ASTROBIOLOGY
Volume 10, Number 7, 2010

DOI: 10.1089/ast.2009.0439

Low-Temperature Ionizing Radiation Resistance of *Deinococcus radiodurans* and Antarctic Dry Valley Bacteria

Lewis R. Dartnell,[1,2] Stephanie J. Hunter,[3] Keith V. Lovell,[4] Andrew J. Coates,[5] and John M. Ward[3]

Abstract

The high flux of cosmic rays onto the unshielded surface of Mars poses a significant hazard to the survival of martian microbial life. Here, we determined the survival responses of several bacterial strains to ionizing radiation exposure while frozen at a low temperature characteristic of the martian near-subsurface. Novel psychrotolerant bacterial strains were isolated from the Antarctic Dry Valleys, an environmental analogue of the martian surface, and identified by 16S rRNA gene phylogeny as representatives of *Brevundimonas, Rhodococcus*, and *Pseudomonas* genera. These isolates, in addition to the known radioresistant extremophile *Deinococcus radiodurans*, were exposed to gamma rays while frozen on dry ice (−79°C). We found *D. radiodurans* to exhibit far greater radiation resistance when irradiated at −79°C than was observed in similar studies performed at higher temperatures. This greater radiation resistance has important implications for the estimation of potential survival times of microorganisms near the martian surface. Furthermore, the most radiation resistant of these Dry Valley isolates, *Brevundimonas* sp. MV.7, was found to show 99% 16S rRNA gene similarity to contaminant bacteria discovered in clean rooms at both Kennedy and Johnson Space Centers and so is of prime concern to efforts in the planetary protection of Mars from our lander probes. Results from this experimental irradiation, combined with previous radiation modeling, indicate that *Brevundimonas* sp. MV.7 emplaced only 30 cm deep in martian dust could survive the cosmic radiation for up to 100,000 years before suffering 10^6 population reduction. Key Words: Ionizing radiation resistance—Antarctic Dry Valley—*Deinococcus radiodurans*—Mars—Planetary protection. Astrobiology 10, 717–732.

1. Introduction

1.1. The McMurdo Dry Valleys and Mars

The climate of Antarctica is very arid, and the continent contains the most extreme cold-desert regions on Earth. The McMurdo Dry Valleys in Southern Victoria Land cover an area of roughly 5000 km^2, at altitudes above 1000 m, and exhibit extensive areas of rock and soil completely devoid of snow or ice (Horowitz *et al.*, 1972). No plant or animal life is visible within the Dry Valleys, but microbial life persists in the ice-covered lakes and desiccated soils and as endolithic communities in translucent rock (Horowitz *et al.*, 1972; Wynn-Williams and Edwards, 2000; Cowan and Tow, 2004). These desert soils constitute one of the most extreme cold habitats on Earth and witness multiple environmental hazards (Vishniac, 1993). In many respects, the Antarctic Dry Valleys are a meaningful analogue of the environmental conditions prevalent on Mars (Horowitz *et al.*, 1972; McKay, 1993; Wynn-Williams and Edwards, 2000).

Water delivery to the Dry Valleys by precipitation is exceedingly low and falls solely as snow; any exposed ice quickly sublimates away into the desiccating katabatic (downslope) winds that descend off the high Antarctic plateau (Horowitz *et al.*, 1972). Lakes (both freshwater and saline) and glacial meltwater streams, however, are found in the Dry Valleys (Vishniac, 1993; Cowan and Tow, 2004). The low precipitation rates and low temperatures mean that transient summer meltwater is often the sole source of moisture in the valley soils. Air temperature ranges between −15°C and 0°C in the summer months and drops to −40°C in the winter. Such low temperatures not only restrict enzyme activity rates and cellular membrane integrity (see recent reviews in Feller, 2003; Chintalapati *et al.*, 2004; Chattopadhyay, 2006; D'Amico *et al.*, 2006) but further limit the

[1]Centre for Mathematics and Physics in the Life Sciences and Experimental Biology (CoMPLEX), University College London, London, UK.
[2]The Centre for Planetary Sciences (CPS) at UCL/Birkbeck, University College London, London, UK.
[3]Research Department of Structural and Molecular Biology, University College London, London, UK.
[4]Department of Applied Science, Security & Resilience, Cranfield University, Shrivenham, UK.
[5]Mullard Space Science Laboratory, University College London, London, UK.

availability of water in liquid state for the hydration of biomolecules and as a solvent for biochemical processes (Wynn-Williams and Edwards, 2000). In addition to the low availability of water, salt concentrations are often high (Vishniac, 1993), which exerts additional osmotic stress on microbial populations and makes the attainment of cellular water energetically demanding (Wynn-Williams and Edwards, 2000). A further environmental factor that limits microbial life is the presence of only low levels of organic material in the soil of the McMurdo Dry Valleys. In fact, the primary source of the soil organic material is not immediately obvious (Vishniac, 1993), given the absence of higher plants (Burkins *et al.,* 2000). This habitat contains only endolithic lichen and cyanobacteria as primary producers (Wynn-Williams, 2000). Additionally, seasonal depletion of stratospheric ozone, which creates the so-called "Ozone Hole" (Farman *et al.,* 1985; Jones and Shanklin, 1995), means that the surface environment of the McMurdo Dry Valleys is exposed to elevated levels of solar UVB radiation (Lubin and Frederick, 1991; Madronich *et al.,* 1998).

The McMurdo Dry Valleys, therefore, combine multiple environmental hazards to the survival of microbial life: cold, desiccation, high salt content, limited availability of organic material, and elevated levels of UV radiation. These emulate many of the harsh characteristics of the martian surface and so provide a valuable research site into microbial survival for the interests of astrobiology.

1.2. Martian ionizing radiation environment

In addition to the aforementioned environmental hazards, the martian surface experiences an ionizing radiation environment significantly greater than that of Earth. The thin martian atmosphere and absence of an ozone shield offer practically no protection against solar UV. This energetic radiation readily photolyzes biomolecules such as amino acids and DNA and inhibits chlorophyll (Cockell, 2000a; ten Kate *et al.,* 2005); bacterial spores lying exposed on the martian surface would be inactivated within minutes (Schuerger *et al.,* 2006). The direct effects of UV are a concern only on the very surface of Mars, however, in that the penetration of UV photons is limited to only a few micrometers (Parnell *et al.,* 2007), and cryptoendolithic bacterial communities could survive within the more clement microenvironment and UV protection of porous rocks, just as they do within the Antarctic Dry Valleys (Cockell, 2000b).

Beyond the penetration depth of UV, the ionizing radiation of solar energetic protons (SEP) and galactic cosmic rays (GCR) dominate in the top few meters of the martian near-subsurface. SEP are accelerated by flares and coronal mass ejections, typically up to energies of several hundred MeV, so the flux is dependent on the 11-year solar activity cycle. The peak flux of GCR particles, at around 500 MeV/nucleon, is about 4 orders of magnitude lower than that of SEP, but the power law tail of the spectra extend up to 10^{20} eV at extremely low fluxes. The GCR spectrum is composed of 85% protons, 14% alpha particles, and a small fraction of heavy ions (fully ionized atomic nuclei, *e.g.,* Fe^{26+}) and is thought to be mainly accelerated by Type II supernovae. GCR below about 1 GeV/nucleon are modulated by the heliosphere (Klapdor-Kleingrothaus and Zuber, 2000), so their flux is anticorrelated with the solar activity cycle. Thus, these two populations of cosmic ray particles are complimentary in nature: SEP possess a high flux but relatively low energy; GCR possess a much lower flux but extend up to very high energy levels. Both, however, vary with the solar cycle.

In the terrestrial atmospheric column, energetic SEP or GCR particles produce extensive showers of propagating secondary particles. When a primary particle strikes an atmospheric nucleus, energetic secondary mesons (pions and kaons), gammas, nucleons, and nuclear fragments are produced, which then decay or interact with other nuclei. Secondary mesons decay over a short timescale to produce muons, gamma rays, and electrons. Thus, the air shower is composed of a central "hard component" core of nuclear fragments within a spreading "soft component" cone of the electromagnetic cascade (Eidelman *et al.,* 2004). With little atmospheric shielding depth, similarly structured cascades occur in the top meters of the martian regolith.

The ionizing radiation field produced by both SEP and GCR is harmful to life (Nelson, 2003) through both direct and indirect mechanisms. Direct damage occurs when deposited energy excites electrons within biomolecules, which leads to ionization or radiolysis. However, radiation primarily interacts with water, as water comprises 40–70% of cells and 20% in bacterial spores, and this creates highly reactive species, such as the hydroxyl free radical or hydrogen peroxide, which then diffuse and attack biomolecules (Baumstark-Khan *et al.,* 2001). The total amount of energy deposited by ionizing radiation in the target material per unit mass is measured in J/kg, or grays (Gy). Different particles of cosmic rays are not equally hazardous to cells. Gamma radiation is weakly ionizing; and, on scales larger than micrometers, the energy of a given dose is deposited uniformly throughout the target. The protons and heavy ions of GCR primary particles and the secondaries generated in hadronic cascades as they interact with matter are, however, highly ionizing. Such high-charge/high-energy particles deposit energy in a dense track that can cause clusters of nearby DNA strand breaks, and so are particularly detrimental to cellular survival.

The biota on the surface of Earth is effectively shielded from the cosmic radiation environment by the combination of charged particle deflection by the geomagnetic field and particle absorption by the thick atmospheric column [1033 g/cm^2 total shielding depth at sea level (COESA, 1976)]. The present martian surface, however, receives no significant radiation protection from either atmospheric shielding or magnetic field deflection. Mars generates no detectable global dipole magnetic field, although the distribution of strong anomalous localized crustal magnetic fields in the ancient southern highlands suggests Mars did once possess an internal dynamo that failed early in the planet's history (Acuña *et al.,* 1998, 1999). The martian atmosphere is also much thinner than the terrestrial shield [16 g/cm^2 at the datum altitude (Simonsen and Nealy, 1993)] and offers no significant shielding of higher-energy SEP or GCR primaries.

While the annually averaged radiation dose delivered to the martian surface by cosmic rays would not be lethal to even radiosensitive terrestrial bacteria (Dartnell *et al.,* 2007a), only metabolically active cells are able to repair radiation damage and reproduce. The current freezing conditions in the martian near-subsurface imply that any extant life will be held dormant for long periods of time, so it is the total dose

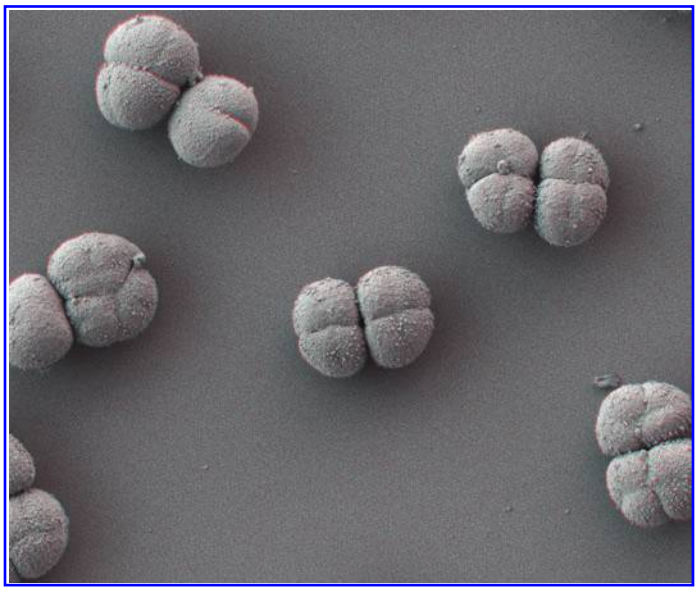

FIG. 1. Scanning electron micrograph of the tetrads of *D. radiodurans* cells. This figure was created as a stereo-pair anaglyph image: viewing the online color version of this figure through red-green stereoscopic glasses will offer a three-dimensional view of the cells (available online at http://www.liebertpub.com/ast). Method: cells grown in liquid culture to late log phase, washed and pipetted onto carbon-coated glass slide and allowed to settle. Sample fixed with glutaldehyde and dried with washes of ethanol and HMDS before gold coated. Left and right eye stereoscopic views obtained with a 5° tilt of the sample stage, and anaglyph composite produced using Adobe Photoshop. Color images available online at www.liebertonline.com/ast.

accumulated over these periods that will be crucial in determining cell survival.

A further consideration for the biological effects of the martian radiation environment is the very low temperature at which cells would be held while they are irradiated and what effect this may have on their survival response. Low temperatures are known to increase resistance to ionizing radiation due to reduced intracellular diffusion of free radicals and thus suppression of the indirect mechanism for biological damage (Baumstark-Khan *et al.,* 2001). Previous studies have looked at the radiation sensitivity of microbes as a function of irradiation temperature. For example, Stapleton and Edington (1956) X-ray irradiated *E. coli* in oxygenated ice and found a 20% increase in radiation resistance at −72°C compared to −12°C and a doubling of resistance at −196°C (77 K). More recently, Sommers *et al.* (2002) found that *Yersinia enterocolitica,* a food-borne pathogen, exhibits an increase in gamma-ray radiation resistance of almost 3-fold at −76°C compared to 0°C. Powers and Tallentire (1968) performed the most comprehensive investigation of the temperature dependency of bacterial spore irradiation survival, which will be treated in the Discussion.

1.3. Desiccation resistance and irradiation survival

There is a further link between the desiccation survival of microorganisms in arid environments, such as the Antarctic Dry Valleys, and survival of the ionizing radiation environment on the martian surface.

The most intense natural radiation environment on Earth is reported to be that of the monazite sand deposits along certain beaches in Brazil, which receive a dose rate of 0.4 Gy/year (Malanca *et al.,* 1993). Terrestrial species such as *Deinococcus radiodurans* can recover from doses 4 orders of magnitude greater than this annual level with no detectable loss in viability, so extreme radioresistance of terrestrial bacteria is therefore not thought to have evolved as an adaptation to the natural radiation environment *per se* (Battista, 1997). Desiccation causes a large number of DNA double-strand breaks, a mode of DNA damage that is also produced by ionizing radiation. *Deinococcus radiodurans* is a mesophilic, non-sporulating, Gram-negative bacterium and the most radiation resistant species known. Figure 1 displays a scanning electron micrograph of the tetrads of *D. radiodurans* cells. Gamma-irradiated populations can survive 5 kGy without measurable loss of viability despite extensive DNA fragmentation (Cox and Battista, 2005), and 1% survival is still found after 10 kGy. The typical gamma radiation survival curve for *D. radiodurans* exhibits a shoulder of resistance to about 5 kGy, after which the population suffers exponential decline with increasing dose exposures (Battista, 1997).

Thus, *D. radiodurans* can survive doses of ionizing radiation substantially higher than that which naturally occur on Earth, and this astounding survival has been linked to their adaptation for desiccation resistance (Mattimore and Battista, 1996). Indeed, many of the same genes are upregulated in *D. radiodurans* during recovery from a sublethal dose of ionizing radiation as are upregulated when reviving after desiccation (Tanaka *et al.,* 2004). This extreme radioresistance is believed to be mediated by several very effective DNA damage recovery mechanisms, including fragment reconstruction that involves multiple genome copies (Minton, 1994; Battista *et al.,* 1999; Zahradka *et al.,* 2006), accumulation of high concentrations of intracellular manganese ions (Ghosal *et al.,* 2005; Daly, 2006), and novel DNA repair enzymes (Cox and Battista, 2005). There is also the growing suspicion that protection of proteins, rather than DNA, subsequent to irradiation is the primary mechanism of extreme radioresistance (Daly, 2009). This link between adaptation to desiccation and radiation survival suggests that bacteria isolated from the arid environment of the Antarctic Dry Valleys could be predicted to exhibit a reasonable level of radiation resistance.

In addition to exhibiting high resistance to ionizing radiation, *D. radiodurans* is also considered to be extremely resilient with respect to many other environmental parameters of vital significance that are inherent to martian surface conditions. This organism is said to be a polyextremophile and is resistant to the hazards of desiccation, UV radiation, hydrogen peroxide, and electrophilic mutagens such as nitrosoguanidine

(Richmond *et al.*, 1999). Members of the *Deinococcus* genus are among the dominant bacteria in mineral soils of the Wright Valley in Antarctica (Aislabie *et al.*, 2006) and are also found in south polar snow (Carpenter *et al.*, 2000). Other organisms have since been discovered to exhibit comparably high radioresistance, such as the actinobacterium *Rubrobacter radiotolerans* (Ferreira *et al.*, 1999) [members of this genus have also been discovered in Antarctic Dry Valley soils (Smith *et al.*, 2006)], the hyperthermophilic archaeon *Thermococcus gammatolerans* (Tapias *et al.*, 2009), as well as other members of the *Deinococcus* genus (Ferreira *et al.*, 1997). It is *Deinococcus radiodurans*, however, that has emerged as the *de facto* radiation-resistant polyextremophilic model organism used in astrobiological studies.

In the present study, we cultured bacterial isolates from the Antarctic Dry Valleys and identified them, using sequencing of the 16S rRNA gene. The fastest-growing strains of these, along with the known radiation-resistant species *D. radiodurans*, were exposed to increasing gamma-ray doses, while frozen at −79°C, to determine their irradiation survival responses under temperature conditions characteristic of the martian surface.

2. Materials and Methods

2.1. Sampling

The Antarctic soil sample utilized in this study was collected from the Miers Valley, within the McMurdo Dry Valleys region, during the Antarctic summer of January 2000. The sample was collected from GPS coordinates 78°05.868′S, 163°47.903′E. The sample was noted to be a surface sediment from a shallow (2–5 cm deep) glacial run-off stream, located 300 m from the western end of Lake Miers (Whiting, 2004). Collection was performed aseptically into an autoclaved polypropylene tube (Nalgene). Throughout sampling and transit, the soil sample was maintained at temperatures below 0°C and stored at −80°C prior to analysis.

For this study, microbes were cultured in October 2006 from pristine surface samples that had remained frozen within polypropylene sampling tubes since collection. Several grams of frozen surface material were removed from the polypropylene sampling tube under sterile conditions and placed into a sterile universal tube. This smaller sample was allowed to thaw slowly overnight at 4°C to emulate the natural warming of Antarctic surface life in the environment and minimize the thermal stress imposed on the microbes. Once thawed, 10 ml of sterile phosphate buffer solution (PBS; Dulbecco's Phosphate Buffer Solution, Sigma) was added to the sample and vortexed briefly to dislodge cells and hold them in suspension.

The likely abundance of culturable bacteria within this soil sample was not known [although Gilichinsky (2002) reports 5.2×10^2 to 6.4×10^4 cells/g for viable aerobes in the Dry Valleys], so a 100-fold dilution series of the buffer was performed. Fifty-microliter volumes of each dilution were spread evenly on plates of quarter-strength nutrient broth agar (¼NB; CM0001 Nutrient Broth, Oxoid; 3.25 g per 1 L deionized water) and quarter-strength Czapek-Dox agar (¼CZD; Difco; 8.75 g per 1 L of deionized water). An extra sterile plate of both medium types was designated as a control and stored with the spread plates to assess contamination. The plates were incubated at 4°C for 15 days, by which point a diverse range of colonies had appeared. Pure isolates were obtained by subculturing individual colonies onto fresh media twice more. Agar plates were harvested by flooding with 5 ml of the corresponding broth, scraping colonies from the surface, and pipetting into an Eppendorf tube. Samples were vortexed to break up the colonies and stored in 20% glycerol solution (final concentration) at −20°C to serve as stocks for further work.

Twenty-nine separate pure cultures were isolated in this way. Of these, three isolates were selected for further investigation due to the morphological diversity of their colonies and the fact that their large colonies identified them as fast growers at 4°C. These three experimental strains were designated as MV.7, MV.10 (both cultured on ¼NB), and MV.27 (cultured on ¼CZD). The well-characterized radioresistant bacterium *D. radiodurans* was also cultured on ¼NB.

2.2. Identification of isolates

The cultured Antarctic soil isolates were identified by 16S rRNA gene sequencing. One hundred microliters of the pure culture glycerol stocks were used to inoculate 5 ml of the appropriate broth in 22 ml universal tubes. They were incubated at 4°C for 16 days with constant agitation until the liquid cultures were turbid. Samples of each (1.5 ml) were extracted and centrifuged (13,000*g*, 5 min) to pellet bacterial cells. The CTAB Chromosomal DNA Preparation protocol (Bailey *et al.*, 1995) was followed to extract genomic DNA from each of these cell pellets.

The bacterial pellet was incubated at 55°C for 30 min in 500 μl of lysis buffer (20 μg/ml proteinase K, 0.5% w/v SDS, in water). Prewarmed CTAB solution (80 μl; 0.7 *M* NaCl, 10% hexadecyl trimethyl ammonium bromide) and NaCl (100 μl, 5 *M*) were then added, and the samples were further incubated for 10 min at 65°C. Isoamyl alcohol:chloroform (1:24, 680 μl) was added and the sample shaken vigorously to form an emulsion. The organic and aqueous phases were separated by centrifugation (13,000*g*, 5 min), and the aqueous phase collected. DNA was precipitated by the addition of isopropanol (0.6 volumes), mixing, and incubation at room temperature for 10 min. Further centrifugation (13,000*g*, 5 min) pelleted the DNA, which was subsequently washed with 70% cold ethanol (v/v) and resuspended in 200 μl molecular biology–grade water (Sigma).

The 16S rRNA gene was amplified by polymerase chain reaction (PCR) with use of the DNA extractions as a template. The primers 27 F (Weisburg *et al.*, 1991) and Un1492R (DeLong, 1992) were used (Table 1). PCR amplification was performed with a Techne TC-512 thermal cycler machine. All reactions were carried out in a 50 μl total volume, comprised as follows: 1 μl template DNA; 5 μl Taq buffer (Sigma); 0.5 μl Taq (5 U/μl; Sigma); 0.5 μl deoxynucleotide triphosphates (dNTPs, 25 m*M* of each; BioLine); 0.5 μl 27 F primer (100 pmol/μl); 0.5 μl Un1492R primer (100 pmol/μl); 42 μl molecular biology–grade water (Sigma). The PCR conditions used were an initial denaturation step of 95°C for 4 min, followed by 25 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. A final elongation step of 72°C for 5 min was used.

After PCR, the products were run on an electrophoresis gel comprised of 1% (w/v) agarose in TBE buffer (90 m*M* Tris borate, 2 m*M* EDTA) at 80 V for 45 min with Hyperladder 1 (BioLine) reference marker to confirm that the

Table 1. Primer Pair Used in PCR Amplification of Bacterial 16S rRNA Gene

Primer	*Sequence 5′–3′*	*Position*[a]	*Reference*
27 F	AGTTTGATCCTGGCTCAG	7–27	Weisburg *et al.* (1991)
Un1492R	GGTACCTTGTTACGACTT	1492–1510	DeLong (1992)

[a]*E. coli* numbering of 16S rRNA gene.

Antarctic samples had indeed been amplified to yield DNA fragments of appropriate length.

Polymerase chain reaction products were excised from the agarose gel electrophoresis under UV illumination. DNA was extracted from the gel slices with use of the QIAquick Gel Extraction Kit Protocol (QIAGEN) by following the manufacturer's instructions. The DNA was stored at −20°C.

The purified PCR products of the 16S rRNA genes were ligated into the pGEM-T easy plasmid vector (Promega). A 10 μl ligation reaction was prepared for each of the DNA samples by using reagents supplied with the vector, that is, 0.5 μl pGEM-T easy plasmid; 5.0 μl ligation buffer; 1.0 μl T4 DNA ligase enzyme; 2.0 μl PCR product; 1.5 μl d H_2O. Ligations were incubated overnight at 4°C.

Competent cells of *E. coli* Top10F′ were prepared for transformation according to Hanahan (1983) and stored in 75 m*M* calcium chloride and 15% glycerol at −80°C, in accordance with Morrison (1977). The ligation product (2 μl) was mixed with 50 μl competent cells and incubated on ice for 20 min. Cells were heat shocked at 42°C for 45 s and returned to ice for 2 min. LB broth (450 μl) was added and the cells incubated for 1.5 h at 37°C, with constant shaking at 150 rpm. Transformants were selected on LB agar plates containing 50 μg/ml ampicillin (Sigma), 80 μg/ml X-gal (Sigma), and 0.5 m*M* IPTG (Sigma). Recombinant *E. coli* colonies (white) were selected and used to individually inoculate 5 ml nutrient broth containing 50 μg/ml ampicillin, which were incubated overnight at 37°C. Plasmid DNA was extracted with QIAprep Spin Miniprep Kit (QIAGEN), in accordance with the manufacturer's instructions.

The plasmids containing the rRNA gene inserts were sequenced with vector-specific primers M13F and M13R (Pearce *et al.*, 2003) to produce end sequences of the 16S genes. Roughly 400 bases of good gene sequence were recovered from each of the ends. These sequences did not overlap; therefore the entire 16S rRNA gene sequence (which is around 1500 bases long, depending on bacterial strain: 1541 for *E. coli;* NCBI sequence ref. J01859) was not obtained. Thus, forward and reverse sequences were treated individually.

Identification of each isolate based on its forward and reverse 16S rRNA gene sequence was confirmed by using both the Ribosomal Database Project, RDP (http://rdp.cme.msu.edu) (Cole *et al.*, 2009), and BLASTn 2.2.21 (NCBI) sequence search utility (www.ncbi.nlm.nih.gov/BLAST) (Zhang *et al.*, 2004). RDP reports the identity of an entered 16S rRNA gene sequence to the genus level, and BLASTn can be used to search for the closest relatives of a sequence, which can also give identification to the genus level.

2.3. Gamma irradiation

The four bacterial strains studied here for their gamma-ray resistance were the three novel strains isolated from the Miers Valley (MV.7, MV.10, and MV.27) and the known radiation-resistant bacterium *D. radiodurans*.

Cell populations were prepared for the irradiation experiment by growing in 200 ml agitated broth appropriate to each strain in 1000 ml conical flasks at room temperature. The extent of cellular radioresistance depends on physiological conditions, such as the growth/irradiation medium and age of the culture (Minton, 1994). Thus, growth of the liquid cultures was followed by measuring the optical density at 600 nm by a spectrophotometer. A 100 ml aliquot was drawn by pipette from the culturing vessel during the mid-late log phase of the growth curve. Regular optical density measurements of the liquid culture were continued to confirm that sampling had indeed occurred during the mid-late log phase.

One-milliliter volumes of this sample were pipetted into 2.0 ml borosilicate clear glass vials (2-CV, Chromacol, purchased through Fisher Scientific; sterilized by autoclaving). These were stoppered with 11 mm polyethylene snap caps (11-PEC1, Chromacol). These polyethylene caps are not thermostable, so they had been sterilized by 5 h exposure to 254 nm UV lamps. The sealed irradiation vials were arranged, widely spaced, in a plastic holder and frozen rapidly in a −80°C freezer. This procedure was designed to emulate the change in environmental conditions of martian subsurface aquifer water being disgorged onto the exposed surface and freezing (although we are not mimicking the reduced pressure).

Two further samples of the growth culture for each bacterial strain were serially diluted with sterile PBS and 100 μl of each dilution spread onto appropriate media plates in duplicate. Colony counts of these plates reveal the cell population at the point of sample freezing and before irradiation and are thus used for the calculation of freeze-thaw survival for each strain.

The radiation exposures were carried out by using the cobalt-60 radionuclide gamma-ray source at Cranfield University, Shrivenham, UK. The glass vials of microbial cultures remained frozen throughout irradiation by fixing them within thin-walled polystyrene boxes and packing dry ice behind them (so as to minimize shielding of the gamma rays). Dry ice, at −79°C, ensures that cellular metabolism and DNA repair are prevented during irradiation and recreates a temperature characteristic of the midlatitude martian surface (Carr, 1996).

Distance from the cobalt-60 rods determines the dose rate that the samples experience, so the sample vials were precisely positioned inside the refrigeration boxes along circular arcs about the cobalt-60 rods. Vials were removed at hourly intervals to produce an increasing series of total dose exposures. To confirm the assumption that cellular survival of irradiation is independent of dose rate at the low temperature of −79°C, the experiment was designed so that the dose

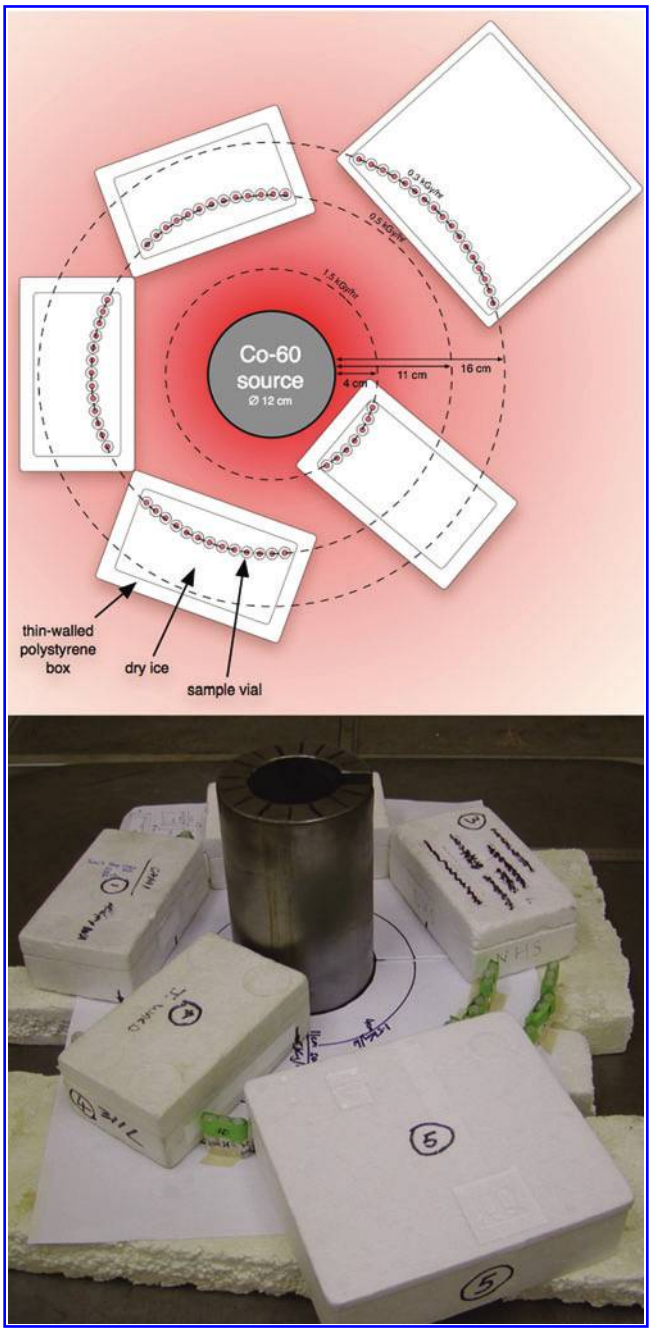

FIG. 2. Top: Scale diagram of the experimental setup for one of the gamma irradiations, showing the concentric dose-rate contours and sample vials positioned along them inside the refrigeration boxes. Bottom: photograph of the exposure setup. Color images available online at www.liebertonline.com/ast.

ranges covered by vials positioned along different dose-rate contours overlapped to allow comparison of data sets.

Dosimetry of the cobalt-60 source was performed periodically with a calibrated ion chamber to confirm the central dose rate and at several distances away, with the dose rate at intervening ranges calculated by interpolation. The accuracy of doses delivered to samples was ±5%, which includes error in the timing of the exposure, positioning of the sample, and dosimetry.

Figure 2 shows a scale diagram and photograph of one irradiation experiment. Data reported here were gathered across two separate irradiation sessions.

After irradiation, each culture vial was slowly thawed, a dilution series performed with sterile PBS, and 100 μl of each dilution spread onto an appropriate media agar plate in triplicate. These plates were incubated at room temperature, and colonies were counted to determine the surviving cell population after that radiation dose. For the very highest doses, and correspondingly high death rates, it was necessary to concentrate the vial contents even more than the neat sample. The 1 ml sample was pipetted from the glass irradiation vial into a sterile microfuge tube and centrifuged at 9000*g* for 2 min. The supernatant was discarded and the cell pellet resuspended by vortex in 100 μl of fresh broth, which thus yielded a 10-fold concentration of the cell sample. In this case, the cell count could only be accomplished in duplicate, with 50 μl of the sample pipetted and spread onto each agar plate.

Control frozen culture vials had also been prepared and were treated identically to the experimental vials with regard to handling, transportation, and thawing. They were not irradiated, however. Colony-counting assays by dilution series and plating (as described above) were performed in triplicate on three control vials for each of the four bacterial strains. These cell counts represent cellular survival after the freeze-thaw procedure but not irradiation, and so provide the initial population number from which irradiation-induced cell death is calculated. This allows the determination of the population survival fraction as a function of gamma-ray dose received.

3. Results

3.1. Identification of isolated strains

The colonies of isolate MV.7 were observed to be bright yellow and shiny, often with well-defined boundaries; MV.10 produced milky-white colonies with a gloopy appearance; MV.27 produced large cream-colored well-defined colonies; and *D. radiodurans* colonies were distinctively bright pink.

Growth experiments performed at room temperature revealed that these isolates are not strict *psychrophiles* [defined as exhibiting maximum growth rate at a temperature lower than 15°C (Rothschild and Mancinelli, 2001)], but *psychrotolerant,* as these fast growers at 4°C actually grew more rapidly to form larger colonies after 14 days at room temperature. Subsequent broth cultures and plated colony counts were thus incubated at room temperature, as this allowed colony counting after 2–4 days rather than 14–15 days when incubated at 4°C.

The 16S rRNA gene sequencing identified the genera of the three novel Antarctic strains as follows: *Brevunidmonas* sp. MV.7, *Rhodococcus* sp. MV.10, and *Pseudomonas* sp. MV.27. These correspond to two bacterial phyla; Proteobacteria (*Brevundimonas* and *Pseudomonas*) and Actinobacteria (*Rhodococcus*). These classifications can be considered to be robust, as they were confirmed by the sequences obtained from both ends of the 16S rRNA gene and by both RDP identification and BLASTn nearest relative search.

Table 2 displays close relatives of these Antarctic isolates from similar low-temperature environments, as identified

Table 2. Table of the Novel Antarctic Species Isolated in This Work, Listed with Close Relatives Discovered in Similar Low-Temperature Environments, as Identified by BLASTn Searches (September 2009) of 16S rRNA Gene Sequences (Both Forward and Reverse)

	Psychrotolerant relatives		
Strain	*Sequence similarity*	*Sampling description*	*GenBank Accession #*
***Brevundimonas* sp. MV.7**	100%	Uncultured; moraine lakes and glacial meltwaters, Mount Everest	DQ675501[a]
	100%	Isolated; La Gorce Mountains soil, Antarctica	DQ351732[b]
	99%	Uncultured; 100K class clean room, Kennedy Space Center	DQ532317[c]
	99%	Uncultured; 10K class clean room, Johnson Space Center	DQ532179[c]
	99%	Psychrotolerant isolate; Qinghai-Tibet Plateau permafrost	DQ108394[d]
	95%	Isolated; beneath John Evans Glacier, high Arctic, Canada	DQ628967[e]
	95%	Isolated; microbial mat from Lake Fryxell, McMurdo Dry Valleys	AJ440993[f]
***Rhodococcus* sp. MV.10**	100%	Isolated; deep-sea sediment; east Pacific	AM111011
	100%	Isolated extremophile; deep-sea sediment	AJ551166
	99%	Uncultured; beneath John Evans Glacier, high Arctic, Canada	DQ228410[g]
	99%	Isolated; Kafni Glacier soil, Himalayas	EF459533
	99%	Isolated; water and soil of Crater Lake, Antarctica	FJ447542
	99%	Isolated; fuel-contaminated Arctic soil, Ellesmere Island	AF230876[h]
	97%	Psychrotolerant isolate; Arctic soil, Finnish Lapland	DQ234488[i]
***Pseudomonas* sp. MV.27**	99%	Isolate; deep sea sediment, Prydz Bay, Antarctica	AM111061[j]
	99%	Uncultured; Antarctic topsoil	AY267367
	99%	Isolated psychrophile; Antarctic deep-sea sediment	AJ519791
	99%	Cold-tolerant isolate; Alpine soil	AY263470[k]
	99%	Isolated extremophile; deep-sea sediment	AJ551160
	99%	Psychrotrophic isolate; Himalayas	AM746975
	98%	Psychrotolerant isolate; Arctic soil, Finnish Lapland	DQ234520[i]

References as follows: [a]Liu *et al.,* 2006; [b]Aislabie *et al.,* 2006; [c]Moissl *et al.,* 2007; [d]Zhang *et al.,* 2007; [e]Cheng and Foght, 2007; [f]Van Trappen *et al.,* 2002; [g]Skidmore *et al.,* 2005; [h]Thomassin-Lacroix *et al.,* 2001; [i]Männistö and Häggblom, 2006; [j]Zhang and Zeng, 2008; [k]Meyer *et al.,* 2004.

by BLASTn search of both forward and reverse sequences. Not all of these related strains shown in Table 2 were successfully cultured as pure isolates by the researchers; many were identified directly from metagenomic environmental samples by PCR and 16S ribosomal gene sequencing. It can be seen that close relatives of the psychrotolerant strains isolated in this work have been identified from a broad range of cold locations, from deep-sea sediments to mountain permafrosts. They also appear to be globally distributed, having been identified at latitudes between the Arctic and Antarctic. This is of interest in terms of environmental microbiology and demonstrates these novel isolates to be members of a truly cosmopolitan group that is distributed globally among diverse cryogenic environments and not limited to the Antarctic locale they were isolated from here. Furthermore, these close similarities to psychrotolerant strains identified from Antarctic locations in previous studies confirm that the isolates cultured here were not lab contaminants.

Figure 3 displays phylogenetic trees of the three novel Antarctic isolates and their relationship to these close relatives from similar low-temperature environments listed in Table 2. Figure 3 was plotted by using the RDP and the reverse sequences of the strains.

Of particular interest is the discovery that one of the strains isolated here, from the Antarctic Dry Valley, *Brevundimonas* sp. MV.7, shows 99% homology of the sequenced 16S rRNA gene to an uncultured bacterium identified within a spacecraft assembly clean room at Kennedy Space Center. The significance of this result to astrobiology will be treated in the Discussion.

3.2. Freeze-thaw survival

Before undergoing gamma-ray exposure, culture samples of the four bacterial strains were frozen to −80°C to emulate the surface temperature conditions on Mars. Colony count assays were performed on samples of both the pre-freeze cultures and control samples that had been frozen and later thawed in an identical manner to the experimental vials, but not irradiated. Thus, comparison of these pre-freeze and post-thaw control populations allows assessment of freeze-thaw survival for each of the four bacterial strains.

Table 3 displays the plate count data for both the pre-freeze cultures and post-thaw control populations for the four bacterial species. The ratio between the means of the pre-freeze population and the post-thaw population yields the survival fraction of the freeze-thaw process.

It can be seen that the Antarctic isolates *Brevundimonas* sp. MV.7 and *Rhodococcus* sp. MV.10 both exhibited excellent survival characteristics of freeze-thaw, maintaining a surviving population of 84.7% and 97.7%, respectively. *Pseudomonas* sp. MV.27 exhibited a one-in-three survival of the freeze-thaw process (33% survival). The freeze-thaw survival of *D. radiodurans* found by our work was marginally greater than unity due to slight experimental sampling noise in the colony counting assays, but it clearly indicates that this hardy environmental bacterium can survive

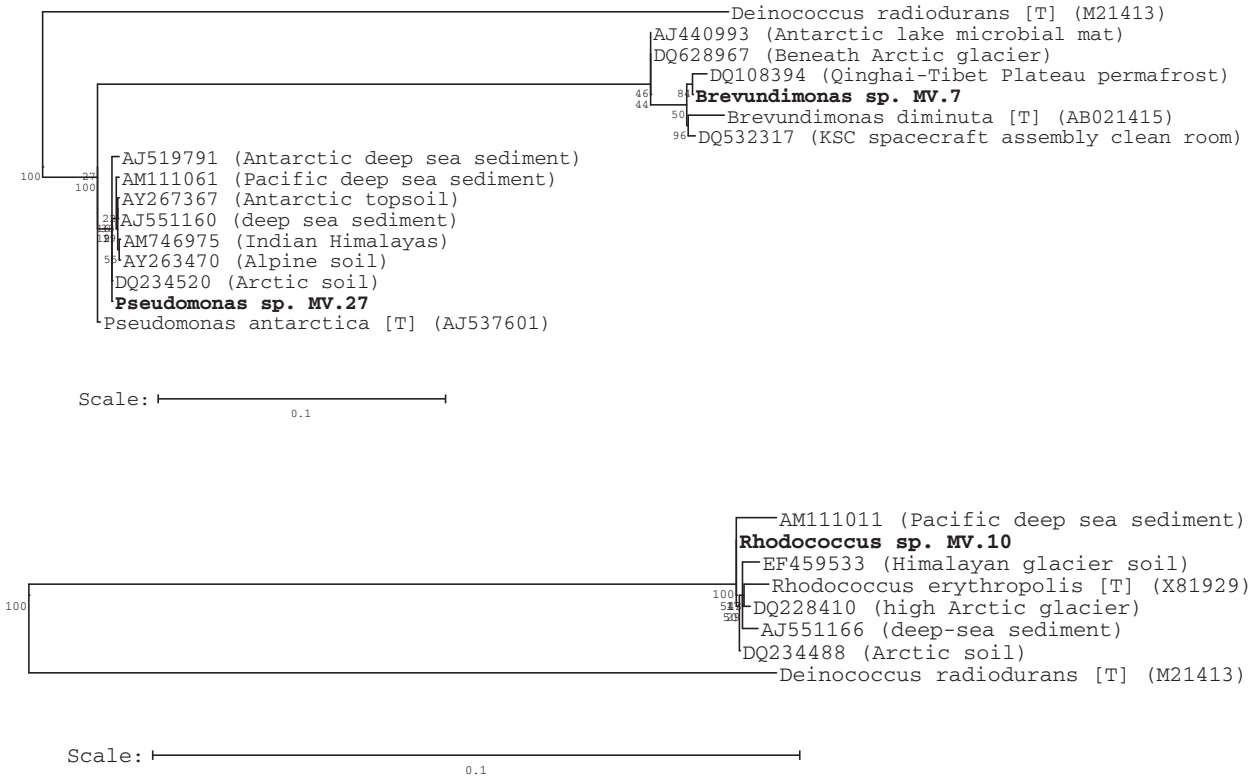

FIG. 3. Phylogenetic trees plotted for the three novel Antarctic bacteria isolated here (bold), closely related strains discovered in similar low-temperature environments, and representative type strains. Top: Proteobacteria phylum (*Brevundimonas* sp. MV.7 and *Pseudomonas* sp. MV.27); Bottom: Actinobacteria phylum (*Rhodococcus* sp. MV.10). Trees plotted using the RDP, with sampling location and GenBank accession numbers of strains given, and type strains indicated with [T]. The outgroup strain used to improve tree topology in both cases was *Deinococcus radiodurans* (GenBank accession M21413) (Weisburg *et al.,* 1989). References for type strains as follows: *Brevundimonas diminuta* (GenBank accession AB021415) (Anzai *et al.,* 2000); *Pseudomonas antarctica* (GenBank accession AJ537601) (Reddy *et al.,* 2004); *Rhodococcus erythropolis* (GenBank accession X81929) (Ruimy *et al.,* 1995).

freezing with negligible loss of population viability. These post-thaw counts of the control samples were the population numbers from which all irradiation survival fractions were calculated.

3.3. Gamma-ray survival

Figure 4 shows the population survival as a function of gamma-ray dose for the four irradiated bacterial strains. The logarithmic survival scale on the *y* axis shows the ratio of viable population number (in colony-forming units per milliliter) after exposure to the given gamma-ray dose to the unirradiated control, and runs from 1 (100% survival) to 10^{-8} (one cell in ten million survival). Error bars are displayed as the minimum and maximum colony count obtained and in general are so small as to be obscured by the datum point itself. The data points are color-coded to the dose delivery rate (*i.e.,* distance from the cobalt-60 source) at which the sample was irradiated. *Deinococcus radiodurans* samples were irradiated at three of these four dose rates, other cells at a subset of two. The lines of best fit (solid lines) are exponential functions, fixed to the initial point (0, 1).

Table 3. Survival of the Freeze-Thaw Process for the Four Bacterial Strains Used in the Irradiation Experiments

Bacterium	*Pre-freeze population count (cfu/ml)*	*Post-thaw population count (cfu/ml)*	*Freeze-thaw survival*
Brevundimonas sp. MV.7	3.00×10^{8}	2.54×10^{8}	84.7%
Rhodococcus sp. MV.10	6.03×10^{7}	5.89×10^{7}	97.7%
Pseudomonas sp. MV.27	7.57×10^{8}	2.55×10^{8}	33.7%
Deinococcus radiodurans	6.89×10^{7}	7.87×10^{7}	114%

The table gives the viable population densities in liquid culture prior to being frozen to −80°C, the thawed post-freeze population counts, and thus the calculated survival rate of the freeze-thaw process for each strain. Displayed population densities are the averaged results of plate counts performed in triplicate. The post-thaw population counts are used as the starting point for the irradiation survival calculations. cfu, colony-forming units.

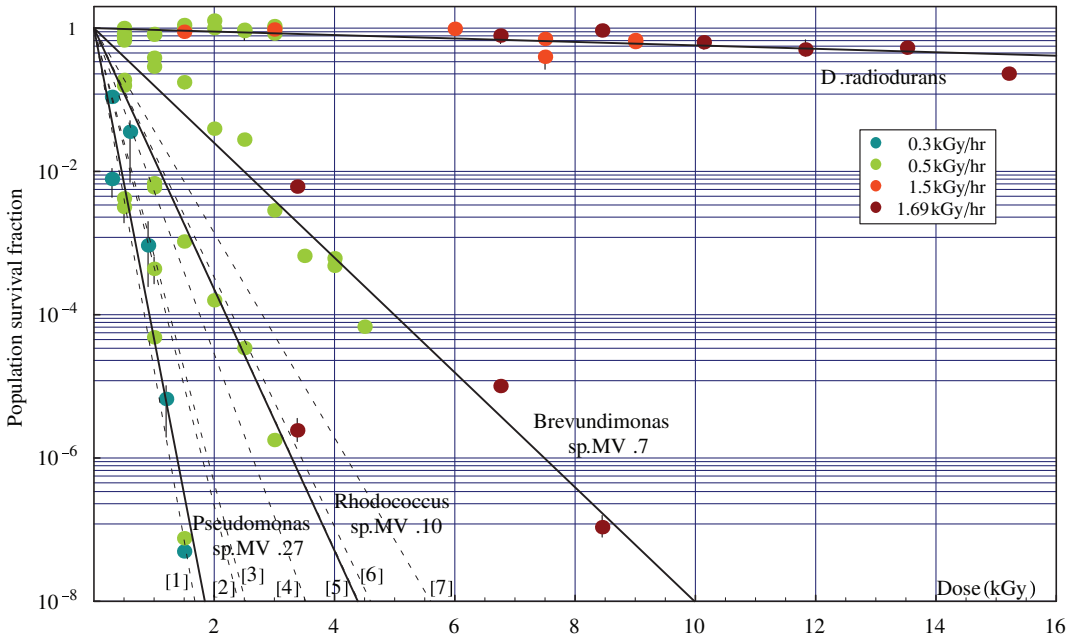

FIG. 4. Gamma-ray survival characteristics of the four irradiated bacterial strains. The dose rate at which each radiation dose was delivered is color coded, as indicated in the key, and exponential lines of best fit are shown (solid lines). Also plotted are similar low-temperature bacterial irradiation survival responses from the literature (dashed lines), labeled as follows: [1] *Aeromonas hydrophilia* (−20°C), Rashid *et al.* (1992); [2] *Vibrio vulnificus* (−20°C), Rashid *et al.* (1992); [3] *Campylobacter jejuni* (−30°C), Lambert *et al.* (1992); [4] *Vibrio parahaemolyticus* (−20°C), Rashid *et al.* (1992); [5] *Yersinia enterocolitica* (−76°C), Sommers *et al.* (2002); [6] *Salmonella typhimurium* (−20°C), Thayer and Boyd (1991); [7] *Listeria monocytogenes* (−20°C), Rashid *et al.* (1992). Color images available online at www.liebertonline.com/ast.

Table 4 shows the fitted parameter, γ, of the exponential dose-effect survival function ($y = e^{-\gamma \cdot x}$) for the three Antarctic bacterial strains irradiated here. The *D. radiodurans* population had yet to reach the shoulder of the survival curve by the maximum total dose available in this experiment and so was not exhibiting exponential decline. The γ parameter corresponds to the gradient of the best-fit line in log-linear space and is known as the sensitivity or inactivation constant of the cell type under irradiation (Powers and Tallentire, 1968). The inverse of this fitted exponent, γ^{-1}, is defined as D_{37} or D_0 and corresponds to the radiation dose required to produce a dose required to produce a 1/e (36.8%) reduction in cell population. At this dose, each cell has, on average, experienced one lethal event, and those that survive must have been balanced by other cells taking two or more lethal events (Cox and Battista, 2005). The D_0 is a standard metric of radioresistance and allows comparison between different bacterial strains or irradiation conditions (Baumstark-Khan *et al.*, 2001). Comparing the D_0 values given in Table 4 shows that the most resistant Antarctic bacterium studied here, *Brevundimonas* sp. MV.7, was more than 5.4 times more resistant to gamma radiation than *Pseudomonas* sp. MV.27. Also shown in Table 4 is the dose required to produce a 10^6 reduction in viable population number, the threshold taken here as complete population inactivation of an environmental sample.

Table 4. Gamma Ray Survival Characteristics Determined for Each of the Three Antarctic Bacterial Strains

Strain	*Survival exponent, γ*	*D_0 (kGy)*	*10^{-6} survival (kGy)*
Brevundimonas sp. MV.7	1.845	0.542	7.49
Rhodococcus sp. MV.10	4.199	0.238	3.29
Pseudomonas sp. MV.27	9.990	0.100	1.38

The three Antarctic bacterial strains: γ, the fitted exponent of the irradiation survival response, of the form $y = e^{-\gamma \cdot x}$; D_0, the dose necessary for a 37% viable cell number reduction; and the dose necessary to produce a million-fold population reduction.

As expected, Fig. 4 shows *D. radiodurans* to be by far the most radiation resistant of the four strains tested here; and, after a dose of 10 kGy, which reduced all the Antarctic bacteria populations to less than 10^{-8} of the initial population, the *D. radiodurans* population still exhibited almost total survival.

This study also found *D. radiodurans* to exhibit a far greater degree of radiation resistance when irradiated at −79°C than when chilled on ice during irradiation (*e.g.*, Battista, 1997; Ferreira *et al.*, 1997; Richmond *et al.*, 1999; Chen *et al.*, 2004; Daly *et al.*, 2004; Omelchenko *et al.*, 2005).

4. Discussion

4.1. Psychrophily and freeze-thaw survival

The discovery that the bacterial strains isolated here from the very cold Dry Valley environment grow more quickly at room temperature than at 4°C, and are thus not strict psychrophiles, concurs with the findings in previous studies. Gilichinsky (2002) also reported that the number of viable aerobic bacteria cultured from Antarctic Dry Valley sands peaks at 20°C, roughly a factor of 10 higher than at either 4°C or 30°C. Vishniac (1993) noted that psychrophily is not

required for survival in Dry Valley habitats and, contrary to the case for constant cold environments such as the deep sea, may in fact be disadvantageous in the surface soils due to the extreme temperature fluctuations driven by insolation and wind.

Table 3 shows that the Antarctic isolates *Brevundimonas* sp. MV.7 and *Rhodococcus* sp. MV.10 exhibited excellent survival of the experimental freeze-thaw process. This behavior may be expected, as freeze-thaw is a regular occurrence during the Antarctic summer diurnal cycle, although over a longer timescale than the rapid-freeze conditions used in this protocol. Similar experiments have found negligible mortality from freeze-thaw of *Exiguobacterium antarcticum* DSM 14480, isolated from a lake microbial mat in the McMurdo Dry Valleys (Vishnivetskaya *et al.,* 2007); a 40–60% survival in another *Pseudomonas* species, *Pseudomonas paucimobilis,* isolated from north-eastern Colorado soil exposed to repeated freeze-thaw cycles during winter (Morley *et al.,* 1983); a 16% survival of a mixed culture derived from soil samples collected from a Chinook wind zone; and 0.5% survival exhibited by *E. coli* (Walker *et al.,* 2006).

4.2. Use of gamma radiation

As noted in the Introduction, the cosmic ray environment in the martian near-subsurface, in contrast to the ionizing radiation field of the terrestrial surface, is characterized by the highly ionizing SEP and GCR primaries and their secondary showers.

Previous attempts to expose biological samples to such a complex mixed radiation field, spanning a wide energy range, have involved either launching very limited samples into low Earth orbit [such as the Long Duration Exposure Facility (Horneck *et al.,* 1994) or BIOPAN (Horneck *et al.,* 2001)] or irradiating with an accelerated ion beam at a high-energy particle accelerator. This second, Earth-based option still only emulates a small component of the complete cosmic ray environment, with a very narrow particle beam, and experimental time at appropriate facilities is tightly limited. Available radionuclide sources of ionizing particles, such as alpha emitters, are generally limited in the particle fluence produced and number of samples that can be exposed at one time; furthermore, alpha particles (helium nuclei accelerated to only around 5 MeV) have a penetration of just a few centimeters in air.

Consequently, a source of ionizing radiation very commonly used as a good compromise in space radiobiology studies is gamma rays [*e.g.,* gamma-ray data used in recent studies including Clark *et al.* (1999), Richmond *et al.* (1999), Kminek *et al.* (2003), Kminek and Bada (2006), Pavlov *et al.* (2006)], as this offers a high flux, high penetration, and practicality of experimentation, even though it is acknowledged that the ionization it causes does not fully recreate the effects of accelerated ions. Furthermore, the dose deposited in the martian subsurface by weakly ionizing secondaries of the soft core of the cosmic ray cascades, such as muons, electrons, and gamma rays, begins to dominate by 2 m depth and is particularly significant in a water-ice column rather than regolith material due to the much lower abundance of heavy nuclei (Dartnell *et al.,* 2007b). Thus, this approximation of the cosmic ray field by gamma irradiation becomes better with increasing depth underground or icy surface material.

4.3. Radiation resistance

In Fig. 4 it can be seen that, for all four bacterial strains, the color-coded data points all lie along the same response line. This indicates that at −79°C the cellular survival response is independent of the dose rate at which the total gamma-ray dose was delivered. This is especially clear in the *D. radiodurans* data, with survival results collected over a broad range of dose rates (0.5–1.69 kGy/h) all clustering tightly along the same line. Cells that are rendered metabolically inactive by freezing cannot repair cellular damage during the course of the irradiation, so it is only the final accumulated dose, and not the rate at which it is delivered, that determines subsequent survival rates.

Figure 4 also shows the measured survival, as reported in the literature, of bacteria from similar experiments in which irradiation was performed on samples in a frozen state (from −20°C to −76°C). Little work has been published on the gamma-ray exposure survival of non-sporulating bacteria irradiated at very low temperatures (*i.e.,* down to −79°C used in this study), and much of this has been performed in the interests of food decontamination (*e.g.,* see review in Farkas, 1998). It can be seen that, while the irradiation survival responses of *Rhodococcus* sp. MV.10 and *Pseudomonas* sp. MV.27 lie within the range of non-radioresistant strains, *Brevundimonas* sp. MV.7 exhibits a far higher degree of radiation resistance.

This study also found an enhanced radiation resistance of *D. radiodurans* exposed at low temperature.

4.4. Enhanced radiation resistance at low temperatures

Figure 5 displays a combined plot of the gamma-ray survival characteristics of *D. radiodurans* determined by several different studies. Included are results of experimental irradiation at −79°C from the present work and another recent irradiation study performed on dry ice (Richmond *et al.,* 1999) (solid lines), alongside several irradiation experiments performed with the *D. radiodurans* samples at room temperature or chilled on ice so as to suppress cellular metabolism and DNA repair during irradiation (dashed lines).

Figure 5 shows that there is notable variability in the observed dose-survival curve of the same organism, *D. radiodurans,* in this case, when irradiated in an unfrozen state (dashed lines). The radioresistance of microorganisms is known to vary, depending on both growth conditions, such as oxygen, nutrient abundance, and growth phase upon irradiation (Minton, 1994; Venkateswaran *et al.,* 2000; Cox and Battista, 2005), and irradiation conditions, such as abundance of oxygen, temperature, and dose rate [reviewed recently in Baumstark-Khan *et al.* (2001)]. Despite variability in the measured survival response, however, Fig. 5 shows that the crucial metric, the accumulated radiation dose that produces a 10^6 reduction in surviving microbial population, deviates by only 60% between the minimum response listed here (Omelchenko *et al.,* 2005) and the maximum (Battista, 1997). It is worth noting that, in all the *D. radiodurans* irradiation experiments displayed in Fig. 5, the major difference in the observed survival characteristics is the dose at which the viable population begins to decline: the shoulder of the survival curve. Beyond this threshold, the gradients of the exponential declines (the inactivation constant, γ) are observed to be roughly equivalent.

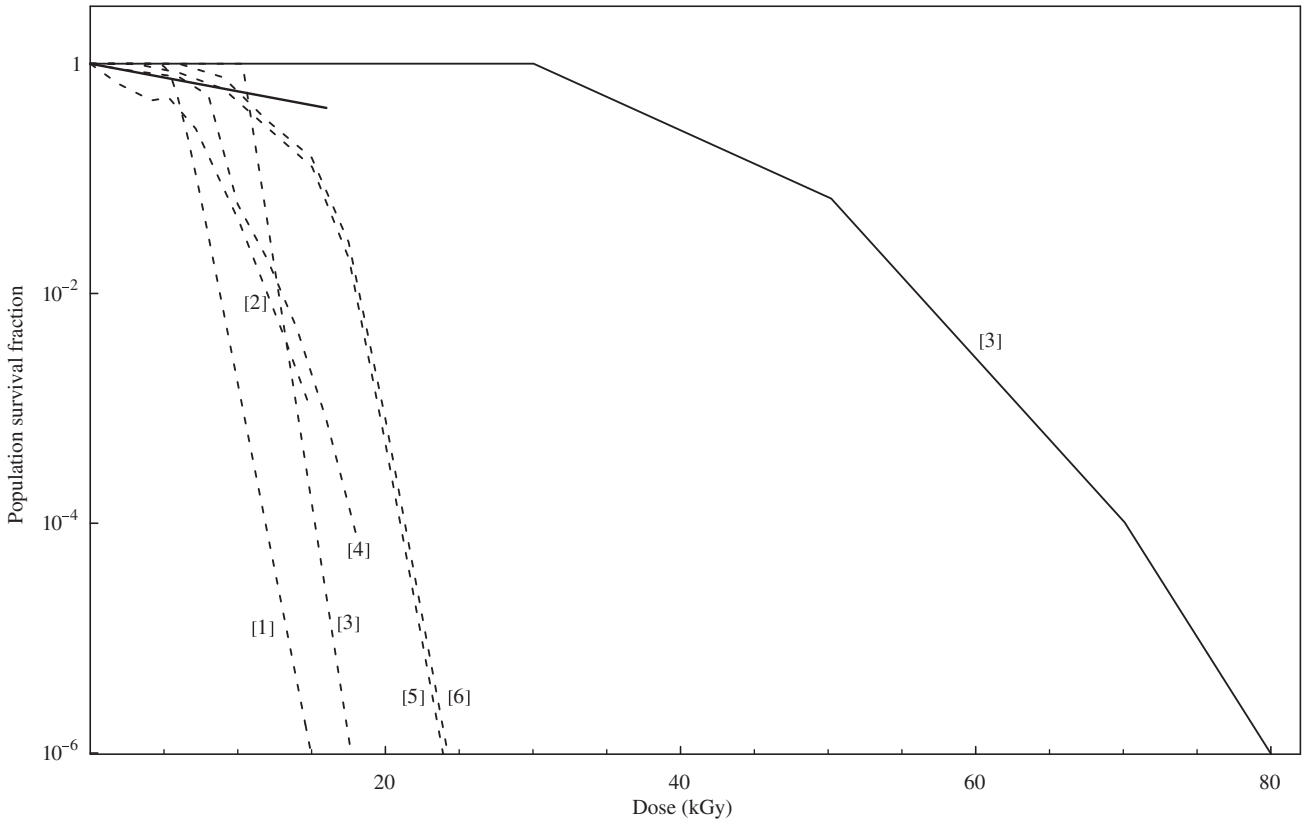

FIG. 5. Plot showing collated results from several published gamma-ray exposure experiments on *D. radiodurans* survival. Two studies, including this present work (bold line), performed at −79°C are shown (solid lines), alongside several irradiation experiments performed with *D. radiodurans* at room temperature or chilled on ice (dashed lines). Published studies numbered as follows: [1] Battista (1997); [2] Ferreira *et al.* (1997); [3] Richmond *et al.* (1999); [4] Chen *et al.* (2004); [5] Daly *et al.* (2004); [6] Omelchenko *et al.* (2005).

Regardless of this experimental variability, however, the clear observation is that *D. radiodurans* survival when irradiated at −79°C is substantially higher than it is at room temperature or when chilled on ice. This current study probed survival up to a maximum dose of 15.2 kGy and found only a slight decrease in *D. radiodurans* viable population. Richmond *et al.* (1999) irradiated *D. radiodurans* populations frozen at −79°C up to 90 kGy and found the shoulder in the survival curve to occur at around 30 kGy with the 10^{-6} survival threshold reached by 78 kGy.

The gamma-ray exposure facility used in our study could provide a maximum dose rate of 1.69 kGy/h, which is achievable when the refrigerated sample vials are positioned right up against the housing of the cobalt-60 rods, at 3 cm distance. Previous modeling work has found that the dose deposition rate from GCR beneath 30 cm of dry martian soil is around 0.065 Gy/year (Dartnell *et al.*, 2007b). Thus, the maximum dose rate achievable by this irradiation exposure, 1.69 kGy/h, represents a delivery rate over 2×10^8 times faster; every second of experimental irradiation at the 1.69 kGy/h contour recreates 7 years of exposure in the martian surface. However, exposing the dry-ice refrigerated samples to a total dose of 90 kGy, as reported by Richmond *et al.* (1999), would have required over a 50 h exposure, which was impossible in our case.

This enhanced survival is due to temperature-dependent processes in radiochemistry. Radiation-induced degradation of biomolecules occurs through both direct and indirect mechanisms: ionization from a particle hit (dominant in dry irradiation) and attack from diffusible free radicals generated by the radiolysis of water (dominant in dilute aqueous solution), respectively (Hutchinson, 1963). Both direct and indirect radiolysis are relevant with regard to the potential for microbial survival in the subsurface martian permafrost. Under gamma irradiation at room temperature, roughly 80% of DNA damage is caused indirectly by irradiation-induced diffusible reactive oxygen species (Ghosal *et al.*, 2005). These are created by radiolysis of water molecules and within a cell diffuse a distance on the order of 3–5 nm from their generation site to attack biological molecules (Hutchinson, 1963).

While the direct mechanism of ionizing radiation damage is independent of temperature, the mobility of different free radical species through the target substrate is temperature dependent. Electron spin resonance spectroscopy studies have shown that radiolytic free radicals such as OH and O_2H are stable in ice at 77 K (−196°C) and do not diffuse to recombine or attack substrate molecules. The diffusion of these free radicals becomes significant above 120 K. The hydrogen atom, H, is only stabilized at 4.2 K and retains high mobility and, consequently, a short lifetime at 77 K (Henriksen, 1962). Thus, below 77 K only H is free to diffuse and attack substrate molecules; above about 120 K other free radicals generated by radiolysis of the water solvent are free to diffuse and chemically attack molecules they encounter, which adds

to the damage inflicted by direct radiation action (see Ferradini and Jay-Gerin, 1999, in French, for a recent review). A comprehensive study of the temperature dependence of ionizing radiation survival was performed on a species of sporulating bacterium (Powers and Tallentire, 1968). Powers and Tallentire (1968) exposed *Bacillus megaterium* spores to X-rays across a great range of temperatures (5–300 K) and calculated the population inactivation constant (the gradient of the exponentially declining dose-survival plot) at each temperature. They found that, at very low temperatures, the inactivation constant, and thus the radiosensitivity of the spores, was independent of temperature, with cell death being mediated predominantly by the direct mechanism of ionizing radiation damage. Above 125 K, however, radiosensitivity increases linearly with temperature due to increasing diffusion of radiogenic free radicals (Powers and Tallentire, 1968). At the irradiation temperature used in this study, −79°C (194 K), radiolytic radical species such as H, OH, and O_2H are mobile but still restricted in their diffusion by the low temperature. By comparison, temperatures of the martian midlatitude (±60°) near-subsurface regolith during summer are steady at the diurnal mean of about 210–220 K, whereas, during winter, polar temperatures drop to 150 K (Carr, 1996).

With regard to the present study and that of Richmond *et al.* (1999), the ramifications of these findings of enhanced irradiation survival of *D. radiodurans* frozen at −79°C, due to restricted free radical diffusion as explained above, are important for astrobiology as it pertains to Mars. Several recent publications (*e.g.,* Mileikowsky *et al.,* 2000; Pavlov *et al.,* 2002; Dartnell *et al.,* 2007a) have attempted to assess the likely survival times of bacteria emplaced in martian meteorites or the planetary surface by combining modeling results on the cosmic radiation environment and experimental results on the radiation dose survival response of different microorganisms, including *D. radiodurans*. Mileikowsky *et al.* (2000) used gamma-ray survival data for *D. radiodurans* presented in Minton (1994); Pavlov *et al.* (2002) and Dartnell *et al.* (2007a) used the representative *D. radiodurans* survival response reported in Battista (1997). The results discussed here, however, suggest that the enhanced radioresistance conferred by irradiation at low temperatures is significant for the survival of *D. radiodurans* on Mars. The published data collated in Fig. 5 indicate that *D. radiodurans* held frozen at −79°C, a temperature characteristic of the martian surface, suffers a 10^6 population decrease after an accumulated gamma-ray dose of between three and five times higher than it does under conditions of irradiation while merely chilled to suppress cellular metabolism. This temperature dependency is expected to be more significant under weakly ionizing gamma irradiation than it would under highly ionizing accelerated ion irradiation because most of the DNA damage induced by gamma irradiation at room temperature is caused indirectly by diffusible reactive oxygen species (Ghosal *et al.,* 2005) that are suppressed at lower temperatures. As already discussed, the radiation field in the martian subsurface becomes dominated by weakly ionizing secondaries at greater depth, especially in water-ice shielding material.

The temperature dependency is much less significant in the irradiation survival of dormant bacterial spores, such as those of *Bacillus* sp., as they are internally desiccated and their DNA well protected from diffusible free radicals (Setlow, 1995). Powers and Tallentire (1968) found only 36% increase in inactivation constant between 125 K and 300 K for the bacillus spores they studied.

Future authors assessing cellular survival of irradiation at low temperatures should be aware of this temperature dependency.

The three novel Antarctic bacterial strains isolated here were irradiated in this study only at the low temperature of −79°C. The expectation would be that these cells also exhibit greater radiosensitivity if exposed while chilled on ice or at standard growth temperature. Such an irradiation survival experiment was not performed in this current study, however, as (i) higher temperatures would not be characteristic of the martian midlatitude near-subsurface conditions of interest to astrobiology, and (ii) complete cessation of cellular metabolism and repair of radiation-induced damage during long exposures cannot be guaranteed in unfrozen samples.

4.5. Implications for microbe retrieval and planetary protection

The radiation survival experimentation performed here on the novel isolate *Brevundimonas* sp. MV.7 is of particular relevance. This was the most radiation resistant of the three novel strains to be tested, and Table 2 shows this bacterium from the Antarctic Dry Valleys to share 99% 16S rRNA gene sequence similarity (based on 440 nucleotide sequence) with an uncultured bacterium (Genbank accession number DQ532317.1) identified from the microbial community within a clean room used for spacecraft assembly at Kennedy Space Center (KSC) (Moissl *et al.,* 2007). The sampling location, designated KSC-4 by the authors, was a $1\,m^2$ area of floor by the bay doors of a 100K classification clean room (certified to less than 100,000 particles of size $\geq 0.5\,\mu m/ft^3$) within the KSC Payload Hazardous and Servicing Facility (KSC-PHSF). Bacteria of the phylum alphaproteobacteria, like *Brevundimonas* sp. MV.7, were discovered to constitute over a third of the clones identified at this sampling location. *Brevundimonas* sp. MV.7 also shares 99% sequence similarity with clones detected at other locations on the floor in the same facility (DQ532298.1 sampled from location KSC-2 and DQ532304.1 from KSC-3) (Moissl *et al.,* 2007). Furthermore, *Brevundimonas* sp. MV.7 also shares 99% sequence similarity with a clone (Genbank accession number DQ532179.1) sampled from the geographically distant Johnson Space Center Genesis Curation Laboratory, identified from the subfloor of a more stringent, class 10K, assembly clean room. The authors note that, in terms of desiccation and nutrient availability, the artificially maintained environments within such clean rooms are "extreme" in the context of microbial survival (Moissl *et al.,* 2007), so it is not surprising that strains surviving there are the same as those isolated from the Antarctic Dry Valleys in the work reported here.

The significance is that this species of *Brevundimonas* was discovered by the research here to survive in the cold, desiccating, environment of the Miers Valley, an analogue site for the martian surface, and is also a contaminant of spacecraft construction clean rooms. There is the potential, therefore, for this organism to be inadvertently transported to the surface of Mars aboard a lander probe despite the best efforts of planetary protection protocols to prevent the forward contamination of extraterrestrial environments with

terrestrial microorganisms (NASA *Planetary Protection Provisions for Robotic Extraterrestrial Missions,* 2005). The Phoenix lander and the Mars Exploration Rovers Spirit and Opportunity were assembled for final launch testing in the KSC-PHSF (Kasthuri Venkateswaran, Biotechnology and Planetary Protection Group, Jet Propulsion Laboratory, personal communication). Mission failure during the landing sequence could emplace contaminant cells sufficiently far beneath the surface for protection from rapid inactivation by solar UV or the flux of SEP, significant in only the top tens of centimeters of subsurface (Mileikowsky *et al.,* 2000; Dartnell *et al.,* 2007a). Even a successfully landed, but imperfectly sterilized, probe could shield bacteria on its underside or deposit them in a shaded enclave for them to persist for significant periods (Moores *et al.,* 2007; Osman *et al.,* 2008) or be widely redistributed and buried by dust storms.

This research assessed the ionizing radiation survival characteristics of this Antarctic Dry Valley isolate. Under irradiation conditions representative of the martian subsurface temperature, *Brevundimonas* sp. MV.7 was found to suffer a 10^6 population decline after exposure to an accumulated dose of 7.49 kGy. The 10^{-6} threshold used here to denote the sterilization of a bacterial population by ionizing radiation is not an arbitrary value. If martian microbial life is indeed similar to that surviving in terrestrial analog sites such as the Antarctic Dry Valleys, a 10^6 population inactivation is a functionally meaningful threshold for population survival in the martian near-subsurface. The justification is as follows.

The Dry Valleys constitute one of the harshest dry environments on the planet and are generally considered to be able to support only low microbiological populations (Vincent, 1988; Vishniac, 1993; Wynn-Williams and Edwards, 2000). Gilichinsky (2007) reported a total cell count (using epifluorescent microscopy) of only 10^3 to 10^4 cells/g dry weight (d.w.) from the surface layer of the Antarctic Dry Valleys, increasing to 10^5 to 10^6 cells/g d.w. in the underlying permafrost 1.5–3.6 m deep, of which only ~0.1% could be successfully cultured. Vorobyova *et al.* (1997) collated information from a number of studies that report total counts of 10^7 to 10^8 cells/g d.w. from Antarctic permafrost sediments and 10^7 to 10^9 cells/g d.w. from Arctic permafrost sediments (>300 m depth), again finding a very low percentage of viable culturable cells. Cowan *et al.* (2002) found total population numbers in the Dry Valleys consistent with this, using a bioluminescent ATP method: 5×10^7 to 4×10^8 cells/g wet weight (w.w.) in the surface mineral soil of Miers Valley, decreasing to 5×10^5 to 8×10^6 cells/g w.w. 25 cm deep in permafrost in Taylor Valley. The total cell count has been observed to be several orders of magnitude higher in Arctic than Antarctic permafrost, but the Antarctic Dry Valley environment is considered to be a better analogue of the martian surface due to the lower temperatures and desiccating environment (Gilichinsky, 2007).

Although unambiguous detection of extinct martian microbes through relic biosignatures would be profound, even more significant would be the opportunity to characterize living cells, either *in situ* or from a sample return mission. Indeed, the Viking landers relied upon culturing metabolically active cells for the Gas Exchange (Oyama, 1972) and Labeled Release (Levin and Straat, 1976) experiments of their life-detection package. Assuming an estimate for surface-dwelling culturable microorganisms of the order of 10^3 to 10^4 cells/g, as above, the population survival threshold of 10^{-6} selected here corresponds to a single surviving cell per 100 g to kilogram of topsoil or regolith rock. Considering the constraints on life-detection instruments [the Viking Gas Exchange and Labeled Release experiments used a soil sample of only a few grams (Oyama, 1972; Levin and Straat, 1976)] or sample return capabilities [a future Mars Sample Return mission is currently envisioned to deliver only between 0.5 kg and a few kg of martian surface material (Wercinski, 1996; O'Neil and Cazaux, 2000; Mattingly *et al.,* 2004)], this population survival threshold represents a meaningful cutoff between the potential recovery or non-recovery of viable cells capable of being roused to a metabolically active state.

5. Conclusions

Combining the findings of the experimental irradiation reported here with the results of our previous modeling of the martian surface and subsurface ionizing radiation environment (Dartnell *et al.,* 2007b) allows the assessment of the likely survival time of a microbial population to cosmic rays in the martian near-subsurface. The radiation model calculates that GCR deliver a dose of around 0.065 Gy/year beneath 30 cm of dry martian soil (Dartnell *et al.,* 2007b), a depth at which cells would not experience rapid deactivation by solar UV or SEP flux. Comparing this with the experimental irradiation results reported here indicates that, under a martian surface temperature of −79°C (194 K), an indigenous martian or terrestrial contaminant cell population of *Brevundimonas* sp. MV.7 could persist at a shallow depth of only 30 cm for up to 117,000 years before experiencing 10^6 inactivation. Species of *Deinococcus* were also discovered in all three spacecraft-associated clean room facilities sampled by Moissl *et al.* (2007). In the event a population of radiation-resistant *Deinococcus* cells were present or delivered to a 30 cm depth in the martian subsurface, they could persist for 1.2 million years before experiencing 10^6 inactivation.

Acknowledgments

L.R. Dartnell was supported by an EPSRC Doctoral Training Centre studentship. The authors thank the two anonymous peer reviewers whose comments helped improve the quality of this paper.

Author Disclosure Statement

No competing financial interests exist for any of the authors.

Abbreviations

¼CZD, quarter-strength Czapek-Dox agar; ¼NB, quarter-strength nutrient broth agar; d.w., dry weight; GCR, galactic cosmic rays; KSC, Kennedy Space Center; KSC-PHSF, KSC Payload Hazardous and Servicing Facility; PBS, phosphate buffer solution; PCR, polymerase chain reaction; RDP, Ribosomal Database Project; SEP, solar energetic protons; w.w., wet weight.

References

Acuña, M.H., Connerney, J.E.P., Wasilewski, P., Lin, R.P., Anderson, K.A., Carlson, C.W., McFadden, J., Curtis, D.W.,

Mitchell, D., Reme, H., Mazelle, C., Sauvaud, J.A., d'Uston, C., Cros, A., Medale, J.L., Bauer, S.J., Cloutier, P., Mayhew, M., Winterhalter, D., and Ness, N.F. (1998) Magnetic field and plasma observations at Mars: initial results of the Mars Global Surveyor mission. *Science* 279:1676–1680.

Acuña, M.H., Connerney, J.E.P., Ness, N.F., Lin, R.P., Mitchell, D., Carlson, C.W., McFadden, J., Anderson, K.A., Reme, H., Mazelle, C., Vignes, D., Wasilewski, P., and Cloutier, P. (1999) Global distribution of crustal magnetization discovered by the Mars Global Surveyor MAG/ER experiment. *Science* 284: 790–793.

Aislabie, J.M., Broady, P.A., and Saul, D.J. (2006) Culturable aerobic heterotrophic bacteria from high altitude, high latitude soil of La Gorce Mountains (86 degrees 30'S, 147 degrees W), Antarctica. *Antarct. Sci.* 18:313–321.

Anzai, Y., Kim, H., Park, J., Wakabayashi, H., and Oyaizu, H. (2000) Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. *Int. J. Syst. Evol. Microbiol.* 50:1563–1589.

Bailey, M., Lilley, A., Thompson, I., Rainey, R., and Ellis, R. (1995) Site-directed chromosomal marking of a fluorescent pseudomonad isolated from the phytosphere of sugar beet: stability and potential for marker gene transfer. *Mol. Ecol.* 4:755–763.

Battista, J. (1997) Against all odds: the survival strategies of *Deinococcus radiodurans. Annu. Rev. Microbiol.* 51:203–224.

Battista, J., Earl, A., and Park, M.-J. (1999) Why is *Deinococcus radiodurans* so resistant to ionizing radiation? *Trends Microbiol.* 7:362–365.

Baumstark-Khan, C., Facius, R., and Horneck, G. (2001) Life under conditions of ionizing radiation. In *Astrobiology: The Quest for the Conditions of Life,* edited by G. Horneck and C. Baumstark-Khan, Springer-Verlag, Berlin, pp 260–283.

Burkins, M., Virginia, R., Chamberlain, C., and Wall, D. (2000) Origin and distribution of soil organic matter in Taylor Valley, Antarctica. *Ecology* 81:2377–2391.

Carpenter, E.J., Lin, S., and Capone, D. (2000) Bacterial activity in South Pole snow. *Appl. Environ. Microbiol.* 66:4514–4517.

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

Chattopadhyay, M. (2006) Mechanism of bacterial adaptation to low temperature. *J. Biosci.* 31:157–165.

Chen, M., Wu, S., Lin, G., Lu, C., Lin, Y., Chang, W., and Tsay, S. (2004) *Rubrobacter taiwanensis* sp nov., a novel thermophilic, radiation-resistant species isolated from hot springs. *Int. J. Syst. Evol. Microbiol.* 54:1849–1855.

Cheng, S.M. and Foght, J.M. (2007) Cultivation-independent and -dependent characterization of bacteria resident beneath John Evans Glacier. *FEMS Microbiol. Ecol.* 59:318–330.

Chintalapati, S., Kiran, M., and Shivaji, S. (2004) Role of membrane lipid fatty acids in cold adaptation. *Cell. Mol. Biol.* 50:631–642.

Clark, B., Baker, A., Cheng, A., Clemett, S., McKay, D., McSween, H., Pieters, C., Thomas, P., and Zolensky, M. (1999) Survival of life on asteroids, comets and other small bodies. *Orig. Life Evol. Biosph.* 29:521–545.

Cockell, C.S. (2000a) The ultraviolet history of the terrestrial planets—implications for biological evolution. *Planet. Space Sci.* 48:203–214.

Cockell, C.S. (2000b) The ultraviolet environment of Mars: biological implications past, present, and future. *Icarus* 146:343–359.

COESA (U.S. Committee on Extension to the Standard Atmosphere). (1976) *U.S. Standard Atmosphere 1976,* published jointly by the National Oceanic and Atmospheric Administration, the National Aeronautics and Space Administration, and the U.S. Air Force, Washington DC.

Cole, J.R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R.J., Kulam-Syed-Mohideen, A.S., McGarrell, D.M., Marsh, T., Garrity, G.M., and Tiedje, J.M. (2009) The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Res.* 37:D141–D145.

Cowan, D., Russell, N., Mamais, A., and Sheppard, D. (2002) Antarctic Dry Valley mineral soils contain unexpectedly high levels of microbial biomass. *Extremophiles* 6:431–436.

Cowan, D. and Tow, L.A. (2004) Endangered Antarctic environments. *Annu. Rev. Microbiol.* 58:649–690.

Cox, M. and Battista, J. (2005) *Deinococcus radiodurans*—the consummate survivor. *Nat. Rev. Microbiol.* 3:882–892.

D'Amico, S., Collins, T., Marx, J., Feller, G., and Gerday, C. (2006) Psychrophilic microorganisms: challenges for life. *EMBO Rep.* 7:385–389.

Daly, M.J., Gaidamakova, E.K., Matrosova, V.Y., Vasilenko, A., Zhai, M., Venkateswaran, A., Hess, M., Omelchenko, M.V., Kostandarithes, H.M., Makarova, K.S., Wackett, L.P., Fredrickson, J.K., and Ghosal, D. (2004) Accumulation of Mn(II) in *Deinococcus radiodurans* facilitates gamma-radiation resistance. *Science* 306:1025–1028.

Daly, M.J. (2006) Modulating radiation resistance: insights based on defenses against reactive oxygen species in the radioresistant bacterium *Deinococcus radiodurans. Clin. Lab. Med.* 26:491–504.

Daly, M.J. (2009) A new perspective on radiation resistance based on *Deinococcus radiodurans. Nat. Rev. Microbiol.* 7:237–245.

Dartnell, L., Desorgher, L., Ward, J., and Coates, A. (2007a) Modelling the surface and subsurface martian radiation environment: implications for astrobiology. *Geophys. Res. Lett.* 34, doi:10.1029/2006GL027494.

Dartnell, L.R., Desorgher, L., Ward, J.M., and Coates, A.J. (2007b) Martian sub-surface ionising radiation: biosignatures and geology. *Biogeosciences* 4:545–558.

DeLong, E.F. (1992) Archaea in coastal marine environments. *Proc. Natl. Acad. Sci. U.S.A.* 89:5685–5689.

Eidelman, S., Hayes, K., Olive, K., Aguilar-Benitez, M., Amsler, C., Asner, D., Babu, K., Barnett, R., and Beringer, J. (2004) Cosmic Rays. Chapter 24 in *The Review of Particle Physics,* published in *Phys. Lett. B* 592.

Farkas, J. (1998) Irradiation as a method for decontaminating food: a review. *Int. J. Food Microbiol.* 44:189–204.

Farman, J., Gardiner, B., and Shanklin, J. (1985) Large losses of total ozone in Antarctica reveal seasonal ClOx/NOx interaction. *Nature* 315:207–210.

Feller, G. (2003) Molecular adaptations to cold in psychrophilic enzymes. *Cell. Mol. Life Sci.* 60:648–662.

Ferradini, C. and Jay-Gerin, J.-P. (1999) La radiolyse de l'eau et des solutions aqueuses: historique et actualite. *Can. J. Chem.* 77:1542–1575.

Ferreira, A.C., Nobre, M.F., Rainey, F.A., Silva, M.T., Wait, R., Burghardt, J., Chung, A.P., and da Costa, M.S. (1997) *Deinococcus geothermalis* sp. nov. and *Deinococcus murrayi* sp. nov., two extremely radiation-resistant and slightly thermophilic species from hot springs. *Int. J. Syst. Bacteriol.* 47:939–947.

Ferreira, A., Nobre, M., Moore, E., Rainey, F., Battista, J., and da Costa, M.S. (1999) Characterization and radiation resistance of new isolates of *Rubrobacter radiotolerans* and *Rubrobacter xylanophilus. Extremophiles* 3:235–238.

Ghosal, D., Omelchenko, M.V., Gaidamakova, E.K., Matrosova, V.Y., Vasilenko, A., Venkateswaran, A., Zhai, M., Kostandarithes, H.M., Brim, H., Makarova, K.S., Wackett, L.P., Fredrickson, J.K., and Daly, M.J. (2005) How radiation kills cells: survival of *Deinococcus radiodurans* and *Shewanella oneidensis* under oxidative stress. *FEMS Microbiol. Rev.* 29:361–375.

Gilichinsky, D.A. (2002) Permafrost model of extraterrestrial habitat. In *Astrobiology: The Quest for the Conditions of Life,* edited by G. Horneck and C. Baumstark-Khan, Springer-Verlag, Berlin, pp 125–142.

Gilichinsky, D.A. (2007) Microbial populations in Antarctic permafrost: biodiversity, state, age, and implication for astrobiology. *Astrobiology* 7:275–311.

Hanahan, D. (1983) Studies on transformation of *Escherichia coli* with plasmids. *J. Mol. Biol.* 166:557–580.

Henriksen, T. (1962) Radiation-induced free radicals in frozen aqueous solutions of glycine. *Radiat. Res.* 17:158–172.

Horneck, G., Bücker, H., and Reitz, G. (1994) Long-term survival of bacterial spores in space. *Adv. Space Res.* 14:41–45.

Horneck, G., Rettberg, P., Reitz, G., Wehner, J., Eschweiler, U., Strauch, K., Panitz, C., Starke, V., and Baumstark-Khan, C. (2001) Protection of bacterial spores in space, a contribution to the discussion on panspermia. *Orig. Life Evol. Biosph.* 31:527–547.

Horowitz, N.H., Cameron, R.E., and Hubbard, J.S. (1972) Microbiology of the Dry Valleys of Antarctica. *Science* 176:242–245.

Hutchinson, F. (1963) Radiation effects on macromolecules of biological importance. *Annu. Rev. Nucl. Sci.* 13:535–564.

Jones, A. and Shanklin, J. (1995) Continued decline of total ozone over Halley, Antarctica, since 1985. *Nature* 376:409–411.

Klapdor-Kleingrothaus, H.V. and Zuber, K. (2000) Chapter 8: cosmic radiation. In *Particle Astrophysics*, 2nd ed., edited by H.V. Klapdor-Kleingrothaus and K. Zuber, Institute of Physics Publishing, Bristol, pp 223–247.

Kminek, G. and Bada, J. (2006) The effect of ionizing radiation on the preservation of amino acids on Mars. *Earth Planet. Sci. Lett.* 245:1–5.

Kminek, G., Bada, J., Pogliano, K., and Ward, J. (2003) Radiation-dependent limit for the viability of bacterial spores in halite fluid inclusions and on Mars. *Radiat. Res.* 159:722–729.

Lambert, A., Smith, J., Dodds, K., and Charbonneau, R. (1992) Microbiological changes and shelf life of MAP, irradiated fresh pork. *Food Microbiol.* 9:231–244.

Levin, G.V. and Straat, P.A. (1976) Labeled release—an experiment in radiorespirometry. *Orig. Life Evol. Biosph.* 7:293–311.

Liu, Y., Yao, T., Jiao, N., Kang, S., Zeng, Y., and Huang, S. (2006) Microbial community structure in moraine lakes and glacial meltwaters, Mount Everest. *FEMS Microbiol. Lett.* 265:98–105.

Lubin, D. and Frederick, J. (1991) The ultraviolet radiation environment of the Antarctic Peninsula: the roles of ozone and cloud cover. *Journal of Applied Meteorology* 30:478–493.

Madronich, S., McKenzie, R., Björn, L., and Caldwell, M. (1998) Changes in biologically active ultraviolet radiation reaching the Earth's surface. *J. Photochem. Photobiol. B, Biol.* 46:5–19.

Malanca, A., Pessina, V., and Dallara, G. (1993) Assessment of the natural radioactivity in the Brazilian state of Rio Grande Do Norte. *Health Phys.* 65:298–302.

Männistö, M. and Häggblom, M. (2006) Characterization of psychrotolerant heterotrophic bacteria from Finnish Lapland. *Syst. Appl. Microbiol.* 29:229–243.

Mattimore, V. and Battista, J. (1996) Radioresistance of *Deinococcus radiodurans:* functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. *J. Bacteriol.* 178:633–637.

Mattingly, R., Matousek, S., and Jordan, F. (2004) Continuing evolution of Mars Sample Return. In *2004 IEEE Aerospace Conference,* Institute of Electrical and Electronics Engineers (IEEE), Los Alamitos, CA, doi:10.1109/AERO.2004.1367630.

McKay, C.P. (1993) Relevance of Antarctic microbial ecosystems to exobiology. *Antarctic Microbiology,* Wiley-Liss, New York, pp 593–601.

Meyer, A., Lipson, D., Martin, A., Schadt, C., and Schmidt, S. (2004) Molecular and metabolic characterization of cold-tolerant alpine soil *Pseudomonas* sensu stricto. *Appl. Environ. Microbiol.* 70:483–489.

Mileikowsky, C., Cucinotta, F., Wilson, J., Gladman, B., Horneck, G., Lindegren, L., Melosh, J., Rickman, H., Valtonen, M., and Zheng, J. (2000) Natural transfer of viable microbes in space. 1. From Mars to Earth and Earth to Mars. *Icarus* 145:391–427.

Minton, K. (1994) DNA repair in the extremely radioresistant bacterium *Deinococcus radiodurans*. *Mol. Microbiol.* 13:9–15.

Moissl, C., Osman, S., La Duc, M., Dekas, A., Brodie, E., DeSantis, T., and Venkateswaran, K. (2007) Molecular bacterial community analysis of clean rooms where spacecraft are assembled. *FEMS Microbiol. Ecol.* 61:509–521.

Moores, J., Smith, P., Tanner, R., Schuerger, A., and Venkateswaran, K. (2007) The shielding effect of small-scale martian surface geometry on ultraviolet flux. *Icarus* 192:417–433.

Morley, C., Trofymow, J., Coleman, D., and Cambardella, C. (1983) Effects of freeze-thaw stress on bacterial populations in soil microcosms. *Microb. Ecol.* 9:329–340.

Morrison, D. (1977) Transformation in *Escherichia coli:* cryogenic preservation of competent cells. *J. Bacteriol.* 132:349–351.

NASA. (2005) *Planetary Protection Provisions for Robotic Extraterrestrial Missions,* NPR 8020.12C, National Aeronautics and Space Administration, Washington DC.

Nelson, G.A. (2003) Fundamental space radiobiology. *Gravit. Space Biol. Bull.* 16:29–36.

O'Neil, W. and Cazaux, C. (2000) The Mars Sample Return project. *Acta Astronaut.* 47:453–465.

Omelchenko, M., Wolf, Y., Gaidamakova, E., Matrosova, V., Vasilenko, A., Zhai, M., Daly, M., Koonin, E., and Makarova, K. (2005) Comparative genomics of *Thermus thermophilus* and *Deinococcus radiodurans:* divergent routes of adaptation to thermophily and radiation resistance. *BMC Evol. Biol.* 5:57.

Osman, S., Peeters, Z., La Duc, M.T., Mancinelli, R., Ehrenfreund, P., and Venkateswaran, K. (2008) Effect of shadowing on survival of bacteria under conditions simulating the martian atmosphere and UV radiation. *Appl. Environ. Microbiol.* 74:959–970.

Oyama, V. (1972) The gas exchange experiment for life detection: the Viking Mars lander. *Icarus* 16:167–184.

Parnell, J., Cullen, D., Sims, M.R., Bowden, S., Cockell, C.S., Court, R., Ehrenfreund, P., Gaubert, F., Grant, W., Parro, V., Rohmer, M., Sephton, M.A., Stan-Lotter, H., Steele, A., Toporski, J., and Vago, J. (2007) Searching for life on Mars: selection of molecular targets for ESA's aurora ExoMars mission. *Astrobiology* 7:578–604.

Pavlov, A., Blinov, A., and Konstantinov, A. (2002) Sterilization of martian surface by cosmic radiation. *Planet. Space Sci.* 50: 669–673.

Pavlov, A., Kalinin, V., Konstantinov, A., Shelegedin, V., and Pavlov, A. (2006) Was Earth ever infected by martian biota? Clues from radioresistant bacteria. *Astrobiology* 6:911–919.

Pearce, D., Gast, C., Lawley, B., and Ellis-Evans, J. (2003) Bacterioplankton community diversity in a maritime Antarctic lake, determined by culture-dependent and culture-independent techniques. *FEMS Microbiol. Ecol.* 45:59–70.

Powers, E. and Tallentire, A. (1968) The role of water in the cellular effects of ionizing radiations. Reprinted from *Actions Chimiques et Biologique des Radiations,* Vol. 12, Masson, Paris, pp 3–67.

Rashid, H., Ito, H., and Ishigaki, I. (1992) Distribution of pathogenic vibrios and other bacteria in imported frozen shrimps and their decontamination by gamma-irradiation. *World J. Microbiol. Biotechnol.* 8:494–499.

Reddy, G., Matsumoto, G., Schumann, P., Stackebrandt, E., and Shivaji, S. (2004) Psychrophilic pseudomonads from Antarctica: *Pseudomonas antarctica* sp. nov., *Pseudomonas meridiana* sp. nov. and *Pseudomonas proteolytica* sp. nov. *Int. J. Syst. Evol. Microbiol.* 54:713–719.

Richmond, R., Sridhar, R., and Daly, M.J. (1999) Physicochemical survival pattern for the radiophile *Deinococcus radiodurans:* a polyextremophile model for life on Mars. *SPIE Conference on Instruments, Methods, and Missions for Astrobiology II* 3755:210–222.

Rothschild, L. and Mancinelli, R. (2001) Life in extreme environments. *Nature* 409:1092–1101.

Ruimy, R., Riegel, P., Boiron, P., Monteil, H., and Christen, R. (1995) Phylogeny of the genus *Corynebacterium* deduced from analyses of small-subunit ribosomal DNA sequences. *Int. J. Syst. Bacteriol.* 45:740–746.

Schuerger, A., Richards, J., Newcombe, D., and Venkateswaran, K. (2006) Rapid inactivation of seven *Bacillus* spp. under simulated Mars UV irradiation. *Icarus* 181:52–62.

Setlow, P. (1995) Mechanisms for the prevention of damage to DNA in spores of *Bacillus* species. *Annu. Rev. Microbiol.* 49:29–54.

Simonsen, L.C. and Nealy, J.E. (1993) Mars surface radiation exposure for solar maximum conditions and 1989 solar proton events. NASA Technical Paper 3300, National Aeronautics and Space Administration, Washington DC.

Skidmore, M., Anderson, S., Sharp, M., Foght, J., and Lanoil, B. (2005) Comparison of microbial community compositions of two subglacial environments reveals a possible role for microbes in chemical weathering processes. *Appl. Environ. Microbiol.* 71:6986–6997.

Smith, J., Tow, L., Stafford, W., Cary, C., and Cowan, D. (2006) Bacterial diversity in three different Antarctic cold desert mineral soils. *Microb. Ecol.* 51:413–421.

Sommers, C.H., Niemira, B.A., Tunick, M., and Boyd, G. (2002) Effect of temperature on the radiation resistance of virulent *Yersinia enterocolitica. Meat Sci.* 61:323–328.

Stapleton, G.E. and Edington, C.W. (1956) Temperature dependence of bacterial inactivation by X-Rays. *Radiat. Res.* 5:39–45.

Tanaka, M., Earl, A., Howell, H., Park, M.-J., Eisen, J., Peterson, S., and Battista, J. (2004) Analysis of *Deinococcus radiodurans's* transcriptional response to ionizing radiation and desiccation reveals novel proteins that contribute to extreme radioresistance. *Genetics* 168:21–33.

Tapias, A., Leplat, C., and Confalonieri, F. (2009) Recovery of ionizing-radiation damage after high doses of gamma ray in the hyperthermophilic archaeon *Thermococcus gammatolerans. Extremophiles* 13:333–343.

ten Kate, I., Garry, J., Peeters, Z., Quinn, R., Foing, B., and Ehrenfreund, P. (2005) Amino acid photostability on the martian surface. *Meteorit. Planet. Sci.* 40:1185–1193.

Thayer, D.W. and Boyd, G. (1991) Effect of ionizing radiation dose, temperature, and atmosphere on the survival of *Salmonella typhimurium* in sterile, mechanically deboned chicken meat. *Poult. Sci.* 70:381–388.

Thomassin-Lacroix, E., Yu, Z., Eriksson, M., Reimer, K., and Mohn, W. (2001) DNA-based and culture-based characterization of a hydrocarbon-degrading consortium enriched from Arctic soil. *Can. J. Microbiol.* 47:1107–1115.

Van Trappen, S., Mergaert, J., Van Eygen, S., Dawyndt, P., Cnockaert, M., and Swings, J. (2002) Diversity of 746 heterotrophic bacteria isolated from microbial mats from ten Antarctic lakes. *Syst. Appl. Microbiol.* 25:603–610.

Venkateswaran, A., McFarlan, S., Ghosal, D., Minton, K., Vasilenko, A., Makarova, K., Wackett, L., and Daly, M. (2000) Physiologic determinants of radiation resistance in *Deinococcus radiodurans. Appl. Environ. Microbiol.* 66:2620–2626.

Vincent, W.F. (1988) *Microbial Ecosystems of Antarctica,* Cambridge University Press, Cambridge.

Vishniac, H.S. (1993) The microbiology of Antarctic soils. In *Antarctic Microbiology*, Wiley-Liss, New York, pp 297–341.

Vishnivetskaya, T., Siletzky, R., Jefferies, N., Tiedje, J., and Kathariou, S. (2007) Effect of low temperature and culture media on the growth and freeze-thawing tolerance of *Exiguobacterium* strains. *Cryobiology* 54:234–240.

Vorobyova, E., Soina, V., Gorlenko, M., Minkovskaya, N., Zalinova, N., Mamukelashvili, A., Gilichinsky, D., Rivkina, E., and Vishnivetskaya, T. (1997) The deep cold biosphere: facts and hypothesis. *FEMS Microbiol. Rev.* 20:277–290.

Walker, V.K., Palmer, G.R., and Voordouw, G. (2006) Freeze-thaw tolerance and clues to the winter survival of a soil community. *Appl. Environ. Microbiol.* 72:1784–1792.

Weisburg, W., Giovannoni, S., and Woese, C. (1989) The Deinococcus-Thermus phylum and the effect of rRNA composition on phylogenetic tree construction. *Syst. Appl. Microbiol.* 11:128–134.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J. (1991) 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* 173:697–703.

Wercinski, P. (1996) Mars Sample Return—a direct and minimum-risk design. *J. Spacecr. Rockets* 33:381–385.

Whiting, S.J. (2004) Investigation of genome diversity in Antarctic Dry Valley soils, PhD thesis, University of London.

Wynn-Williams, D.D. (2000) Cyanobacteria in deserts—life at the limit? In *The Ecology of Cyanobacteria: Their Diversity in Time and Space*, edited by B.A. Whitton and M. Potts, Kluwer Academic Publishers, Dordrecht, the Netherlands, pp 341–366.

Wynn-Williams, D.D. and Edwards, H.G.M. (2000) Antarctic ecosystems as models for extraterrestrial surface habitats *Planet. Space Sci.* 48:1065–1075.

Zahradka, K., Slade, D., Bailone, A., Sommer, S., Averbeck, D., Petranovic, M., Lindner, A., and Radman, M. (2006) Reassembly of shattered chromosomes in *Deinococcus radiodurans. Nature* 443:569–573.

Zhang, G., Ma, X., Niu, F., Dong, M., Feng, H., An, L., and Cheng, G. (2007) Diversity and distribution of alkaliphilic psychrotolerant bacteria in the Qinghai-Tibet Plateau permafrost region. *Extremophiles* 11:415–424.

Zhang, J.-W., and Zeng, R.-Y. (2008) Molecular cloning and expression of a cold-adapted lipase gene from an Antarctic deep sea psychrotrophic bacterium *Pseudomonas* sp 7323. *Mar. Biotechnol.* 10:612–621.

Zhang, Z., Schwartz, S., Wagner, L., and Miller, W. (2004) A greedy algorithm for aligning DNA sequences. *J. Comput. Biol.* 7:203–214.

Address correspondence to:
Lewis R. Dartnell
Centre for Mathematics and Physics in the Life Sciences and Experimental Biology (CoMPLEX)
University College London
Gower Street
London WC1E 6BT
UK

E-mail: l.dartnell@ucl.ac.uk

Submitted 12 August 2009
Accepted 25 May 2010