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Are We Alone? The Search for Life on Mars and Other Planetary Bodies

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<http://dx.doi.org/10.5772/intechopen.75437>

Abstract

Extensive research has shown the potential for microorganisms to survive in some of the most extreme environments on Earth. Our current understanding of diverse life on Earth implies that, even though the surface of Mars is very inhospitable to life, it is possible that there may be indigenous microorganisms on Mars, especially in the protective subsurface. Ultimately, a better understanding of microbial diversity on Earth is needed to determine the limits of life to help determine the potential for life on Mars and other exoplanets.

Keywords: microorganisms, extreme environments, life on Mars, exoplanets

1. Introduction

The search for extraterrestrial life is bolstered by our long-standing quest to determine if we are alone in the Universe. Mars and Europa are two likely candidates to target in the search for extraterrestrial life, since both have carbon, potential energy sources, and water in some form [1–4]. The current focus to search for life on Mars is supported by the fact that although Mars is quite cold and dry, current conditions are thought to be analogous to conditions on early Earth when single-celled life was gaining a foothold [5]. Furthermore, because there is a diversity of microorganisms known to thrive in the most inhospitable habitats on Earth, it is not unreasonable to think that microorganisms could live on Mars.

While continuing Mars explorations confirm that all of the basic necessities for microbial life are present, it remains unclear whether microorganisms that are metabolically capable

of living on Mars can actually survive in the Mars environment. The Mars surface presents a very inhospitable habitat for life because of the intense radiation, highly oxidizing conditions, concentrated evaporative salts, and extremely low water activity. Determining if microbes can survive those surface conditions, including tolerance to radiation (both ionizing and non-ionizing), desiccation, and oxidizing environments of microorganisms that utilize the carbon and energy resources available on Mars is vital. Some information is available on the survival of spore-forming microbes in a Mars-like environment, but much more information is needed regarding survival potential of different types of microorganisms. This chapter will focus on the search for life on planetary bodies.

2. The Martian environment

Although Mars is considered to be at the outer edge of the habitable zone of our solar system, the idea that there could potentially be life on Mars, especially in the subsurface, is not unfathomable. Although it can be expected that different areas of Mars would have somewhat different environments dependent on location, overall the Martian environment is quite inhospitable to most life as we know it. Average temperatures on Mars can range from -10 to -76°C with an average surface temperature of -65°C although temperatures can fluctuate from as high as 25°C to -123°C [6–8].

Mars is considered to be quite dry, but recent information suggests otherwise. Studies of the Gale Crater by the rover Curiosity found hydration of soils to be as much as 2.25 wt%. This finding was consistent with findings by both Viking 1 and 2 and the Mars Odyssey [9]. What is unknown is if there is an underground source of water. Geophysical and geochemical features on Mars indicate that there may have been water on the surface at some time in the past but it is unknown to what extent surface water would have existed. Features include alluvial fans in craters, dendritic valley networks, and the presence of specific minerals thought to only form in the presence of water. One hypothesis is that hydrothermal environments associated with craters from impacts and volcanism could have easily provided a source of liquid water on Mars [10].

The Martian atmosphere is much different from that on Earth. Mars has primarily a CO_2 atmosphere (95.3%) compared to the CO_2 content in Earth's atmosphere (0.04%). Earth's atmosphere consists mainly of N_2 (78.1%) while there is only 2.7% N_2 in the Martian atmosphere. The O_2 concentration on Earth is 20.9% whereas Mars' atmosphere contains only about 0.1% O_2 [7]. Studies by Mumma et al. [11] showed the presence of methane in extended plumes that appeared to be released from discrete regions on Mars. One of the principal plumes contained as much as 19,000 metric tons of methane, an amount comparable to that of a massive hydrocarbon seep in Santa Barbara, California. However, analyses by the Mars rover Curiosity found no detectable atmospheric methane. Although results are contradictory, it is possible that the location of the rover was too far from the methane seeps and prevented the detection of methane in the atmosphere.

The surface of Mars is subjected to both cosmic ionizing radiation and solar UV radiation. Ionizing radiation on Mars is believed to be 100X higher than on Earth, ranging from 100 to

200 mSv/a compared to Earth 1–2 mSv/a [7]. The UV-B and UV-C fluxes on Mars are nearly 5× higher than they are on Earth with fluences of 361 kJ/m² and 78 kJ/m², respectively [12]. On Mars, the high atmospheric concentration of CO₂ neutralizes incoming UV radiation <200 nm, however wavelengths >200 nm still reach the Martian surface [7]. Of note is that some of the UV radiation may be attenuated at times by the presence of dust storms in a particular region.

Data collected during the Viking missions showed that the surface of Mars was highly oxidized compared to the Mars atmosphere [13]. Mapping of hydrogen peroxide (H₂O₂) on Mars using infrared high-resolution imaging spectroscopy indicated H₂O₂ abundance on Mars is 15 ± 10 ppb although prior mapping showed concentrations as high as 40 ppb [14]. The formation of peroxides could occur in the presence of hematite, trace amounts of water, and UV radiation [7]. A more likely scenario is that radiolysis of ice or water would create a larger amount of peroxide formation. It has been reported that the surface ice of Europa contains as much as 1,300,000 ppb H₂O₂ which is generated from radiolysis of ice [15]. Additionally, perchlorate, a strong oxidizing agent, was found by the Phoenix Lander to be present in Martian soils in concentrations of 2.1–2.6 mM [16].

Martian soils contain few nutrients to support life as we know it, and the soils themselves pose a harsh environment. Martian soils were expected to be acidic, but the Phoenix Lander showed that the soils at its landing site were mildly basic with a pH of 7.7 ± 0.5 [17]. Salt tolerance would be required for life to survive and grow on Mars due to the high salt concentrations found in Martian soils in the form of NaCl, MgSO₄, CaSO₄, FeSO₄, MgCl and CaCl₂ [17]. The lack of water, the intense radiation and oxidative conditions make the Martian surface quite inhospitable to life.

3. Special regions on Mars

3.1. Introduction

Mars Special Regions are regions where organisms are likely to survive. NASA Procedural Requirement (NPR) 8020.12D [18] defines these areas as regions that have a high potential for the existence of extant Martian life forms, have sufficient water activity (0.5–1.0 aw) and have sufficiently warm temperatures (–25° C lower limit) to permit replication of Earth organisms. Areas that have observed features that may be associated with the presence of water must also be classified as Special Regions. It is noted that these parameters may need to be changed as our understanding of Mars and life on Earth evolve and as our technological capabilities improve [19, 20].

3.2. Formulating special regions

The COSPAR colloquium on special regions stated that “Preventing terrestrial biological contamination from becoming established and widespread on Mars is essential to our ability to protect high-priority science goals on Mars” [20]. The current standards are based solely on protecting science goals and not on protecting Mars in and of itself. The NRC study takes a

precautionary principle approach by stating that there is insufficient data to determine which regions of Mars should be considered “special” and that all of Mars should be considered “special” until it can be proven otherwise [19, 20]. The COSPAR disagreed and concluded that there was sufficient data to arrive at a conclusion as to which areas of Mars would be defined as “Special Regions” [20].

The COSPAR colloquium concluded with the enactment of the standards that are currently in NPR 8020.12D [18]. Two main standards, water activity and temperature, are the basis for determining which regions should be taken into consideration. One area in need of additional research is that of microbial growth and reproduction at low temperatures. It was noted that most of the work in this area has been performed on laboratory isolates and more environmental data is needed to begin to define the lower temperature for life. It was concluded that investigations were needed to determine if microbial reproduction at water activities of lower than 0.6 is possible, that more studies are needed using Mars simulated environments, and that knowledge of reproduction of communities rather than isolates is essential to improve our understanding of life. COSPAR also noted that a larger phylogenetically diverse array of organisms needs to be studied and diurnal, seasonal and long-term variations in the Martian surface need to be better understood [20].

The NASA Planetary Protection Office made some initial suggestions to try to define special regions. The parameters were set as: (1) the existence of liquid water in “pure” form or in strong brines up to 5.5 M CaCl_2 ; (2) regions of current or active volcanism or enhanced heat flow which is yet unknown; (3) permafrost through 100% water ice, including segregated ground ice, ice-rich frozen ground, polar caps and subsurface ice; (4) subpermafrost groundwater and (5) any gully system that may be indicative of recent water activity within the last <50,000 years. The Special Regions Science Analysis Group (SRSAG) determined that regions should be defined as non-special if the temperature remains below -20°C or the water activity remains below 0.5 for a period of 100 years after spacecraft arrival [21]. Ultimately, the SRSAG developed a map of regions that are considered “significant” and of interest for determining special region boundaries. Of note is that the current definition of special regions mostly takes into account the known and sets the water activity and temperature parameters slightly below what is currently known.

4. The relationship between life on Earth, and the potential for life on Mars

4.1. Life in extreme environments

Despite our limited knowledge of microbes on Earth, everywhere we have looked for microbes and we have been able to find them. It appears that life inhabits all places on Earth including some of the most extreme environments imaginable. Microorganisms have been discovered surviving and reproducing in hot springs, at terrestrial depths exceeding 2 km, in the most arid of deserts, and in hydrothermal vents on the ocean floor. Microbial life has been found in extremely cold places such as in Antarctica and Greenland, and microorganisms have been

described as reproducing and thriving at temperatures as low as -15°C . Many microorganisms can grow in salt at concentrations exceeding 20% NaCl, or 2 M MgSO_4 , and others thrive in either very acidic or very alkaline environments. Microbes can conserve energy by respiring some of the most extreme compounds, such as U, Mn, Se, As, S and Cl-based molecules. Life at either high or low atmospheric pressures has been described, as well as organisms that are highly resistant to radiation and oxidative conditions. Most of the organisms surviving in these types of environments have a symbiotic relationship with other organisms in the same community. For example, methane-oxidizing archaea (MOA) are known to live in symbiosis with sulfate-reducing bacteria (SRB) in deep hydrothermal vents on the ocean floor. MOAs break down methane to CO_2 and H_2 , and the H_2 is then utilized by the SRBs to reduce SO_4^{2-} to HS^- [22]. These types of relationships between organisms are far from uncommon.

Earth microorganisms have developed physiological and biochemical mechanisms to be able to survive in a variety of extreme niches. As previously stated, it would not be unreasonable to expect niches on Mars, although considered extreme, to support microbial life of some sort as well. The remainder of this chapter will discuss what is known about how microorganisms survive some of these extreme environmental conditions and how this information is relative to the potential for life on Mars. Although this portion of the chapter will focus on bacteria, it should always be kept in mind that many of the topics discussed apply to archaea and fungi as well.

4.2. Survival at low temperatures

Average temperatures on Mars can range from -10 to -76°C with an average surface temperature of -65°C although temperatures can fluctuate from as high as 25°C to as low as -123°C [6–8]. For an organism to be able to thrive on Mars it would need to be able to grow and reproduce in these frigid temperatures. An exception would be a subsurface environment that was geothermally heated though no such areas have been discovered on Mars.

A number of psychrophilic (cold-loving) organisms have been isolated from many regions of the Arctic and Antarctic where there are polar ice sheets, glaciers and permafrost. Additionally, microorganisms are known to inhabit the ocean floor where temperatures are $\leq 4^{\circ}\text{C}$ [23]. These organisms are comprised of representatives from the Eukarya (algae, fungi and yeast), Bacteria and Archaea. Morozova et al. [24] identified several methanogenic archaea that were able to survive not only low temperatures ranging from -75 to 20°C , but could also simultaneously survive low humidity and a 95.3% CO_2 atmosphere. The methanogens that survived best under these conditions were isolated from permafrost. Six isolates from permafrost and nine known species of *Carnobacterium* were found to grow not only at 23°C , but also at 0°C , under low pressure and in a CO_2 -enriched anoxic atmosphere [25]. A strain of *Serratia liquefaciens*, a common mesophilic organism often found as a contaminant in bathtubs, was shown to be capable of growth at 0°C as well as at low pressure and CO_2 -enriched anoxic atmospheres [26]. Mykytczuk et al. [27] identified a *Planococcus* isolate that grows and divided at -15°C and is still metabolically active at -25°C .

Despite these organisms being interesting in themselves, what is even more interesting is the ability of these organisms to make both physiological and biochemical modifications to

survive in such environments. *Psychrobacter arcticus* 273-4, a bacterium capable of growing at temperatures as low as -10°C , was found to downregulate genes related to energy metabolism and carbon incorporation, and upregulate genes required for maintenance of membranes, cell walls and nucleic acid motion. Furthermore, this organism turns on the expression of a cold-shock DEAD-box RNA helicase A, a protein that may be key for maintaining life in cold temperatures [28]. *Planococcus halocryophilus* Or1 grew at subzero temperatures by forming encrustations around the cell and increasing the ratio of saturated to branched fatty acids in the cytoplasmic membrane [27]. This is unique because often growth at lower temperatures results in a higher content of unsaturated, polyunsaturated and methyl-branched fatty acids to increase membrane fluidity at these temperatures. In many organisms, enzymes involved in transcription, translation, protein folding and stabilization of DNA and RNA show activity at very low temperatures and are adapted to life in cold environments. Antifreeze-like proteins have been seen in Antarctic lake microbes and trehalose and exopolysaccharides might also provide cryoprotection for psychrophiles [29]. Although scientists are far from having a full understanding of life in cold temperatures, studies like the ones above provide insights as to how these organisms adapt to their extreme environment. Additionally, the microbes are models to further our understanding of how organisms may survive on Mars, and can be useful as we continue the search for life on cold planets and moons.

4.3. Tolerance to high salt

Due to the high salt concentrations found in Martian soils in the form of NaCl , MgSO_4 , CaSO_4 , FeSO_4 , MgCl_2 and CaCl_2 , salt tolerance would be required for life to survive and grow on Mars [17]. Salts can be chaotropic as they influence water activity, affect cell turgor, and are major stressors of cellular systems [30]. It is estimated that 1/4th of the Earth's land is covered by salt and salt water makes up the majority of Earth's water. On Mars, it is estimated that sulfurous salts are more common than chlorinated salts by a ratio of 3:1. On Earth the most common type of salt is NaCl but many brines also contain MgCl_2 , MgSO_4 and other salts [17, 30]. Studying hypersaline environments from Earth increases our understanding of how organisms can adapt to these extreme environments.

Many *Bacillus* sp. are salt-tolerant and thus of special interest with regard to growth under high salt conditions. Previous studies in our laboratory have shown that many different species of *Bacillus*, including *pumilus*, *licheniformis*, *horti*, *mannilyticus* and *cellulosilyticus*, as well as species belonging to other genera including *Paenibacillus*, *Amphibacillus* and *Alkalibacterium*, could grow under salt concentrations as high as 10% NaCl . Several of these organisms also showed growth in media containing 20% NaCl . These isolates were collected from the Alvord Basin in Oregon where the soils are known to have elevated salt concentrations [31]. The ability of *Bacillus* sp. to grow under these conditions is not uncommon and many organisms which have been identified as non-spore formers can also grow in high NaCl concentrations.

A diversity of prokaryotes was discovered residing in deep hypersaline anoxic basins in the Mediterranean Sea; basins that are nearly saturated with MgCl_2 (5 M). In addition to growing in extremely high concentrations of MgCl_2 , the microorganisms were involved in sulfate reduction and methanogenesis, and contributed to the cycling of carbon [32]. Furthermore,

the overall microbial community was unique because the bacteria and archaea identified were not related to organisms normally found in seawater, and the archaea branched deeply within the Euryarchaeota indicating they comprised a new order.

It is estimated that the majority of salt on Mars would likely be MgSO_4 , with lower concentrations of NaCl and CaCl_2 . Studies by Crisler et al. [17] focused on the growth of microorganisms under high MgSO_4 concentrations using microorganisms collected from the Great Salt Plains in Oklahoma. Though the microbes were not identified, it was found that 35% of the organisms from the bacterial collection could grow in medium containing 2 M MgSO_4 and at least 80% could grow in the presence of 10% MgSO_4 [17]. Studies using isolates collected from the Mars Science Laboratory (MSL) pre-launch showed that a large percentage of the organisms from the MSL were able to grow in media containing 1 M or 2 M MgSO_4 (Smith, unpublished).

Although scientists are still learning more about how life survives in these extreme, high salt environments, we do know that the cells must have special physiological and biochemical properties to survive such environments. The primary factors for surviving these conditions are the amount of energy generated during dissimilatory metabolism and the mode of osmotic adaptation utilized [33]. A review of studies from 1999 concluded that aerobic respiration, denitrification, and both oxygenic and anoxygenic photosynthesis can occur under the highest salt concentrations but autotrophic oxidation of ammonia and nitrate, some forms of methanogenesis and sulfate reduction were never found at salt concentrations $>100\text{--}200\text{ g l}^{-1}$ [33]. Processes identified as occurring, albeit poorly, at salt concentrations $>200\text{ g l}^{-1}$ included fermentation, aerobic autotrophic oxidation of sulfur compounds, sulfate reduction by incomplete oxidizers and some other forms of methanogenesis.

Oren hypothesized based on his findings that life at high salt concentrations is energetically expensive, and the upper salt concentration limit at which dissimilatory processes occur is determined partly by bioenergetics constraints. Given this the main factors that determine whether a certain type of organism can make a living at high salt concentrations are the amount of energy gained during its dissimilatory metabolism and the mode of osmotic adaptation used. Based on his review of halophiles, Oren stated that the energy cost associated with salt exclusion and pumping ions out was unfavorable and that the "salt-in" strategy was energetically favored. Given this the following types of metabolism are most likely to occur under high salt concentrations: (i) those that use light as the energy source, (ii) aerobic respiration, denitrification, and other highly exergonic dissimilatory processes coupled with large production of ATP and (iii) types of metabolism performed by organisms that use the "salt-in" strategy even when the amount of ATP obtained in their dissimilatory processes is low [33]. Oren hypothesizes that the salt-in option would be energetically favorable to organisms, and it is clear that organisms have made adaptations to their molecules to thrive under high salt conditions and allow for the "salt-in" option. Studies by Tehei et al. [34] identified a malate dehydrogenase and tRNA molecules, from the archaeon *Haloarcula marismortui*, that are protected in the presence of high salt. The salt protected the tRNA molecules from thermal degradation while the malate dehydrogenase was protected from thermal denaturation. While studying the lipid composition of *Halobacillus halophilus*, Lopalco et al. [35] found that the organism increased the number of shorter chains and incorporated unsaturated chains in the lipid core structures.

It was believed that these changes compensated for an increase in phospholipid packing and rigidity, and sulfoglycolipid polar heads. It is believed that these changes allowed for homeostasis of membrane fluidity and permeability under high salt stress conditions.

Although many more studies need to be conducted to have a full understanding of how organisms survive these high salt environments, these studies do show that life under these conditions is possible and even, in some cases, protective. Given this, it would not be unreasonable to think that such microorganisms would be able to thrive on Mars in the salty Martian soils. Oren includes organisms using light as the energy source, however this would be unlikely on Mars since organisms living on this planet would also have to survive other conditions on the surface such as desiccation, and high radiation (to be discussed later). It is more likely that organisms on Mars would utilize exergonic dissimilatory processes or utilize types of metabolism which allowed for the “salt-in” strategy [33].

4.4. Tolerance to pH extremes

The ability of organisms to withstand alkaline pH is a factor to consider when discussing life on Mars. Initially, it was thought that the Martian soil was likely to be acidic but results by the Phoenix Lander showed that the soils at that site were mildly basic with a pH of 7.7 ± 0.5 [16]. Although the pH at the Phoenix Lander study site was only slightly basic, it is possible that other soils on Mars are more basic.

Alkaliphiles are organisms which grow above neutral pH whereas extreme alkaliphiles generally grow in the pH range of 10.0–14.0. Studies on alkaliphilic organisms have mostly focused on *Bacillus* sp. with the most extensive studies having been performed on *B. halodurans* and *B. pseudofirmus* [36]. The biggest hurdle facing alkaliphilic organisms is the ability to maintain homeostasis and maintain chemiosmosis. Alkaliphiles use transporters to help catalyze proton transport and these transporters include proton-pumping respiration chains, proton-coupled ATPases, and secondary active transporters. Often the uptake of protons is unequal where 2H^+ are exchanged for one Na^+ ion. Studies have shown that even in extreme alkaliphiles, the pH remains relatively neutral to slightly alkaline in the cytoplasm even though the surrounding medium might be extremely alkaline. There is still much to be learned but it is clear that organisms have easily adapted to alkaline environments thus it would not be difficult for organisms to grow in Martian soils.

4.5. Surviving desiccation

Surviving desiccation is absolutely necessary if a microorganism is to survive on Mars as organisms must be able to survive the desiccating environment until they can come into contact with a water source suitable for growth. Only after finding suitable water activity, such as a polar ice cap or subsurface water sources, could the organisms then potentially become active.

As previously discussed, Mars is considered to be quite dry, and soils contain only 2.25 wt% water [9]. However, this analysis was performed on soils on the Mars surface so we do not know what the soil water content is at deeper depths. It is not known if there is a source of subsurface water, but geographical features of Mars indicate that there may have been

water on the surface at some time in the past. It is not unreasonable to think that the water would have seeped into the subsurface and may still be present to some degree. Additionally, hydrothermal environments on Mars associated with craters from impacts and volcanism could have easily provided a source of liquid water, and crater impacts generating water are a potential concern today [11]. It may be possible for an organism to remain dormant for an extended period of time, then flourish after a wind storm has transferred the organism to a water source or water flows from a crater impact.

Several studies have shown that desiccation resistance in microorganisms is far from rare, and not only includes spore-forming microorganisms such as *Bacillus*, but non-spore-forming organisms such as *Moraxella* and *Staphylococcus* as well [37, 38]. Overall, dehydration of cells leads to severe cell damage by causing structural changes to lipid membranes and proteins, cross linking and polymerization of DNA molecules, inhibiting or altering enzyme activity, changing membrane permeability, and altering or mutating genetic information. DNA in the cell is at most risk to the desiccating environment since loss of water can lead to partial DNA denaturation [39]. Spore-forming organisms such as species belonging to the genera *Bacillus* and *Clostridium* are more likely to resist desiccation as the spore coat provides protection against a desiccating environment. The water content of spores is reduced to 25–45% of the cell's wet weight causing proteins to become immobile and ceasing enzymatic activity altogether [39]. However, the overall resistance of the spore to the desiccating environment is mostly due to protection of the dehydrated core by the cortex and spore coat layers while the DNA is protected by small DNA binding-acid soluble which protect the DNA from chemical and enzymatic reactivity [39].

Many non-spore-forming organisms have been shown to be resistant to desiccation. Studies by La Duc et al. [40] identified several isolates of *Pseudoaltermonas*, *Psychrobacter* and *Acinetobacter* that survived a 7-day incubation at a Rh of $18 \pm 3\%$. Several *Moraxella* sp. have been shown to survive a 30°C incubation for 35 days under dry conditions [37]. *Staphylococcus aureus* can survive on dry plastic surfaces for more than 1097 days [38]. The methanogens, *Methanobacterium wolfeii*, *Methanosarcina barkeri* and *Methanobacterium formicicum* survived desiccation for 90–120 day incubation periods [41]. Studies on Amazonian oxbow lake sediments showed that desiccation for 1 year at 4°C not only increased the overall abundance of *Methanocellales* and *Methanosarcinaceae*, but also increased the rates of CH₄ production after rewetting [42].

Although it is clear that the spore coat protects spore-forming organisms from a desiccating environment, it is relatively unclear how non-spore-formers survive similar environments. Studies by de Goffau et al. [45] have shown that cells can maintain intracellular water activity above that in their environment as long as the microbes can generate more water metabolically than is lost to the environment. However, this would require that the organisms were metabolically active which would be questionable under most desiccating environments such as the case of *Staphylococcus aureus* residing on a dry surface where there would be little to no nutrients [38]. Studies by Chaibenjawong and Foster [38] showed that the mutants *clpX*, *sigB* and *yjbH* were required for desiccation resistance in *Staphylococcus auerus*. *ClpX* and *yjbH* are both important for protein turnover while *sigB* plays a role in overall stress resistance [38]. It is likely that there are several factors involved in the desiccation resistance of non-spore-forming organisms but more studies on these unique organisms will need to be performed before we have a comprehensive understanding of these systems.

4.6. Exposure to an oxidative environment

Data from the Viking missions showed that the surface of Mars was highly oxidized compared to its atmosphere [13]. Additional studies of Mars have shown that H_2O_2 abundance can range from 15 ± 10 ppb to 40 ppb [14]. The formation of peroxides can occur in the presence of hematite, trace amounts of water, and UV radiation, and radiolysis of ice or water can create even larger amounts of peroxide formation approaching 0.13% as seen on Europa [7, 15]. For an organism to survive on Mars it would need to have mechanisms to protect itself from this oxidizing environment.

A number of microbes collected directly from spacecraft assembly facilities or pre-launch spacecraft are highly resistant to 5% H_2O_2 . An isolate of *Acinetobacter radioresistens*, collected from the Mars Odyssey spacecraft, showed only a 2 log reduction after exposure to 100 mM H_2O_2 . Even after exposure to 320 mM H_2O_2 there was still incomplete killing of all of the microbes [44]. Studies by Kempf et al. [43] have shown recurrent isolation of H_2O_2 -resistant *Bacillus pumilus* from the JPL spacecraft assembly facility. Both vegetative cells and spores of these isolates survived exposure to 5% H_2O_2 . Spores were less susceptible to killing showing only a 1–5 log reduction compared to vegetative cells which experienced a 5–8 log reduction. The examples just mentioned are far from a comprehensive list of organisms that have resistance to H_2O_2 , but they demonstrate that organisms are able to withstand these types of exposures.

There have been numerous attempts to try to understand how microorganisms protect themselves from H_2O_2 exposure. Most of these studies have been performed in *Bacillus* species although there is some knowledge overall about how bacteria cope with this stress. Three well studied mechanisms are the peroxide responsive regulators OxyR, PerR and OhrR that also act as transcription regulators. OxyR and PerR are mainly involved in the detection of H_2O_2 whereas OhrR is involved in the sensing of organic peroxides and sodium hypochlorite. When exposed to peroxides, specific cysteine residues on OxyR and OhrR and histidine residues on PerR are oxidized by an Fe-catalyzed reaction. These transcriptional regulators are not only involved in H_2O_2 sensing, but also serve in the formation of biofilms, host immune response evasion, and antibiotic resistance [46].

Beyond general sensing of H_2O_2 , genes involved in protein protection, such as groES, dnaK and clp tend to be upregulated thus also serving to protect the cell [47]. These proteins may be important for stabilizing the enzymes involved in the actual conversion of H_2O_2 to water and O_2 , including catalases, peroxiredoxins, and peroxidases [48]. Studies in *Bacillus subtilis* have identified σ^B -dependent stress genes that are also involved in resistance to oxidative stress. Ultimately, the work performed by Reder et al. [49] identified 47 general stress response genes that were required for survival to superoxide, 6 genes required for protection from H_2O_2 stress and 9 genes that were required to protect against both.

Studies of the highly resistant strain, *Bacillus pumilus* SAFR-032, collected from JPL's spacecraft assembly facility, have identified many genes involved in H_2O_2 resistance overall [48]. Checinska et al. [50] looked further into the role of two manganese catalase proteins in the SAFR-032 spore coat, YjqC and BPUM_1305, which had been previously identified by others. It was concluded that the synergistic activity of YjqC and BPUM_1305, along with

other coat oxidoreductases, contributes to the increased resistance of SAFR-032 to H₂O₂ over other *Bacillus pumilus* strains. This work has greatly improved our knowledge of the resistance of SAFR-032 to H₂O₂.

4.7. Exposure to radiation

The ability of an organism to survive radiation is paramount if the organism is to survive near the surface of Mars and pose a planetary protection threat. The radiation exposure on Mars is much more intense than it is on Earth because Mars lacks a magnetic field to deflect incoming charged particles and the atmosphere is <1% that of Earth [51]. There are 2 major types of radiation to be concerned with on Mars. The first type of radiation, Galactic Cosmic Rays (GCR), originates outside of our solar system and is formed from events such as supernovas. The second type of radiation, Solar Cosmic Radiation (SCR), originates from the sun and consists of both a constant flow of radiation as well as brief bursts [39, 51]. In the past, the overall radiation level on Mars has been based solely on calculations and modeling. New studies using data collected from the MSL found that the radiation in flight to Mars is approximately two times higher than the radiation on the surface of Mars (0.21 mGy/day vs. 0.48 mGy/day). The lower radiation level on the Mars surface is due in part to some atmospheric shielding by the Martian atmosphere, which is not provided to the spacecraft en route, and because radiation from GCR is modulated by SCR [51].

SCR can consist of both ionizing (e.g. gamma radiation) and non-ionizing radiation (e.g. UV radiation). This section will focus mostly on UV radiation since that has been the focus of the majority of previous studies. It is of note that ionizing radiation can be of more concern since it can penetrate through the Martian soils thus potentially making the first meter of soil inhabitable [51]. Solar UV radiation is divided into 3 spectral ranges; UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (200–280 nm). UV-B and UV-C radiation are of the most concern since DNA has high absorption at those wavelengths and can be mutated leading to cellular inactivation [39]. Radiation of biological cells can cause breaks in molecular bonds including single and double strand breaks in DNA and photolysis of amino acids [52]. Calculations have suggested that DNA weighted irradiance on the Martian surface would be three orders of magnitude greater than that on Earth meaning that microbes would need to be resistant to much higher levels of UV radiation to sustain life on the surface of Mars [53].

Most of the research on radiation resistance and/or survival of microorganisms have been performed on spore-forming organisms since they are of the most interest to planetary protection and tend to be hardy due to their protective spore coat. Studies by Wassman et al. [54] exposed *Bacillus subtilis* spores to low Earth orbit and simulated Martian conditions for 559 days aboard the ESA's EXPOSE-E facility. Although results showed that there was 100% survival of *Bacillus subtilis* MW01 spores to simulated Martian conditions (UV $\lambda \geq 200$ nm), only a $\leq 8\%$ of spores survived low Earth orbit conditions (UV $\lambda \geq 110$ nm). Studies on *Bacillus pumilus* spores showed 10–40% viability on the EXPOSE facility versus a survival rate of 85–100% under dark simulated Martian atmospheric conditions. However, when the same studies were performed on the super tolerant *Bacillus pumilus* SAFR-032 strain, a 7 log reduction in viability was observed [55]. Overall SAFR-032 spores showing UVC resistance remain

viable even after exposures up to 2000 J/m² [56]. Comparative proteomic studies showed that superoxide dismutase was present in higher concentrations in the space exposed isolates and exhibited higher UV-C resistance than the ground control counterparts [55]. Tauscher et al. [57] studied the effects of *Bacillus subtilis* spores exposed to simulated Mars solar radiation for an equivalent of 42 min of Mars solar radiation. Radiation exposure reduced spore viability by 3 logs but measure of germination metabolism was only reduced by <1 log. They concluded that the spores can retain the ability to initiate germination-associated metabolic processes and produce viable signature molecules despite being rendered nonviable.

It has been estimated that spores are 10–50 times more resistant than growing cells to UV radiation at 254 nm. This is due to a difference in the UV photochemistry of the DNA as well as error-free repair of any photoproducts formed by the UV light. Instead of forming thymine dimers as a photoproduct, spores tend to form thymine adducts instead; furthermore, small acid soluble proteins (SASPs) appear to suppress cyclobutane pyrimidine dimers [26]. Relative to gamma radiation, spores are significantly more resistant due to the decreased levels of water in the spore coat compared to vegetative cells which may reduce the amount of hydroxyl radicals formed overall [58]. SASPs do not appear to play a role in γ -radiation resistance [26].

Many non-spore-forming organisms have also been identified as being UV-resistant. Studies by Montero-Calasanz Mdel et al. [59] identified an isolate of *Geodermatophilus tzadiensis* that showed resistance to UV light at 254 nm. A highly radiation resistant isolate from the *Moraxella-Acinetobacter* group showed increased survival after a repeated exposure to UV light. Ultimately, this isolate was able to withstand a UV dose of 5940 J/m² with a 48% survival rate [60]. Antarctic Dry Valley bacteria closely related to *Brevundimonas*, *Rhodococcus*, and *Pseudomonas*, all showed resistance to γ -radiation. Surprisingly, these organisms, along with *Deinococcus radiodurans*, all showed increased resistance to γ -radiation when irradiated at -79°C [52].

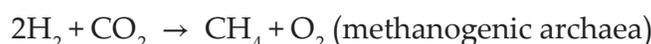
Although the ability of non-spore-forming organisms to survive radiation appears to be poorly understood, there are some studies which have given clues to how these organisms survive. Keller et al. showed that the UV light resistance mechanism for survival was not associated with increased mutagenesis when the *Moraxella-Acinetobacter* isolate was repeatedly exposed to UV [60]. Studies on several strains of *Staphylococcus aureus* showed that UV-C resistance increased as the organisms entered into stationary growth phase, a characteristic that was attributed in part to the expression of σB during this phase [61]. Exposure of the lipids and proteins of *Acinetobacter* sp. PT511.2G and *Pseudomonas* sp. NT511.2B to ultraviolet radiation caused an increase of methyl groups that were associated with lipids, causing lipid oxidation, and alterations in lipid composition in addition to changes in propionylation, glycosylation, and/or phosphorylation of cell proteins [62]. The authors concluded that these changes may account for differences in UV sensitivity.

Ultimately, there are many microorganisms, both spore-forming and non-spore-forming, that are able to survive exposure to radiation and could potentially survive on Mars. For example, *Deinococcus radiodurans* would only be eradicated from the top several meters of Martian soil after a period of a few million years based on the radiation that currently reaches Mars. However, if the organism were to start growing again, then the clock would start over, and organisms could continue to stay dormant and survive up through today. This has implications for the potential for life to exist on Mars.

4.8. Conservation of energy

Unlike Earth, the Martian environment provides very little nutrients to sustain life. Any microbes that may already be on Mars would have to make a living using the limited nutrients that are available. As previously discussed, Mars has a mostly CO₂ atmosphere (95.3%) with low amounts of N₂ (2.7%) and O₂ (0.1%) [7]. However, studies by Mumma et al. [11] have shown the presence of methane in extended plumes that appeared to be released from discrete regions containing as much as 19,000 metric tons of methane. Additionally, previous studies have shown high amounts of salts including MgSO₄ and FeSO₄ [17]. Two of the most abundant compounds on Mars are Fe and S and there is evidence that there are large concentrations of sulfur in the Martian regolith [65]. Perchlorate, a strong oxidizing agent, was shown by the Phoenix Lander to be present in Martian soils in concentrations of 2.1–2.6 mM [16]. All of these compounds are potential chemical energy sources that can be used by microorganisms to survive.

The large methane plumes on Mars are of unknown origin. These plumes seasonally fluctuate but the amount of methane produced is on par with methane plumes on Earth that are known to be of biotic origin. Although the Mars rover Curiosity has found no detectable atmospheric methane, it is possible that the location of the rover prevented the detection of methane in the atmosphere since these methane plumes have been seen at polar regions rather than mid-latitude regions. Methanogenesis has become a well-known method for microorganisms to conserve energy. Many archaea, such as *Methanosarcina*, can use various carbon compounds to produce methane [63]. H₂ can readily be oxidized with the large amounts of CO₂ in the atmosphere to generate energy via methane production [64]. Once this methane is available, it could be oxidized by methanotrophic archaea in the presence of sulfate-reducing bacteria to complete a methane cycle which would support at least 3 types of organisms [65]. An overview of the reaction might look something like this:



The electron donor H₂ could easily be generated by photochemical dissociation of water [66] and it has already been determined that there are large amounts of sulfate, especially in the form of MgSO₄ and FeSO₄ in the Martian soils [17, 67].

More likely energy sources fairly abundant in near surface soils on Mars are inorganics such as iron or sulfur [8]. An electron donor such as H₂ could be used to reduce Fe(III) or sulfate during respiration, with utilization of CO or CO₂ as a source of carbon. Sulfate and iron reduction by organisms on Earth have been very well studied. These organisms play very important roles in the biogeochemical cycling of carbon, nitrogen, sulfur, and other metals [68]. Studies by Karr et al. [69] identified a group of sulfate-reducing bacteria residing in the permanently frozen freshwater lake, Lake Fryxell, in Antarctica. These organisms are able to utilize the reduction of sulfate to conserve energy under very cold conditions (4°C). There have

also been studies showing that Fe respiration under alkaline conditions is possible. Studies by Williamson et al. [70] identified organisms that could easily reduce Fe(III) at pH 10. These studies show that it is possible for these reactions to occur under cold or alkaline conditions. Once Fe or S has been reduced it is available for oxidation by other organisms.

Perchlorate, detected in soils by the Phoenix Mars Lander, is one of the more interesting potential electron acceptors recently discovered on Mars [16, 71]. More than 50 microorganisms on Earth are known to respire perchlorate coupled to the oxidation of H_2 or small organic acids, a metabolism that has been intensely studied over the past decade [72, 73]. This group of organisms is quite diverse and many have been found in environments that might seem, on the surface, to be inhospitable such as paper mill waste. Studies by Ju et al. [74] showed bacteria in sludge that were capable of oxidizing both Fe^0 and S^0 while reducing perchlorate. The enrichment culture was also able to oxidize S^{2-} and $S_2O_3^{2-}$ to support the reduction of perchlorate, and they also confirmed the disproportionation of S^0 to S^{2-} and SO_4^{2-} . Thus perchlorate reduction would tie in neatly to both the Fe and S cycles.

Although Mars seems inhospitable and lacks an abundant supply of nutrients, there are plenty of nutrients available to support anaerobic life on the red planet. The studies discussed above show that the organisms could work together to supply nutrients for one another within a complex ecosystem. Additionally, many of the organisms discussed above can survive in extreme environments on Earth while still making a living as evidenced by many of these processes still taking place at low temperatures or in alkaline environments.

5. Conclusions

Despite all that we know, there is still much to be learned with regard to the absolute limits for life. In order to answer these questions, we must have a better understanding of life on Earth. With regard to the potential for indigenous populations on other planets and moons, research has shown repeatedly that life can exist in the harshest of environments. Although this was not covered in depth in this chapter, life has been found in some of the most dry or frigid environments on Earth such as the Atacama Desert or Antarctica. It is not unreasonable to believe that microorganisms, similar to those found on Earth, could be thriving on locations such as Mars or Europa, especially in the subsurface where radiation would be lower and there would be a better chance for the existence of liquid water. While searching for life on other planets and moons, we look for the signs of life that are already known such as the presence of carbon and water. It may be possible that if we find life in these distant places that we may discover new limits to life in extremis.

Acknowledgements

This work was funded by NASA ROSES award #15-PPR15-0006 NASA EPSCoR award # NNX11AQ30A. We wish to thank members of the NASA and JPL Planetary Protection groups for the financial and collaborative support of our research work. The opinions expressed in this article are the authors' own and do not reflect the view of JPL or NASA.

Conflict of interest

The authors do not have any conflict of interest to report.

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