

**PHYSICO-CHEMICAL SURVIVAL PATTERN FOR THE RADIOPHILE  
*D. radiodurans*: A POLYEXTREMOPHILE MODEL FOR LIFE ON MARS**

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## I. INTRODUCTION

The two Viking missions of the 1970's are a testimony to the success of our technological capability when it is driven by consuming curiosity and sense of adventure. In the case of Viking, the national spirit in the United States supported an assemblage of equally spirited expertise within NASA to determine if life existed on Mars, and within the defined science of those missions to establish if conditions on Mars might support life. The technological successes of Viking led to a confusion of interpretations for the issue of life on Mars. This confusion in turn led to polarities in the scientific community and a subsequent resting period of some years for the enthusiasm required to support continued investigation of the potential existence of life on Mars, and indeed elsewhere in our solar system.

The spirit for continued investigation of the potential for life on Mars is now revived in part by the provocative evidence reported from analysis of the martian meteorite ALH84001 (1). That provocation has been supported by the persistent logic of life-allowable signatures from the Viking Lander experiments (2), by the growing awareness of how remarkably adaptable life is on Earth to a variety of extreme environments that feasibly mimic past and/or present habitats available at various locations throughout our solar system, and by the growing acceptance of the multifactorial merger of knowledge regarding the creation and evolution of the Inner Planets. Some of the more provocative factors regarding the latter are: knowledge of early emergence of life on Earth, even during its late stages of accretion up to 4 billion years ago; probable similarities of both Earth and Mars habitats during early geologic times up to 3 billion years ago or less; accumulating evidence supporting cross-fertilization of planetary material between Mars and Earth as was initially proposed in the panspermia hypothesis (3); a growing evidence and acceptance of subsurface aqueous reservoirs on Mars; recent firm estimates of large water-ice reservoirs in both polar ice-caps on Mars (4); recent evidence for young (within approximately the last 300,000 years) substantial martian volcanism that supports a high probability for continuing volcanism (5).

The latter two recently discovered features of Mars are revolutionary for thinking related to the probability of extant life on Mars. Both of those discovered features derive from telemetry from the recently placed Mars Global Surveyor (MGS). That satellite promises to improve our knowledge of Mars such that decision making for realistic outcomes will be forthcoming much like the knowledge of the DNA double-helix led to the reality of present-day genetic engineering. One of the principal realistic outcomes anticipated from the MGS is life-probability selection of landing sites for Mars' probes planned for 2003, 2005, and 2008, as well as the manned exploration mission anticipated for 2018. If the primary goal of our exploration of Mars is to establish the existence of past and/or present life on Mars, then selection of the landing site(s) that will provide the highest probability for such discovery is clearly by far the most important requirement of our entire effort. It is largely in support of this requirement that we introduce here a polyextremophilic model for life on Mars in anticipation that simulations of martian environments supporting or allowing survival of that model can be programmed as signatures into the MGS-based search for the best landing site(s).

A key issue upon which support or not for Mars exploration has hinged has been the presence or not of adequate water to support life as we know it. Even before the revolutionary findings from the MGS described above, consensus has been growing that substantial reservoirs of water and water-ice is likely on Mars as underground sources in brine deposits and permafrost (e.g., 6, 7) and in effluent released from occasional thermal and thermoclastic events on the surface of Mars (e.g., 8). Adding to this growing consensus of martian water supply is the recent release of Viking Lander photographs showing probable water-snow on Mars (8), where this pictorial evidence is now provided in a temporal sequence suggesting that the Viking Lander actually experienced snowfall (URL [http://wundow.wustl.edu/vlander/vl\\_0002/browse/i0xx.htm](http://wundow.wustl.edu/vlander/vl_0002/browse/i0xx.htm)). Assuming that rapid shifts in atmospheric temperature are possible on Mars, and knowing that a partial pressure of water averaging approximately 15 precipitable microns exists in its atmosphere (9), then snowfall on Mars can be hypothesized at the leading edge of a cold front when rapidly decreasing temperature merges with a somewhat warmer zone of relatively high humidity (similar to RCR personal observations in central New Hampshire, most often on star-filled nights, with snow accumulations at times approaching half an inch).

The portrayal of martian habitat has often been one of friendly to life during early planetary evolution (as "friendly" as the early thermophilic habitat on Earth that is known to provide us with records of life), but unfriendly to life at present (as "unfriendly" as the extreme habitats on Earth often assumed previously not to harbor life). The growing awareness that there is hardly a potential habitat on Earth not harboring life, however, is now changing our consensus of consequences for life within the unfriendly habitats on Mars, especially for potential habitats existing below its surface.

Quite apart from any arguable assumptions of life on Mars, however, is the fact that the probable similarity of thermophilic habitats on Earth and Mars 4 billion years ago has evolved now into unarguable planetary differences such that cold, low atmospheric pressure, impinging solar and cosmic radiations, and oxidative soil properties make the surface-involved habitats of Mars a multiply extreme environment for sustaining life. Also unarguable for extreme environments described on Earth is the extent, and at times rapidity, with which existing life can adapt to fill them. The question then may be, could ancient life on Mars adapt over the hundreds of millions of years presumably allowed about 3 billion years ago during the transition of a hot and fulminating habitat to a cold and rather nondynamic habitat? The answer to that question at this time can only be a probability, either way. If the answer is probably yes, then such evolutionary outcome needs to consider seriously the probability of creation of polyextremophiles, given the multiplicity of extreme changes involved during this paleogeologic maturation of Mars.

Extremophiles on Earth are nearly always defined with a singular characteristic that allows existence within a singular extreme environment. However, species of the genus *Deinococcus* demonstrate a suite of extreme survival advantages similar to that needed to survive the currently understood multiple challenges for life on Mars. One of these species, *Deinococcus radiodurans*, is proposed here as a model for such a potential polyextremophile. Not only should this model best survive the extreme environments of cold, low atmospheric pressure, impinging solar and cosmic radiations, and oxidative soil environments mentioned above, but also might survive the overwhelming radiation doses accumulating from internal and environmental radioisotopes during eons of cryptobiotic inactivity. Most, if not all, of the survival characteristics summarized as required for life forms on the surface of Mars (10) are embodied by *D. radiodurans*. Such a polyextremophile would seem to be required for extant life on Mars existing near the surface, and thus accessible by robotic and human explorations planned within the foreseeable future.

## II. A POLYEXTREMOPHILE MODEL

*D. radiodurans* (11) is likely the first polyextremophile to be identified that is not provided with a cryptobiotic phase of its cell cycle. This ubiquitous ancient nonpathogenic chemoorganotrophic mesophilic catalase-positive Gram-positive carotenoid-containing coccus was isolated due to one of its extremophilic characters not likely involved in any current earthly survival advantage, which is extraordinary resistance to ionizing radiation. The amorphous appearance and biochemical makeup of this isolate also led to its initial misclassification as *Micrococcus* rather than as *Deinococcus* (12). Despite complex growth requirements as a chemoorganotroph, *Deinococcus* is perhaps the most ancient of the eubacteria (13). Despite ubiquitous distribution and ancient derivation, only six species of *Deinococcus* are currently described, two of which are halotolerant (14) and two of which are thermophilic (15).

*D. radiodurans* is an extremophile in its resistance to damage caused by desiccation, ionizing radiation, ultraviolet radiation, hydrogen peroxide, and electrophilic mutagens such as nitrosoguanidine. Although it is classified as a mesophile, it can grow between temperature ranges of 4 to 42° C (14). Sublethal exposure to hydrogen peroxide has also been noted to induce additional extreme resistance to hydrogen peroxide that is coupled to increased catalase synthesis, and that concomitantly increases also the already extreme resistance to ultraviolet radiation and ionizing radiation (16). *D. radiodurans* is also inducible for thermotolerance, such that heat shock has been observed to increase subsequent resistance to both heat and salt (17).

*D. radiodurans* is highly redundant in haploid genomic copies, with 8-10 copies and 4 copies per cell found during log phase and stationary phase growth, respectively (18). It has been hypothesized that these genomic copies are held in register within *D. radiodurans* such that recombinational repair of potentially lethal DNA double-strand breaks is efficiently accomplished (19), thus explaining its known survival of high doses of a wide range of genotoxins. In this regard, it is known that DNA double-strand breaks may accumulate from damage caused by most of the physico-chemical insults to which *D. radiodurans* is most notably resistant. It is therefore hypothesized here that the known highly efficient repair of DNA strand-breaks in *D. radiodurans* accounts for its polyextremophile profile involving resistance to genotoxic damage. This hypothesis is offered on the basis that a single efficient repair mechanism would more likely be evolved to provide polyextremophilic resistance than would a series of separate efficient repair mechanisms be evolved for each extremophilic character noted for *D. radiodurans*.

Resistance to ionizing radiation is not thought to provide *D. radiodurans* with survival advantage within Earth's present day ecosphere; rather, it has been suggested that this radiation resistance is an incidental consequence of its resistance to desiccation (14). It has also been hypothesized that the high concentration of carotenoids within the core of the cell contributes to effective scavenging of oxidative free radicals thereby contributing to resistances against both hydrogen peroxide and ionizing radiation (20). However, desiccation and ionizing radiation are both known to induce DNA double-strand breaks that are rapidly repaired in *D. radiodurans* (e.g., 20). This latter repair pathway is hypothesized to be an opportune mechanism evolved in response to the selection pressure of desiccation that provides then for the extreme resistance to genotoxic damage caused by ionizing radiation (19). Ample opportunity exists on Mars for the desiccation of life forms.

Both ionizing radiation and hydrogen peroxide have been shown to cause DNA strand-breaks (22). Although single-strand breaks predominate in DNA damage caused by both agents, at high doses overlapping interactive domains of single-strand breaks are thought to lead to creation of double-strand DNA breaks (23). Thus, the hypothesized efficient repair of DNA double-strand breaks (19) may account for the pronounced resistance to hydrogen peroxide noted for *D. radiodurans*. Resistance to peroxides and superoxides should benefit existing life on Mars, at least within any habitat similar to its rather homogeneous surface, which is established to be oxidative (e.g., 2).

Restitution of the bulky lesions in DNA created by the action of ultraviolet radiation, i.e., principally pyrimidine dimers (24), and nitrosoguanidine is not directly related to DNA repair pathways that reconstitute frank strand-breaks and base-damage caused by ionizing radiation. Excision repair is a predominant restitution process of bulky DNA adducts, and that repair process indeed is suggested to cause some production of DNA strand-breaks ultimately in UV-irradiated *E. coli* cells (25). Thus, the same repair mechanism of DNA double-strand break repair hypothesized for *D. radiodurans* (19) is also hypothesized to explain its observed extreme resistance to killing by alkylating agents and UV irradiation.

It is clear that *D. radiodurans* provides a remarkable, possibly unique, capability to withstand a range of physico-chemical potentially lethal environmental insults. This suite of resistances has allowed for its ubiquitous distribution on Earth, and emphasizes its value as a model for projecting extinct, and possibly extant, life on Mars where such a suite of resistances would provide survival advantage.

### III. SUPPORTING RESULTS

#### A. HEAT RESISTANCE

The cold dry surface and probable semifrozen subsurface permafrost of Mars may harbor life evolved from hot wet beginnings. In such a case, it may be that some early-evolved thermal resistance remains important because periodic thermal melts of some magnitude are suggested in the superficial geologic record of the dry surface of Mars. These records of aqueous melts are in the form of splash craters formed by meteorite impacts, by tectonic activity, and by volcanic activity (8). Psychrophilic or frozen microbes normally cycling within the ecological niche of permafrost or the martian surface may periodically benefit from revival in and/or resistance to thermal destruction within these occasional thermal and/or thermoclastic events. It has also been estimated that moderately energetic impacts of ca.  $10^{26}$  joules frequently heat the entire martian surface to the melting point, and that relatively large energetic impacts of  $>10^{28}$  joules will uniformly heat the martian surface such that thermophiles will be the only survivors (26).

Concave-upward survival curves are at times observed for heat inactivated populations of microorganisms. This is true for *D. radiodurans* as we report here. This resistance to heat inactivation that develops at extended times of heating is similar to decreasing rate of cell killing with increasing time of heating reported for *Staphylococcus aureus* (27), for example. Various hypotheses have been offered for developing thermal resistance during heating, such as clumping and emergent subpopulations (28). One of the more appealing hypotheses to explain a concave upward thermal death curve is heat shock-induction of thermotolerance (29).

In Figure 1 we show that although log-phase *D. radiodurans* heated in Tryptose-Glucose-Yeast Extract (TGY) growth media is only moderately resistant to initial killing by heat at 46° C, it then appears to develop tolerance during continued heating. The reason for development of this resistance to heat inactivation that we report here for *D. radiodurans* is not yet established, but the observation adds to its polyextremophilic potential that is thought to provide a useful model for survival during past and present conditions on Mars.

It is also interesting to speculate on possible biological effects related to the isotopic fractionation of hydrogen on Mars, which is known to favor accumulation of HDO and D<sub>2</sub>O within the planet's water balance relative to on Earth (9). Information on the HDO/H<sub>2</sub>O ratio for martian hydrology is estimated at about 0.1% from both atmospheric (9) and SNC meteorite (30) analyses, or about 6-fold higher than the terrestrial value. Despite the fact that the D<sub>2</sub>O fraction is appreciably less than HDO, it is nonetheless provocative to consider that only a few percent of D<sub>2</sub>O in H<sub>2</sub>O contributes to observed heat resistance in phage, virus, yeast, bacteria, and mammalian cells, and that

effects of small fractional D<sub>2</sub>O replacements in cells, especially in the form of chronic exposures, is largely unstudied.

The first reported study on D<sub>2</sub>O-induced protection of cells from thermal killing (31) is extended here to demonstrate similar protection by the presence of 5% D<sub>2</sub>O against thermal killing in *D. radiodurans* (Figure 1). Conversely, the development of heat-shock response leading to development of thermotolerance is also reported to be inhibited by D<sub>2</sub>O (32, 33). Thermotolerance, i.e., heat resistance induced by prior exposure to some sublethal temperature, appears to require protein synthesis that is inhibited by D<sub>2</sub>O (32). Therefore, the concave-upward shape of the thermal death curves of Figure 1 may not be due to classic thermotolerance in this case.

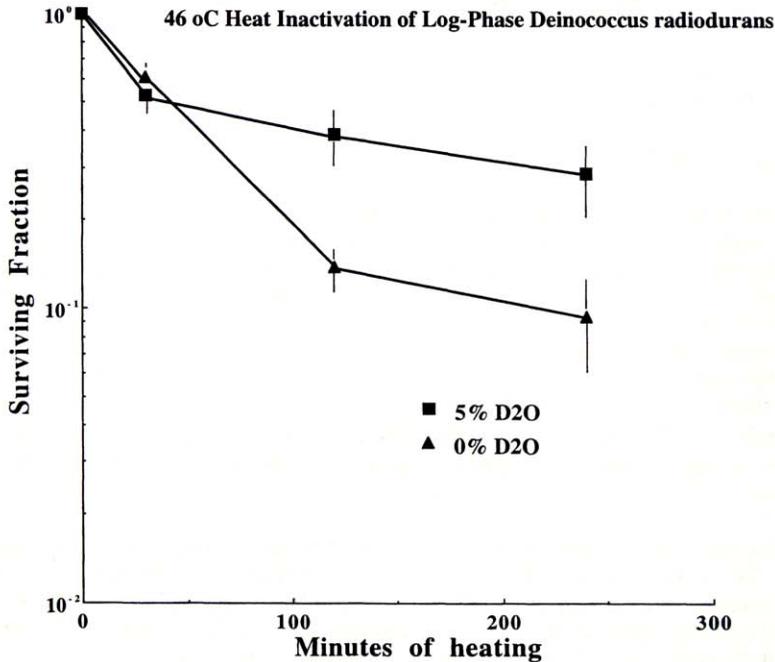


FIGURE 1: Heat resistance of *D. radiodurans* cells to 46° C as influenced by 5% D<sub>2</sub>O. Log-phase cultures growing in Tryptose-Glucose-Yeast Extract media, with or without 5% D<sub>2</sub>O, were heated to 46° C for various lengths of times followed by plating and colony formation with incubation at 37° C. Surviving fraction was calculated as the number of colonies growing from heated samples divided by the nonheated control colony number, and corrected then by the appropriate dilution factor.

## B. RADIATION RESISTANCE

Considerations of survival following radiation-induced damage by potential life on Mars need to include the influence of genetic adaptive response for an evolving polyextremophile. One genetic vector within bacterial populations on Earth that provides facile development and relatively rapid conference of resistance factors is exchangeable transforming DNA elements. This versatile genetic transfer is central to present day genetic engineering of *D. radiodurans* by experimental design rapidly in the laboratory, reflecting the possibility of a somewhat slower rate of genetic engineering by natural selection in the wild.

Transformation- and plasmid-based mutation in DNA followed by DNA-exchange amongst cells is one way in which adaptation to extreme environments might occur. The proposed model of *D. radiodurans* supports this genetic utility in that plasmid-insertion into transforming DNA leads to competent transfection, and this vector system is currently being used to genetically engineer *D. radiodurans* cells to detoxify metals (34) and organics (35) in radioactive waste sites. This capacity to bioremediate potentially toxic extreme environments obviously could be an important aspect for modeling life and its associated ecosystems on Mars, as well as for contributing to habitat-modification during the human exploration of Mars.

There is no report known for the radiation resistance of vector-transfected *D. radiodurans*, i.e., our proposed model. Therefore, radiation survival curves were constructed for *D. radiodurans* R1 wild-type cells transfected with the large (26 kilobase) vector R1/pMD66 that carries a resistance gene to the antibiotic kanomycin. These R1/pMD66 cells were grown to log-phase in TGY media and then irradiated at a dose rate of 1.0 Mrad/hr ( $10^4$  Gy/hr) in a  $^{60}\text{Co}$ -irradiator either in an ice-bath or frozen at  $-79^\circ\text{C}$ , knowing that frozen conditions would be commonly encountered on Mars.

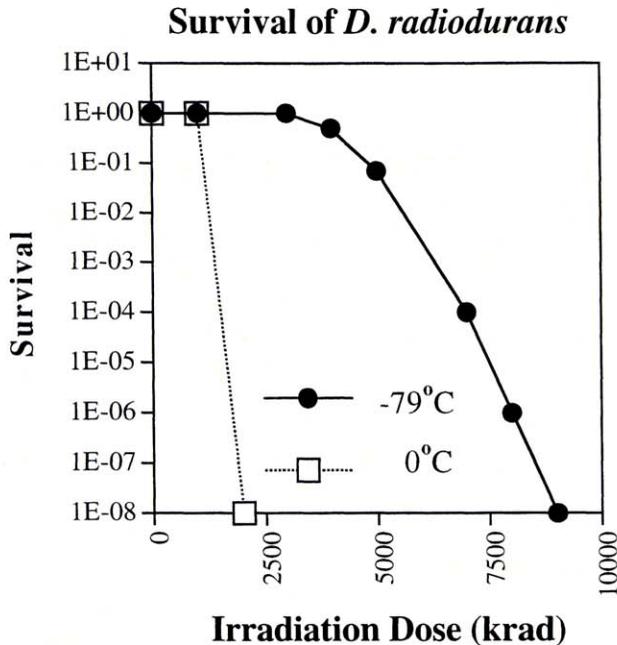


FIGURE 2: Radiation sensitivity in Tryptose-Glucose-Yeast Extract media of stationary-phase *D. radiodurans* R1 wild-type cells. Surviving fraction was determined as described in Figure 1. Radiation sensitivity is the same for cells transformed with R1/pMD66 vector carrying resistance to kanomycin (data not shown). Irradiation at  $-79^\circ\text{C}$  leads to increased radiation resistance. From the ratio of slopes of lines, i.e., enhancement ratio (ER), radiation sensitivity increases by 150% when cells are irradiated at  $-79^\circ\text{C}$  compared to the  $0^\circ\text{C}$  in suspension, i.e.,  $\text{ER}-1 = 1.5$  (36).

The influence of freezing on the radiation sensitivity of *D. radiodurans* R1/pMD66 is shown in Figure 2 comparing the response of stationary-phase cells irradiated in TGY media at ice bath temperature compared to cells similarly irradiated at  $-79^\circ\text{C}$ . The initially aerated frozen state in this case is appreciably more radiation resistant compared to irradiation in the initially aerated liquid state. The estimated slope values of the log-normal survival curves are such that the suspended cells are calculated to be 150% more sensitive (36) to radiation-induced killing than are the frozen cells; the

shoulder of the survival curve for frozen cells is 3-fold more developed in radiation dose than is the shoulder for suspended cells. It should be mentioned that the slope of the survival curve estimated for suspended R1/pMD66 cells here ( $k = 15 \text{ Mrad}^{-1}$ ) is close to that of a previous reported survival curve for suspended *D. radiodurans* wild-type cells ( $k = 13 \text{ Mrad}^{-1}$ ) (14). It is anticipated that genetic engineering of *D. radiodurans* will neither substantially alter its inherent resistance to ionizing radiation nor its inherent resistance to other genotoxins mentioned previously.

The greater radiation resistance noted for frozen *D. radiodurans* has substantial implications for developing this model to anticipate probabilities for extant life on Mars. This is because of the probability that cryptobiotic cells on Mars may need to survive eons of radiation dose accumulating from radioisotope decay both external and internal to those cells. Such radiation dose accumulation is likely the most profound of all the factors that can limit cryptobiotic cell viability on Mars, as further detailed in Discussion.

The physico-chemical modifications of radiation damage are known to be exact and wide-ranging. Complete control of all applicable conditions, including the exclusion of oxygen from irradiated samples, the management of water activity and atmospheric gases during irradiation, and the description of effects of postirradiation conditions, will be required in order to project *D. radiodurans* as a useful model for radiation resistance on Mars. Therefore the two conditions of Figure 2 are offered at this time only as recognition of the effort required to establish *D. radiodurans* as a predictive model for radiation resistance on Mars.

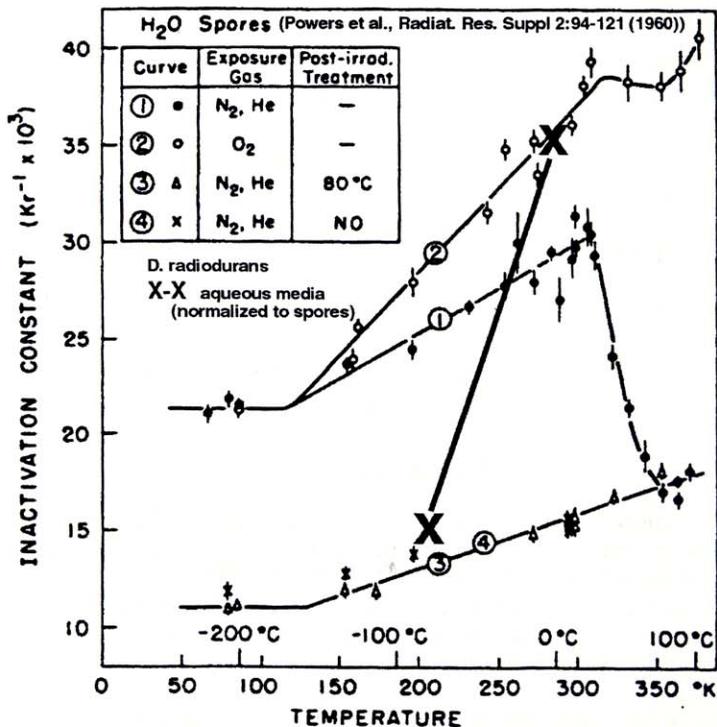


FIGURE 3: Radiation sensitivity of *D. radiodurans* cells (X-X) from Figure 2 normalized to temperature response previously reported for bacterial spores (37) within physico-chemical parameters that are thought to be applicable for conditions affecting radiation sensitivity in off-Earth environments.

The bacterial spore of *Bacillus megaterium* is clearly the best system available (37) at this time for guiding development of the *D. radiodurans* physico-chemical model of radiation resistance. The need to carefully control the irradiation conditions mentioned above can be appreciated in Figure 3 where protiated spore radiation sensitivities across a range of physico-chemical parameters (37) are used to reference the slopes obtained from the irradiated suspended and frozen *D. radiodurans* cells. In Figure 3 the slope value for suspended *D. radiodurans* cells is normalized to the 4<sup>o</sup> C sensitivity for oxygenated spores that are immediately plated after irradiation; the appropriately adjusted slope value for frozen cells is then placed at the -79<sup>o</sup> C coordinate of radiation sensitivity. The relative increase in radiation resistance for *D. radiodurans* cells irradiated at -79<sup>o</sup> C is then seen to approach the anoxic resistance of the bacterial spore model.

#### IV. DISCUSSION

##### 1. Experimental approach to the use of *D. radiodurans* as a model for the search for life on Mars

The topmost surface layer is likely the most damaging environment for microbial survival because of the high flux of UV (38), incident solar and cosmic rays, and known oxidizing properties of the martian soil. However, this is also the region on Mars that is most easily searched during initial exploration. The use of *D. radiodurans* as a model to establish an estimate of survival times on the surface of Mars should be obtained by preparing *D. radiodurans* in simulations of this soil and determining then its ability to survive and repair increasing damage caused by ionizing radiation, ultraviolet radiation, freezing, desiccation, and peroxides. Modifications of radiation dose delivery and quality can be estimated from the shielding expected by surface structures, e.g., rocks, and terrain such as rifts in the Highlands, craters, and various depths of topsoil in the plains (39, 40). In this way, dose-dependent, and thus time-dependent, survival and repair estimates of microbial survival on the surface of Mars may be calculated. With such knowledge it is likely that the shielding of potential life from radiation by rocks, rubble, or rifts in accessible life-sustaining harbors on Mars could be estimated prior to exploration, and these estimates would be important for determining both the sites and the efforts planned in any search for life.

A relatively small amount of surface shielding could provide a large degree of protection from solar and cosmic radiation incident upon the martian surface. For example, the Earth's atmosphere provides 10.3 meters of water-equivalent attenuation of solar and cosmic radiation at the Earth's surface (41), and this kind of attenuation can be provided by rocks and soil of substantially less than that thickness, which would also exclude the solar UV flux. Not much beyond the single Earth-atmosphere-equivalent of radiation shielding by martian soil, irradiation of extremophiles would be derived from in situ background radiation.

Water is surmised to reside below the surface of Mars in the forms of permafrost and frozen deposits, as well as possibly fluid aquifers and lakes. Analogies to water-ice transition environments have been established on Earth as glacial ice and permafrost. The distinction between Mars and Earth in these analogies is that on Mars at least some water-ice environments have likely existed for ca. 2 to 3 billion years, whereas on Earth the oldest permafrosts are thought to be ca. 30 to 40 million years old. Permafrost appears to be the best environment for extended preservation of microbial life. To date, recovery of viable microbes has been reported from 3 million year old samples of permafrost (42). In contrast, the oldest samples of water-ice on Earth that contain microbial life are reported to be only about 0.2 million years old, and these have been obtained from the Vostok drilling site on the central Antarctic ice sheet, where the relative yield of viable microorganisms is reported to be substantially less than from permafrost (43). Interestingly, anaerobic bacteria utilizing iron or sulfur minerals as energy sources are found to be rather impervious to aging in permafrost (44).

2. Experimental and philosophical concepts for survival of accumulating radiation dose in cryptobiotic versus cryopreserved microbes

Even on Earth the radiation backgrounds within environments containing extremophiles are not specifically evaluated yet for radiation dose exposures, so far as is known. Nonetheless, some conservative approximations in regard to background radiation can be made for both Earth and Mars. First, the natural background endogenous to planetary crust should be similar since both planets accreted from the same asteroid pool; this pool of external emitters provides the Earth's surface-dwelling organisms with an in situ background of about 0.03 rad/year, and this was likely on the order of 10 times greater ca. 3 billion years ago when martian permafrost was presumably first forming. Second, the only radioisotope significantly concentrated within cells on Earth is  $^{40}\text{K}$ , which remains in significant abundance because of its cosmically relevant half-life of 1.3 billion years. The dose to cells on Earth from this internal emitter is about 0.02 rad/yr (45), and a similar dose rate can be assumed for internal dose exposure in cells on Mars. Third, as stated above, radiation absorption by 10.3 meters of water, i.e., the equivalent shielding provided by Earth's atmosphere, is sufficient to eliminate 99.5% of incident solar and cosmic radiation dose (41) to an incident value of about 0.05 rad/yr; thus, only on the surface of Mars does the appreciable radiation dose of impinging space radiation need to be considered on the order of 100-times greater than endogenous background radiation. For the scenarios of martian microbes locked in water-ice or permafrost below the surface, shielding of space radiation can be assumed to be essentially complete, and that Earth-equivalent background radiation doses will accumulate in those cells.

The mechanism suggested for extended survival of microbes in permafrost relative to solid water-ice is that water dispersed in the alluvial soil mix of permafrost is only ca. 95% frozen, even at  $-30^\circ\text{C}$ , i.e., a likely realistic temperature for much permafrost existing on Mars, and that the remaining ca. 5% aqueous phase is puddled around the textured soil surfaces in a thin film of concentrated brine (46). This brine envelops the microbes in permafrost as well, and appears to equilibrate with those cells to provide a nutrient-containing cryopreservative that maintains microbes in a cryopreserved state, i.e., barely but perceptibly metabolizing (47). It is not unreasonable to think that paleobiological adaptation in permafrost may select out microbes with metabolic capabilities to repair the unavoidable accumulation of background radiation-induced damage.

On Earth, permafrost is also found to conserve sulfides, which could support anaerobic metabolic processes mentioned above, as well as contribute to the reducing conditions associated with permafrost (43). Sulfides and thiols can scavenge oxidative free radicals known to contribute to radiation-induced damage in cells (48-50). Sulfide content in the permafrost on Mars is expected as well, given the high sulfur content determined for that planet's surface (51, 52). Thus, the environment of permafrost may have provided paleobiological resistance to accumulating radiation damage in two ways, one by supporting low metabolic rates within cryopreserved microbes and one by providing the reducing environment that will scavenge the free radical component of radiation-induced cell killing.

If a radiation resistant organism similar to *D. radiodurans* was to be maintained as fully cryptobiotic in some frozen and/or dry habitat at the martian surface, and thereby be unable to repair accumulating background radiation damage, then the theoretical accumulated dose maximally allowed without overwhelming an ecological population might approach 10 to 15 megarads (0.1 to 0.15 megagrays), even assuming the presence of radioprotectors such as carotenoids, thiols, and sulfides. At the background dose rate of about 0.05 rad/yr anticipated below the martian surface, then this theoretical maximum dose would be reached in about 200 to 300 million years. This is to say, some reconstituting event, such as warming and hydration, would be required at least once in that time period to revive the population, allow it to repair accumulated radiation damage, and to repopulate its habitat before re-entering cryptobiosis, in order to survive extinction. Numbers here are used more to serve a philosophic point rather than a quantitative prediction for continuing life on Mars. That is, accumulating radiation damage will eventually overwhelm any form of life as we know it such that if

rejuvenation of populations has not been possible on Mars for the last billion years or so, then there is no possibility today for survival of life. However, as reviewed above and in Introduction, the accumulating data on both recent and paleogeologic records of Mars is showing that rejuvenating events for populations held in the near-surface have indeed been probable over geologic time, and that such rejuvenating events likely continue over modern time.

That accumulating background radiation doses in cryptobiotic, hence nonrepairing, cells will eventually destroy them unless changing conditions, in ways discussed above, occur with a frequency adequate to allow them to repair and repopulate their environments. This assumption is supported to an approximation by the reported low yield of viable microbes from 0.3 to 0.5 million year old glacial ice derived from cores taken from the subterranean site at the Vostok station in Russia (43). In that case, ca. 0.02 rads/yr internal background radiation to those microbes would accumulate to ca. 10 krad-equivalent of acute radiation-induced damage at the time of revival of those microbes, i.e., a dose which would inactivate the majority of vegetative bacteria irradiated under aerobic conditions. However, the reported survival of microbes from 3 million year old terrestrial permafrost (43) is robust enough that survival would be expected even for microbes in permafrost 10 times as old, i.e., ca. 30 million years. It is of interest that this age approximates that of the oldest reported surviving cryptobiotic organism (a bacterial spore) on Earth (53) (Caus and Boruhi 1995). The associated age-related accumulation of radiation damage in that case is beyond the point of survival to be expected for typical cryptobiotic microbes, i.e., other than *D. radiodurans*. It is for that reason that we ascribe to the speculation that the conditions in permafrost on Earth are cryopreservative rather than cryptobiotic due to the presence of boundary films of brine (46). This is to say that some low-level metabolic maintenance of microbes entrapped in martian permafrost may accordingly allow some repair of accumulating radiation-induced damage.

In conclusion it can be surmised that:

1. The greatest challenge to survival of cryptobiotic life forms on Mars, i.e., forms that might reasonably have evolved into the current surface environments, is the management of lethal accumulations of background radiation-induced damage.
2. The greatest scientific benefit for the use of *D. radiodurans* as a polyextremophile model to anticipate extinct and extant life on Mars is that novel multiresistance mechanisms may be discovered and understood.
3. The greatest applied benefit of the use of *D. radiodurans* as a polyextremophile model for advancing science on Mars is that specific environments and landing sites may be identified in which there is the highest probability of finding evidence for life.
4. Finally, in the spirit of anticipation, the greatest applied benefit of the use of *D. radiodurans* as a polyextremophile model for the human exploration and development of Mars will be its pre-established, and pre-genetically engineered, value for providing domestic cultures in martian environments for the purpose then of growing foodstock, biologically mining the martian surface via bioremediation of specific elements, and biologically providing and adapting a local habitat that will support the needs for human survival.

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