

Review

Habitability of Mars: How Welcoming Are the Surface and Subsurface to Life on the Red Planet?

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Abstract: Mars is a planet of great interest in the search for signatures of past or present life beyond Earth. The years of research, and more advanced instrumentation, have yielded a lot of evidence which may be considered by the scientific community as proof of past or present habitability of Mars. Recent discoveries including seasonal methane releases and a subglacial lake are exciting, yet challenging findings. Concurrently, laboratory and environmental studies on the limits of microbial life in extreme environments on Earth broaden our knowledge of the possibility of Mars habitability. In this review, we aim to: (1) Discuss the characteristics of the Martian surface and subsurface that may be conducive to habitability either in the past or at present; (2) discuss laboratory-based studies on Earth that provide us with discoveries on the limits of life; and (3) summarize the current state of knowledge in terms of direction for future research.

Keywords: Mars; habitability; surface; subsurface; water; organics; methane; life; microorganisms; lichens; bryophytes

1. Introduction

“The Earth remains the only inhabited world known so far, but scientists are finding that the universe abounds with the chemistry of life.”—Carl Sagan [1].

The study of the origin, evolution, and distribution of life in the universe allows us to have a deeper understanding of how life began and evolved on Earth and seeks to answer the question: “Are we alone in the universe?” Mars is of great interest in the search for past or present life in our universe, since current conditions on Mars are thought to be analogous to conditions on early Earth, and Mars contains carbon, potential energy sources to support life, and water in some form [2–5].

The numerous discoveries of microbial life in extreme environments on Earth [6], including Siberian permafrost [7], high temperature seafloor hydrothermal vents [8], high pressures in the deep ocean [9], hot springs [10], caves [11], and deserts or hypersaline lakes [12], have broadened our understanding of the limits of life by terrestrial microorganisms. This expansion of our knowledge has led to a broader perception of the survival capabilities of extremophilic microorganisms in the search for life on different solar bodies, among which Mars is a leading destination in the search for signatures of past or present life. The habitability of Mars has been debatable for a very long time, but with new discoveries in the fields of Mars geology, chemistry, and astrobiology, it needs a continuous revision to encompass newly emerging information.

The definition of “habitability” can vary depending on the discipline; however, we use the term as defined by astrobiologists. Cockell et al. define a ‘habitat’ as “an environment capable of supporting the activity of at least one known organism, where activity (and thus living) is metabolic activity allowing for survival, maintenance, growth, or reproduction” [13]. To discuss the habitability of Mars, we rely on this definition and on our knowledge of life forms on Earth. The habitability of Mars for humans is not considered in this review. Cockell et al. [13] lists the requirements for habitability, which include:

(1) solvent (water); (2) temperature and physiochemical conditions; (3) an energy source; and (4) major and trace elements. These requirements strictly intertwine with the limits of life based on our current understanding [14]. Life forms, whether residing on the surface or in the subsurface, must survive within specific ranges of physical and chemical factors which include pH, temperature, water activity, radiation, pressure, composition of the atmosphere, salinity, and nutrients. The goal of this review is to discuss the environmental characteristics on Mars that may allow for the presence and support of life either in the past or present.

2. Characteristics of the Martian Surface and Subsurface

2.1. Habitability of the Martian Surface

2.1.1. Ice and Water

Even as a structurally simple molecule, water supports complex processes encompassing all molecular, organismal, and ecosystem processes on Earth [15]. Earth life cannot be supported without it. Geological features on Mars indicate that there may have been liquid water on the surface at some time in the past as evidenced by dry river valleys, the appearance of lake formations, alluvial fans, and deltas [16–18]. Additionally, hydrothermal environments on Mars, associated with craters from impacts and volcanism, could have easily provided a source of liquid water [19]. Studies and observational data have provided evidence for sedimentary processes that are likely a result of previous aqueous flows on Mars [3,20,21]. Images from the Mars Global Surveyor (MGS) Mars Orbiter Camera (MOC), and Mars Odyssey Thermal Emission Imaging System (THEMIS) show what appears to be a large drainage basin [3]. There appears to be at least 10 valleys leading to a central valley which enters into the Holden NE Crater. These geological features suggest there was initially a tributary that was likely to have been buried but is now partially exposed by the meteor impact [3]. Ultimately, these features lead to a fan-like formation which contain layers of sedimentary rock and is consistent with water flows. Additionally, what has been interpreted as sedimentary outcrops have been identified at the Valles Marineris, Mawrth Vallis, Arabia Terra, Terra Miridiana, Northern Hellas, and Southern Elysium Planitia [20]. Although aerial deposits cannot be ruled out, it is likely that these features are from lacustrine depositions [20]. Moreover, the mineralogy of several intracrater sedimentary deposits have been described [21–23]. Studies along the Syrtis Major indicate the formation of clays, carbonates, and sulfate during the Noachian–Hesperian transition. This transition is believed to have been responsible for the changes in water pH from alkaline to acidic [23]. It is believed that this change was caused by volcanic activity and the outgassing of volatiles such as sulfur [19].

Mars' surface sedimentary alluvial fans from the Sharp Crater contain Fe–Mg and aluminum phyllosilicates, and the Sharp–Knobel watershed contains eight chloride deposits. Many of the phyllosilicates are layered above the chloride deposits. The phyllosilicates are minerals associated with hydrothermal activity in Mars' past. Alternatively, Fe–Mg phyllosilicates at the Gale Crater are in both sediments and bedrock, and sulfates have been the only salts detected in this region. These differences in sedimentary deposits indicate that aqueous activity may have contributed to this variety in rock composition [21].

Previously, the presence of water on Mars was debatable, but current research has shown that water does exist as polar ice caps, subsurface ice deposits, frosts, water vapor, hydrated salts, groundwater [2,24], and a recently discovered subglacial lake [25]. Water in ice form is ubiquitous on Mars, and the polar regions contain ice caps. The North polar region dome's layers (Planum Boreum) consists of basal unit layers, polar layered deposits (PLD), and an ice cap consisting of mostly water ice [26]. These PLDs are composed of ice–dust fractions and were shown to contain high purity water ice [27]. By contrast, the PLDs of the south polar region (Planum Australe) contain large amounts of carbon dioxide (CO₂) ice deposits [28]. Moreover, water exists in the perennially frozen underground, forming a layer referred to as the cryosphere, which can be up to 9 km deep at the Mars equator and up to 10–22 km deep at the poles [29].

The presence of liquid water on Mars has been a subject of vivid discussions in the scientific community [30]. The current physical parameters lead to complexity in its retainment on the surface. Simply, a very low atmospheric pressure of 6 mbar (6 hPa) leads to immediate water sublimation or water remains frozen due to very low temperatures on the Red Planet [31]. In the study by McEwen et al. [32], the analysis of images from the Mars Reconnaissance Orbiter High Resolution Imaging Science Experiment (HiRISE) showed recurring slope lineae (RSL), which are dark streaks up to 5 m long on relatively steep slopes. The explanation for their seasonal variation, when they are darker during spring and summer and lighten in winter months, led to the theory that they are the result of seasonal water flows [32,33]. This theory has been challenged by a more recent study, where it was proposed that the RSL do not represent seeping liquid water, but rather granular flow [34]. The authors state that water can play a role in their genesis, but their occurrences on only steep flows, and the lack of analogous processes in Earth gullies, might lead to the revision of their formation mechanism from the water flow theory [34].

Radar measurements by the Mars Advanced Radar for Subsurface and Ionosphere Sounding (MARSIS) carried by the Mars Express spacecraft led to the discovery of the first large liquid water body presently on Mars [22]. The 20 km wide lake is overlying the South Martian pole under the PLDs. The temperature of the subsurface and the pressure from the ice deposit, along with the high concentrations of salts, are features that maintain the water in liquid state. This discovery has many implications for the search for life on Mars, as microbial cells have been found in analog environments on Earth, including in the briny Antarctic Lake Vida, which has a temperature of -13°C , under a permanent ice cover [35].

There is other evidence for transient liquid water under the surface of Mars. In the study by Martín-Torres et al. [36], relative humidity and air and ground temperature data collected by the Curiosity rover at the Gale crater were analyzed. The scientists concluded that liquid brines can appear in a 5 cm deep layer of the subsurface during nighttime but evaporate shortly after sunrise. Another conclusion from the same study shows that at a depth of 15 cm and below, the combined conditions of water activity and temperature permanently retains hydrated Ca and Mg perchlorates [36]. Transient water activity was also identified within the Martian slope streaks [37], even though the mechanisms behind their formations are still not understood. The authors suggested that the lithological composition of the slope streak regions consists of high concentrations of Fe, Cl and Ca that can form various brines leading to the formation of the slope streaks as well as a diurnal exchange of water between the Martian regolith and atmosphere.

The transient presence of aqueous activity is connected with the deliquescence of hygroscopic salt formation of brines on its surface and subsurface with low eutectic temperatures [36,38–40]. Experimental measurements and modeling on the stability of Mg perchlorate and Na perchlorate show that the brines may be stabilized due to the low eutectic temperatures around 206 K (-67°C) [41]. The Mg perchlorate brines and their stability can even allow for water reabsorption after evaporation periods. Rivera-Valentín et al. [42] used measurements of ground and in-air temperatures, and relative humidity, collected over the years at the Gale Crater by the Rover Environmental Monitoring Station (REMS) carried by the Curiosity rover, to study deliquescence of Ca perchlorate and formation of brines in the surface and subsurface. The results showed that surface perchlorate brines were favorable; however, modeling of environmental data showed brines could form in the shallow subsurface [42]. In a study carried out by Davila et al. [43], climate models were prepared using temperature and relative humidity data collected from various Mars regions. The regions, which showed potential for habitability due to deliquescence, were modeled for various chloride salts. The deliquescence of Na chloride allows for solutions with water activity that would be conducive to microbial growth, but Ca and Mg chloride solutions had water activity below the growth limit.

Water, whether in the form of transient water, brines or ice, is critical for the habitability of Mars. The current state of water in combination with other environmental factors (discussed below) is not

enough to support life on the surface. Studies indicate that early Mars was likely to have been more active and habitable than present-day Mars [44].

2.1.2. Organic Compounds

Organic compounds, those in which one or more atoms of carbon are covalently linked to atoms of other elements such as nitrogen, oxygen or hydrogen, are associated with life as we know it. Organics can also be produced by abiotic processes. The first discovery of organic molecules was reported by Freissinet et al. [45]. Among discovered molecules by the Sample Analysis at Mars (SAM) instrument on the Curiosity rover were chlorobenzene (150–300 ppb) and C₂ to C₄ dichloroalkanes (up to 70 ppb). The SAM instrument included gas evolved analysis (EGA) followed by gas chromatography coupled with a mass spectrometer (GC–MS). In the study by Eigenbrode and colleagues [46], organic matter was identified in lacustrine mudstones of the 3.5 billion year old Murray formation located at Pahrump Hills, at the Gale Crater. The compounds included the organic sulfur compounds thiophene, methylthiophenes, methanethiol, dimethylsulfide, benzothiophene, carbonyl sulfide, CS₂, H₂S, and SO₂, in addition to O₂, CO, and CO₂. Only the presence of thiophene and 2- and 3-methylthiophene was confirmed by GC–MS. EGA also detected aliphatic compounds and aromatic compounds such as benzene, toluene, alkylbenzenes, and the possible presence of chlorobenzene and naphthalene. By comparison, studies of lacustrine sites on Earth showed that the presence of sulfides on Mars might indicate microbial activity [47]. This further supports the idea that analog Martian sites containing the identified sulfides might be a target in the search for microbial life. The search for organics in Martian sediments by the SAM instrument was a second attempt at detecting organics, after a first attempt performed during the Viking mission only detected organics at a level of a few parts per billion in near surface samples [48]. In between the Viking landers and Curiosity rover missions, the scientific community debated if organic compounds can be detected at all due to their possible degradation by ionizing and ultraviolet radiation, and the highly oxidizing conditions resulting in the formation of perchlorates [49]. However, even with the discovery of organic compounds, it is still unknown whether their source is from biogenic or abiogenic processes or if they have current functions in terms of habitability [46].

2.1.3. Salts

As a part of the Microscopy, Electrochemistry, and Conductivity Analyzer (MECA) suite carried on the Phoenix Mars Lander, three soil samples were analyzed using the Wet Chemistry Laboratory (WCL) instrument [50]. The results indicated that the average pH of Martian soil is 7.7 ± 0.5 , which is close to the optimal pH for microbial growth, even though acidic and alkaline pH values are also tolerated by microorganisms on Earth [51]. The WCL also measured the concentration of various ions: Ca²⁺, Mg²⁺, K⁺, NH₄⁺, Na⁺, Cl[−], Br[−], and I[−] [50]. Salts found in Martian soils include NaCl, MgSO₄, CaSO₄, FeSO₄, MgCl₂, and CaCl₂ [52]. It is estimated that sulfur-containing salts are more common than chlorinated salts on the Martian surface by a ratio of 4:1 [53]. Salts influence water activity and have major impacts on the potential for life, as high salt concentrations can stress cell systems, including cell turgor [54]. Water activity of pure water is equal to 1.0, and the more saline it is, the less water is available [55]. The current known limit of water activity for terrestrial microorganisms was found to be 0.586 for *Aspergillus penicillioides* cell differentiation and cell division [56], while a recent study by Steinle et al. [57] showed that sulfate reduction by microorganisms could still occur in saturated MgCl₂ solution with only a water activity of ~0.4. The study of Martian data from Meridiani Planum and other brine environments on Mars surfaces indicated at first that the water activity at these locations might be a limiting factor for microbial growth and survival [58]. However, in light of recent studies [56,57], the barrier of low water activity may be overcome.

WCL results also showed high concentrations of perchlorates (~0.6 wt%) [50]. Further analysis of the WCL results showed that the perchlorates form salts with an ~3:2 ratio of Ca and Mg salts [59]. Theoretical studies by Chevier et al. [41], using conditions found at the Phoenix landing site, showed that perchlorates are probably in liquid form part of the time during the summer. Additional studies

showed that Mg perchlorate eutectic solutions would likely be stable during a few hours of the day. Moreover, these perchlorate salts are likely to have very low evaporation rates on the order of 0.07 to 0.49 mm h⁻¹ depending on the type of perchlorate salt (Mg versus Na) and temperature [41]. Perchlorates are highly oxidizing compounds, soluble in water and generally considered toxic to humans in low concentrations, yet a number of microorganisms capable of utilizing perchlorates as electron acceptors have been described [60]. Perchlorate respiration has been found to be coupled to the oxidation of fatty acids, and small organic acids [61]. Studies have also shown that some perchlorate reducers are capable of oxidizing both Fe and S, S²⁻, and S₂O₃²⁻ in conjunction with perchlorate reduction [62].

The presence of salts does not seem to create a barrier for surface habitability, as halophilic microorganisms are common on Earth and known to survive and grow under high salt concentrations. However, combined with other factors, such as radiation, adverse conditions might still render habitability on the Mars surface impossible.

2.1.4. Radiation

Habitability on the Martian surface is highly dependent on protection from UV and ionizing radiation [63,64]. Mars does not have a magnetic field to deflect energetically charged particles, including galactic cosmic rays and solar energetic particles. These particles penetrate the thin Martian atmosphere and react with the regolith, resulting in the creation of other particles leading to an oxidizing environment on the Martian surface. The radiation dose received by Mars was measured over 300 sols by the Radiation Assessment Detector (RAD) carried by the MSL rover [65]. The galactic cosmic rays on the Mars surface were found to be 0.210 ± 0.040 mGy/day (average total dose), while the solar energetic particle dose was 50 µGy. These data were used for modeling the radiation dose in the Martian subsurface for determining the potential for microbial survival and assessing how radiation influences the preservation of organic compounds. The galactic cosmic ray radiation was found to diminish 3 m below the surface. When considering the potential survival of microorganisms, models showed predictions that an ~1 m drill would be enough to access radioresistant microbial cells, while organic matter would be best detected in rocks and soil (due to preservation) at sites without aqueous activity, which have been recently exposed (such as through erosion or impact craters) [65].

Additionally, UV-B and UV-C fluxes on Mars are nearly five times higher than they are on Earth, with fluences of 361 kJ/m² and 78 kJ/m², respectively [66]. This poses another challenge for habitability on Mars. However, the high atmospheric concentration of CO₂ neutralizes incoming UV radiation at <200 nm, but wavelengths >200 nm still reach the Martian surface [67]. Interestingly, UV radiation may be at times attenuated due to the presence of dust storms [68].

The adaption and survival of microorganisms to surface radiation would be an important factor for Mars to be habitable. Otherwise, even short-term survival, as evidenced by laboratory-based studies (Section 3.1 in this review), is difficult as the radiation on the surface is too strong for any life forms to thrive. This makes it more plausible that life may exist or has existed in the Martian subsurface.

2.1.5. Atmosphere

The Martian atmosphere is anoxic. Initial measurements showed that the atmosphere consisted of 95.3% CO₂, 2.7% N₂, 1.6% Ar, and 0.1% O₂ [69], but recent measurements from the SAM on the Curiosity rover show it to be composed of 96% CO₂, 1.93% Ar, 1.89% N₂, 0.145% O₂, and <0.1% CO [70]. Many terrestrial microorganisms require growth in strictly anoxic atmospheres or can switch from aerobic metabolism if no oxygen is available (facultative anaerobes). Some microorganisms isolated from the spacecraft assembly facilities (SAF) are facultative anaerobes including but not limited to: *Bacillus pumilus*, *Bacillus thuringiensis*, *Brevibacillus invocatus*, *Enterococcus faecium*, *Paenibacillus jamilae*, *Paenibacillus xylanilyticus*, *Staphylococcus epidermidis*, *Pseudomonas oryzihabitans*, *Blastococcus aggregatus*, *Curtobacterium luteum*, and some species of *Bacillus*, *Serratia*, and *Georgenia* [71]; while among obligate anaerobes (species that may be tolerant to low oxygen levels but conduct anaerobic metabolism) were

Propionibacterium acnes, *Clostridium perfringens* [72], *Corynebacterium pseudogenitalium*, *Propionibacterium aoidum* [73], *Clostridium colicanis*, *Clostridium sacharolyticum*, *Clostridium sporogenes*, and *Paenibacillus wynnii* [74]. Whether these microorganisms can grow in a rich CO₂ Martian atmosphere is not known, but their capability to survive and replicate under anaerobic conditions might suggest this possibility.

The Martian atmosphere also contains small quantities of water. Water vapor in the Martian atmosphere has a pressure of 10^{−3} mbar that is dependent on seasonal water removal from ice caps at both polar regions, exchange with water bounded in the surface regolith, and transport by winds [31]. Whether water from the atmosphere can be utilized by organisms is unknown, although there are reports of fungi that can survive and grow with limited precipitation in low relative humidity conditions in the arid Namib Sand Sea [75].

Another characteristic of the Martian atmosphere that is extremely distinct from the Earth's atmosphere is a very low pressure of ~6 mbar [76]. Low-atmospheric pressure Earth environments include Mount Everest (36.6 kPa) [77] and low Earth orbit (10^{−7}–10^{−4} Pa) [78]. However, there are reports that indicate the survival of microorganisms at low pressure, such as *Carnobacterium* spp. [79] and *Serratia liquefaciens* [80], and even cell proliferation by *Vibrio* spp. [81] tested under laboratory conditions (Section 4 in this review). While low pressure is considered a limiting factor for habitability, examples of these microorganisms surviving low pressure may suggest that the Martian atmosphere might be easily overcome for surface, and in particular subsurface, habitability.

2.2. Mars Subsurface as a Potential Habitat for Life

The subsurface of Mars is the most probable place where life is likely to survive and thrive, due to protection from the extreme environment found on the Martian surface. For present-day Mars, it is estimated that life could exist in the subsurface at depths up to 8 km [82]. The subsurface also provides protection from radiation. Moreover, the low atmospheric pressure is considered a limiting factor for Mars surface habitability, but subsurface pressure is expected to be higher [80]. Apart from escaping these challenging environmental conditions on the surface, the subsurface physicochemical characteristics are favorable for harboring present chemolithoautotrophic life [83] through the use of inorganic compounds for the production of metabolic energy, as has been shown in Earth's deep biosphere [84]. Fisk and Giovanonni [85] stated that conditions exist in the Mars subsurface to sustain life. These include water, a temperature from 0 °C to 120 °C (the known upper temperature limit for microbial life is 120 °C [86]), major elements, and energy sources. Similar conditions for Mars subsurface habitability are discussed by Cockell [87]. Moreover, as previously discussed (Section 2.1.1), there is evidence for the presence of subsurface water in the form of groundwater or ice, as evidenced by the recently discovered subglacial lake [25].

The major elements needed for life (C, H, N, O, P, and S) are present, and CHONPS-based chemistry associated with life may be concentrated in the Martian subsurface. Carbon is associated with organic compounds [46], atmospheric CO₂ and CO [70], and carbonates [88]. Hydrogen can be produced during serpentinization processes [89] or radiolysis [90] (discussed below). Nitrogen is present as a gas at a low concentration in the atmosphere (1.89%) [70] and as nitrates [91]. The source of oxygen may be from perchlorates and other oxidized compounds [59]. Apatite and merrillite are primary minerals of phosphorus [92,93], while sulfur has been detected as sulfur salts and organics [46].

The most commonly recognized potential electron donor in the Mars subsurface is H₂, which can be used by microbes in methanogenesis and iron or sulfate reduction. Other possible electron donors are Fe²⁺, S, and CO [13]. Moreover, the discovery of methane and hydrogen on Mars suggests the potential for past or present habitability and life on Mars [94]. Methane was discovered independently by three research groups; in the Martian atmosphere by the Planetary Fourier Spectrometer on board the Mars Express Spacecraft [95], by Fourier Transform Spectrometer at the Canada–France–Hawaii Telescope [96], and infrared spectrometers [97]. A significant methane plume was observed in summer 2003 and measured again in 2006 by the high dispersion infrared spectrometers in Hawaii [98]. Later studies showed a strong pattern of seasonal variations as measured by the Tunable Laser

Spectrophotometer on the Mars Science Laboratory (MSL) Rover [99]. These measurements were performed for 5 years at the Gale Crater, and results showed methane was present in the range of 0.24–0.65 ppbv. The spike of methane was also independently detected by the Mars Express orbiter at the Gale Crater a day after data were reported by MSL [100]. However, the most recent study report by Korablev et al. [101] did not detect traces of methane in the atmosphere by the ExoMars Trace Gas Orbiter even though it was one of the goals of this mission. The authors suggested an unknown process that might remove methane or sequester it from the lower atmosphere.

On Earth, methane sources are mostly associated with microbial activity conducted by species from the domain Archaea [102] and by analogy may provide an explanation for methane on Mars [103]. If life does not exist on present-day Mars, the released methane might have been produced by now-extinct microbial organisms. However, abiotic sources of methane can be another explanation for its origin on Mars. Geological methane production is attributed to the Fisher–Tropsch type reactions in which hydrogen and carbon monoxide or carbon dioxide are converted into methane in the presence of catalyst metals such as Ni, Fe, Cr or Ru [104]. Methane can be produced in volcanic degassing, UV irradiation of organics, and geothermal reactions requiring high temperatures [86,95]. Another possible explanation is methane release as a result of thermal degradation of organic matter of abiotic or possible ancient biological origins [104].

The presence of molecular hydrogen is strongly intertwined with the presence of methane. Carbon dioxide and hydrogen are utilized by hydrogenotrophic methanogens as a source of carbon and electrons, respectively [105]. Hydrogen is also utilized in abiotic Fisher–Tropsch reactions [104]. The sources for hydrogen on Mars have been assigned to serpentinization of ultramafic rocks in the Martian subsurface [89,106], volcanic degassing [104] or radiolysis [90]. The latter process is more ubiquitous as it does not require high temperatures and occurs on the interface of water with rock and sediments [107]. Dzaugis et al. [108] quantitatively assessed radiolytic H₂ production in wet Martian environments, which included past wet surfaces and present wet subsurfaces, as a possible source of energy. The scientists chose 11 sites (including the final landing site for the Mars 2020 mission, Jezero Crater, and control sites of lowest and highest H₂ production) to calculate H₂ production based on their radionuclide composition data obtained from the γ ray spectrophotometer (GRS) on Mars Odyssey. The outcomes from the analysis of fractured hard rock and sediment models showed that the radiolytic H₂ production rates (nM/year) are from 0.12 to 1.2 nM/year in low porosity (<35%) fine-grade sediment, 0.03 nM/year in high-porosity sediment, and <0.71 nM/year in microfractured hard rock (1 μ m). In comparison, the subseafloor basalt in the South Pacific has values from 0.02 to 0.6 nM/year and are sites where microbial activity has been identified [109].

When compared with the requirements for habitability listed by Cockell et al. [13], the subsurface of Mars meet all of the requirements in its current state; therefore, it can be recognized as habitable. Even with this assumption, we do not know if there are any life forms that presently reside deep in the Martian subsurface. It is the presence of both methane and hydrogen that is an important drive for consideration that life might exist now or might have existed in the past [110]. However, more research is needed to determine the source of methane and hydrogen. Once their origin is assigned either to abiotic or biotic activity or a combination of both, then assessment of Mars habitability will be more defined.

3. Mars Analog Sites on Earth

The studies of Mars analog sites on Earth are aimed to characterize physical, chemical, and geological characteristics of Mars by utilizing the knowledge of similarities between Earth and Martian environments [111]. The applications of research conducted at such sites are numerous; therefore, in this review, we only focus on the analog sites that relate to habitability.

There is no environment on Earth that has all of the current distinctive characteristics of Mars combined, including low atmospheric pressure, a CO₂-saturated atmosphere, high radiation, low temperatures, and a lack of liquid surface water. The limits of these analog sites constrain research

related to Mars habitability and astrobiology. However, these sites do present an opportunity for studying natural environments that might result in interesting scientific findings.

3.1. Dry Valleys of Antarctica

Antarctic Dry Valleys represent a unique environment on Earth. They are cold and arid deserts with low snow precipitation [112]. The high-altitude valleys (1000–2500 m) are especially relevant as Mars analogues. Ice-cemented grounds are found at a depth up to over 50 cm [113], while summer day temperatures do not rise above freezing. Multiple studies have been carried out on samples from the McMurdo region to learn about the composition of microbial communities and their functions [114]. However, a recent study of permafrost samples from the University Valley [115] showed that the biomass of microbial communities was extremely low, and only six isolates were retrieved. Moreover, even though the community composition was elucidated, RNA extractions did not yield any detectable molecules, suggesting that no microbial activity occurs under these conditions [115].

3.2. Atacama Desert

The Atacama Desert in Chile is a Mars analog environment on Earth due to its aridity and extremely rare rain events. It is considered the oldest desert on Earth [116]. Even though it is a hot desert, the Atacama soil resembles Mars soil in its composition and low organic content [117,118]. A study of cyanobacteria in the Atacama Desert showed that microorganisms conduct their metabolism due to salt deliquescence [119], suggesting that a similar process may occur in Martian conditions.

3.3. Lava Tubes

Lava tubes are present both on Earth and Mars [120]. They are caves or channels that are formed during or after volcanic eruption as lava flows harden on the “roof” while the hot lava is still inside. The interests in lava tubes as astrobiological sites with implications for Mars is based on the possibility of life being present beneath the surface of Mars [82]. The importance of studying lava tubes, with regard to possible habitation on Mars, stems from the possibility that they may protect life forms from harmful radiation, desiccation, and weather events (e.g., winds and dust storms) and might harbor signatures of past or present life or evidence of aqueous activity [120]. The study of microbial communities in lava tubes in Lava Beds National Monument showed that they have lower diversity and include many unidentified species in their communities, which makes them distinct from microbial communities found on the surface [121]. In a study by Gonzalez-Pimentel et al. [122], the microbial communities of lava tubes on the Canary Islands were mostly represented by Proteobacteria and Actinobacteria, with the latter found to be metabolically active. Both studies show that the lava tubes on Earth harbor their own microbial communities. Even though it is not possible to say if life exists or has existed on Mars, Martian lava tubes are potentially habitable considering that most requirements for habitability are met on the subsurface (Section 2.2 in this review).

4. Studies on Survival of Organisms in Simulated Martian Conditions

Even with the presence of analog sites on Earth with implications for astrobiological discoveries on Mars, none of the sites represent a wide range of the environmental conditions found on present-day Mars. Therefore, laboratory-based studies that expose various life forms to simulated Martian conditions carry great importance for learning about the survival mechanisms used by terrestrial organisms and the overall potential for habitability of the Red Planet.

4.1. Microorganisms

Microorganisms are considered the most likely organisms to colonize the surface or subsurface of Mars due to their high tolerance to extreme environmental conditions. Additionally, transfer of organisms to Mars may occur through transfer either on mission vehicles [123,124] or as explained

by the panspermia theory, which is the distribution of microbial forms of life by various means such as through space dust [78]. If life existed or currently exists on Mars, it would most likely be in the form of microbial communities that could be supported by Martian conditions, especially in the deep subsurface [24]. Studies on the survival of microorganisms in simulated Martian conditions include experiments on microbial survival in Mars soil analogs [125,126], on spacecraft surface materials [124], and in predefined laboratory media [127]. Even though the latter two do not refer directly to the surface or subsurface of Mars and their associated parameters (pH, oxidation, temperature, water activity), they are discussed in this review to cover the spectrum of microorganisms and their survival capabilities in simulated Martian conditions.

4.1.1. Archaea

Archaea are very abundant in extreme environments; however, their growth is challenging under laboratory conditions, and therefore, studies on Archaea in simulated conditions are limited. Methanogens are of great interest for studying the potential for survival on Mars due to their ability to use hydrogen and carbon dioxide as the only energy and carbon sources, respectively [104]. They are often present in high numbers in the most extreme of environments and have been shown to survive and grow under low temperatures, high salinity, and both acidic and alkaline environments [128]. Moreover, some of their representatives, such as *Methanosarcina* spp., can use various carbon compounds to produce methane, which may provide an explanation for the detection of methane on Mars. As discussed by Smith et al. [129], H₂ can readily be oxidized with atmospheric CO₂ to generate energy, thus resulting in methane production [130]. The resulting methane could be oxidized by methanotrophic archaea in the presence of sulfate-reducing bacteria to complete the methane cycle [131]. H₂ could be generated by photochemical dissociation of water and serve as an electron donor [132]. There are large amounts of sulfate salts in the Martian soils which could support this methane cycle and thus microbial life.

Under laboratory conditions, a few species of methanogens were studied by exposing the isolates to single or simultaneous Martian physical and chemical environmental stressors. *Methanothermobacter wolfeii*, *Methanosarcina barkeri*, and *Methanobacterium formicicum* were tested for survival upon exposure to low pressures of 400 mbar and 50 mbar in JSC-1 Mars regolith analog. The studies showed that methane was produced under these conditions although this production was reduced at 50 mbar [133]. Furthermore, these three strains and an additional strain of *Methanococcus maripaludis* were desiccated at 1 bar and 6 mbar pressure. While *M. barkeri* survived 330 days of desiccation at 1 bar, the other two strains did not survive as long; *M. wolfeii* only survived 180 days and *M. formicicum* survived 120 days. The survival of organisms during desiccation at 6 mbar of pressure was much lower; *M. barkeri*, *M. wolfeii*, and *M. formicicum* only survived for 120 days, while *M. maripaludis* did not survive at all in the 1 bar pressure desiccation experiment but surprisingly survived for 60 days at 6 mbar pressure [133]. However, each of the four species were able to survive 6 mbar of pressure while in an aqueous environment [134]. In the other experiments, the same strains were exposed to combined conditions of low pressure (6 mbar) and desiccation for 90 or 120 days while exposed to various Martian regolith analogs [135]. The results showed that *M. barkeri* survived under various combinations of these conditions. *M. wolfeii* and *M. formicicum* survived the combination of desiccation and low pressure but not in the presence of any Martian regolith analogs. *M. maripaludis* did not survive any conditions [135]. The same strains were also exposed to perchlorate salts (Mg, Na, and Ca) at concentrations of 0.5%, 1%, 2%, 5%, and 10% [136]. All species survived concentrations of 5%, while *M. wolfeii* and *M. barkeri* exhibited survival up to 25% salt concentrations for up to 72 h. Just recently, Mickol and Kral [137] showed that *M. barkeri* not only produced methane at 100 mbar, but the culture also increased in density at 50 and 100 mbar.

As detection of methane was an important discovery, it will be crucial to find out if a biological source is responsible for production on Mars. Therefore, the study of methanogens and their survival in simulated Martian conditions are extremely important for learning if life might exist on the surface

or subsurface. The results from laboratory studies show it might be possible for some methanogens to inhabit the subsurface of Mars due to their tolerance to low pressure, desiccation, and perchlorate salts.

4.1.2. Bacteria

Bacteria have been commonly studied for their potential survival in simulated Martian conditions. They are commonly isolated from natural extreme environments, but also from spacecraft-associated facility (SAF) surfaces, using traditional and molecular techniques. The most researched bacteria used in space studies are from the genera *Bacillus*. Interest and focus on *Bacillus* spores and other spore formers are due to their high resistance to various extreme environmental conditions. Moreover, their long-term survival mechanisms might support their transfer to Mars and survival under present-day Martian environmental conditions. Spores can survive high concentrations of H₂O₂, long exposure to UV radiation, low temperatures, and desiccation—all of which are characteristics of Mars [138–140].

Many studies have been undertaken to better understand the ability of bacteria to survive in simulated Martian environments. In one study, spores of *Bacillus subtilis* were inoculated on aluminum coupons at 2.46×10^6 per sample and exposed to a combination of the following conditions in a Mars Simulation Chamber: temperatures of -80 °C, -40 °C, -10 °C or 23 °C, pressure of 8.5 mbar or 1013 mbar of air, pure N₂, pure CO₂ or a Mars atmosphere mix, and UV-VIS–NIR [124]. The results showed that 99.9% of spores were inactivated under normal Earth conditions of pressure, temperature, and atmosphere with the Mars UV-VIS–NIR spectrum after 30 seconds, but 15 min was required for total spore inactivation. When other environmental conditions were tested, a pressure of 8.5 mbar was found to have the highest spore reduction under the Mars UV spectrum and lowered the spore level by 20%–30%.

The effect of Mars UV irradiance was studied on spores of multiple microbial isolates, including strains originating from spacecraft surfaces destined for Mars [141]. In the first step, screening for resistance to UV at 254 nm preselected 19 isolates that survived the exposure. From this group, seven isolates with the highest LD₉₀ (lethal dose when 90% of population is inactivated) were further screened for survival under Mars simulated UV radiation: UVA (315–400 nm), UVA + B (280–400 nm), and UVA + B + C (200–400 nm) with 590 W m^{-2} as the total solar irradiance at the Mars simulated level. The spores were exposed to UV irradiation while in water, and among seven tested strains, spores of *Bacillus pumilus* SAFR-032 (an isolate from spacecraft assembly facilities) showed the highest survival at the full UV spectrum (200–400 nm). In a recent study conducted by Cortesão et al. [142], the spores of wild type and mutant *Bacillus subtilis* strains deficient in various spore coat components or DNA repair mechanisms were exposed to a simulated Martian atmosphere with or without exposure to Martian UV radiation for 8 hours. The study showed that the survivability of spores not exposed to UV was much higher than that of those directly exposed. The survival strategies of spores not exposed to UV under simulated Martian conditions relied on protection mechanisms provided by the spore coat as the outermost barrier to this environmental stressor. The spores exposed to UV under simulated Martian conditions retained core dehydration and stronger DNA binding to major small acid soluble proteins and generated spore photoproducts [142]. These results from the exposure showed protection mechanisms that are typical of the exposure to UV radiation by spores [139]. Importantly, the combination of other stressors from simulated Martian conditions did not diminish these capabilities, thus resulting in spore survival despite being exposed to strong UV radiation equivalent to that on Mars.

Martian Soil Analogue Exposure

In a series of studies, the effects of biotoxicity of Martian soil analogs were studied on various microorganisms. Berry et al. [143] tested *Escherichia coli* and *Serratia liquefaciens* for growth and survival at a combination of temperatures (-20 °C, 4 °C, 20 °C, desiccated at room temperature) and time (1 or 7 days). The Mars soil analog (fine-grain volcanic palagonite sourced from Hawaii) was inoculated with bacterial cells in modified Luria–Bertani (LB) medium that did not contain the salt in the original

recipe. The initial testing showed better survival rates for *E. coli* over *S. liquefaciens*. *E. coli* was then treated under simulated Martian conditions (0.71 kPa pressure, Martian atmosphere, UVC radiation, and temperature within the 20 to -50°C range) with and without UV exposure at various levels of salinity (0%, 5% MgSO_4 , and 15% salt mix). Generally, the cells survived the 7-day exposure but did not show growth above the initial inoculum levels. The authors concluded that *E. coli* might survive but not grow under Martian conditions. However, it is unknown what the survival rate would be if no media (modified LB) was used as it would have provided nutrients for the cells. Another study looked at survival of *Enterococcus faecalis* ATCC 29212 and spores of *Bacillus subtilis* HA101. These isolates were selected since they are the most resistant to desiccation among the other screened strains [125]. Monolayers of spores or vegetative cells were deposited on aluminum coupons and covered by a 1 mm layer of soil analog under a Martian similar atmosphere (6.9 mbar, -10°C , CO_2 atmosphere). The soil analogs included basalt (control) and soils composed by mixing various minerals to obtain acidic, alkaline, high salt, aeolian (i.e., global Mars analog soil) and perchlorate soil (Phoenix landing site analog soil). The results showed a slight reduction in spore numbers in acidic and high salt soil after a 7-day exposure to Martian conditions, while *E. faecalis* cells were reduced by 7-log in high salt soil and 2–3 log in other soil analogs. In follow-up experiments, the same microorganisms were used for survival testing in aqueous solutions of the six soil analogs obtained by suspending 50 g of each soil in 100 ml of double deionized water and shaking it at 250 rpm for 2 h [126]. The spores of *B. subtilis* and cells of *E. faecalis* were suspended in these solutions for up to 28 days at various temperatures: 24°C , 0°C or -70°C . There was only a slight reduction in spores, but *E. faecalis* cells were killed under high salt and acidic solutions. The soil analog of the Phoenix landing site regolith composition was also used to assess germination and outgrowth of *Bacillus subtilis* 168 and *Bacillus pumilus* SAFR-032 spores [144]. There was no suppression on the germination and outgrowth up to 5 hours in soil analog extract amended with LB media; however, long-term exposure to the same media effectively inhibited their growth after 20 h of incubation.

Exposure of Bacterial Isolates from Spacecraft Assembly Facilities

Studies utilizing isolates collected from SAF surfaces, including those from spacecraft surfaces, are of value when considering the potential of microorganisms to inhabit the surface or subsurface of Mars. The ongoing research and more advancement instrumentation might find evidence of past life or signs of present life. Advanced instrumentation combined with knowledge of terrestrial microorganisms' survival and capabilities might lead to the discovery and confirmation of Mars habitability. Considering that there is a high chance for transfer of microorganisms to the Martian surface from spacecraft bound for Mars, studies on survival and growth of the most common isolates from spacecraft surfaces are beneficial for assessing survival and growth potential, both to expand the knowledge of survivability on Mars and to determine if spacecraft associated isolates may interfere with life detection on Mars. Microorganisms can potentially be transferred from external spacecraft surfaces as described in the study on *B. subtilis* spores by Kerney and Schuerger [123]. *Bacillus subtilis* HA101 endospores were first applied to a simulated rover wheel, then exposed to simulated Martian UV. Spore reductions of 94.6% and 96.6% were observed after UV exposure for 3 and 6 h, respectively. Even though there was a reduction in the number of viable spores, there are still enough spores remaining to potentially contaminate Mars and survive Martian conditions. Moreover, spores have a potential to be encapsulated in polymeric spacecraft materials, and recent studies have shown that these spores can retain their viability [145]. Nonspore-forming species have been recovered in low numbers in the epoxy adhesive used on spacecraft (*Acinetobacter radioresistens*, *Deinococcus radiodurans*, and *Staphylococcus xylosus*) [146]. These results show that vegetative cells of certain microorganisms are hardy enough to survive encapsulation in spacecraft materials and possibly travel to Mars.

Multiple isolates collected from various phases of the Phoenix mission preparation (before, during, and after spacecraft assembly) were isolated based on survival in conditions of 5% hydrogen peroxide, UVC radiation, alkaline pH of 11, acidic pH of 3.0, salinity of 25% (NaCl), high (65°C) and low (4°C)

temperatures, and spore formation (NASA standard spore assay) [71]. The study resulted in isolation of 8 thermophiles, 18 psychrophiles, 23 alkali-tolerant, and 6 UV-tolerant strains. Spore-formers were isolated under multiple conditions except low pH and high salinity. *B. pumilus* was a strain that was isolated under multiple conditions except low temperature. *B. pumilus* is one of the most often isolated strains from the SAF with high tolerance of both its vegetative cells and spores to UV and hydrogen peroxide [147]. In a study by Smith et al. [127], the isolates that were retrieved from surfaces of the MSL ($n = 358$) were tested for growth and survival under aerobic conditions with exposure to various environmental factors, such as low temperature (4 °C), high salt concentrations (0.5%, 5%, 10%, 20% NaCl), pH ranges (7, 8, 9, 10, 11, 12), desiccation, UVC, hydrogen peroxide, and growth under anaerobic conditions with various sources of energy (perchlorate, sulfate, and arsenate). Various microorganisms were found to be resistant when exposed to single environmental stresses, but there were also microorganisms that survived as many as four conditions independently. These species included *Bacillus safensis*, *Bacillus aerius*, *Bacillus* sp. T14, and *Staphylococcus pastuerii* (20% NaCl, 4 °C, 5% H₂O₂ or at pH 9.0), *Staphylococcus epidermidis* (20% NaCl, 4 °C, 5% H₂O₂, pH 8), and *Paenibacillus lautus* (20% NaCl, 4 °C, 5% H₂O₂, pH 10.0) [127].

In a study by Schuerger and Nicholson, a low-pressure, low-temperature, and high-CO₂ atmosphere was tested on seven isolates collected from prelaunch spacecraft [148]. Both spores and vegetative cells of *Bacillus* species, which are common contaminants of spacecraft assembly facilities, were exposed to a combination of various levels of pressure, atmospheres, and temperatures. The results showed that spores were not able to germinate when exposed to 25 mbar at 30 °C under CO₂ conditions, while vegetative cells showed growth under the same conditions, but not at pressures lower than 25 mbar. In another study, six permafrost isolates identified as *Carnobacterium* sp. and nine type strains from the same genus were found to grow at 0 °C under low pressure (7 mbar) and a CO₂ atmosphere [79]. One of the isolates, WN1359, showed significantly better growth under these conditions than under Earth's atmosphere or pressure.

Collectively, these studies show that isolates collected from spacecraft assembly facilities and spacecraft could potentially survive transfer to Mars and survive under Martian environmental conditions.

Survival of Bacteria in Brines

Al Soudi and colleagues [149] tested 18 isolates from Hot Lake, WA and the Great Salt Plains of Oklahoma for growth in a high concentration of Na, K, Mg chlorate, and perchlorate salts. Moderate to strong growth was observed in media containing up to 1 M of perchlorate salt, while for chlorate salts, growth was observed in solutions containing up to 2.75 M. Among the tested isolates, the most common species included *Halomonas venusta*, *Planococcus salinarum*, and *Bacillus licheniformis*. Isolates that were collected from the surface of the MSL were tested for growth under a 10 mM concentration of Na perchlorate salt with acetate or lactate as an electron donor (20 mM) [127]. These isolates included *Bacillus* sp. (mostly *B. subtilis*) and *Gracilibacillus dipsosauri*. The bacterial isolates from Big Soda Lake, NV representing *Bacillus*, *Alkalibacillus*, and *Halomonas* spp. were found to be halophilic and had a high tolerance to perchlorate salts (5% NaClO₄) [150]. Oren et al. [151] exclusively focused on the archaeal species from *Halobacteriaceae* and the bacterium *Halomonas elongate*. The authors found that although cells could grow in the presence of 0.4 M perchlorate, microscopic observation showed that cells were swollen or distorted. Prolonged incubation (2 weeks) showed that *Halobacterium salinarum* can tolerate up to a 0.2 M concentration of perchlorate in medium. The tolerance to perchlorate salts and observation of growth indicated the potential of microorganisms to withstand the presence of perchlorate salts in the Mars regolith. However, in a study by Wadsworth and Cockell [152], the exposure of *Bacillus subtilis* vegetative cells to perchlorates, iron oxides, and hydrogen peroxide under Martian UV (254 nm) resulted in a 10.8 increase of cellular death when compared to the control. Tolerance to salts is an important adaptation for Mars habitability, as the majority of liquid water on present-day Mars probably exists as eutectic solutions as described earlier in this review. Wilks et al. [153] found that *Halomonas* sp. strain BLE7 (Basque Lake, BC isolate) grew in an eutectic epsomite solution of 43% MgSO₄ at −4 °C.

The same isolate and three other strains of *Nesterenkonia* sp. HL76, *Bacillus* sp. JPL10, and *Staphylococcus* sp. JPL27 grew in 3% KCl at -3°C . The latter two strains were isolates from spacecraft assembly facilities and represent the most common contaminants residing on spacecraft surfaces [74,154,155], and as such, these organisms could potentially be transported to Mars on spacecraft surfaces.

Contradictory results on the limits of habitability of microbial communities in simulated Martian brines were obtained by Fox-Powell et al. [156]. Briefly, microbial communities were collected from various natural habitats: >5 cm top soil (Edinburg, UK), 1.1 km subsurface samples evaporite deposit (Whitby, UK); mud from an acidic hydrothermal pool at Kverkfjöll Volcano, Iceland; and brines and sediments from Basque Lakes, British Columbia. The resulting microbial suspensions in brines were incubated at 30°C for 30 days, then transferred to fresh brines. This was repeated three times. Assessment of cell viability was performed by measuring turbidity at 600 nm or by counting cells after staining with SYBR gold or DAPI dyes. Out of eight simulated Martian brines, only three supported microbial community growth for all types of communities studied, while the remaining five did not lead to microbial growth even though water activity was not a limiting factor. This led to the conclusion that the ionic strength of brines that contain multivalent ions prevents habitation by microorganisms even though water in a biologically available form is present [156]. Importantly, this study was unique because it tested microbial communities composed of multiple microorganisms, which represents a more natural state of occurrence of terrestrial microorganisms over testing survival of single microorganisms.

Conclusions on Growth of Bacteria Under Martian Conditions

The described studies show that the organisms with the highest potential for survival of Martian conditions are likely to be spore-forming bacteria which show resistance to multiple extreme physicochemical factors. It is important to determine if vegetative cells of spore-formers and nonspore-formers could withstand long-term simulated Martian conditions. The microorganisms isolated from various Earth environments show this potential, but more research is needed on studying the limits of life for bacteria in the context of Mars habitability.

4.1.3. Fungi

The tolerance developed by fungi to extreme conditions is of particular interest as they are eukaryotic organisms [157]. Fungal representatives have been found in hot and cold deserts [158], alpine elevations [159], the deep sea [160], and at the Chernobyl nuclear power plant and its 30-km Exclusion Zone [161]. The presence of melanins (dark pigments) protects against UV radiation and other stressors [162]. Additionally, fungi have been growing towards sources of ionizing radiation near the Chernobyl nuclear power plant [161]. This shows the immense potential of these microorganisms to potentially inhabit the surface of Mars.

More studies on fungi exposed to simulated Martian conditions are emerging. The most studied fungi, *Cryomyces antarcticus* CCFEE 515 and *Cryomyces minteri* CCFEE5187, are black psychrophilic fungi which are resistant to UV and desiccation. The fungi were isolated from the McMurdo Dry Valleys of Antarctica [163]. These organisms were exposed to simulated Martian conditions aboard the International Space Station for 18 months in the EXPOSE-E facility [164]. The samples were kept under a Martian atmosphere at 1000 Pa, a temperature range from -21.7 to 42.9°C , and exposed to various ranges of solar UV radiation (200–400 nm) at 0, 0.63 or 475 MJ/m², and galactic cosmic rays of 220–238 mGy. After exposure, colonies did not show changes in shape and color. However, a viability test showed that colony recovery was very low; $1.48\% \pm 0.26\%$ CFU and $0.08 \pm 0.06\%$ CFU for unexposed samples (0% UV), and $0.87 \pm 0.18\%$ CFU and $0.30 \pm 0.02\%$ CFU for exposed samples (100% UV) for *C. antarcticus* and *C. minteri*, respectively. The samples exposed to 0.1% UV showed higher variability of $8.40 \pm 1.65\%$ CFU and $2.07 \pm 0.33\%$ CFU, although the authors could not present an explanation. When the samples were treated with propidium monoazide (PMA) to test for cell integrity followed by qPCR, the number of *C. antarcticus* cells was equally undamaged under all

conditions (~66%), while *C. minterii* showed the highest number of undamaged cells at 0.1% UV ($51.12 \pm 3.34\%$) followed by the 100% UV-exposed samples ($45.66 \pm 1.07\%$). In this test, the influence of UV was also tested on a cryptoendolithic microbial community, which showed only a 10.72% survival at 100% UV radiation.

In another study, *C. antarcticus* CCFEE 515 was grown in lunar and Martian regolith analog pellets and exposed to simulated Martian conditions [165]. The fungal CFU recovery was the highest for samples exposed to a Martian atmosphere and radiation in early Mars analog phyllosilicatic Mars regolith simulant (P-MRS) (78%), while growth in the present-day sulfatic Mars regolith simulant was 40%.

Blachowicz et al. [166] tested conidia of various fungi that were isolated from Chernobyl nuclear reaction sites and from the environment of the International Space Station. A suspension of 100 μ L of 10^5 conidia per mL was applied on aluminum coupons followed by desiccation. The coupons of four fungal strains were exposed to simulated Martian conditions for 5 min and 30 min. All four strains, *Apiospora montagnei* IMV 01851, *Cladosporium herbarum* IMV00034, *Cladosporium cladosporioides* IMV 00236, and *Aspergillus fumigatus* ISSFT-021, survived a 5-minute exposure, but only the last two survived a 30-minute exposure. The two long-exposure survival strains were further examined for secondary metabolite production in control and Martian simulated conditions. There were no differences in secondary metabolite profiles generated, but only changes in the secreted quantity of these compounds. Proteomic profiling showed increased expression of translation proteins, ribosome biogenesis proteins, and carbohydrate metabolism proteins that might be responsible for resistance to these conditions [166].

Based on the study results, the fungi studies survived exposure to simulated Martian conditions in various capacities. Their resistance to radiation might be an important advantage over other microbial forms with regard to survival under Martian conditions.

4.2. Lichens

Lichens are symbiotic microorganisms composed of a fungal component and algae or cyanobacteria (10% of total), and their extreme tolerance to desiccation [167] and low temperatures [168] make them another candidate organism for growth and survival in space and on Mars [169]. Various species of lichens were tested for resistance under simulated Martian conditions. The lichen *Xanthoria elegans* was exposed to Mars-like conditions for 22 days [170]. The results showed a lack of significant reduction in vitality as determined by confocal scanning electron microscopy. Upon exposure to 95% of the Earth's pressure, photosynthetic activity was reduced, but when the pressure was lowered further, photosynthetic activity was comparable to that at a normal level. Sanchez et al. [171] investigated the resistance of the lichen *Circinaria gyrosa* to simulated Martian conditions. The study showed that after 120 hours of exposure to Martian conditions (atmosphere, temperature, pressure, and UV irradiation), the lichen was performing photosynthesis at the same level, thus showing a very high resistance to extreme conditions.

Although Mars would present a harsh environment for sustaining life on its surface due in part to the high amount of radiation, a study by Cockell and Raven [172] showed that an environment protective against high levels of radiation could be present on the Martian surface, which may allow for the survival and proliferation of photosynthetic organisms. These protective environments would need to contain iron, encrustations of halite, polar snows and crystalline rocks. These environments may allow for photosynthetic growth by lichens and other photosynthetic organisms on the surface, while allowing for a protective niche from the harsh environment.

4.3. Bryophytes

Bryophytes are considered pioneers among plants due to their habitation of very dry environments [173]. Research on the limits and survivability of bryophytes under simulated Martian and space conditions were assessed recently for the first time by Huwe et al. [174] as a part of

the complex experiment BIOMEX (Biology and Mars Experiment), outside the International Space Station [175]. Bryophytes of the genus *Grimmia* were collected from an alpine environment from the Swiss Alps and exposed to simulated Martian conditions (Mars atmosphere gas mixture, 370 ppm H₂O, 1 kPa for 31 days with 17-day or without (control) exposure to UV_{200–400 nm} radiation for a total dose of 5×10^5 kJ/m²). The exposure to a simulated Martian atmosphere did not affect viability of the bryophyte samples, but the UV radiation decreased photosynthetic activity by 36% as measured as a yield from the photosystem II. Exposure to temperature ranges of −25 to 60 °C had no effect on the samples. This exposure was short-term, and these effects might be different when bryophytes are exposed to the same temperatures for longer periods of time [174]. These results showed that bryophytes have a high potential for survival in Martian conditions, although more research is needed. Even though UV exposure did not inhibit photosynthesis completely, it would be necessary to determine if bryophytes can conduct photosynthetic activity for extended periods under these conditions.

5. Considerations for Future Studies

The view on past or present habitability of Mars will change as information on Mars and the limits of life expand. Our current knowledge is strictly based on technology development and the constant and steady improvement of instruments used for life detection. The increased limit of detection (sensitivity) is one example of the advancements in engineering and instrumentation. Even though the Viking lander had a pyrolysis gas chromatograph coupled with a mass spectrophotometer, the instrument did not accurately detect levels of organic compounds because of its limits [176]. The Sample Analysis at Mars instrument suite on the MSL Rover, which constituted a gas chromatograph and mass and laser spectrometers, led to the discovery of organic compounds such as methane [99], organic sulfur molecules [46], chlorobenzene, and C₂ and C₄ chloroalkanes [45]. The new NASA Mars 2020 rover is based on the design of the MSL with certain improvements and carries seven instruments, including an advanced camera system (Mastcam-Z), Mars Environmental Dynamics Analyzer (MEDA), Mars Oxygen ISRU Experiment, Planetary Instrument for X-Ray Litochemistry (PIXL), Radar Imager for Mars' Subsurface Exploration (RIMFAX), UV Raman spectrometer SHERLOCK, and a SuperCam [177]. With these instruments on board, the goals of the Mars 2020 mission are to search for signs of past microbial habitats and any associated biosignatures, collect samples and prepare them for their return mission to Earth, and assess oxygen production in Martian conditions in preparation for a future human mission. Concurrently with Mars 2020, ExoMars rover's mission has science objectives aimed at seeking signatures for life [178]. These currently planned missions will also continue to provide us with a more profound understanding of Martian conditions and the possibility for past or present life on Mars; however, challenges for the exploration of Mars habitability remain. To look for signatures of present or past life, more samples must be collected, especially from the deeper subsurface, while simultaneously maintaining protection from forward contamination with Earth microorganisms or biosignatures [179]. Moreover, either known organic compounds or those yet to be discovered must be assessed for their origin, including whether they come from biological or abiotic sources. Therefore, future missions' goals should accommodate these overarching goals by adding more instrumentation and including advanced methods for procuring deep surface samples to obtain answers to these questions.

6. Conclusions

Even though the Martian surface and subsurface are characterized by some of the most extreme and life-limiting physical and chemical characteristics, there are study-based indications that Mars may indeed be habitable for microbial life forms. Although both the surface and subsurface can provide the basic needs to meet habitability requirements, the subsurface is more likely to provide a protective niche from radiation and low temperatures. The novel discoveries on Mars-related habitability reassures researchers to continue the path of seeking signatures of past or present life. Whether any of these will be discovered, and in what forms, is yet to be determined.

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