Forside

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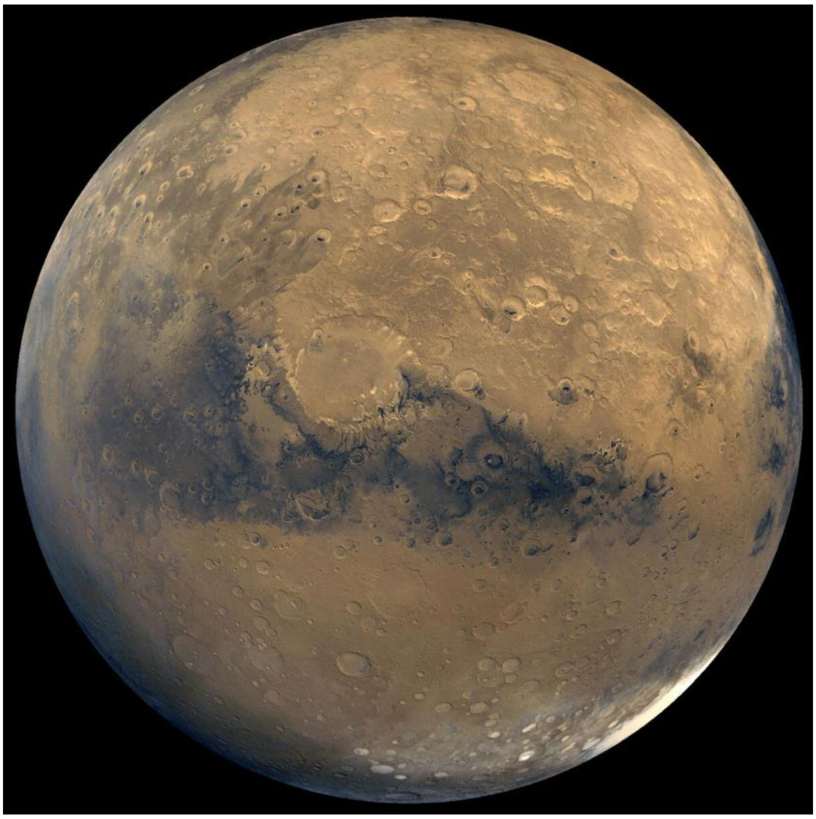
KØBENHAVNS UNIVERSITET
BIOLOGISK INSTITUT

Accessing the Habitability of the Martian Surface and Subsurface Based on Analogs Found on Earth

Bachelorprojekt



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Indhold

1. Abstract

So far, the only life we know of is on Earth, but the search for life on other planetary bodies is still ongoing. Mars is particularly interesting, mainly because it is more practical to study compared to many other planets, and evidence suggests that the Martian environment may have been habitable once. Whether life could exist on present Mars is unknown. Technological advancements have increased the ability to study the planet's environment and extremophiles that may be able to survive in that environment. In this review, the aim is to compile the data from years of research to hopefully accomplish three goals: (1) Discuss the properties and characteristics of the environment on Mars; (2) discuss the survival capabilities of extremophiles in Martian analogs, namely Atacama Desert and the Antarctica Dry Valleys, and under Martian simulated conditions; (3) discuss the habitability of Mars based on the extremophiles found in the Martian analogs. Based on the literature used for this review, it is evident that further technological advancements still need to be made to fully understand the environment on Mars and therefore to fully assess the habitability. However, based on

the current sum of knowledge, it is not unreasonable to believe that habitable niches may exist in the subsurface.

2. Introduction

The search for extraterrestrial life encompasses many of our fundamental scientific questions. The question of whether life exists elsewhere in the universe has been around since at least the ancient Greeks. Currently no direct evidence has been gathered on life on other planetary bodies, and which basic requirements are universally needed to sustain living systems, and which are contingent is still unknown. Searching for extraterrestrial life is a difficult task, firstly because it is difficult to define what life is. Our definition of life is bound to what we can observe on Earth, and it is not evidently clear what boundaries should differentiate between living and non-living things. Several definitions have been proposed. Although many of them differ from one another, they are still predominantly based on important biological properties such as reproduction, metabolism, growth etc(1–14).

The difficulties in reaching a consensus on a definition of life is mostly bound to the abstract nature of life or intractable counterexamples(15). Based on what we know of life on Earth, life on other planetary bodies is most likely microbial, since analog environments on Earth are predominantly inhabited by microbial life and it can survive under a wider array of environmental parameters. When looking at Earth's history multicellular organisms are very much latecomers, with fossil records around 635 million years old, compared to evidence of microbial life existing 4.1 billion years ago(16).

Mars has been of particular interest in the search for extraterrestrial life. This is mainly due to accessibility and it being the most similar planet to Earth in the solar system. Evidence suggests early Mars had more hospitable conditions than today including liquid H_2O, higher temperatures, and lower radiation levels(17). The planet may have hosted an environment with the potential for life originating. However, for life to originate, the essential elements must be present at a long enough timescale for the assemblage from macromolecules to cells. Hereafter the cells would need a favorable environment for a long enough period to evolve adaptations to changes in the habitability. Today Mars is dry, cold, and exposed to high levels of UV radiation, and if life still exists it must have adapted survival strategies to overcome such stressors. Therefore, to determine whether Mars is habitable for life today, it is logical to look at life in Martian environmental analogs on Earth. For this literature review, both Atacama Desert and Antarctica Dry Valleys will be studied due to them being some of the most Mars-like environments on Earth. Our ability to gain a complete grasp on extremophilic capabilities is still limited by the low cultivability of some extremophiles.

It is not necessarily needed to directly observe life but need only to detect signs of life. In general, for a planet to be habitable it should contain environments that can support activity of an organism. This means metabolism for survival, growth, and reproduction is possible in at least a part of the year. Therefore, the environment needs to be able to sustain a suitable temperature so that liquid H_2O is available, protect the organisms from outside radiation and have enough available energy sources.

In this review, the near surface and subsurface environment of Mars will be outlined, including liquid H_2O, temperature, radiation, atmospheric composition, nutrient availability, and the presence of organic molecules. To assess the habitability on Mars, extremophilic organisms found on Martian analogs will be compared to the environmental conditions on Mars.

3. Method

The aim of the review paper is to compile data from several studies describing different aspects of the Martian environment, survival in extreme environments and limitations in studying Mars, with the goal of gaining a more complete insight into the habitability of Mars. To fulfill this purpose, multiple procedures were followed to ensure a high-quality review of the literature. This includes a comprehensive search of peer-reviewed journals, from which data from newer measurements was prioritized compared to a similar dataset from older studies. However, older studies were still included but since technological development since the Viking landers has increased sensitivity, data from newer rovers has been prioritized. Studies with longer time frames were likewise prioritized compared to shorter time frames, particularly with studies regarding the survival of organisms under specific conditions.

The literature search was based on a wide range of key terms including, but not limited to, *polyextremophiles, microorganisms, Mars, surface environment, subsurface environment, survival, growth, geochemistry, and habitability.* In efforts to obtain a narrowly defined search with relevant articles the various terms were combined with "AND" commands. These search terms were selected based on their appropriateness and relevance towards the purpose of this literature review. Identifying the source studies was initially done through Google Scholar and the Royal Library of Denmark to gain a general overview of the different primary source and peer-reviewed research articles that currently exist on the topic. From the articles derived by the initial search, other databases were utilized to specify the search topics. These include but are not limited to Journal of Microbiology, Frontiers in Microbiology, MDPI Journal of Geoscience, Journal of Astrobiology and Frontiers in Astronomy and Space Sciences. Articles were assessed based on their relevance to the topic of this literature review, being published in peer-reviewed journal sources and being primary source research.

In addition to the database search a snowball approach was also utilized, a method in which several articles are located through the reference lists in relevant articles. The initial search provided the start set for the snowball approach. These start sets were primarily research review articles based on the specific topics. For this approach, bias towards a specific publisher was minimized by primarily utilizing google scholar to find the start set. This enabled the start set to comprise articles from a variety of publishers and years, ensuring greater diversity in the start set.

4. Martian near surface environment

Meteorological data have been obtained from Mars since the 1970s, and understanding the near-surface environment is a high priority of the NASA Mars Exploration Program. Many aspects of the Martian environment are unfavorable for sustaining life, including lack of liquid H_2O, low temperature, high levels of ionizing radiation, and high salt concentrations. Due to the increasing interest in sending rovers and potentially people to Mars, environmental conditions have been a popular subject of study. With the fascination in life's potential on other planetary bodies, understanding the climate on Mars has surged in importance. To date, no direct evidence suggests life exists or has existed on Mars, but evidence suggests that the Noachian period may have had a suitable surface environment.

Much of the work in the search for life has been to understand the surface and subsurface environment. A lot of work has also been conducted on known extremophiles from different environments on Earth that resemble Mars, both in situ and in Mars simulated chambers.

4.1 Near Surface Temperature

The surface temperature and the temperature variability are mostly governed by the surface radiation budget. As will be further detailed under section 4.4, the levels of surface radiation vary over the year and depends on location, atmospheric opacity, dust, albedo, and other physical properties of the surface. The surface temperature has been measured by several rovers including the Mars Exploration Rover (MER), the Pheonix lander (PHX) and the Mars Science Laboratory (MSL). Daily minimum, mean and maximum ground temperature has been recorded by the MSL during the first 1526 solar days on Mars (sols) of the MSL mission. The results show a very low interannual variability of the daily mean ground temperature. The mean daily ground temperature changes through the seasons, with a minimum temperature between approximately -98°C and -63°C and a maximum temperature between approximately -23°C and 16°C. The seasonal temperature variability follows the seasonal surface solar insolation, with the lowest temperatures and surface solar insolation readings occurring during the fall and the highest during the spring. The MSL readings were taken at different locations at the Gale crater. Difference between daily maximum and minimum temperatures are governed by the thermal inertia, and locations with lower thermal inertia show significant greater difference than locations with higher thermal inertia. Temperature readings from PHX show a maximum daytime temperature of approximately -14°C and a minimum nighttime temperature of -93°C, and the MER readings are similar(18).

In general, cells living in very low temperature environments must overcome a range of environmental stressors. In any given organisms, a distinction can be made between the temperature needed to complete its life cycle, the temperature needed to metabolize and the temperature in which it can survive. Stressors associated with low temperatures includes a reduction in membrane fluidity, changes in intracellular pH, loss of macromolecular integrity, decreased efficiency of transport proteins and intracellular ice formation(19–25). An absolute limit for how low temperatures microbes can survive in is still unknown. In 2013, *Planococcus halocryophilus* strains Or1, isolated from high Arctic permafrost, have shown to grow and divide at -15°C and be metabolically active at -25°C. *P. halocryophilus* is specifically adapted to withstand arctic conditions due to a combination of protein flexibility, resource efficiency and genomic plasticity(26). Biofilms of *Deinococcus geothermalis* DSM11300 can survive in -25°C and sustain viability and culturability for 2 months when stored in a desiccated state(27). It has been suggested that the theoretical boundaries for a minimum temperature for which metabolism is possible is -40°C(28). Some methanogens have even been shown to survive but not grow after prolonged period of -80°C exposure and thawing(29).

4.2 Atmosphere

The Viking Landers first identified the composition of the near-surface Martian atmosphere. The Curiosity rover later updated the measurements. The most prominent gas in the atmosphere was identified as carbon dioxide, with a concentration of 96%(30) (Table 1).

Gas	**Concentration**	**Measured by**
CO_2	96% (± 0.7%)	Curiosity
^{40}Ar	1.93% (± 0.03%)	Curiosity
N_2	1.89% (± 0.03%)	Curiosity
O_2	0.145% (± 0.009%)	Curiosity
CO	0.07%	Viking
H_2O (gas)	0.03%	Viking
Neon	2.5 ppm	Viking
Krypton	0.3 ppm	Viking
Xenon	0.08 ppm	Viking
Ozone	0.03 ppm	Viking

Table 1: Overview of the atmospheric composition on Mars. The four-curiosity measurement have also been measured by Viking with concentrations of CO_2 (95.32%), N_2 (2.7%), Ar (1.6%), and O_2 (0.13%)(31). The curiosity measurements are however more recent(30).

This makes the Martian atmosphere anoxic compared to Earth with its 21% oxygen concentration. Many organisms are known to live and grow under anoxic conditions, including a range of organisms isolated from the spacecraft assembly facilities (32).

Besides being anoxic, a limiting factor the atmosphere possesses is the low atmospheric pressure at only approximately 6.1 mbar(33). This, in combination with low temperatures, inhibits H_2O from being stable in liquid form, at least on the surface. This might not concern the entire planet. Pressure readings from the Viking Lander indicate that liquid H_2O, if present, might be able to remain stable at different locations for at least 5% of the Martian year. These locations include large areas on the edge of the northern lowlands, in the Isidis, Argyre, and Hellas plains(34). In general, low pressure does not have direct inhibitory effects on the survival of microbial communities. Several prokaryotes, fungi, and lichens can survive under spacelike conditions, including low-pressure levels at 10^{-7} to 10^{-4} Pa(35). Likewise, the bacterium *Deinococcus geothermalis* have shown to survive under a simulated space and Martian environment. These simulations included a combination of stressors that were desiccation, vacuum, and UV for the space simulation, and desiccation, Martian-like atmosphere, and UV for the Martian simulation. *D. geothermalis* populations remained viable post-exposure to each stress condition, and the formation of biofilms proved advantageous for the survival rate(27).

4.3 Absence of liquid H_2O

One of the main limitations of life on Mars is the absence of liquid water (defined as liquid H_2O). H_2O is generally inhibited from staying liquid because it either remains frozen due to the low average temperature or undergoes sublimation due to the low atmospheric pressure averaging 6 mbar, which is about 0.6% of the Earth's atmospheric pressure(36,37).

Most of the known H_2O on Mars exists as ice. A small amount exists as water vapor in the atmosphere and possibly in low-volume liquid brines in shallow soil areas. Much of the ice is located at the poles and mid-latitude of Mars, where a reservoir with a known volume of $\geq 5 \cdot 10^6\ km^3$ is located(38–40).

During the earlier geological history of Mars, liquid H_2O might have been more prevalent. Several lines of evidence support this, one of the main ones being the geological features of Mars. The 2001 Mars Odyssey mission was launched by NASA on April 7, 2001, to make a global map of the amount and distribution of the chemical and mineral makeup of the Martian surface. Since then, images from the Mars Odyssey Thermal Emission Imaging System (THEMIS) shows the development of branching valley networks that, like many drainage basins on Earth, are dendritic with tributaries beginning near the peak of topographic divides(41–43). This is also shown from images by the Mars Global Surveyor (MGS), Mars Orbiter Camera (MOC), and the Viking Orbiter camera(44). Chloride deposits found by the 2005 Mars Reconnaissance Orbiter across the southern highlands of Mars suggest that the surface H_2O may have been present in small volumes into the early Amazonian period(45). These images are one of the main pieces of geological evidence suggesting pre-existing flowing H_2O. Other evidence supporting this includes crater lakes interlinked with the valley networks also point toward higher precipitation rates and a wetter climate on early Mars(41,46,47), and sedimentary processes that likely result from a previous aqueous environment(44,48,49).

Although the consensus historically has been that H_2O cannot remain liquid on Mars for an extended period, if at all, newer findings have opened for debate if H_2O exists in liquid form on Mars. NASA's Mars Global Surveyor space probe produced images of a liquid that could indicate the possibility of flowing H_2O being temporarily present on the surface(50). It has also been argued that the deuterium to hydrogen ratio (D/H) on Mars, being 5-6 times higher relative to terrestrial values(51,52), requires a H_2O reservoir of much greater volume than the amount of hydrogen and deuterium lost over the past several hundred million of years(53,54).

In 2018, radar evidence first identified what was thought to be a stable body of liquid H_2O on Mars. Located 1.5 km underneath the south polar ice cap and with a length of 20 km, the pressure from the ice above and dissolved salts supposedly keeps the H_2O liquid. The reasoning behind the evidence is that anomalously bright subsurface reflections are evident within the zone and are surrounded by much less reflective areas. The brighter zone also showed, through quantitative analysis, dielectric permittivity (>15) above what is usually associated with dry materials and closer to that of H_2O-bearing materials(55,56). Whether these observations result from a stable liquid brine or clay deposits is still unknown. Following the first detection in 2018, a study from January 2021 tried to analyze a broader dataset to establish if it indeed was liquid H_2O. The original study was only based on 29 observations from 2012 to 2015, which have been criticized as insufficient to conclude. The 2021 study then expanded the observation set to 134 from 2012 to 2019. This resulted in the authors drawing the same conclusion as in the 2018 study and finding three other possible bodies of liquid H_2O nearby. It is suggested that they are hypersaline perchlorate brines(57). These conclusions have been controversial due to a lack of agreement on the composition of the bright spot. In June 2021, a research letter was published describing how the bright radar reflections might consist of hydrated, frozen-solid smectite clay deposits instead of liquid H_2O (58). Earlier observations from The Global Surveyor have also been suggested to indicate the possibility of flowing H_2O being temporarily present on the surface(50).

Brines have been suggested to form and remain liquid for a prolonged period. Perchlorate salts have been identified as a widespread salt, with concentrations varying from 0.5 to 1%, at the gale crater on equatorial Mars by the Curiosity rover(59,60) and at the phoenix landing site(61). Perchlorate has been shown to lower the freezing point of aqueous solutions depending on the concentrations of different perchlorates, the lowest being -74.6 degrees for calcium perchlorate ($Ca(ClO_4)_2$) (62,63), and by deliquescence form stable, hydrated compounds and liquid brines under Mars simulated conditions(64–67). The formation and stability of brines differ in different locations. It is suggested that metastable brines can exist from the equator to high altitudes on the Martian surface for at least a couple of hours, a few percent of the year(68). These transient liquid brines may even be abundant and widespread on present-day Mars, in equatorial regions with higher atmospheric humidity and lower temperature(69,70). Calcium chloride brines have also been suggested to exist in the deep subsurface of Mars(71).

Although much of the H_2O only exists as ice, it is not necessarily inaccessible to utilize. Algae blooms of Zygnematophycean from the Greenland Ice Sheet, also known as the glacier algae, have been found to lower the bare ice albedo. On the Greenlandic ice sheet, the algae direct between 48 and 65% of incident energy from the captured ultraviolet and short-wave radiation towards melting the ice surface. This results in melting the ice sheet by amplifying energy absorption at the surface, allowing the algae to thrive on the bare ice. To withstand the harsh irradiation, the algae are highly pigmented, which allows the algae to survive while also directing the captured UV and short-wave radiation into the ice below. Creating a habitable microenvironment might be a possible strategy to overcome the lack of liquid H_2O (72).

4.4 Radiation

In some forms, radiation can be very harmful to an organism when exposed to excessive amounts of ionizing and UV radiation. Ionizing radiation is radiation that has sufficient energy to remove electrons from atoms. This includes gamma rays, x-rays, and higher energy ultraviolet light. Ultraviolet light can be classified into three wavelength ranges: UV-C (100 nm to 280 nm), UV-B (280 nm to 315 nm), and UV-A (315 nm to 400 nm). When discussing harmful ultraviolet light, the focus is UV-B and especially UV-C since UV-A does not have nearly as much energy as UV-B or UV-C. Most of the radiation reaching Earth is UV-A, but some UV-B also reaches the surface, and in some areas, it is enough to inhibit life. The ozone layer generally stops UV-C. The UV-B flux on Mars is almost five times higher than on present Earth, and Mars is also exposed to UV-C, which Earth is not in any significant way(73–75). This makes it difficult for organisms to live on the surface since excess levels of UV-B and UV-C can be harmful. High levels of UV-B and UV-C can inactivate microorganisms by forming dimers in RNA (uracil and cytosine) and DNA (thymine and cytosine)(76). Higher energy X-rays can break the sugar phosphate backbone(77). Radiation-resistant organisms have been found in the Bacteria, Archaea, and Eukarya domains. In bacteria, *ionizing radiation resistance* is defined as needing more than 1 kGy of acute ionizing radiation to achieve a 90% reduction of non-spore-forming bacteria(78). For reference, the LD_{50} at 30 days ($LD_{50/30}$) from ionizing radiation exposure for humans is between 2.5 – 4.5 Gy(79), $LD_{50/7\text{-}14}$ is between 8 to 10 Gy(80), and increased cancer risks can be seen in as low as ≈10-50 mGy acute exposure(81). The thermophilic bacterium *Thermococcus gammatolerans* EJ3 and mesophilic bacterium have been shown to resist an accumulated exposure of 30 kGy of γ-radiation at a rate of 60 Gy/min(82,83). The xerotolerant *Psychrobacter pacificensis* L0S3S-03b have shown to withstand to UV254 irradiation doses of >1000 Jm^{-2}, 0.5 Mrad γ-radiation, heat-shock, desiccation, and 5% liquid H_2O_2. Under Mars simulated radiation exposure, isolates of L0S3S-03b had an LD_{90} of 30s and a 100% lethal dose of 2 min(84). Radiation-resistant bacteria also often express polyextremophilic traits, probably because radiation resistance is a fortuitous consequence of developing survival mechanisms towards other environmental stressors(85,86). Protection against UV damage often involves expressing antioxidants, improved DNA repair mechanisms, and producing pigments. The DNA repair mechanisms include direct repair of the damage that UV imposes, either by photoreactivation or removal of damaged bases, UV damage endonuclease, or nucleotide excision repair. These mechanisms can often be seen in microorganisms living in some extreme environments on Earth(87,88).

NASA launched the Mars Curiosity Rover on 26 November 2011 to investigate the climate and geology of Mars. Built into the rover was the Radiation Assessment Detector to detect the amount of high-energy radiation reaching the Martian surface. During the first 300 sols, the galactic cosmic rays dose rate varied between 180 and 225 μGy/d(89), and later measurements showed an average radiation dose received at the surface to be 233 ± 12 μGy/d(90). UV-C and UV-B doses have been estimated to have a combined average value of 361 kJ/m^2 at the equator of Mars, which is much higher than that of Earth at 39 kJ/m^2 (75). These values vary between different areas, times of day, season, dust storms, solar flares, and latitude. Interannual variability of the atmospheric opacity creates differences in solar insolation between the apsides of Mars orbit. In general, the radiation values are higher in the perihelion than in the aphelion. Average temperatures, dust concentrations in the atmosphere, and opacity are all greater during the perihelion(18,75,91–93).

In the early periods of Earth's history, the surface was exposed to UV-C and higher UV-B levels than today and was comparable to the present-day UV flux on Mars(75). Since the UV flux on early Earth has been a significant evolutionary selection pressure to life on Earth(94–96), it would be easy to imagine it playing a similar role on Mars. Since the UV conditions are comparable to the Archaean Earth, it may not alone constrict the formation or existence of life. However, the high UV levels, combined with the other stressors, can be very destructive to life. One main difference between present-day Mars and Archaean Earth is the H_2O content. Though liquid H_2O may have been common on Mars before the Amazonian era (3 billion years ago to present) and the Hesperian era (3.8 billion years ago to 3 billion years) (97–99), it is not as readily available in the current environment. If life have existed on Mars, it would have needed to adapt to an environment without much if any H_2O and high UV radiation.

4.5 Organic materials

Organic compounds are essential for life since they are the primary components of all known life. The detection of organic compounds is intriguing since they might be remnants of biosynthesis by ancient life, although organic compounds might also be the result of abiotic geological processes. The Curiosity Rover has multiple times discovered organic materials on Mars. In 2015 both chlorobenzene and C_2 to C_4 dichloroalkanes was identified upon heating samples from the sheepbed mudstone, a rock stratum in Yellowknife Bay, Gale Crater (Figure 1). Chlorobenzene was found in concentrations of

150-300 parts per billion by weight (ppbw) and C_2 to C_4 dichloroalkanes at 70 ppbw(100). In 2021, discovery of benzoic acid and ammonia by the Curiosity Rover was reported at the Bagnold Dunes(101), as well, as several nitrogen-bearing molecules, phosphoric acid, and phenol were present. Although ammonia is an inorganic compound, it is still of considerable interest because it is a possible biosignature for biotic ammonia synthesis(102). In 2018, complex organics were identified to be preserved in lacustrine mudstone at the base of the 3.5-billion-year-old Murray formation at Pahrump Hills, Gale crater(103). Several different reduced carbons have been identified from Martian meteorites, including graphite, polyaromatic hydrocarbons (PAHs), macromolecular carbon (MMC), and aromatic organics (104).

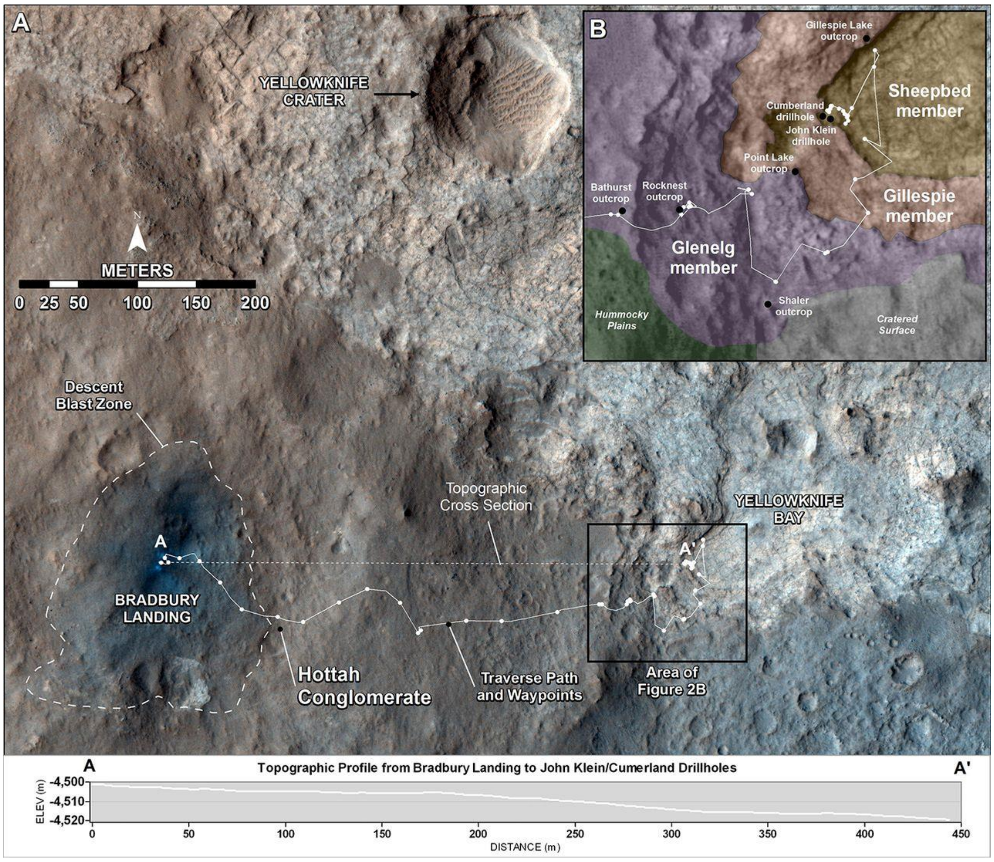

Figure 1: (A) Geological map of Bradbury Landing within Gale Crater, showing Curiosity's path from the landing site to Yellowknife Bay, from which chlorobenzene and C_2 to C_4 dichloroalkanes was identified. (B) Geological map detailing the southwest part of Yellowknife Bay where the sheepbed mudstone is located from which samples was taken(105).

The origin of the organic material is debatable, whether it is of biotic or abiotic origin. Multiple meteorites have been found on Earth with proposed Martian origin. They provide a valuable view of the rock and soil composition on Mars since they are physical samples that can be studied.

Allan Hills 84001 found in the Allan Hills in Antarctica is a fragment of a Martian meteorite that, upon further analysis, was proposed to be crystallized from molten rock 4.091 ± 0.030 billion years ago, making it one of the oldest Martian meteorites(106).

Chemical analysis suggests that it originated when an aqueous environment was present(107), and isotopic analysis showed that it contained polycyclic aromatic hydrocarbons(108), carbonate globules(109) precipitated at 18 °C with H_2O and carbon dioxide from the Martian atmosphere(106,107,110) and nitrogen containing organics(111–113). It is proposed that the organic compounds found in the meteorite are abiotically synthesized by serpentinization, a mechanism whereby basaltic rock reacts with aqueous fluid and produce serpentine minerals, magnetite, and hydrogen(114,115). The organics in the meteorite are collocated with magnetite that in a sample portion coexists with a talc-like phase, which then indicates the serpentinization synthesis(116). Other portions of the sample showed magnetite within an area containing only amorphous silica, carbonate, and organic carbon, which indicates that Martian mineral carbonation reactions are also responsible for the formation of the organics(116).

Nakhla, found on June 28, 1911, in the Egyptian desert, is a Martian meteorite and was the first meteorite to define the Nakhlite meteorites, which are meteorites rich in augite pyroxenes formed from basaltic lava flows(117). It is estimated that it was formed 1.3 billion years ago(118). In situ measurements of D/H in Nakhla have hinted at the presence of liquid H_2O in the past. The H_2O on Mars and Earth may have originated from the same source material(119). Protein and nonprotein amino acids, including aspartic and glutamic acids, glycine, alanine, β-alanine, and γ-amino-*n*-butyric acid (γ-ABA) as the most abundant, have also been detected in the Nakhla meteorite. The concentrations ranged from 20 to 330 parts per billion of bulk meteorite, and the distribution of the amino acids is comparable to what is expected from bacterial degradation of organic matter(120). Biomorphic ovoid structures composed mainly of iron-rich clay have been discovered within the meteorite. The origin, however, is most probable abiotic since the evidence is lacking for a biotic origin. However, the team behind this discovery noted that "*it is evident that the Martian subsurface contains niche environments where life could develop*"(121). Microtunnels in Fe-Mg silicate minerals have been identified in the Nakhla meteorite, with morphological similarity to bioerosion textures in terrestrial Fe-Mg silicates. Although evidence is insufficient to claim a result of biotic processes, since morphology alone is an incomplete tool for detecting primitive life due to its inability to differentiate between biotic and abiotic geological conditions(122), the presence of such formations can potentially be biosignatures for present or past life(123).

4.6 Nutrient availability

Extensive research has gone into understanding the Martian soils and rocks. Data from both meteorites and rovers has made it possible to get a detailed mineral compositions at several different sites on Mars. Based on the sum of gathered data, it has been suggested that all basic elements needed for microbial life is present on Mars and that the basalt of Mars contain enough resources for the growth of cyanobacteria due to their nitrogen-fixing- and photosynthetic abilities and their lithotrophic lifestyle(124). Basalts is particularly common in both Earth's and Mars crust(125). The pores of basalt vesicles may be the most likely place for present day Martian life to exist(126). Oligotrophic ocean crusts on Earth, mainly consisting of basaltic rock(127), have been shown to host a diverse and stable microbial community. Ferrous iron (Fe(II)) oxidizing *Pseudomonas* and ferric iron (Fe(III)) reducing *Shewanella sp.* have been isolated from the oligotrophic basaltic substrates at Loihi Seamount off the southeast coast of the Big Island of Hawai'i. In these environments, the microbes can utilize reduced iron and oxidized iron as electron- donor and acceptor respectively(128). This is in line with previous studies on microbial communities in basaltic environments(129–131). Both Fe(III) and sulfur (S), have been identified to be potentially globally widespread on Mars and significantly more enriched in Martian soils and rocks compared to Earth(132–136). Fe- and S-reducing microorganisms may be able to use a variety of electron donors such as organic molecules if present on Mars(100,111,112,137–139), and molecular hydrogen (H_2) with CO_2 as a carbon source(140).

4.7 Salts

High salt concentration significantly impacts the potential for life to exist in the environment since salt can affect the water activity and cause heightened osmotic stress, which inhibits non-halophilic bacteria(141). Microbial community structures and composition has been shown to be impacted by salinity gradients found in hypersaline environments such as the Salton Sea at the southern end of the U.S. state of California(142,143). Halophiles can be found in all three domains of life. The current highest salinity in which an organism has been cultivated is 35% NaCl for the bacterium *Halarsenatibacter silvermanii* strain SLAS-1^T (144). In 2020, a study investigated the survivability and adaptability of the extremotolerant bacterium *Planococcus halocryophilus* when exposed to one of six different chloride and mars-relevant perchlorate salts at 25°C and 4°C in aerobic conditions. *P. halocryophilus* were exposed to an incremental increase in salt concentrations of 1 percent by weight (wt%), to establish the maximum halotolerance for growth (Table 2).

Salt	**Maximum halotolerance at 4°C**	**Maximum halotolerance at 25°C**
NaCl	11 wt%	14 wt%
$MgCl_2$	10 wt%	11 wt%
CaCl2	10 wt%	8 wt%
$NaClO_4$	7 wt%	12 wt%
$Mg(CIO_4)_2$	4 wt%	5 wt%
$Ca(CIO_4)_2$	3 wt%	3 wt%

Table 2: *P. halocryophilus* maximum halotolerance towards different salt in which it can grow. The salts include sodium chloride (NaCl), magnesium chloride ($MgCl_2$), calcium chloride (CaCl2), sodium perchlorate ($NaClO_4$), magnesium perchlorate $(MgCIO_4)_2$ and calcium perchlorate ($Ca(CIO_4)_2$) (145).

It is evident that lower temperatures decrease the halotolerance of *P. halocryophilus*, except in the case of $CaCl_2$ which is in accordance with earlier studies(146) and an increase in halotolerance at low temperatures has also been observed in *Clostridium* sp. and *M. soligelidi*(147,148). It is still mechanistically unclear how this enhance occurs. The result from this study shows the ability of *P. halocryphilus* to survive in concentrations of Mars-relevant salt solutions(145). The increase in tolerance in lower temperatures is particularly interesting since Ca^{2+} rich brines might be present in the Martian subsurface(71). The same year, a new record in perchlorate tolerance was found in the halotolerant yeast *Debaryomyces hansenii*, which showed growth in liquid growth medium containing 2.4M sodium perchlorate ($NaClO_4$)(149). Earlier studies showed a maximum perchlorate tolerance in the bacteria and archaea domains, in which the bacterium *Planococcus halocryophilus* and *Halomonas venusta* tolerated 1.1M(145) and 1.0M(150) respectively, and the archaea *Halorubrum lacusprofundi* and *Haloferax mediterranei* tolerated 0.8M(151) and 0.6M(152) respectively. The authors behind the study investigating *D. hansenii* notes that if life exists in perchlorate brines, it would likely have evolved even higher tolerance due to a long-time exposure to a natural selection pressure(149).

Most microorganisms do not grow at a water activity below 0.91 and most molds at 0.80, however, some species of xerophilic archaea, bacteria, and fungi can tolerate a range of water activities of ~0.650–0.600(153), and even as low as 0.585 for the fungus *Aspergillus penicillioides*(154). The osmotic pressure of organisms in highly saline environments is generally achieved by excluding salts by synthesizing or accumulating organic osmotic solutes such as amino acids. A second strategy involves accumulating molar concentrations of potassium and chloride in the cytoplasm, but this is not as readily seen in halophiles(155,156). Due to its impact on microbial communities, it is of interest to understand the salt concentrations of the Martial soils.

The goal of the Microscopy, Electrochemistry, and Conductivity Analyzer (MECA) on the Phoenix Mars Lander was to analyze and characterize the soil composition and pH on Mars. MECA's wet chemistry lab (WCL) analyzed three different soil samples from the Pheonix lander site. Perchlorate and several ions were identified from these samples(157) (Table 3).

Ion identified by WCL	**Average concentration**
Na^+	1.4 mM (± 20%)
K^+	0.38 mM (± 20%)
Ca^{2+}	0.52 mM (± 50%)
Mg^{2+}	3.3 mM (± 50%)
Cl^-	0.54 mM (± 20%)
ClO_4^-	2.4 mM (± 20%)

Table 3: Ions identified by the WCL analysis of three different soil samples gathered from the Pheonix lander site. Beside these, perchlorate, I^-, Br^-, NH_4^+ and H^+ was also identified(157).

Likewise, analyses of outcrops from Meridiani Planum by the Opportunity rover have found various salts, including NaCl, $MgSO_4$, $CaSO_4$, $FeSO_4$, $MgCl_2$, and $CaCl_2$, and it is estimated that sulfur-containing salts are more abundant than chlorinated salts on the Martian surface(158,159). Data from the Phoenix WCL soil samples at its landing site have also been used to measure the average pH to 7.7 ± 0.1, suggesting the soil may not be, at least globally, inhospitable since only being just slightly basic(160).

Perchlorate is a common salt on Mars, found at the Phoenix landing site(157), Gale crater(161) and at the two Viking landing sites(138) at average concentrations of 0.5 - 1%. Perchlorate could be globally distributed in the top centimeters of the regolith since the measured abundance at the various sites matches the total abundance of Chlorine measured from orbit. In general, the concentration of perchlorate in Martian soil is several times higher than typical concentrations found in soil on Earth(61). Several bacterial strains are known to use perchlorate as a sole electron acceptor in anaerobic environments(162–164) , and some halophilic bacteria have been shown to tolerate perchlorate concentrations comparable to those that occur in the Martian regolith(149,165,166). In addition, perchlorate reduction using organic substrates as electron donors has been reported in halophilic euryarchaeotes(152,167). Carbon monoxide might be the most abundant organic substrate that is readily available with a concentration of about 700 parts per million (ppm). In 2017, the first evidence of microbe-mediated perchlorate coupled oxidation was reported. The euryarchaeal halophiles *Haloarcula* sp. PCN7 and *Halobaculum* sp. WSA2 was shown to couple CO oxidation with perchlorate and chlorate under anaerobic conditions and in a 3.8 M NaCl brine with perchlorate concentrations ranging from 0.01 to 1 M. Growth was however not observed at CO concentrations above 100 ppm(162). CO is relatively abundant in the atmosphere at about 700 ppm(30,168), and due to the scarcity of organic carbon it may be the most readily available(162). A perchlorate coupled CO oxidation may be able to support metabolism in Martian brines(162,166).

4.8 Subsurface of Mars

Compared to the surface, the subsurface might be more hospitable to life. The subsurface might be the most probable place to find life due to the natural protection from some of the stressors that exist on the surface. Living in the subsurface provides natural protection from the harmful UV radiation and low atmospheric pressure. It has been suggested before that the subsurface would be the most logical place to look for biosignatures, both to look for signs of life on Mars and to understand the origin of life(169). The physicochemical conditions in the subsurface on Mars have been proposed to be sustainable for chemolithoautotrophic life, even when the surface conditions became inhospitable(170). The subsurface might also be the most stable environment on Mars(171). This would help the preservation of life and biomarkers and lower the amount of environmental variation that a lifeform needs to overcome.

As discussed earlier, H_2O was most probably present on Mars and may still be below the surface. Fisk and Giovannoni published a paper in 1999, arguing that If liquid H_2O is indeed present in the subsurface, the significant conditions needed to satisfy a biosphere may exist in the subsurface. This includes H_2O, a carbon source, temperature between 0 and 120 °C, nutrients in the form of basaltic crust, gradients in oxidation state, chemical energy for the metabolism of chemolithotrophs, and fluid flow (172). When talking about the conditions for life, six significant elements are considered the most important: Carbon, Hydrogen, Nitrogen, Oxygen, Phosphorus, and Sulfur (commonly known as CHNOPS). These may all be present in the subsurface. Carbon in organic compounds is suggested to be preserved and widespread below the surface(103). Hydrogen potentially exists in H_2O and can be produced during serpentinization processes(173). Evidence suggests biochemically accessible nitrogen exists on the surface of Mars in the form of nitrate(174), and nitrogen also exists in the atmosphere at relatively low concentrations (1.89%) (30). An active nitrogen cycle on Mars has been suggested, in which nitrogen is transported from the surface to the subsurface (≈10 m) in transient thin liquid films, providing nitrogen for a potential biotic layer in the subsurface(175). Perchlorate and chlorate are a common salt on Mars and may be a source of oxygen(138,139,157,161) as well as oxygen in the atmosphere (0,145 %)(30). Phosphorus has been measured as merrillites(176) and in the soil, where it shows a correlation with sulfur in soils from Meridiani Planum and Gusev Crater and with chlorine of rocks and soils in the Gusev plains(177–179). Through analysis of meteorite data, sulfur seems to be relatively common, with an average concentration of 6% sulfur trioxide (up to 37%) in Martian soil(132). Sulfur has also been detected as an organic compound(103).

Studying the subsurface introduces the obstacle that our rovers can only drill a couple of meters, meaning that we only have access to the near-surface subsurface and do not have access to a significant potential habitat. However, we can still theories on the subsurface's environments and study the near-surface subsurface to get an idea of the habitability. When deciphering whether there exists a habitable place on Mars, the subsurface may be the most probable.

5. Life in extreme environments on Earth

Many places on Earth act as an analog for studying survival under Martian-like conditions. Looking at the organisms surviving under these conditions can help grow our knowledge base of what life is like under extreme environments and thereby hint at what life might look like on Mars. No natural environment mimics the Martian environment completely, but some will have specific parameters that resemble it closely enough for the organisms to be an interesting benchmark. Together with laboratory simulations, it gives us the best way to study the potential habitability of other planetary bodies. However, it should be noted that the conditions that cellular life has adapted to survive in do not necessarily support the evolution of prebiotic chemistry. Extremophiles may well be irrelevant to the origin of life since the actual origin may require specific environmental and geological conditions (180). Martian analogs exist for the several different time periods of Mars. Some of the analogs we have for the present Martian environment include but are not limited to; The Atacama Desert, The Antarctic Dry Valleys (181).

5.1 Atacama Dessert

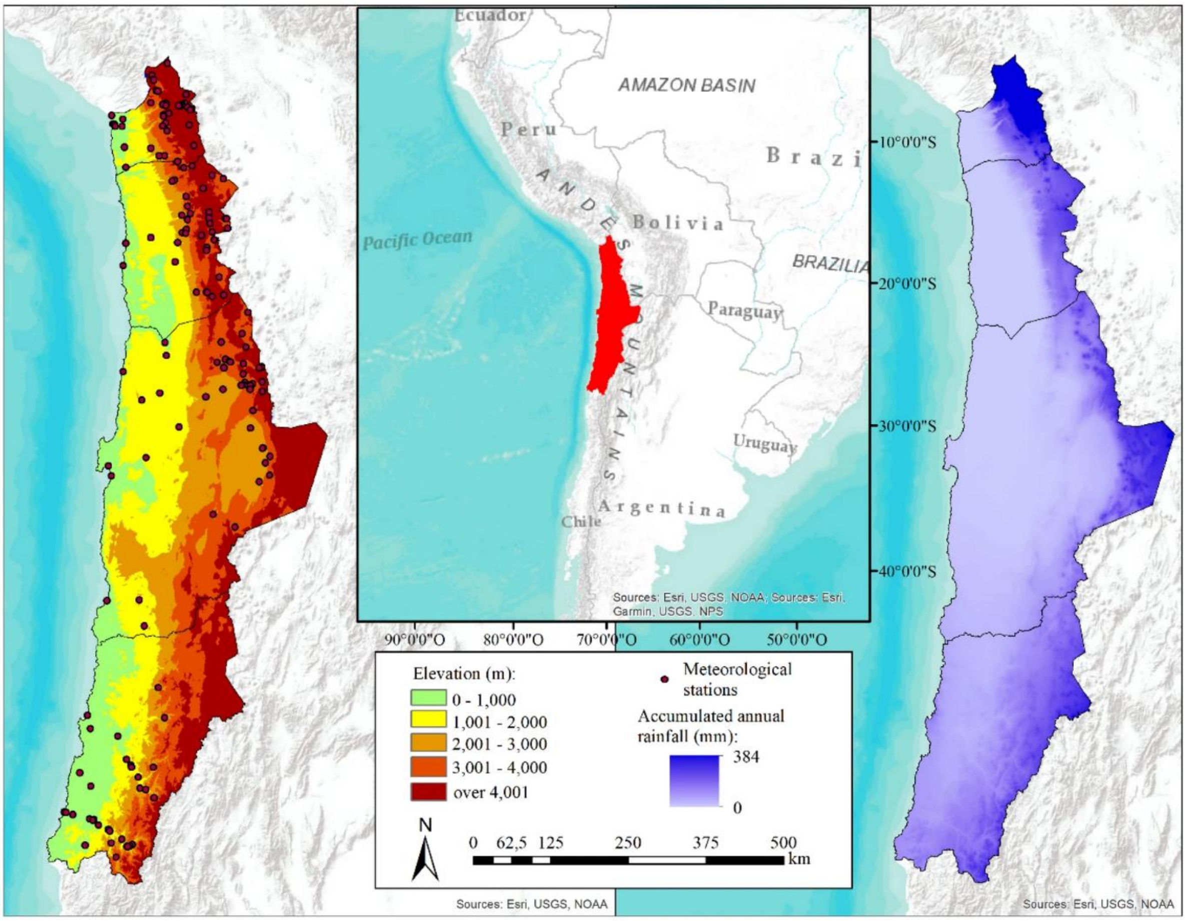

Figure 2: Map over Atacama Desert and the locations of the meteorological stations (left) and the distribution of the mean accumulated annual rainfall between 1950 and 2000 (right)(182).

The Atacama Desert in South America is one of the oldest deserts on Earth that, for the past 150 million years, has been hyperarid and quite unfavorable for life (183). For the last 20 years, it has been one of the most studied regions as a Mars analog model. The thorough study allows us to understand how life can adapt to the hyperarid environment and understand the potential dry limits for life. Besides studying the organisms adapted to survive in the desert, it is also used as a testing ground for rovers and other detection instruments before they are sent to Mars. The main reason it works as a Martian analog environment is due to the extreme levels of dryness at <2mm precipitation per year(184) (Figure 2), volcanic soils rich in perchlorate, and an annual range of UV-B and UV-A radiation doses of about 3.5 kWh/m^2 and 130 kWh/m^2 in coastal areas to 5 kWh/m^2 and 160 kWh/m^2 on the Andean plateau (Figure 3)(185). It can therefore be interesting looking at the microorganisms living under these conditions to get an idea of what organisms might be able to survive in Mars-like soils. Small poikilohydric life forms dominate in desert environments, especially cyanobacteria and chlorophytes, fungi, heterotrophic bacteria, lichens, and mosses(186). Organisms living in desert environments must generally be adapted to overcome low moisture availability, ultraviolet irradiation, and either very high or low temperature stressors. In such dry environments, H_2O from non-rainfall sources has an increasingly important role in shaping the biome. Fog- and dew-derived has an effect that is three orders of magnitudes greater than rainfall in the moisture levels at Atacama Desert (187) . Due to the unfavorable conditions in desert environments, most microorganisms lay dormant to reduce their metabolic activity and minimize the fitness reduction of unfavorable conditions (188). When we first began studying the Atacama Desert, we thought that no photosynthetic life would be able to survive under the polyextreme conditions of the Atacama Desert, but further analysis of samples shows that some microorganisms have adapted to the hyperarid environment.

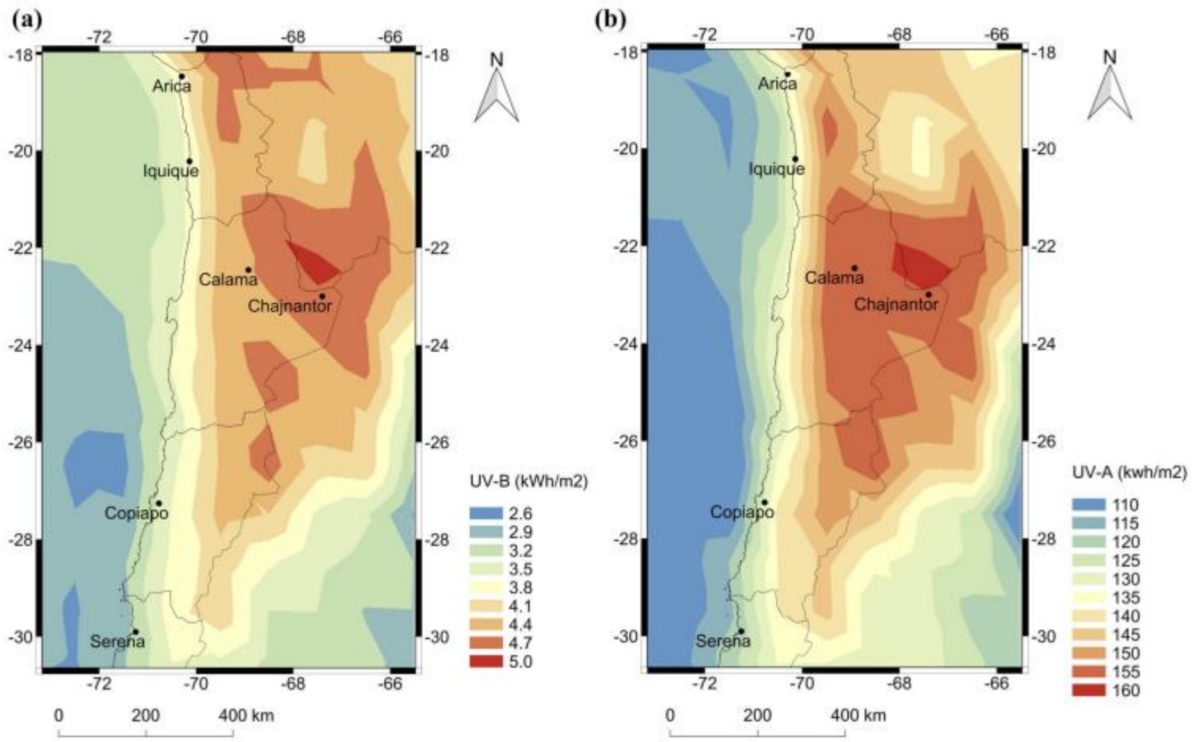

Figure 3: Map over the annual dose of surface UV estimated for all sky conditions in the Atacama Desert. (a) UV-B; (b) UV-A(185).

The Hyperarid Core and The Coastal Range regions of the Atacama Desert are particularly suitable analogs for Martian-like environments. A study from 2001 tried to assess the survivability of several desiccation-tolerant microorganisms on the surface at the hyperarid core. Spores of *Bacillus subtilis*, conidia of *Aspergillus niger*, *Versicolor* and O*chraceus*, and cells of *Deinococcus radiodurans* were subjected in the dark to the harsh conditions of the Atacama Desert for up to 15 months. Of all the organisms, *Bacillus subtilis* and *Aspergillus niger* were the only ones to survive during the entire test period, with a survival rate of 15% and 30%. The rest of the species did not survive, and both *Bacillus subtilis* and *Aspergillus niger* did not survive for more than a few hours in direct sunlight (189). Additional studies have been able to identify microbial communities in the Hyperarid Core. In 2006 a study of rocks in the Yungay area identified a dark gray layer 2-5 mm thick and 3-7 mm beneath the rock surface. Through further study of these gray spots under the stereoscopic microscope, they were identified to be an endolithic monospecific population of *Chroococcidiopsis* and heterotrophic bacteria (190). In 2015, endolithic communities of chlorophototrophic and eukaryotic algae and chlorophototrophic and prokaryotic cyanobacteria lived within gypsum deposits. The authors found that a few millimeters beneath the gypsum surface, the cryptoendolithic colonization occurred with a succession of organized algae and cyanobacteria. By taking advantage of the gypsum deposits, the microorganisms could limit the exposure to excess solar irradiation and maintain lower evapotranspiration rates by adopting an endolithic strategy (191). In 2020, samples from a layer enriched in smectites located 30 cm below the surface of the hyperarid core showed the clay-rich layer to be wet with a constant humidity of 78% (a_w 0.780). Through 16S rRNA amplification and sequencing from samples of smectite-rich subsurface layers in the hyperarid region, the authors also identified a diverse microbial community consisting of at least 30 metabolically active halophilic bacteria and archaea (192). This is particularly interesting since similar clay deposits may exist on Mars, and if life is present, biosignatures could exist within these clay deposits (192,193).

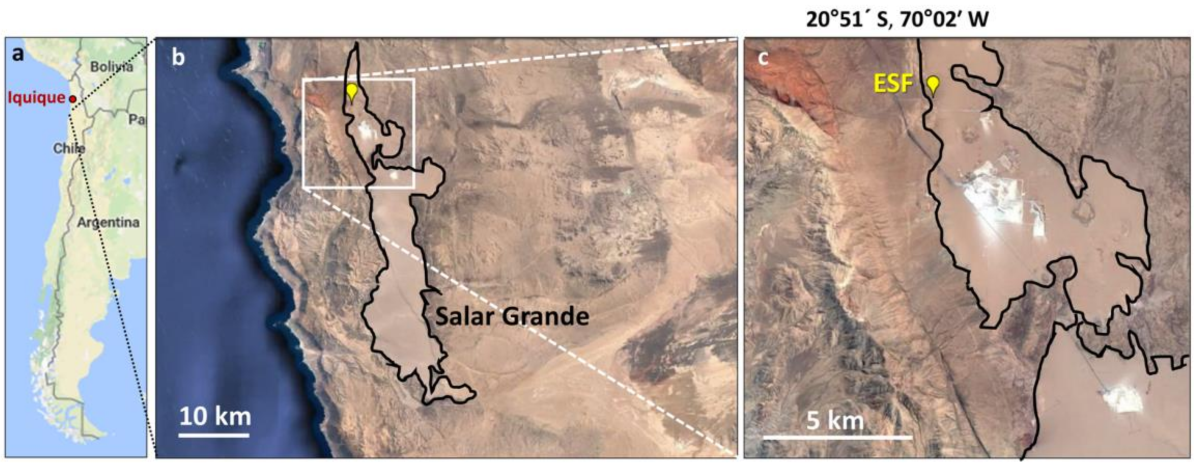

Figure 4: (a) map over Chile, (b) Location of Salar Grande within the Coastal range, (c) location of the drillings in which biomarkers have been identified (194).

The second region, The Coastal Range, is a 3,000 km long mountain range with an altitude of 3,100 m. Salar Grande is a salt lake located within the Coastal Range that is used as a Martian analog due to the high salt concentrations (95% NaCl average) (195,196). In 2018, the halite deposits were assessed by drilling to 100 m depth to look for potential biomarkers. From these drilling samples, unsaturated fatty acids, and mono-methyl n-alkanes were found in relatively large quantities near the surface in halite-rich samples working as biosignatures for Cyanobacteria. In samples from deeper drillings, crocetane was found for methanogenic and methanotrophic Archaea, squalane for halophilic Archaea, and branched alkanes with quaternary carbon for sulfur oxidizers, fatty acids for bacteria, and lastly, alkanes for lichen (194). The Coastal Range also contains caves that have been used to study if organisms could survive in some of the Martian caves. Martian caves have been suggested to be possible habitable environments on Mars since they provide natural protection against the harmful UV irradiance while allowing enough radiation to comply with the minimum required for phototrophs on Earth (197). In 2009, biofilms of *the red algae Cyanidium* were found in a cave at the Coastal Range, gaining protection from excess harmful radiation and surviving with 0.06% (1 μE) of the outside photosynthetic active radiation (198). In 2010, *Dunaliella* was found to thrive on spiderwebs across the walls of a cave in the Atacama Desert, using the moisture buildup on the webs for its H_2O source (199).

Since the Atacama Desert stretches between 1,000 to 1,100 km from north to south, the aridity exists on a gradient over the Atacama Desert. The mean rainfall declines from 21 mm year^{-1} in the relatively less arid regions to ≤2 mm year^{-1} in the hyperarid regions. This dramatically affects the abundance of organisms thriving across the desert, with a significantly decreased abundance and molecular diversity in the hyperarid regions (187). It is evident from the identified samples of microorganisms, especially from the hyperarid regions, that endolithic strategies are a systematic way of adapting to high UV radiation and low moisture levels. Adapting to an endolithic lifestyle can be a potential strategy for protecting from lethal UV irradiation, excessive photosynthesis, and enhancing moisture availability (200). From looking at the lifeforms we find throughout the Atacama Desert, it is fair to suggest that an endolithic adaptation or cave living would be a likely strategy to decrease UV exposure if life were to exist on Mars. One unclarity regarding the microbial communities found in places like Atacama Desert, is whether the environments can support survival and growth for a prolonged period, or if they are the result of sporadically introduction by atmospheric transport. In April of 2015 a soil sample from the surface and near subsurface (<30cm) was taken from six different locations in the Atacama Desert with a decreasing moisture gradient. These samples were taken shortly after a rain event, with the goal characterizing the microbial composition over time. Subsequent samples were taken again in February of 2016 and January of 2017. The microbial composition in the soil surface was dominated by desiccation and UV-resistant species, and deeper layers was dominated by halophilic bacteria and archaea. Both microbial biomass and diversity decreased with an increased in aridity, but locations with the lowest precipitation still supported microbial communities by allowing episodic increase in metabolic activity following an increase in moisture with events of rain. This implies the possibility that if microbes evolved on Mars, they may have been able to endure increasing aridity cycles during its environmental transition towards a more inhabitable place(201).

5.2 Antarctic Dry Valley

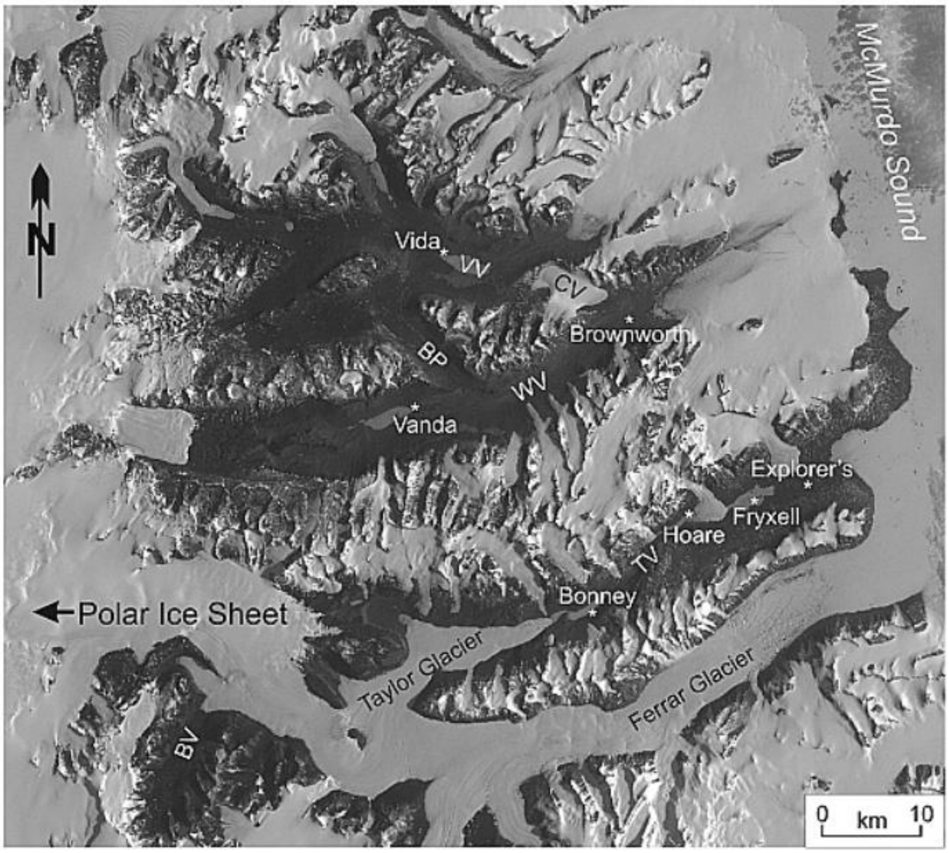

Figure 5: Map over the dry valleys(202).

The Antarctic Dry Valleys, also known as McMurdo Dry Valleys, are located between the East Antarctic Ice Sheet (EAIS) and the Ross Sea (Figure 5). It is one of the coldest, with a mean annual near surface air temperature ranging from −14.8 °C to −30.0 °C(200,203), and most arid regions on Earth. The temperature variability between summer and winter is high due to its relatively low albedo compared to the surrounding snow and ice-covered areas. With an annual mean total solar radiation at 400-1100nm from 78 Wm^{-2} to 113Wm^{-2} (202,204), together with the low temperatures and high aridity, it is one of the most used analogs for the Martian environment. Both the annual mean temperatures and radiation levels fluctuate without any significant trends. From 1987 to 2015, the mean annual levels of shortwave radiation increased from 78 Wm^{-2} to 86 Wm^{-2}, with fluctuating values in the years between and a maximum of 105 Wm^{-2} in 2009 and 103 Wm^{-2} in 2001. This is suggested to mainly result from changing global sources of sulfur aerosols(204). Variation also exists between different locations in the Dry Valleys in terms of mean annual air temperature and solar radiation(202). Most surfaces are free of ice and covered with sandy gravel soil due to the Transantarctic Mountain Range blocking EAIS flow and creating a precipitation shadow (202). Most of the precipitation occurring at the Dry Valleys is snowfall, with a maximum of approximately 50 mm of H_2O equivalence near the coast and decreasing to about 3mm inland, classifying the Dry Valleys as a hyper-arid region(205).

Lake Fryxell is a 4.5 km long lake located in the Taylor Valley, the southernmost of the three Dry Valleys, which serves as one of the central locations for the Long-Term Ecological Research Network. Lake Fryxell is permanently covered by a 4- to 6-meter-thick ice cover. The upper part of the water column consists of freshwater supersaturated with dissolved oxygen and an anoxic and increasingly sulfidic environment below 10 meters in the water column (206). In 2003 a study set out to characterize the bacterial communities contributing to anoxygenic photosynthesis in the frozen lakes in Antarctica Dry Valleys. By denaturing gradient gel electrophoresis (DGGE) analysis of the pufM gene, a gene that encodes a pigment binding protein in the photosynthetic reaction of purple phototrophic bacteria, several purple nonsulfur bacteria were identified to inhabit the water column (207). The presence of psychrophilic nonsulfur bacteria, specifically Rhodoferax antarcticus strain Fryx1, was again observed in 2004 (208). In 2005, a study concluded that a diverse group of sulfate-reducing bacteria (SRB) inhabit Lake Fryxell. These findings were based on the fact that Lake Fryxell is highly sulfidic, which could result from SRB reducing sulfate to sulfide. Several psychrophilic SRBs have been isolated from permanently cold, anoxic marine environments (209). Based on 16s rRNA gene analysis, six groups of SRBs were identified to inhabit Lake Fryxell throughout the water column, specifically *Desulfovibrio*, *Desulfosarcina* group, *Desulfotomaculum*, *Desulfobulbus*, *Desulfobacter*, and *Desulfobacterium* (210). In 2003, the cyanobacterial diversity of Lake Fryxell was attempted to be mapped from a field microbial mat sample. By microscopy and genetic-based analysis, much greater diversity and abundance of Antarctic endemic species were discovered compared to what was previously thought (211). Within and surrounding the Dry Valleys exist several paleolakes with lacustrine deposits that are upwards of millions of years old. This is particularly interesting due to the possible lacustrine deposits existing or having existed on Mars (212). In 2019, large quantities of ancient life were found to be preserved in the paleolakes, including intact microbes, possible dormant spores, and well preserved non-vascular plants (213). This is interesting since it opens the possibility of remains still being somewhat preserved, despite the extreme cold and hyperarid environment, if life were present in paleolakes on Mars. If life evolved on early Mars when the environment had more favorable conditions, locations such as paleolakes might be a potential place to find traces of such life. The University Valley, as part of the Upper Dry Valley, is one of the coldest and driest parts of the Dry Valleys with a mean annual air temperature of -23.4°C and mean annual soil surface temperature between -23.5°C and -26.5°C based on readings from December 2009 to February 2013. These soil samples were highly oligotrophic (0.01%-0.05% total carbon, undetectable to 0.09% total nitrogen) and near neutral pH (7.5-8). These samples were studies for microbial presence, and showed very low microbial biomass of six different heterotrophic isolates ($1.4 – 5.7 \times 10^3$ cells per g soil)(214). This indicates that the combination of temperatures significantly below freezing, aridity and oligotrophy conditions of the University Valleys permafrost soils is nearing the limits for what microbes can adapt to.

5.3 Mars simulated conditions

When studying the survival capabilities of an organism, it can be advantageous to simulate the conditions compared to in situ testing. The most obvious advantage is that the conditions can be controlled, which can be beneficial when looking for the limits of an organism's survival capabilities, or it is of interest to isolate specific stressors. Unfortunately, not all organisms are equally easy to study under simulated conditions since there can be challenges when removing organisms from their natural habitats. This is known as The Great Plate Count Anomaly, coined by Staley and Konopka (215). It is the observation that most microbes seen under a microscope cannot necessarily be grown under laboratory conditions, either because they are nonviable or viable but nonculturable. Several reasons exist that can explain this imbalance. For example, if the organism is slow-growing or has a low prevalence, it can be easy to overlook in laboratory cultures. It can also be that the species cannot adjust to the laboratory conditions due to dormancy or other factors. When trying to decipher if any known organisms could survive on other planetary bodies, it can be a good idea to combine simulated conditions and in situ studies to utilize the advantages of both.

Several organisms have been studied under conditions that simulate those found on Mars. In 2002 *Escherichia coli* and *Serratia liquefaciens* were tested for survival and growth under simulated Mars conditions, including high salinity, low temperature, and low pressure both in combination and alone. Both *Escherichia coli* and *Serratia liquefaciens* are known spacecraft contaminators, despite thorough sterilization measures taken to reduce contamination (216). In the study, a Mars Simulation Chamber (MSC) was used for the *E.coli* to test its survivability and growth potential. The MSC is a low-pressure cylindrical chamber made of stainless steel with internal dimensions of 70 cm in length and 50 cm in diameter. Five parameters were tested for in the MSC: pressure levels down to 0.01 kPa, UV irradiation from 190 to 400 nm, dust loading, temperatures from -100 to 30 °C, and composition of gasses. Only *E.coli* was subject to the MSC since the earlier analysis of growth under desiccation showed *E.coli* to be more resistant. Results showed an increased growth penalty from exposure to any of the stressors. Growth was significantly impaired when combining several stress factors and, in many cases, resulted in the bacteria not being able to grow at all. *E.coli* failed to grow in the MSC. Especially the combination of multiple stressors was shown to have a nonlinear adverse effect, meaning the combination of stressors impacts the survival and growth more than just the sum of impacts from single stressors. In summary, the authors conclude that *E.coli* might be able to persist on Mars for a short period, but the combining stressors would inhibit growth. Since *S. liquefaciens* is more sensitive to desiccation, it would last even shorter under Martian conditions (217). Likewise, in 2020 five facultative anaerobic bacteria strains were isolated from extreme environments and subjected to Mars simulated conditions. The five strains were *Buttiauxella* sp. MASE-IM-9, *Clostridium* sp. MASE-IM-4, *Halanaerobium* sp. MASE-BB-1, *Trichococcus* sp. MASE-IM-5, and *Yersinia intermedia* MASE-LG-1. The specific stressors were low water activity, oxidizing compounds, and ionizing radiation. The results show that tolerance towards the condition stressors was species-specific. However, as the previous study described, the combination of stressors affects the organisms more than the sum of single stressors. Higher exposure to sodium perchlorate resulted in a greater sensitivity to desiccation, and exposure to desiccation resulted in a greater sensitivity to ionizing radiation in some species. However, an enhanced radiation tolerance was found in *Clostridium* sp. MASE-IM-4 and *Trichococcus* sp. MASE-IM-5. In summary, the authors conclude that the survival on Mars of some of the anaerobic bacteria is, in principle, possible because of the effects desiccation has on some bacteria, in which it increases the tolerance toward other stressors (218). In 2019 fungi and bacteria samples inside the Microbes in Atmosphere for Radiation, Survival and Biological Outcome Experiment (MARSBOx) payload, where an artificial Martian atmosphere and pressure were maintained, were launched to the middle stratosphere. The goal was to observe the effects of radiation levels. The specific species launched were *Salinisphaera shabanensis*, *Staphylococcus capitis* subsp. *capitis*, and *Buttiauxella* sp. MASE-IM-9 for the bacteria and *Aspergillus niger* for the fungi. Some samples were not exposed to UV radiation, while others were exposed to UV (280-400 nm) doses of 1148 kJm^{-2}, with five hours of exposure to the stratosphere. The results showed that *A. niger* and gram-negative *S. shabanensis* were the two most radiation-tolerant species, with a 2- and 4-log reduction, respectively. The rest were either inactivated in both situations or only survived under the UV shielded conditions (219). In the same way, *Fuligo septica* spores were tested for survivability in the stratosphere, and likewise, *F. septica* survived a 9-hour exposure (220). The high tolerance of radiation in fungi is likely due to the pigmentation. Biofilms and planktonic cells of *Deinococcus geothermalis* DSM 11300 has been exposed to Mars- and space-like conditions as part of the internationally collaborated EXPOSE-R2 mission on the International Space Station. The purpose of this mission was to assess the long-term survival of biofilm and single cells under Mars- and space-like conditions. The space-like conditions were achieved by filtering exposure to shortwave radiation (<110 nm) and vacuum storage. The Mars-like conditions were achieved by exposure to a simulated Mars atmosphere with a gas mixture and pressure levels closely resembling that of Mars, and filtering exposure to shortwave radiation (>200 nm). In-depth details on the mission are provided with the basic description of EXPOSE-R2 mission(221). The results published in July 2019 showed that *D. geothermalis* did remain viable in a desiccated state and survived an almost 16-month long exposure to Mars-like conditions both in biofilms and as single planktonic cells. The biofilm cells did however preserve better cultivability compared to planktonic cells, which is due to the better stress tolerance from biofilm protection(222).

Some methanogens have been exposed to either single parameters or a combination of parameters resembling the Martian environment. Methanogens are particularly interesting due to the presence of methane on Mars. Three strains of methanogens from the Siberian Permafrost (*Methanosarcina soligelidi SMA-21,* Candidatus *Methanosarcina SMA-17,* and Candidatus *Methanobacterium SMA-27)* and two strains from non-permafrost environments (*Methanosarcina mazei,* and *Methanosarcina barkeri*) have been subjected to simulated Mars-like conditions. The permafrost strains were isolated from active layer of permafrost-affected soils in the Lena Delta, Siberia. The non-permafrost strains were isolated from a peat bog in northern Germany and sewage sludge plant in California. Pure cultures were grown at 28°C and were frozen to -80°C +-2°C when they reached exponential growth phase. They were exposed for subfreezing temperatures up to 315 days. Besides subfreezing temperatures, pure cultures were exposed to Mars regolith simulants, which were based on the chemical and mineral composition of Martian meteorites, as well as Mars-like gas mixture to simulate the atmospheric environment. Lastly 10 mL pure cultures were exposed to 2.4, 25, 100 and 500 mM of magnesium perchlorate ($MgCl2O8$) for 50 days. The result showed that three of the strains, on permafrost and the two non-permafrosts, had no noticeable reduction in the number of viable cells after the 315 days of desiccation. The methanogenic activity increased or was not impacted after the 315 days. This shows that some methanogens are particularly adapted to extreme environments and could possibly withstand the Martian environment(29). Methanogens have adapted several ways to withstand cold temperature such as increased regulation of membrane fluidity and expression of cold-shock-proteins(223–226).

6. Discussion

The ability to assess the habitability of Mars is limited by current technological limitations. A lack of sensitivity and ability to gather information makes an assessment difficult. With an improvement in analytical and detection capabilities by technological development, predictions on whether life inhabited or do still inhabit Mars will become more accurate. The Viking lander pyrolysis procedure and gas chromatograph/mass spectrometer have shown an inability to accurately detect organic compounds (227), and it could in theory miss a cellular concentration of up to ~30 million bacterial cells from 1 gram of Martian soil due to detection limits (228). It is evident that greater sensitivity than with the Viking lander is needed to properly evaluate the presence of organic compounds and to overcome the detection limits. Since the Viking 1 & 2 mission, several rovers have been launched. Both the Phoenix mass spectrometer and the Mars Science Laboratory have had a substantial improvement to its sensitivity and precision compared to Viking(100,229,230). Besides sensitivity, luck is also a factor when taking samples since the sample sizes are limited. Depending on how the lifeforms would be distributed in the soil, it could be more or less likely that any would present in the sample in an adequate amount. The Mars 2020 Perseverance rover was launched on july 30, 2020, with the goal of seeking signs of ancient life and collecting samples of rock and regolith for a possible return to Earth. It will be the first mission to return rock samples to Earth, which will hopefully enable a more accurate absolute chronology of Mars, including when H_2O flowed on the surface or the age of the Mars' crust(231). Studying the subsurface is affected by the restrictions in drilling abilities. Both environmental and technological challenges exist that affect our ability to understand the subsurface. Environmental challenges include, but are not limited to, the lack of an adequate atmosphere to support liquid cooling of the bit and stability during the drilling, high temperature fluctuations impairing the drilling tools and regolith abrasiveness being higher than terrestrial counterparts imposing premature wear on the drilling equipment. Technological challenges include, but are not limited to, optimal rotational speed can be difficult to achieve since the higher speed causes more heat generation, the power budget of the space drilling tool is limited since fluid constrictions inhibits its supply of power to solar resources, and transportation restrictions(232,233). Some of the technological challenges with the current drilling systems places a limitation on drilling depth, that inhibits the ability to study physical samples of deeper levels in the subsurface(234).

Many extremophiles are likewise difficult to study because their growth is often challenging under laboratory conditions. archaeal isolation is particularly difficult and bacterial isolation is generally much more successful(235). Several factors can be involved in making archaeal cultivation difficult. Metabolic substances, growth factors and signaling factors can have reduced availability during in vitro cultivation, due to a dependency on synthesis of such substances from other organisms in situ, meaning that interspecific material exchange is inhibited(236,237). Archaea in general have very slow growth rates making it difficult to observe archaeal growth(238–241). In situ conditions can be difficult to emulate in transport and in vitro, leading to incubation failure or incubation of viable but nonculturable state Archaea(242). These limitations in studying extremophiles can limit our ability to fully understand extremophilic survival and the physical boundaries in which life can exist. With improvements in cultivation techniques, the proportion of archaea that can be readily cultivated is increasing.

7. conclusion

Whether life could originate, evolve, and survive on Mars is difficult to answer, since there still is a lack of knowledge on the conditions on Mars. It is not possible with our current knowledge to conclude whether life could originate on Mars. However, there are study-based indications that life may be able to exist on Mars. Several microorganisms have been shown to survive a prolonged period in Mars simulated environments. Based on geological and mineralogical indications, early Mars was properly plentiful of liquid H_2O and had a more protective atmosphere with higher temperatures and lower radiation levels. It may therefore be possible that life could originate on Mars. The main problem with such a conclusion is in the uncertainty with our predictions of how Mars looked several billion years ago. Whether life could adapt to the changing environments is still unsure, but the possibility that life could withstand the conditions on present-day Mars is not non-existing. Looking at extremophiles on Earth may give indications to how life on Mars may have adapted. The conditions on Mars would place great evolutionary pressure towards specific survival strategies. Even though both the surface and subsurface poses some of the basic requirements for being habitable, the subsurface are more likely to provide a suitable niche for sustaining life. It would provide natural protection from the high radiation levels, low temperatures, and high temperature fluctuations. Various caves may also provide a suitable habitat. One of the most defining uncertainties is whether H_2O can remain liquid in any location. If any environment shows capability to hold liquid H_2O or contains ice, such an environment should be of high priority in the search for life. If liquid H_2O indeed exists in the subsurface, it will enforce the likelihood of habitable niches existing. Other traits that most likely will be present in Martian life include high levels of pigmentation, spore formation, anaerobic metabolism, endolithic living, or accumulation of protective intracellular molecules that protect the information storing systems (DNA, RNA, etc.) from oxidative damage.

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