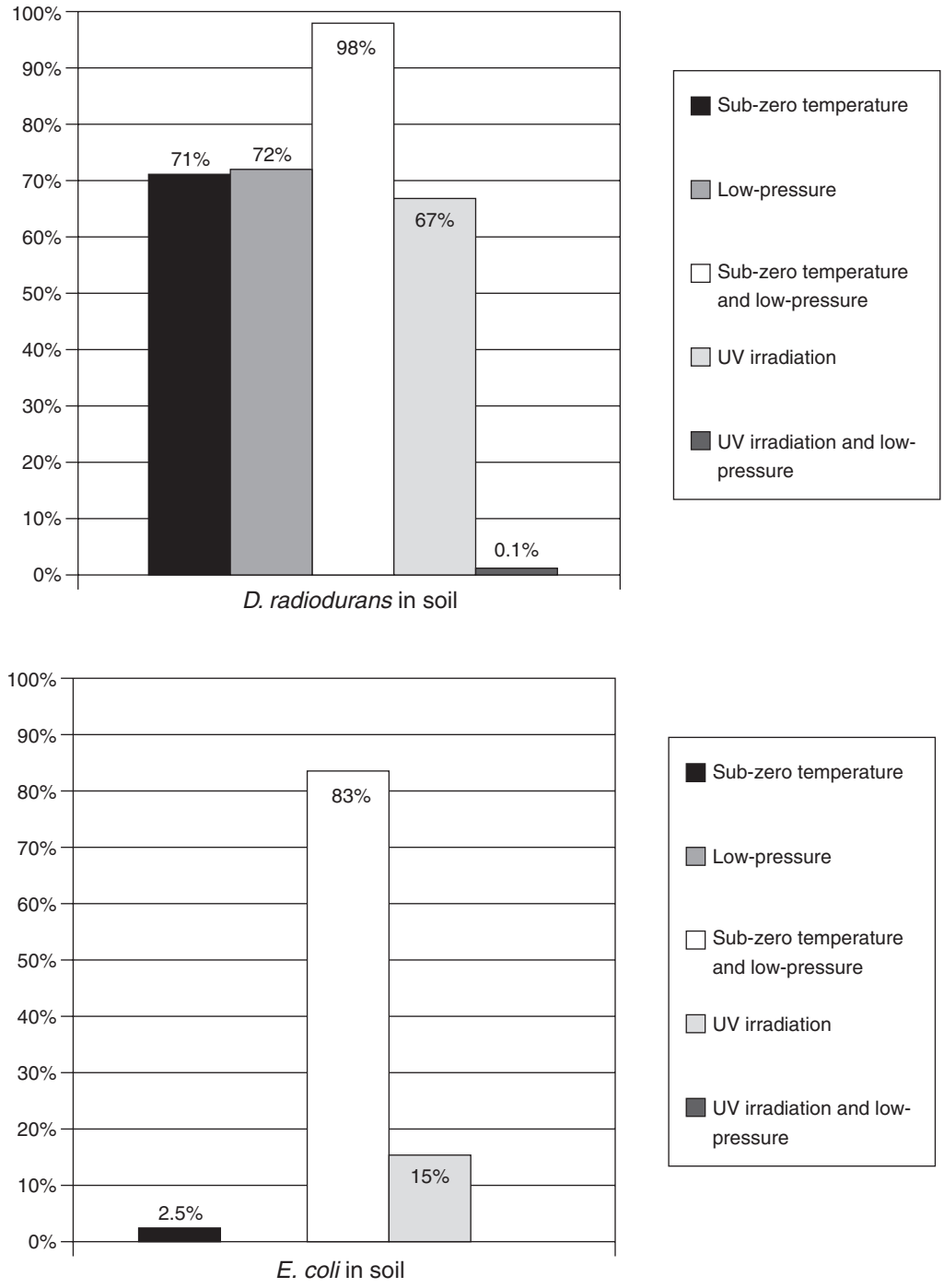

FIG. 3. Comparisons of microbial viabilities gathered after environmental stress treatments in saltwater soil and freshwater soil at a depth of 1 cm for (top panel) *D. radiodurans* and (bottom panel) *E. coli*. Percent viability was gathered from the 1-cm-depth results from both types of soils. Note that the stresses were applied in Experiments 1–3 (subzero temperature, low pressure, and subzero temperature + low pressure, respectively) for 10 days, while in Experiments 4 and 5 (UV irradiation and UV irradiation + low pressure) for 24 h.

radiodurans was able to repair associated damage. UV irradiation may have produced free radicals that attack DNA and microbial membranes, while low-pressure treatment removed any water needed for UV protection and the stability of chemical reactions and chemical bonds such as hydrogen bonds in DNA, proteins, and membranes.

TABLE 8. COMPARISONS OF MICROBIAL VIABILITY IN SOIL AND WATER SAMPLES AFTER TREATMENT WITH ENVIRONMENTAL STRESSES

	Viability of organism in medium					
	E. coli			D. radiodurans		
	Soil				*Soil*	
Treatment	*Depth*	*Viability (%)*	*Seawater*	*Depth*	*Viability (%)*	*Seawater*
Subzero temperature	0 cm	1.5%	20%	0 cm	56%	57%
	1 cm	2.5%		1 cm	71%	
	5 cm	2%		5 cm	64%	
Low pressure	0 cm	0%	73%	0 cm	73%	100%
	1 cm	0%		1 cm	72%	
	5 cm	0%		5 cm	47%	
Subzero temperature + low pressure	0 cm	59%	20%	0 cm	92%	100%
	1 cm	83%		1 cm	98%	
	5 cm	60%		5 cm	89%	
UV	0 cm	1.5%	34%	0 cm	27%	59%
	1 cm	15%		1 cm	67%	
	5 cm	50%		5 cm	97%	
UV + low pressure	0 cm	0%	69%	0 cm	0.3%	91%
	1 cm	0%		1 cm	0.1%	
	5 cm	52%		5 cm	56%	

Soil values are averaged for different salinities (saltwater and freshwater soil) and are noted for 0 cm (surface), 1 cm, and 5 cm depths, respectively. Any viability percentage above 100% was equated to 100% in the calculations. Environmental stresses used in Experiments 1–3 were applied for 10 days, while environmental stresses used in Experiments 4 and 5 (involving UV irradiation) were applied for 24 h.

When comparing microbial viabilities in soil and water (Figs. 3 and 4 and Table 8), it was observed that *E. coli* and *D. radiodurans* microbial populations both significantly improved their chances for survival in the seawater habitat, which indicates that water provides an excellent protection from external stresses. Of course, liquid water as medium also removes the dehydration stress associated with low-pressure conditions and possibly UV irradiation. In general, *D.*

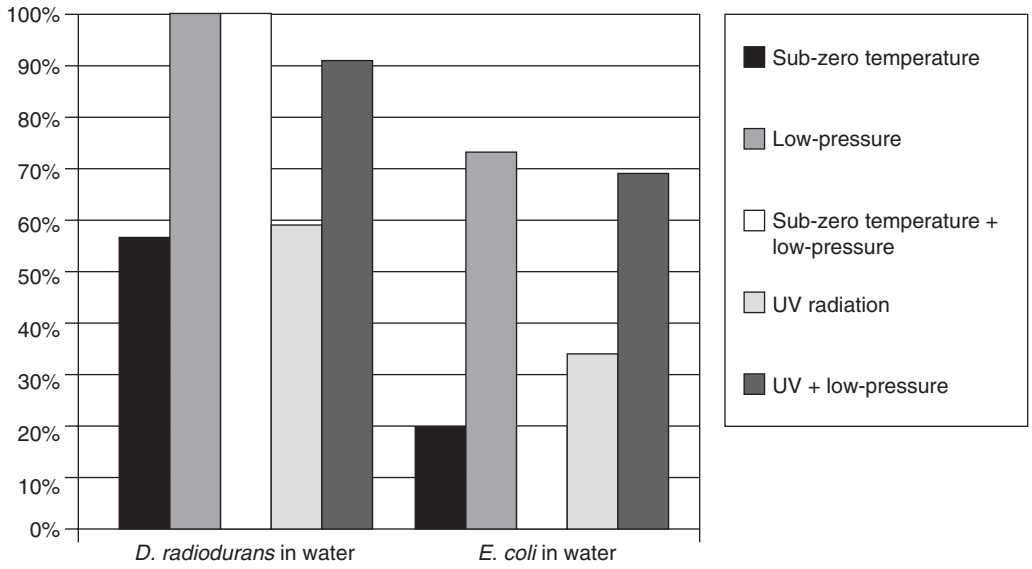

FIG. 4. Comparison of microbial viabilities of *D. radiodurans* and *E. coli* after treatment to environmental stresses in a seawater medium. Stresses were applied in Experiments 1–3 for 10 days, while only for 24 h in Experiments 4 and 5. Samples from seawater were taken at a depth of 1 cm.

radiodurans populations experienced higher survival rates in the saltwater medium than *E. coli*.

We also compared microbial viability rates in saltwater soil with freshwater soil after treatment to environmental stresses. The rationale for using saltwater soil was to simulate and provide martian analogs for saltwater conditions in soils such as ancient seabeds and evaporate deposits (Cockell *et al*., 2000). Rothschild (1990) and Mancinelli *et al*. (1998) proposed that martian evaporates could provide UV protection for organisms. When averaging soil results from all depths, however, no trend was observed whether microbial populations in saltwater soils or freshwater soils had higher viability rates (data not shown). Surprisingly, when averaging only the results from the surface and the shallow depth of 1 cm, for which the environmental stresses were most severe, higher viability rates were mostly observed in freshwater soil (with the noted exception of surface exposure and UV irradiation for both organisms; Table 6). It is likely that a higher salt content increased the osmotic stress for the organisms tested, offsetting any beneficial shielding effect inherent in the higher salt content (Table 9).

Microbial viabilities were measured at the surface and at depths of 1 cm and 5 cm. There was no trend observable between viability rates at the surface and different depths for the environmental stresses of low-temperature and low-pressure conditions (Table 8). Although the low-pressure environment was expected to dehydrate some organisms and add additional stress, it appears that the effect was offset by easily accessible nutrients on the surface at the surface–atmosphere interface. However, the situation was quite different when UV irradiation was added as an environmental stress. The highest microbial survival rates were measured at a depth of 5 cm, and for the surface environment generally very low viability rates were observed (Table 8). Thus, our experiments confirmed that subsurface environments provide a suitable microhabitat that protects microbes from UV protection.

Although our experimental conditions did not simulate martian environmental conditions (Table 1), we did simulate the same major stresses that organisms on or near the surface of Mars would be exposed to. In particular, our tested low-pressure conditions (83.3 kPa) did not reach the low-pressure conditions on Mars (~600 Pa; Table 1). However, the tested microorganisms were already extremely sensitive to low-pressure stresses of 83.3 kPa (Table 4). Thus, we demonstrated the negative effect that low pressure has on *E. coli* and *D. radiodurans* microbial populations, and probably any other Earth organisms intrinsically dependent on the availability of water. The comparison of microbial viability rates between soil medium and salt-water medium strongly indicated that the low-pressure effect was mostly due to dehydration stress. At pressures of about 600 Pa these stresses would be much more dramatic given the sensitivity observed here, and any putative organisms on Mars would be driven much further into the martian subsurface in order to survive. Our UV experimental conditions were roughly comparable to martian environmental conditions, but our testing period was only 24 h due to experimental limitations. The tested low-temperature conditions are in the range of average temperature conditions on Mars, but did not simulate any changes of temperature with time (Table 1).

Table 9. Comparisons of Microbial Viability Near the Surface (0 cm and 1 cm in Depth) of Saltwater and Freshwater Soil Samples After Treatment to Environmental Stresses

	E. coli		D. radiodurans	
Treatment	*Saltwater soil*	*Freshwater soil*	*Saltwater soil*	*Freshwater soil*
Subzero temperature	2%	2%	45%	83%
Low pressure	0%	0%	48%	97%
Subzero temperature + low pressure	58%	84%	92%	98%
UV	17%	0.35%	44%	50%
UV + low pressure	0%	0%	0%	0.4%

Soil values are averaged over surface and 1 cm depths. Any viability percentage above 100% was equated to 100% for these calculations. Environmental stresses used in Experiments 1–3 were applied for 10 days, while environmental stresses used in Experiments 4 and 5 (involving UV irradiation) were applied for 24 h.

Considering the possibility of microorganisms on Mars brought by spacecraft from Earth, any long-term survival on or near the martian surface seems to be unlikely because of the observed sensitivities to UV irradiation and dehydration stress associated with low-pressure atmospheres. Although *D. radiodurans* populations generally exhibited higher viability rates than *E. coli* under the tested environmental conditions, it should be noted that the mesophilic microbe *E. coli*, certainly not known for its presence in extreme environments, showed remarkable survival rates under the tested conditions. Still, whether these organisms or other microbes from Earth would be able to thrive under surface or near-surface conditions for longer periods of time [other than perhaps in a dormant spore-like form as suggested by Schulze-Makuch *et al*. (2005)] is doubtful given the observed sensitivities. In more general terms, this study supports the hypothesis that Earth-like microbial life beneath the surface is much more likely to be the rule than the exception on other worlds, especially on planetary body with thin atmospheres (Schulze-Makuch and Irwin, 2004).

CONCLUSION

We tested responses of *E. coli* and *D. radiodurans* to some of the major environmental stresses that occur on Mars. Chances of survival for both *E. coli* and *D. radiodurans* significantly improved when they were protected in a microhabitat 5 cm below the surface or when they were suspended in liquid water. Desiccation stress associated with low-pressure conditions and UV irradiation eliminated or significantly reduced microbial populations at and near the surface. Given that the experimental conditions did not reach the ambient 600 Pa that exists in the martian atmosphere, the martian low-pressure conditions and consequently very low availability of water would be highly detrimental to any microbial organisms near the martian surface. Survival of microbial organisms may be possible at depths not reached by desiccation stresses advancing from the surface to the deeper soil layers. The outcome of this study suggests that life at the microbial level may be able to survive outside the existence and confines of Planet Earth, if sheltered and protected from the stresses that challenge it. However, planetary surfaces that possess little to no atmosphere and have low water availability (or generally solvent availability) would not be favorable environments for microorganisms.

ACKNOWLEDGMENTS

The JSC-1 Martian stimulant soil was provided by Carlton C. Allen, at the NASA Johnson Space Center, Houston, TX, and the described study was in part supported by NASA grant NAG5-9542. We appreciate the input on earlier versions of the manuscript provided by Susan Childers, Andrew Schuerger, David Gaylord, Kent Keller, and two anonymous reviewers.

ABBREVIATIONS

cfu, colony-forming units; JSC, Johnson Space Center; UV, ultraviolet.

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