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Radiation-Dependent Limit for the Viability of Bacterial Spores in Halite Fluid Inclusions and on Mars

Gerhard Kminek^{a,1}, Jeffrey L. Bada^a, Kit Pogliano^b, and John F. Ward^c

^aScripps Institution of Oceanography, University of California, San Diego, California

^bDivision of Biology, University of California, San Diego, California

^cDepartment of Radiology, University of California, San Diego, California

Abstract

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When claims for the long-term survival of viable organisms are made, either within terrestrial minerals or on Mars, considerations should be made of the limitations imposed by the naturally occurring radiation dose to which they have been exposed. We investigated the effect of ionizing radiation on different bacterial spores by measuring the inactivation constants for *B. subtilis* and *S. marismortui* spores in solution as well as for dry spores of *B. subtilis* and *B. thuringiensis*. *S. marismortui* is a halophilic spore that is genetically similar to the recently discovered 2-9-3 bacterium from a halite fluid inclusion, claimed to be 250 million years old (Vreeland *et al.*, *Nature* 407, 897–900, 2000). *B. thuringiensis* is a soil bacterium that is genetically similar to the human pathogens *B. anthracis* and *B. cereus* (Helgason *et al.*, *Appl. Environ. Microbiol.* 66, 2627–2630, 2000). To relate the inactivation constant to some realistic environments, we calculated the radiation regimen in a halite fluid inclusion and in the Martian subsurface over time. Our conclusion is that the ionizing dose of radiation in those environments limits the survival of viable bacterial spores over long periods. In the absence of an active repair mechanism in the dormant state, the long-term survival of spores is limited to less than 109 million years in halite fluid inclusions, to 100 to 160 million years in the Martian subsurface below 3 m, and to less than 600,000 years in the uppermost meter of Mars.

INTRODUCTION

Bacterial spores exhibit a remarkable resistance to adverse environmental conditions like desiccation, temperature extremes, pH variation, UV radiation and ionizing radiation. They have developed strategies to deal with the threat of irreversible damage to their DNA by reducing the water content in the cell, binding their DNA to small, acid-soluble proteins and using an efficient repair mechanism that operates during spore germination (1).

The isolation of an ancient viable halotolerant bacterial spore from a halite fluid inclusion in the Permian Salado Formation in New Mexico sparked new interest and controversy around the question of how long bacterial spores can survive in adverse environments here on Earth, and potentially on Mars (2, 3). At the moment, no upper limit for the survival of bacterial spores has been established. The ultimate limit for their survival might be the accumulation

of damage that overwhelms the repair mechanism when germination is induced. We propose that the ionizing radiation dose in different environments could well be the Achilles heel for the long-term survival of bacterial spores. To test this hypothesis, we have investigated how the radiation environment in halite fluid inclusions and in the Martian subsurface would affect the survival of viable bacterial spores. The work we present here consists of two parts: experiments to measure the radiation inactivation constant of bacterial spores, and calculations to evaluate the radiation dose in fluid inclusions and on Mars.

MATERIALS AND METHODS

Bacterial Strains Used

The bacterial strains used in this investigation were *Bacillus subtilis* (PY79), *Salibacillus marismortui* (ATCC No. 700626^T), and *Bacillus thuringiensis* subsp. *kurstaki* (BGSC No. 4D11). *S. marismortui*, formerly *Bacillus marismortui*, is a moderately halophilic spore former that was first isolated from Dead Sea water samples by Volcani in 1936 (4, 5). Based on the 16S rDNA phylogenetic tree, *S. marismortui* is very similar (99% S) to the isolate 2-9-3 that has been cultivated from a halite fluid inclusion (2, 6). Moderately halophilic bacteria initiate sporulation when the salt concentration of the brine reaches saturation. *B. thuringiensis* is a soil bacterium that is genetically close to *B. anthracis* and *B. cereus*. The divergence in DNA between *B. thuringiensis*, *B. anthracis* and *B. cereus* is less than 5% (7). *B. thuringiensis* is used as insecticidal toxin, while *B. anthracis* is the cause of the disease anthrax and is a potent biological warfare agent, and *B. cereus* is a common soil bacterium that causes food poisoning (7). We used a soil bacterium as a model because this class of bacterium usually is more resistant to desiccation, which coincidentally makes them also more resistant to ionizing radiation (8). *B. subtilis* was used to compare the radiation resistance of *S. marismortui* and *B. thuringiensis* with a well-known standard bacterium.

Spore Preparation

Single colonies of *B. subtilis* and *B. thuringiensis* were suspended in 3 ml of DSM [Difco sporulation agar, autoclaved and supplemental with 1 mM FeSO₄, 1 M Ca(NO₃)₂ and 10 mM MnCl₂] sporulation medium at 37°C. *B. subtilis* produced spores in 24 h, *B. thuringiensis* in about 1 week. Single colonies of *S. marismortui* were suspended in 500 ml K medium (750 ml filtered natural sea water, 250 ml distilled water, 0.5 g yeast extract, 2 g peptone per liter; autoclaved, 20 ml Hepes added and pH adjusted to 7.7–7.8) at room temperature. *S. marismortui* produced spores in about 2 weeks. Sporulation was verified by phase-contrast microscopy. The spore count was in the range of 5×10^8 spores/ml. After sporulation, the spores were cleaned with double-distilled water rinses (five times) and centrifugation (14,000 rpm for 5 min each). *B. subtilis* and *B. thuringiensis* were assayed on LB (Luria-Bertani) plates (10 g Bacto-tryptone, 5 g yeast extract, 5 g NaCl, 1 ml 1 M NaOH, 16 g agar per liter), *S. marismortui* was assayed on K-medium plates (same as liquid K medium, plus 15 g agar), all at 30°C. *B. thuringiensis* showed colonies after 7–10 h, *B. subtilis* after about 1 day, and *S. marismortui* after about 3 days.

Spore Irradiation

The spores were not stored but were used immediately for the different kinds of radiation experiments. For the radiation experiments with liquid samples (*B. subtilis* and *S. marismortui*), the spores were suspended in 1 ml double-distilled water with the pH adjusted to either 7 or 4. Aliquots of 200 µl were placed in thin-walled glass vials (inner diameter 4 mm, wall thickness 1 mm). For the experiments with dry samples (*B. subtilis* and *B. thuringiensis*), the spores were suspended in 1 ml of double-distilled water. Aliquots of 200 µl were placed in glass vials and dried down at room temperature and 1.3 kPa pressure. The

glass vials with the liquid and dry samples were flushed with nitrogen overnight and flame-sealed under constant nitrogen flow.

The samples were irradiated with γ rays from a ^{137}Cs source at a dose rate of 13.3 Gy/min to a total dose from 1–8 kGy at the Radiation Medicine Resource of the UCSD Cancer Center. At least three samples of each bacterial strain were exposed to each radiation dose (1, 2, 4, 6, 8 kGy). All experiments were performed twice, so that each data point consists of six individual measurements.

Irradiated spores and nonirradiated standards were diluted in 10 ml 1xT-Base. A dilution series from 2×10^{-2} to 1×10^{-6} covered the surviving fractions for all bacterial strains. The numbers of surviving spores were assayed after plating and growth for 1 day on LB plates (*B. subtilis* and *B. thuringiensis*) and for 3 days on K-medium plates (*S. marismortui*).

RESULTS

We measured the loss of colony-forming ability of spores irradiated with γ rays from a ^{137}Cs source with a total dose between 1–8 kGy. Dose–survival curves for dry *B. thuringiensis* spores and *S. marismortui* spores in pH 4 solution are shown in Fig. 1. The curve characteristics are shown in Table 1. Regression curves are based on a first-order exponential response:

$$\ln(N/N_0) = -kD + n,$$

where N is the number of colonies after irradiation, N_0 is the number of colonies prior to irradiation, k is the inactivation constant (kGy^{-1}), D is the radiation dose (kGy), and n is the extrapolation number.

The results show that the largest difference in radiation inactivation constants is between dry and wet samples. The ratio of the inactivation constants of liquid and dry spores ($k_{\text{liquid}}/k_{\text{dry}}$) of *B. subtilis* strain PY79 is 1.21. Differences between *B. subtilis* and *S. marismortui* spores in a pH 7 solution are within the statistical error. The same is true for differences in the inactivation constant of dry *B. subtilis* and *B. thuringiensis* spores. Noteworthy is the reduced radiation sensitivity at low pH. A comparison of *S. marismortui* in solutions of pH 7 and pH 4 shows a ratio of inactivation constants of 1.08 ($k_{\text{pH 7}}/k_{\text{pH 4}}$).

To put the radiation inactivation constant of spores into a geochemical context, we calculated the effect of natural radiation on the viability of spores in halite fluid inclusions and on Mars.

Halite Fluid Inclusions

Halite fluid inclusions can be used to study the composition of sea or lake water through time (9). Essential for the interpretation of the chemical composition of fluid inclusions is an understanding of the sedimentary and diagenetic environment in which the evaporate evolved. In addition, any halite crystal that contains fluid inclusions has to show features of primary evaporates so that more recent exchange with ground water through dissolution and recrystallization processes can be excluded (10, 11). Only then will the age of the deposit be equal to the age of a fluid inclusion and everything it contains.

We investigated what effect ionizing radiation in a halite fluid inclusion would have on the viability of bacterial spores. The only radionuclide of significance in a halite fluid inclusion is ^{40}K , which accounts for 0.0117% of natural potassium today. ^{40}K has a half-life of $1.28 \times$

10^9 years and decays by β -particle emission to calcium ($\langle E \rangle = 0.455$ MeV, 89.3%) and by an electron capture to argon ($E_\gamma = 1.46$ MeV, 10.7%). The size of a 10- μ l fluid inclusion was chosen because it is large enough to determine the chemistry of the brine and to extract samples for cultivation. About 83% of the β electrons are emitted in the energy range from 0.1 to 1.3 MeV. Fluid inclusions with a density of 1.25 g/cm³ (12), and a dimension of 10 μ l, will absorb most of the β -particle decay energy. However, only about 1% of the γ -ray energy emitted from inside the fluid inclusion is actually absorbed in the inclusion. Gamma radiation from the surrounding crystal might contribute to the internal radiation dose, but the amount is low compared to the internal radiation dose from the β -particle decay because the abundance of potassium in the halite crystal is usually two orders of magnitude smaller than its abundance in the brine. Therefore, we did not include the energy from the emitted γ radiation in our calculations. Potassium concentration in halite fluid inclusions can vary significantly (12–14). We calculated the dose for a lower potassium concentration of 2.2 g/liter, and an average potassium concentration of 7 g/liter. The isotopic abundance of ⁴⁰K changes over time, from 0.0117% today to 0.0134% 250 million years ago. Because of the wide range of different potassium concentrations in halite fluid inclusions, this isotopic change is of minor significance and will not be considered in the radiation dose estimates.

The dose rate is proportional to the concentration of the radionuclide, the decay rate and the energy release per decay. The specific activity of ⁴⁰K is 2.0905×10^5 Bq/g, and the energy released from the β -particle decay only (89.3%), after correction for the energy loss in neutrino emissions, is 0.455 MeV per decay or 2.6787×10^{12} MeV/year per gram of ⁴⁰K. A concentration of 2.2 g/liter potassium (0.257 ppm ⁴⁰K, with an isotopic abundance of 0.0117%) gives a dose rate of 110 μ Gy/year. A concentration of 7 g/liter potassium (0.819 ppm ⁴⁰K) gives a dose rate of 350 μ Gy/year. The accumulated dose over 250 million years is between 28 kGy and 88 kGy, depending on the potassium concentration.

The accumulated dose over time can be compared to the radiation sensitivity of bacterial spores. We measured the radiation inactivation constant for the halophilic *S. marismortui* spore in a deoxygenated solution (Table 1). Most of the halite fluid inclusions have a pH of 4–5 (15). Therefore, we used the inactivation constant of *S. marismortui* in a pH 4 solution ($k = 1.14$ kGy⁻¹), which is slightly different from the inactivation constant of *S. marismortui* in a pH 7 solution ($k = 1.23$ kGy⁻¹) (Table 1). Radiation inactivation constants of *B. subtilis*, *B. pumilus* and *B. cereus* in solution (mostly around pH 7) measured by other authors are in the range of 1.32–2.15 kGy⁻¹ (16).

To calculate the loss of viability of bacterial spores in a fluid inclusion, we hypothetically put them in a 10- μ l fluid inclusion. Assuming a number of 4000 CFU/ml in a brine (17) and an $N/N_0 = 10^{-6}$, the chances of finding viable spores is about 1 in 25,000 after 17–34 million years for 7 g/liter potassium and 55–109 million years for 2.2 g/liter potassium, depending on the radiation inactivation constant used (Fig. 2).

The situation of retrieving ancient viable bacterial spores in a halite fluid inclusion on Mars is similar to the terrestrial case. The abundance of potassium ions on Mars, based on analysis of the Martian meteorite Nakhla, is similar to that in terrestrial seawater (18). Hence it would be highly unlikely to find viable spores in fluid inclusions from an extinct early biosphere on Mars.

Mars

For centuries, scientists believed that Mars might be a planet almost like ours, habitable for life as we know it and occupied by an advanced civilization that struggled for their survival (19). However, in 1965, Mariner IV showed us a very different world (20). Mars today is a cold, dry desert planet, bathed in UV radiation and with an atmospheric pressure that is

1/100 that of the Earth. However, the climate on Mars is thought to have been more clement during the transition from the Noachian to the Hesperian epoch (21, 22), a transition period around 3.1 billion years ago (23). If life ever evolved on Mars, would it be possible to see the remnants of a long-extinct biosphere today? Or even more interesting, would it be possible to find Martian bacterial spores that survived for billions of years on Mars?

In the course of this study, we calculated the effect of the ionizing radiation environment on Mars on the survival of viable bacterial spores. There are two distinct radiation environments on Mars: The first few meters of the Martian subsurface is dominated by the effect of galactic cosmic radiation (GCR), while below about 3 m, the radiation environment is characterized by radiation from the radioactive isotopes ^{40}K , ^{232}Th , ^{235}U and ^{238}U .

We will address the radioactivity of the Martian subsurface below 3 m first. The abundance of potassium, thorium and uranium on Mars is about two orders of magnitude smaller than on Earth. This reflects the lower degree of differentiation of the Martian crust. Based on the SPB (Shergotti Parent Body) model, the abundance of potassium, thorium and uranium is 315 ppm, 5.6×10^{-2} ppm and 1.6×10^{-2} ppm, respectively (24). The isotopic abundance today is 0.0117% for ^{40}K , 100% for ^{232}Th , 0.7200% for ^{235}U , and 99.2745% for ^{238}U . The decay energy corrected for neutrino loss is 0.71 MeV for ^{40}K , 39.9 MeV for ^{232}Th , 47.1 MeV for ^{238}U , and 44.2 MeV for ^{235}U (25).

The dose rate from internal radioactivity can be calculated from the concentration of each radionuclide and its decay parameters:

$$D \text{ (Gy/year)} = \left[c \text{ (ppm)} \times E \text{ (MeV/decay)} \times \lambda \text{ (year}^{-1}\text{)} \times 6.022 \times 10^{23} \right] \div \left(A \times 6.24181 \times 10^{15} \right), \quad (1)$$

where D is the dose rate, c is the concentration of the radionuclide, E is the energy per decay corrected for neutrino loss in β -particle decay; λ is the decay constant, 6.022×10^{23} Avogadro's constant in mol^{-1} , A is atomic mass, and the conversion factor for 1 Gy is 6.24181×10^{15} eV/g.

The calculated dose rate using Eq. (1) from the decay of radionuclides ranges from 350 $\mu\text{Gy/year}$ at 3.1 billion years ago to 130 $\mu\text{Gy/year}$ today. The total accumulated dose over the last 3.1 billion years in the Martian subsurface is close to 740 kGy.

The accumulated dose over time can be compared to the radiation sensitivity of bacterial spores. We measured the radiation inactivation constant of dry *B. thuringiensis* soil spores in a nitrogen atmosphere. The inactivation constant, of *B. thuringiensis* is 1.00 kGy^{-1} (Table 1). The radiation inactivation constant of dry *B. subtilis* and *B. pumilus* spores measured by other authors is in the range of $0.66\text{--}0.92 \text{ kGy}^{-1}$ (26). Dry bacterial spores show a temperature dependence in their radiation sensitivity, following a vant'Hoff-Arrhenius law above 130 K (27). The temperature-corrected inactivation constant for a Martian subsurface temperature of 220 K is $0.59\text{--}0.90 \text{ kGy}^{-1}$ for the referenced and measured dry bacterial spores.

To calculate the loss of viability of bacterial spores, we hypothetically put them in the Martian subsurface 3.1 billion years ago (Fig. 3). The chances of finding viable bacterial spores in a 1-g sample of Martian soil is less than 1 in a million after 100–160 million years, depending on the radiation inactivation constant used, assuming an optimistic original bacterial concentration of $1 \times 10^8/\text{g}$ of soil (28) and a reduction of the population by 14 orders of magnitude (D_{14}).

The probability is even lower when considering the Martian subsurface that is accessible to *in situ* landers (with present technology <3 m). The oldest Noachian terrain on Mars had an extremely low erosion rate during the subsequent Hesperian and Amazonian periods of just several 10^{-2} to 10^{-1} nm/year (29). At the same time, about 95% of all channel features on Mars are located on the old Noachian terrain (30). Thus over 3.1 billion years, or since the time that Mars had a more clement period, only several centimeters of material has been eroded away in Noachian net deflation areas. These areas represent the best location to access samples from this early time. Unfortunately, the first couple of meters of the Noachian terrain have been exposed to the GCR for the same period. The GCR field on Mars is derived from results of theoretical calculations describing the radiation attenuation of GCR behind Martian regolith (31). The model was generated by solving the Boltzmann equation using a HZETRN system with the 1977 Solar Minimum environmental model (31). With a density of 2.6 g/cm^3 (32), the depth-dependent dose rate ranges from 0.19 Gy/year at the surface to 600 $\mu\text{Gy/year}$ at a depth of 3 m. The accumulated dose over 3.1 billion years within the first meter is of the order of 200–800 MGy. The probability of finding viable bacterial spores in a 1-g sample of Martian soil within the first meter is 1 in a million within 600,000 years using an average radiation inactivation constant for dry bacterial spores (Fig. 4).

DISCUSSION

It might be argued that the much higher dose rate used for the present experiments is not a good model for the long-term exposure at very low dose rates. To evaluate the validity of our experiment, it is necessary to briefly discuss the effect of water content and oxygen on the radiation damage of spores. There are three classes of damage to bacterial spores from ionizing radiation (33, 34): Class I damage is the direct damage to spores by short-lived radicals in the absence of oxygen, class II is the damage with short-lived radicals in the presence of oxygen during irradiation, and class III is the damage by long-lived radicals in the presence of oxygen after irradiation. Liquid water completely anneals class III damage so that any spores in anoxic solution will only show class I damage. In that sense, we suggest that our experiment can indeed simulate the long-term radiation exposure of spores in anoxic solutions. Any oxygen present in a real fluid inclusion would only increase the inactivation constant and shorten the survival time for viable spores (35). The situation is different in dry spores. Irradiated dry spores develop class I and III damages when they are exposed to oxygen after irradiation (33). In our experimental procedure, we measure the inactivation constant of class I and III damage. However, thermorestitution can prevent the latent oxygen effect by annealing the long-lived radicals, reducing the inactivation constant (36). This thermorestitution requires temperatures of 50°C or higher for maximal restoration. At lower temperatures and longer anaerobic storage times, the efficiency of this process is strongly reduced. In addition, the thermorestitution constant is strongly dependent on temperature. Thus we assume no substantial restoration effect at Martian subsurface temperatures of 220 K and below. Therefore, we suggest that we can simulate the long-term radiation exposure of spores in the anoxic dry state. Since the spores are dormant and damage will build up at both dose rates with no interaction with any enzymatic repair processes, no dose-rate effect should occur.

One obvious question is how and to what extent the radiochemistry of a brine could affect our interpretation. Radiolysis of deoxygenated pure water results in a 10^{-8} – 10^{-7} mol/liter steady-state concentration of hydrogen, hydrogen peroxide and oxygen (37), while oxygenated pure water shows a buildup of hydrogen peroxide and hydrogen in the 10^{-5} mol/liter range and a depletion of oxygen (37). Radiolysis of sea water leads to numerous radical reactions but is dominated by the radiation chemical reactivity of the chloride ion (38, 39). The cytotoxicity of irradiated sea water is most likely derived from hydrogen peroxide and

hypochlorite (40-42). Bacterial spores are protected from the detrimental effect of hydrogen peroxide at low concentrations by a thick spore coat (43). The sporicidal effect of hypochlorite on bacterial spores, however, is much more pronounced (42). To avoid the sporicidal effect of hypochlorite, which is strongly dependent on concentration, we used pure water in our experiments. Deoxygenated pure water also limits the contribution of hydrogen peroxide (low concentration for a short time) in spore inactivation. Both cytotoxins will be present after irradiation of brine inclusions—hypochlorite from the chloride in the brine for a very short time and hydrogen peroxide from water hydrolysis at a low steady-state concentration. Therefore, the time we calculated to inactivate bacterial spores in a halite fluid inclusion can only be seen as an upper limit, ignoring any sporicidal effect of the irradiated medium.

Our results do not totally exclude the survival of ancient bacterial spores in halite fluid inclusions. They are valid to the extent that bacterial spores have indeed been dormant and unable to repair any damage in this state. However, it does set limits on the long-term viability of bacterial spores based on the chemistry of the fluid inclusion, which, if the inclusion is a primary feature, should have remained constant over time.

Several studies investigated the survival of bacterial spores under simulated Martian conditions (e.g. 44, 45). The conclusion of these studies is that bacterial spores show significant survival as long as they are protected from the incident UV radiation. However, no consistent upper limit for the long-term survival is given. Our results show that even in the absence of any other chemical or physical degradation process, ionizing radiation severely limits the long-term survival of viable bacterial spores in the Martian subsurface. If any cycle on Mars allows dormant life forms to awake from their dormant state, repair the accumulated damage, and multiply, then the period of this cycle must be shorter than the time until the viability of spores falls below a certain value. In that case, radiation will not limit the long-term viability of dormant life forms other than setting the upper limit for the time between dormant/active cycles. This period has to be shorter than about 100 to 160 million years for the deeper subsurface and less than about 600,000 years for the uppermost few meters, assuming a D_{14} until recovery of dormant spores. The only established quasi periodic changes on a global scale are the obliquity oscillations with a period of 120,000 years and an amplitude modulation of 2 million years (46, 47). The obliquity is the angle between the spin axis and the normal to the orbit plane. On Earth, the presence of the Moon has stabilized the rotation axis. Mars does not have a large moon, and so the obliquity can change from 13° to 42° (47). This strongly affects the climate on Mars. The thermal wave of these obliquity changes can penetrate several hundreds of meters into the subsurface and, depending on obliquity and on the latitude, can either lower or raise the temperature in the Martian subsurface (48). However, the peak temperatures where permafrost is stable close to the surface (>60° latitude) are still limited to those below 200 K. Although there are cryptoendolithic communities that can thrive at temperatures below freezing, this ability depends on a nanometer-thin layer of liquid water around them (49). The thickness of this water layer goes down steadily with decreasing temperature. It might be possible to have some extremely slow metabolic activity at temperatures of about 250 K, which, however, is still 30 to 50 K higher than the Martian subsurface temperature. Thus, although the period of obliquity changes are within the limit for D_{14} , it is questionable whether they could change the environmental conditions enough to allow spores to germinate. Another possible source of periodic changes in the Martian environment would be local volcanic activity. Unlike Earth, hot spots on Mars are likely to be confined to the same locations for long periods (50). If such activity occurred throughout the history of Mars, it would allow very local ecological niches in the Martian subsurface. There are indications for recent (order of 100 million years) volcanism on Mars (51).

However, even if viable bacterial spores cannot be found on Mars, it should still be possible to detect their molecular remains. Amino acids would be stable enough to still give a detectable signal assuming about 10^8 cells/g of soil 3.1 billion years ago. For a detection limit of parts per billion at present, the search would have to drill to a depth of at least 1 m into the Martian subsurface. At shallower depth, amino acids are destroyed by the GCR over 3.1 billion years (Kminek, unpublished data).

Are there other models than bacterial spores that show similar long-term survival capabilities? The extremophile *Deinococcus radiodurans* might be a potential candidate. *D. radiodurans* has been described as a polyextremophile model organism for long-term survival on Mars (52). Based on its radiation resistance alone (53), dormant *D. radiodurans* could survive at least as long in the Martian subsurface as bacterial spores. However, because *D. radiodurans* does not form spores, it might be more prone to other physical and chemical effects. Although *D. radiodurans* is resistant to desiccation, a 6-log reduction in viability is expected after only 3–4 years, and the survival is even lower if alternating cycles of desiccation and partial rehydration are present (8). Bacterial spores show a better resistance to desiccation (54). Potential chemical agents are peroxides in the Martian regolith. There are indications from Viking measurements and theoretical models that at least the surface layer of Mars contains a variety of peroxides (55, 56). While spore-forming bacteria are well protected from peroxides by their spore coat (43), *D. radiodurans* is not. Therefore, bacterial spores are probably still a better model for the long-term survival of life forms on Mars.

CONCLUSION

In the absence of any possibility to repair damage over time, we conclude that the long-term survival of viable bacterial spores is limited by ionizing radiation from the environment. For halite fluid inclusions, the upper limit is less than 109 million years, depending on the potassium concentration. From a radiobiological point of view, it is very unlikely that a 250 million-year-old viable bacterial spore could be isolated from a halite fluid inclusion.

The long-term survival of viable bacterial spores on Mars is limited to less than 100 to 160 million years in the Martian subsurface deeper than 3 m and to less than 600,000 years within the uppermost meter of Mars. It is highly unlikely to find viable bacterial spores from an early Martian biosphere in the absence of frequent revivals of dormant spores through environmental changes.

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FIG. 1.
Dose–survival curves for dry *B. thuringiensis* spores (●) and *S. marismortui* spores (◆) in a pH 4 solution.

FIG. 2.

Calculated surviving fraction of *S. marismortui* spores in a pH 4 solution as a function of time in million years. Radiation dose is calculated for a low and average potassium concentration of 2.2 (—) and 7 (---) g/liter.

FIG. 3.

Calculated surviving fraction of dry bacterial spores in the Martian subsurface as a function of time in million years. The starting point is 3.1 billion years ago. Variations in the radiation inactivation constant have no strong impact on the survival time.

FIG. 4.

The graph shows the calculated depth dependence of the time (in thousand years) until the viability of dry bacterial spores is reduced by a factor of 10^6 (—) or 10^{12} (---) by GCR on Mars. Variations in the radiation inactivation constant have no strong impact on the survival time.

TABLE 1Radiation Inactivation Constants for *B. subtilis*, *S. marismortui* and *B. thuringiensis* Spores

Radiation inactivation constant	<i>B. subtilis</i>		<i>S. marismortui</i>		<i>B. thuringiensis</i> Dry
	Wet pH 7	Dry	Wet pH 7	Wet pH 4	
k (kGy ⁻¹)	1.22 ± 0.04	1.01 ± 0.03	1.23 ± 0.02	1.14 ± 0.03	1.00 ± 0.01