

# Chapter 10

## Experimental Evolution to Explore Adaptation of Terrestrial Bacteria to the Martian Environment



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### 1 Introduction

The question of the possible existence of life beyond the confines of Earth has been a broad topic of philosophical debate for over two millennia (O’Leary 2008; Nicholson 2009). But only relatively recently, beginning in the late nineteenth century, has this question been addressed scientifically. Beginning in the 1970s, scientists began to fully appreciate the broad range of extreme physical conditions under which Earth life can grow, and it rapidly became apparent that single-celled microorganisms of the domains Bacteria and Archaea were capable of growth at the widest extremes of temperature, pressure, pH, and salinity (Horikoshi et al. 2011).

But is Earth unique among planets, or could microbial life actually exist elsewhere in our solar system or beyond? Until recently, the nearly complete lack of data about the environments of planets beyond Earth posed an insurmountable obstacle to answering this question. Major advances in understanding of the physical conditions prevailing on other planets within our solar system have come only since the last half of the twentieth century, with (1) orbiters, landers, and rovers providing detailed analyses of the surface environment of Mars and (2) orbiters around Jupiter and Saturn revealing the existence of subsurface oceans beneath some of their icy moons. In parallel, the discovery of the first planets orbiting other stars was reported, prompting scientists to search for clues of their possibly harboring life. These recent advances in planetary exploration have resulted in the emergence of the field of “habitability” to astrobiology, i.e., studies of the ability of planetary environments to host life [reviewed recently in Cockell et al. (2016), Heller and Armstrong (2014)].

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What makes a planet habitable? Until we discover life elsewhere, “life as we know it” is Earth life. Thus the question immediately becomes constrained to “what makes a planet habitable to Earth life?”. At present it is generally agreed upon among scientists that the requirements for Earth life are:

1. Liquid water (hence, temperature/pressure/solute conditions at which water can exist as a liquid)
2. A number of elements, some needed in bulk (mainly C, H, O, N, P, S), with lesser amounts of Na, Mg, Ca, Cl, K, and Fe and trace amounts of several other elements such as Cr, Co, Cu, F, I, Mn, Mo, Se, and Zn
3. A source of energy (supplied either by sunlight or redox chemistry)

And of course, life needs to be protected from, or be able to mitigate, the lethal effects of bombardment by ionizing or UV radiation or exposure to toxic chemicals.

A substantial amount of environmental data regarding Mars habitability has been gained from robotic exploration missions. For example, orbiters have observed large features indicative of past or present water on the surface of Mars, such as channels, ancient lake beds, and sedimentary features. Mars landers and rovers have also observed sedimentary features at close range and have measured such environmental parameters as air pressure and composition, wind speed, relative humidity, UV and ionizing radiation flux, and the chemical compositions of myriad rocks and soils. Taken together, the data suggest that present-day Mars may be habitable and that conditions in the distant past were even more so. No definitive evidence exists of past or present life on Mars, but future robotic missions are being planned to directly test for signs of life (so-called “biosignatures”).

The potential habitability of icy moons such as Europa and Enceladus derives from orbital observations of the moons’ wobble and the patterns of surface ice cracks and chaotic terrain, coupled with deep-penetrating radar, magnetic, and surface altimetry data. Taken together, these observations indicate the presence of global oceans of liquid water beneath the surface of both moons. In addition, the Cassini spacecraft directly observed jets of material emerging from the south pole of Enceladus and actually flew through and sampled this material with its onboard mass spectrometer—finding predominantly water, CO<sub>2</sub>, N<sub>2</sub>, methane, and traces of acetylene and propane (Waite et al. 2006). It is thus tantalizing to suggest that both Mars and icy moons are places with environments capable of supporting life as we know it.

In this chapter I will attempt to summarize recent attempts at understanding of how terrestrial life forms might be capable of growth in extraterrestrial environments, either due to their natural endowments or due to directed evolution experiments. Most of this discussion will focus on the environment of Mars, because of its proximity and similarity to Earth, its relative accessibility to exploration, and the relative wealth of environmental data we possess. This review updates and expands upon previous reviews, to which the reader is directed for further information (Nicholson et al. 2000, 2005, 2009; Nicholson 2009; Fajardo-Cavazos et al. 2007).

## 2 Evolutionary Trajectories of Earth and Mars

In addressing the question of whether or not a particular form of life can inhabit a particular extraterrestrial environment, first we need to know the physicochemical characteristics of the environment in question. Although current evidence suggests that the environments of ancient Earth and Mars were once rather similar [reviewed in Nicholson (2009)], present-day Mars has a very different environment from that of present-day Earth, summarized in Table 10.1.

The different evolutionary trajectories of Earth and Mars stem from the time of their formation by accretion in the early solar system. Both are terrestrial (rocky) planets, but Mars is only 1/10 as massive as Earth and its gravity is only 38% that of Earth's. Mars is ~1.5 times further from the Sun than Earth, receives only about 40% as much solar radiation, and takes 687 Earth days to orbit the Sun. Both Earth and Mars have roughly 24-hour days, and their axes of rotation are both inclined to the Sun, at 23.5° (Earth) and 25° (Mars); hence, both planets have four seasons. Both planets have moons, but Earth's moon is much more massive than Mars' tiny moons Phobos and Deimos (which are likely small asteroids captured by Mars' gravity). Earth's massive moon stabilizes its inclination to an approximately 40,000-year period of "wobble" between 22.1° and 24.5°, which has a stabilizing effect on our long-term climate. In the absence of a large stabilizing moon, Mars' wobble is much more pronounced, varying between ~15° and ~35° with a period of ~100,000 years. This extreme wobble results in the planet cycling between relatively warm, wet periods and relative cold, dry periods.

Due to the energy released during accretion, the terrestrial planets started their lives as near-molten balls, far too hot for liquid water to form. As bombardment slowed and the planets cooled, they developed dense atmospheres and crusts floating on liquid magma, and the crusts cooled to the point that oceans of liquid water were stable on the surface. Because Mars formed further from the Sun and was less massive than Earth, it cooled at a faster rate, likely becoming "habitable" before Earth did. Both planets originally had an internal dynamo generating a protective magnetic field. However, the faster rate of Mars core cooling led to faster core solidification and subsequent collapse of its protective magnetic field. Losing its magnetic protection from the charged particles of the solar wind, the lighter components of the Mars atmosphere were blown into space, leaving behind a thin, predominantly CO<sub>2</sub> atmosphere.

### 2.1 Atmospheric Composition and Pressure (P)

Earth contains a relative thick (~101.3 kPa), humid atmosphere. Nitrogen comprises the majority gas (~78%), with both oxygen (~21%) and carbon dioxide (~0.04%) to support abundant and diverse photosynthetic and aerobic life (Table 10.1). In stark contrast, the average atmospheric P on Mars (0.7 kPa) is only 1/150th that of Earth

**Table 10.1** Comparison of the physical environments of Earth and Mars<sup>a</sup>

Parameter	Earth	Mars
Temperature:		
Air, global average	+15 °C	−55 °C
Air, measured extremes	−89 to +56 °C	−75 to −2 °C (MSL)
Ground, range at surface	−93 to +94 °C	−91 to +3 °C (MSL)
Ground (~10 m depth)	+4 to +27 °C	Approx. −93 °C
Ocean, range	−2 to +36 °C	n.a.
Air pressure at surface:		
Average	101.3 kPa (sea level)	~0.7 kPa
Range	~29 (Mt. Everest) to ~106 kPa (Dead Sea)	~0.1 to ~10 kPa
Atmospheric composition:	N <sub>2</sub> : 78.1% O <sub>2</sub> : 20.9% Ar: 1% CO <sub>2</sub> : 0.04% H <sub>2</sub> O vapor: (v), up to 100%	CO <sub>2</sub> : 96.0% Ar: 1.93% N <sub>2</sub> : 1.89% O <sub>2</sub> : 0.145% CO: 0.07% H <sub>2</sub> O vapor: ~0.03% (v)
Magnetic field:	Global; ~65,000 nT	Patchy; ~1500 nT
Solar UV:		
Solar constant	1368 W/m <sup>2</sup>	590 W/m <sup>2</sup>
Spectrum at surface	≥290 nm (UV–B + A)	≥190 nm (UV–C + B + A)
Ionizing radiation at surface:		
SEP <sup>b</sup> , episodic	Negligible	Spikes, ~0.3 mGy/day
GCR, continuous	Negligible	0.18–0.23 mGy/day
Organic compounds	Abundant and diverse	Chlorobenzene and chlorinated C <sub>2</sub> –C <sub>4</sub> alkanes (ppb range)

<sup>a</sup>Modified from Schuerger (2004), Nicholson et al. (2005), Rummel et al. (2014), Millan et al. (2016)

<sup>b</sup>Abbreviations: GCR galactic cosmic radiation, nT nanoTesla, ppb parts per billion, SEP solar energetic particles, (v) varies

(Table 10.1). Furthermore, the composition of the martian atmosphere differs greatly from that of Earth; CO<sub>2</sub> makes up the majority (96%) of the atmosphere, followed by N<sub>2</sub> and Ar, each at ~2% of the total. Note that O<sub>2</sub> (0.145%) and water vapor (~0.03%) are only minor constituents of the atmosphere. This has several profound consequences for Mars habitability.

## 2.2 *Temperature (T)*

The surface  $T$  of a planet is determined in large part by its mass, proximity to its parent star, and the greenhouse effect supplied by its atmosphere. Present-day martian surface  $T$ 's remain cold year-round, because (1) Mars orbits farther from the Sun and thus receives only about 40% as much solar radiation as does Earth and, (2) despite its high concentration of the greenhouse gas  $\text{CO}_2$ , the thin martian atmosphere provides a relatively weak atmospheric greenhouse effect.

Earth's global average  $T$  is  $\sim 15^\circ\text{C}$  but can vary dramatically depending on location, season, time of day, and type of material being measured (air, ground, water, ice). Recorded air  $T$ 's on Earth vary over a span of nearly  $145^\circ\text{C}$ , from a record low of  $-89^\circ\text{C}$  (Vostok Station, Antarctica) to a record high of  $+56^\circ\text{C}$  (Death Valley, USA). Mars is clearly much colder than the Earth; by comparison, the global average air  $T$  on Mars is estimated to be approx.  $-55^\circ\text{C}$ , and diurnal  $T$ 's are estimated to vary from lows of approx.  $-153^\circ\text{C}$  at the poles to daytime highs of around  $-10^\circ\text{C}$ . In addition, brief excursions of up to approx.  $+20^\circ\text{C}$  at the summertime equator are estimated (Schuerger 2004; Rummel et al. 2014).

At the surface, ground  $T$ 's can vary even more widely than air  $T$ 's but rapidly stabilize with increasing depth; even so, the  $T$  in the martian near-subsurface is substantially lower than that of Earth (Table 10.1).

## 2.3 *UV and Ionizing Radiation*

The composition and density of the Mars atmosphere have important implications for the radiation environment at the surface. First, the low abundance of oxygen in the atmosphere precludes the development of a UV-absorbing stratospheric ozone layer as found on Earth. The bulk gas in the martian atmosphere is  $\text{CO}_2$ , which is transparent to UV down to  $\sim 190\text{ nm}$  (Table 10.1); thus, the surface is bathed in high levels of lethal UV-C radiation, with roughly 2–3 orders of magnitude higher biologically relevant doses than the Earth (Cockell et al. 2000). Ionizing radiation species ( $X$ -rays,  $\gamma$ -rays, protons, electrons, and high-energy nuclei of atomic number  $\geq 2$ ) originating from the Sun and from outside the solar system (galactic cosmic radiation) are either absorbed by Earth's atmosphere or deflected by Earth's strong global magnetic field (Table 10.1). By contrast, Mars' attenuated atmosphere and weak remnant magnetic field result in the ionizing radiation flux being significantly higher on the martian surface (Table 10.1).

## 2.4 *Liquid Water*

The boiling and freezing points of water are a function of T and P. Most of the Earth's surface is at a T–P regime conducive to the presence of abundant liquid water available to biological processes. However, on the surface of Mars, there exists only a small T–P window within which liquid water can exist stably; thus the vast majority of water on Mars is frozen either underground or in the polar caps, and the remainder is present mainly as vapor or ice crystals in the atmosphere.

Interestingly, there is abundant evidence from orbiters and rovers that liquid water flowed freely on the martian surface in the past, indicating that the ancient Mars environment may have been warmer with a higher-P atmosphere capable of supporting liquid water. How could water remain in the liquid state at the T–P conditions of present-day Mars?

## 2.5 *Salinity*

Mars orbiters, rovers, and landers have sampled various sites associated with ancient sedimentary features and have detected high concentrations of various salts, including chloride, sulfate, and perchlorate salts of Ca, Na, or Mg. In particular, perchlorate salts have been detected at high levels in soil and ground ice samples from various locations on the martian surface (Hecht et al. 2009; Glavin et al. 2013). Dissolved salts can dramatically lower the freezing point of water and slow its evaporation. High salinity is a double-edged sword however; high concentrations of solutes, such as salts or organic compounds (e.g., sugars), lower the water activity ( $a_w$ ), effectively sequestering water away from cells and inhibiting microbial growth. At present, no microbes have been found which are able to grow at  $a_w$  less than 0.61 [reviewed in Rummel et al. (2014)].

## 2.6 *pH*

Knowledge about the pH of martian rocks and regoliths (soils) are by and large poorly constrained. Because direct measurement of pH relies on suspension of sample material in water, most pH estimates have been derived indirectly from dry chemical composition data, yielding qualitative measures such as “acidic” or “alkaline.” In fact, the only direct measurement of martian soil pH was conducted at the Phoenix landing site, where a slightly alkaline pH of  $7.7 \pm 0.5$  was measured (Hecht et al. 2009).

## 2.7 *Organic Compounds*

Early Mars probes failed to find concrete evidence for organic compounds down to the ppb range, and until relatively recently, it was thought that organic compounds were essentially absent on Mars. This finding was paradoxical, as it was widely presumed that organics were constantly being delivered to the surface of Mars in meteoritic dust from space, as it is on Earth. Organic detection experiments rely on heating soil samples in an oven, then separating and identifying the released volatile compounds using gas chromatography/mass spectrometry (GC/MS). Evidence has been mounting that during the heating step, strongly oxidizing compounds (particularly perchlorates) present in the martian soil samples were reacting with organics in the soil, breaking them down to organochlorine compounds and CO<sub>2</sub> (Sephton et al. 2014). Consistent with this notion, the Sample Analysis at Mars (SAM) instrument on the Mars Science Lab recently detected chlorobenzene and C<sub>2</sub>–C<sub>4</sub> dichloroalkanes at 70–300 ppb in martian soil samples (Glavin et al. 2013; Freissinet et al. 2015). More recent sampling by MSL of material collected from the interiors of rocks resulted in detection of both aromatic and aliphatic organic compounds (Eigenbrode et al. 2018). Therefore, Mars soils and rocks do appear to contain organic compounds, but oxidants, mainly perchlorate, present in martian soils make their detection, identification, and quantification difficult.

## 2.8 *Transport of Life from Earth to Mars*

At first glance, it would seem that Mars and Earth are isolated by at least 50 million kilometers of raw space. How could microbes possibly traverse this void? Two mechanisms have been postulated by which Earth microbes could be transported to Mars: natural impacts and human spaceflight activities.

### 2.8.1 *Natural Impacts*

Currently scientists have found over 80 meteorites on Earth that originated from Mars (for details, the reader is referred to the Martian Meteorite Compendium maintained by NASA at <https://curator.jsc.nasa.gov/antmet/mmc/>). These meteorites were ejected into space by large impacts striking the martian surface, and it is reasoned that large impacts on Earth's surface could also transport Earth rocks bearing viable microbes to Mars. Thus it is postulated that Earth and Mars have been continuously exchanging life since the early days of the solar system [reviewed extensively in Mileikowsky et al. (2002), Nicholson (2009), Nicholson et al. (2000)].

### 2.8.2 Human Spaceflight Activities

Beginning in the early 1960s, humans have launched over 40 flyby, orbiter, lander, and rover missions toward Mars (for details, see <http://mars.nasa.gov/programmissions/missions/log/>). Several of these missions were unsuccessful and crashed onto the martian surface. Even a successful lander or rover mission leaves debris strewn across the martian surface, including parachutes, heat shields, back shells, etc. which are discarded during entry and landing operations. Because microorganisms are ubiquitous on Earth, it is virtually certain that they have contaminated flight hardware and been deposited on the surface or near-subsurface of Mars. Spacefaring nations have addressed this concern by international “Planetary Protection” agreements dictating the strict biological cleanliness of spacecraft destined for Mars [for recent extensive reviews, see Rummel et al. (2014), Rettberg et al. (2015)], but it should be stressed that current Planetary Protection protocols do not mandate *sterilization* of spacecraft, but the *reduction* of microbial bioburdens to below specified levels. A major unanswered question remains: is transfer of Earth microbes to Mars ecologically relevant? Just because an Earth microbe is deposited into the Mars environment does not necessarily mean that it can survive, let alone propagate, in that environment. This question has been addressed by the use of simulations of the martian environment as described in Sect. 3.

### 2.8.3 Candidate Microorganisms

A wide diversity of prokaryotes exist in various Earth niches, but which ones would be likely candidates for Earth-to-Mars transport? When one considers the physics of natural impacts, only microbes inside (1) well-consolidated rocks located (2) at or near the surface of (3) a ring-shaped *spallation zone* surrounding the impact site are most likely to be accelerated to escape velocity without shock and heating sufficient to sterilize the rock [discussed in Melosh (1984, 1989), Mileikowsky et al. (2000), Nicholson et al. (2000), Nicholson (2009)].

Considering Earth-to-Mars transport by human spaceflight activities, numerous bacteria have been identified by sampling Mars-bound spacecraft and their ultraclean Spacecraft Assembly Facilities (SAFs) (Venkateswaran et al. 2001; La Duc et al. 2003, 2004, 2007). Spore-formers, mainly *Bacillus* spp., comprise a major fraction of these isolates, and resistance of their spores to extreme disinfecting treatments (UV, ionizing radiation, hydrogen peroxide) has been documented to greatly exceed the resistance properties of common laboratory strains (Kempf et al. 2005; Link et al. 2004; Newcombe et al. 2005). SAFs are maintained in an ultraclean state by HEPA filtration, rigorous cleaning and disinfection, and strict protocols for contamination control. It has been suggested that these very conditions render SAFs excellent selective environments for the very hardiest microorganisms—paradoxically, the very contaminants most likely to survive an Earth-to-Mars transit (Link et al. 2004; Crawford 2005).



### 3 Growth of Terrestrial Microbes in Simulated Mars Environments

Considerable effort has been expended since the 1970s in testing the ability of Earth microbes to survive and grow in the martian environment, driven mostly by the need to mitigate the so-called forward contamination of Mars by Earth microorganisms [reviewed in Rummel et al. (2014), Rummel (2001), Nicholson et al. (2009)]. These experiments have been conducted in various chambers designed to simulate the environmental conditions on Mars. As our understanding of Mars has become more detailed and refined, such simulations have become increasingly more sophisticated and representative of the martian environment. With the exceptions of gravity and ionizing radiation, a myriad of modern Mars simulation chambers have been designed that can more or less faithfully replicate nearly all physical aspects of the martian environment such as T, atmospheric P and composition, and the solar fluence and spectrum at Mars through the UV-visible-near-IR range under atmospheric opacity conditions ranging from clear sky to global dust storms. In addition, our increasing knowledge about the composition and properties of martian soils has enabled the preparation of increasingly accurate Mars soil simulants (Schuerger et al. 2012). Below is a brief summary of our current knowledge regarding the ability of Earth microbes to survive and grow under simulated Mars conditions.

#### 3.1 *UV and Ionizing Radiation*

On the surface of Mars, solar UV is by far the strongest environmental factor limiting bacterial survival [reviewed in Schuerger (2004); Nicholson et al. (2000, 2005)]. However, the results from experiments conducted in low Earth orbit or in Mars simulation chambers have indicated that viable microbes can be shielded effectively from UV by relatively thin layers of UV-opaque materials such as dust, regolith, irregularities in spacecraft surfaces, or even the upper layers of cells deposited in multiple layers (Horneck 1993; Schuerger et al. 2005, 2006). Thus, to avoid the rapidly lethal effects of solar UV, microbes would likely have to reside in subsurface environments (Rummel et al. 2014). Direct measurements of the ionizing radiation flux on Mars have recently been obtained using the Mars Science Lab's Radiation Assessment Detector (MSL RAD) instrument (Hassler et al. 2014). The combined GCR and SEP doses measured at the martian surface by MSL RAD were extrapolated to an annual dose of  $<0.3$  Gy, much lower than the dose required to inactivate microbes by even one order of magnitude (ranging from  $\sim 200$  to  $\sim 12,000$  Gy). These observations led to the conclusion that with minimal shielding, UV and ionizing radiation would exert negligible lethal effects on the viability of microbes on Mars (Rummel et al. 2014).

## 3.2 *The Mars Atmosphere*

Many of the key physical constraints to the growth of terrestrial life on Mars are manifested in its atmosphere.

### 3.2.1 Atmospheric Composition (AC)

The martian atmosphere is CO<sub>2</sub>-rich and oxygen-poor (Table 10.1). These factors per se are not inhibitory to microbes, as numerous prokaryotes are capable of growth under oxygen-limited or completely anaerobic conditions (Horikoshi et al. 2011). In particular, the low concentration of oxygen (~0.145%) is further exacerbated by low P. It has been calculated that the pO<sub>2</sub> in the “average” martian atmosphere (~700 Pa at -10 °C) is ~1 Pa, which corresponds to a dissolved O<sub>2</sub> concentration of ~3 nM; in comparison, the O<sub>2</sub> concentration on sea-level Earth (~101.3 kPa, +25 °C) is ~250 μM. At an O<sub>2</sub> concentration of 3 nM, *E. coli* cells were demonstrated to be capable of growth using only aerobic respiration (Stolper et al. 2010), suggesting that even aerobic microorganisms could utilize the scant O<sub>2</sub> present on Mars (Rummel et al. 2014).

### 3.2.2 Atmospheric Temperature and Pressure

As discussed above in Sect. 2, a key factor for Mars habitability is the presence of liquid water. However, water is largely in the form of either ice or vapor at martian surface conditions, where T and P hover around the triple point. What are the lowest T's and P's at which life can function? A recent exhaustive review of the literature (Rummel et al. 2014) revealed that the lowest temperature at which cell division has been reported to occur in the laboratory is -18 °C, by the yeast *Rhodotorula glutinis* (Collins and Buick 1989). Regarding P, early experiments used an approach in which growth of laboratory bacteria or bacteria isolated from Spacecraft Assembly Facilities was tested in simulation chambers under conditions where T, P, and AC were systematically altered one at a time; in other words, cells were cultivated at fixed T and AC under various P conditions. Results from these experiments indicated that the group of terrestrial microbes tested were unable to grow at P's lower than ~2.5 kPa (Schuerger and Nicholson 2006; Berry et al. 2010; Kral et al. 2011; Smith et al. 2009).

### 3.2.3 Improved Simulations of Temperature, Pressure, and Atmospheric Composition

Changing one parameter at a time in a simulation chamber raises the possibility of missing combined effects. For example, T and P exert opposite effects on

**Table 10.2** Terrestrial bacteria capable of growth under simulated Mars atmospheric conditions<sup>a</sup>

Phylum	Genus	Species	Reference
Actinobacteria	<i>Rhodococcus</i>	<i>qingshengii</i> (1 isolate)	Schuerger and Nicholson (2016)
Firmicutes	<i>Bacillus</i>	3 unclassified isolates	Schuerger and Nicholson (2016)
Firmicutes	<i>Carnobacterium</i>	<i>alterfunditum</i> <i>divergens</i> <i>funditum</i> <i>gallinarum</i> <i>inhibens</i> subsp. <i>gilichinskyi</i> <i>inhibens</i> subsp. <i>inhibens</i> <i>maltaromaticum</i> <i>mobile</i> <i>pleistocenium</i> <i>viridans</i> 5 isolates tentatively classified as <i>viridans</i>	Nicholson et al. (2013)
Firmicutes	<i>Carnobacterium</i>	5 unclassified isolates	Schuerger and Nicholson (2016)
Firmicutes	<i>Clostridium</i>	2 unclassified isolates	Schuerger and Nicholson (2016)
Firmicutes	<i>Cryobacterium</i>	7 unclassified isolates	Schuerger and Nicholson (2016)
Firmicutes	<i>Exiguobacterium</i>	<i>sibiricum</i> (5 isolates)	Schuerger and Nicholson (2016)
Firmicutes	<i>Paenibacillus</i>	<i>antarcticus</i> (6 isolates) <i>macquariensis</i> (1 isolate) 9 unclassified isolates	Schuerger and Nicholson (2016)
Firmicutes	<i>Trichococcus</i>	<i>collinsii</i> (2 isolates) <i>pasteurii</i> (10 isolates) 1 unclassified isolate	Schuerger and Nicholson (2016)
Proteobacteria	<i>Serratia</i>	<i>ficaria</i> <i>fonticola</i> <i>grimesii</i> <i>liquefaciens</i> <i>plymuthica</i> <i>quinivorans</i>	Schuerger and Nicholson (2016), Schuerger et al. (2013)

<sup>a</sup>0 °C, 0.7 kPa, CO<sub>2</sub>-dominated, O<sub>2</sub>-limited atmosphere

membranes: membranes become more rigid as T decreases but become more fluid as P decreases. These effects may result in a microbe being unable to grow at low T or low P applied singly but capable of growth when both P and T are lowered simultaneously. Recently a more holistic approach was pursued in experiments testing the growth of microbes from so-called “Mars analog” environments, i.e., sites such as alpine sites, high deserts, permafrost soils, arctic and Antarctic sites, etc. In this approach, microbial growth was tested in a chamber simultaneously simulating Mars T, P, and AC conditions (0 °C, 0.7 kPa, CO<sub>2</sub>-dominated, O<sub>2</sub>-limited atmosphere). Using these conditions, we discovered a small subset of the total microbial populations originating from environmental samples capable of growth at 0.7 kPa, belonging to a variety of bacterial taxonomic groupings (Table 10.2)

(Nicholson et al. 2013; Schuergel et al. 2013; Schuergel and Nicholson 2016). Examination of Table 10.2 immediately reveals that *Firmicutes* comprise the preponderance of environmental bacteria capable of growth under simulated Mars atmosphere (66 out of 73 total isolates, consisting of 3 *Bacillus*, 20 *Carnobacterium*, 2 *Clostridium*, 7 *Cryobacterium*, 5 *Exiguobacterium*, 16 *Paenibacillus*, and 13 *Trichococcus* isolates). In contrast, only the genus *Serratia* from the phylum *Proteobacteria* was found to be capable of growth under simulated Mars atmospheric conditions; furthermore, two *Serratia* species (*S. marcescens* and *S. rubidaea*) could not grow under these conditions (Schuergel and Nicholson 2016).

### 3.3 Nutrients, Liquid Water, CO<sub>2</sub>, and Perchlorate Salts

In the experiments described above, some bacteria were able to grow under simulated martian atmospheric conditions; but it must be noted that the samples were cultivated in Petri dishes containing complex rich agar media (LB, R2A, TSA, or TSY) and abundant liquid water. At 0 °C, the water in these plates did not turn to ice, because the presence of solutes and salts in the media depressed its freezing point; nor did the water evaporate rapidly, because the P–T conditions were below the liquid/vapor boundary, again partly due to dissolved organic solutes and salts. Clearly the Mars surface poses a distinctly different environment than that provided by a well-hydrated Petri dish.

#### 3.3.1 Potential Nutrients, Including CO<sub>2</sub>

Mars appears to contain essentially all of the bulk elements, macro- and micronutrients necessary for life (see Sect. 1). Carbon and oxygen are present in the atmosphere as CO<sub>2</sub> and CO, as carbonates in the soil, and as the parent compounds to the organics detected by the MSL SAM instrument (see Sect. 2). The martian atmosphere contains nitrogen gas, and nitrogen was recently detected in Mars soils heated in the SAM instrument, suggestive of nitrate in the soil samples (Stern et al. 2015). Sulfur is widespread on the martian surface in the form of sulfate-dominated mineral deposits [reviewed in Gaillard et al. (2013)]. Hydrogen is obviously present in water, but direct detection of hydrogen gas, postulated to be an essential component of the subsurface biosphere, is lacking. However, H<sub>2</sub> can be produced by geochemical metamorphism in which water interacts with certain igneous (ultramafic) rocks to produce the mineral serpentine and H<sub>2</sub>. The detection of serpentine mineral deposits from orbit argues in favor of this process having occurred on Mars in the past (Ehlmann et al. 2010). Autotrophic microorganisms are capable of growth using purely inorganic compounds. A large class of photosynthetic microbes, the photoautotrophs, are not considered to be strong candidates for inhabitants of Mars, as their need for exposure to sunlight would necessarily expose

them to the harshly biocidal UV environment (Cockell et al. 2000). However, a category of autotrophic microbes, the methanogenic archaea, has recently gained much attention concerning possible life on Mars. These organisms use  $\text{CO}_2$  as a source of carbon, producing methane ( $\text{CH}_4$ ) in the process. Indeed, the detection of trace amounts of methane in the martian atmosphere, both by remote sensing (Mumma et al. 2009) and by the MSL rover (Webster et al. 2015, 2018), has been taken by some as evidence of methanogenic life, although abiotic sources of methane are certainly possible.

### 3.3.2 Liquid Water and Perchlorate Salts

As discussed in Sect. 2 above, evidence to date indicates that perchlorate salts permeate the soils at various sampling locations on Mars. Chloride ( $\text{NaCl}$ ,  $\text{MgCl}_2$ ) and perchlorate [ $\text{NaClO}_4$ ,  $\text{Mg}(\text{ClO}_4)_2$ ,  $\text{Ca}(\text{ClO}_4)_2$ ] salts can lower the freezing point of water to eutectic points of  $-33\text{ }^\circ\text{C}$  to  $-74\text{ }^\circ\text{C}$  (Stillman and Grimm 2011), and water containing these salts can further be supercooled to even lower temperatures (Toner et al. 2014). Furthermore, measurements of water activities in solutions of  $\text{NaClO}_4$  from  $+25\text{ }^\circ\text{C}$  down to  $-98\text{ }^\circ\text{C}$  revealed that  $a_w$  actually increased with decreasing T, maintaining values of  $>0.6$  even at temperatures as low as  $-73\text{ }^\circ\text{C}$  (Toner and Catling 2016). Therefore, it appears that liquid water at the T–P conditions of the martian surface could be stabilized significantly by high concentrations of perchlorate salts. Although perchlorates are toxic to humans via inhibition of iodine uptake by the thyroid, at least 40 species of bacteria can utilize perchlorate as a redox compound, reducing it sequentially through chlorate and chlorite to chloride [reviewed in Nicholson et al. (2012)]; thus perchlorate could be exploited by putative martian microbes as a redox chemical. To test the possible toxicity of perchlorate to bacteria, a Mars soil simulant was produced based on the chemical composition of martian soil at the Phoenix landing site, where water was detected along with perchlorate (at  $\sim 1.5\text{ wt.}\%$ ) (Schuerger et al. 2012). An aqueous extract of this soil simulant was tested for its ability to inhibit spore germination or vegetative growth of *B. subtilis* and *B. pumilus*; both microbes were found to grow and germinate normally in the presence of the extract, indicating that levels of perchlorate found at the Phoenix landing site were not inhibitory to these two commonly used model spacecraft contaminants (Nicholson et al. 2012).

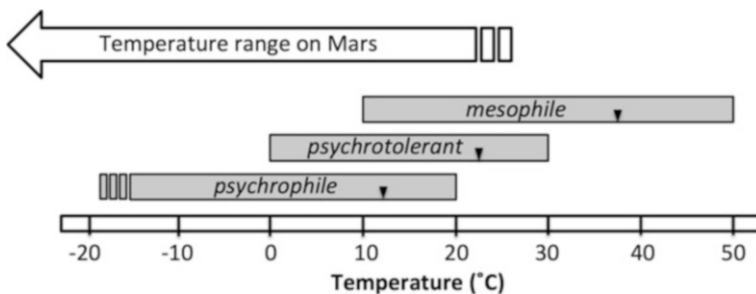
In summary, (1) Mars appears to contain all of the chemical ingredients for life, although perhaps not optimally available; (2) different groups of prokaryotes possess attributes enabling them to deal with different aspects of the martian environment; and (3) some terrestrial bacteria are able to grow under the physical constraints of martian T, P, and AC. However, to date, no single Earth bacterium has been isolated that exhibits all of the attributes necessary for growth under the myriad constraints posed simultaneously by the environments on Mars.

## 4 Experimental Evolution to Mars Environmental Extremes

Our understanding of the question of whether Earth life could grow on Mars has been greatly enhanced through the utilization of chambers simulating various aspects of the martian environment, particularly in identifying various physicochemical factors constraining Mars habitability. It is important to define the limits at which life can function but perhaps even more important to understand *why* some organisms can survive and proliferate under a set of environmental conditions (say, Mars) which would be lethal to other organisms. Increasingly, these studies have aimed toward understanding at the molecular level how microbes sense and respond to factors in the martian environment at the limits of their growth range. And as discussed below, experimental evolution has been used as a tool to use the constraints of the martian environment as selective forces to uncover mechanisms by which cells expand their growth ranges to inhabit new niches.

### 4.1 Low Temperature (*T*)

As mentioned above, Mars presents an extreme cold environment to life. Psychrophiles are cold-adapted microorganisms whose cardinal growth temperatures are minimum  $<0$  °C, optimum  $\sim 15$  °C, and maximum  $\sim 20$  °C (Fig. 10.1). Terrestrial psychrophiles are required to withstand wide fluctuations in temperature (from  $-50$  °C to  $+25$  °C for Antarctic soil organisms), as well as desiccation, low nutrient levels, and high radiation impacts; in contrast, deep marine psychrophiles experience constant low *T*'s ( $\sim 0$ – $3$  °C) and high *P*'s (up to  $\sim 100$  MPa) [reviewed in Casanueva et al. (2010)]. Cellular effects of low *T* include freezing of intracellular water, increased rigidity of membranes, lowering of enzyme catalytic efficiency, and increase in reactive oxygen species (ROS) due to increased solubility of oxygen.



**Fig. 10.1** Approximate temperature ranges for growth of psychrophilic, psychrotolerant, and mesophilic microorganisms (gray boxes). Downward arrowheads denote the approximate optimum growth *T* of each class. The temperature range on Mars is shown for comparison. See text for details

Accordingly, psychrophiles exhibit several adaptations to life in the cold. They import or produce compatible solutes, antifreeze proteins, and ice-binding proteins to prevent intracellular water from freezing. Their membranes typically contain large amounts of unsaturated fatty acids which result in increased fluidity at low T's. The enzymes of psychrophiles show numerous adaptations for efficient function in the cold, and several psychrophilic protein-refolding enzymes (i.e., chaperonins), nucleic acid-binding proteins, and helicases have been identified that improve DNA and RNA function in the cold [reviewed in Casanueva et al. (2010), D'Amico et al. (2006)].

In contrast, psychrotrophs and mesophiles demonstrate minimum growth T's of  $\sim 0$  °C and  $\sim 10$  °C, respectively, but it should be noted that their lower T ranges overlap considerably with the upper T range of psychrophiles (Fig. 10.1). A sudden lowering of T induces a "cold-shock" response in many mesophiles, involving upregulation of a group of cold-shock proteins (Csp's) including small nucleic acid-binding proteins and helicases which enable replication, transcription, and translation at low T. Interestingly, homologs of many of the cold-shock-inducible Csp proteins in mesophiles are also present and expressed constitutively in psychrophiles.

Although the literature lacks actual experiments describing the evolution of mesophiles to psychrophily, it seems reasonable to expect that mesophilic organisms could be induced to evolve enhanced growth ability at low T. For example, *E. coli* is a mesophile whose growth rate drops off dramatically at temperatures lower than  $\sim 20$  °C, and growth essentially ceases at  $\sim 7$  °C (Ferrer et al. 2003). Researchers noted that activity of the *E. coli* GroES/EL chaperonins, involved in refolding of denatured proteins, was inhibited at low T, leading them to propose that inhibition of *E. coli* growth at low T was due to cold sensitivity of its GroES/EL proteins. In support of this notion, expression of the cold-active chaperonins Cpn60 and Cpn10 from the psychrophile *Oleispira antarctica* in *E. coli* allowed the transgenic strain to grow well at temperatures below 4 °C (Ferrer et al. 2003). Similarly, metabolic pathway analyses of mesophiles vs. psychrophiles uncovered that as T decreases, psychrophiles increase ATP production while mesophiles decrease ATP production (Parry and Shain 2011). The researchers noted that in the purine biosynthetic pathways of the two classes of organisms, psychrophiles were enriched in de novo AMP-synthesizing enzymes, whereas mesophiles were enriched in AMP-degrading enzymes. By genetic manipulation, they engineered an *E. coli* strain lacking its native AMP nucleosidase while expressing the AMP-generating PurA enzyme from the psychrophile *Psychrobacter cryohalolentis* and observed that the engineered *E. coli* strain grew 70% faster at low T (Parry and Shain 2011). Thus it is clear from the above experiments that relatively few and simple genetic changes can profoundly alter the minimum growth T of an *E. coli*, common mesophilic bacterium.

## 4.2 Low Pressure (P)

We have undertaken experiments with the aim of exploring the evolution to growth at low P using the Gram-positive spore-forming bacterium *Bacillus subtilis*. We had previously shown that *B. subtilis* could grow (albeit slowly) at 5 kPa but that its growth essentially ceased at P's at or below ~2.5 kPa (Schuerger and Nicholson 2006). To analyze how *B. subtilis* responded to low P, we compared its global transcriptional pattern (i.e., transcriptome) when cultivated at either ~101.3 kPa or at 5 kPa (Waters et al. 2014). We found that incubation of *B. subtilis* at low P resulted in significant alteration in the expression of 10 regulons and most notably resulted in upregulation of 86 transcripts involved in the general stress response (GSR) regulon (Waters et al. 2014). Transcription of GSR genes is controlled by RNA polymerase containing the sigma-B factor (Esig<sup>B</sup>), and we showed that expression of the GSR gene *ctc* was induced at low P in an Esig<sup>B</sup>-dependent manner (Waters et al. 2014).

We were interested to see if *B. subtilis* could evolve to improve growth at low P, using the near-inhibitory low P of 5 kPa as the selective factor (Nicholson et al. 2010). We propagated a wild-type laboratory strain of *B. subtilis*, called WN624, in rich liquid (LB) medium for 1000 generations at 27 °C and 5 kPa, making frozen glycerol stocks from samples of the population at 50-generation intervals for later study. Over the course of 1000 generations at low P, the population exhibited a stepwise evolution to better growth at 5 kPa. We isolated a strain from the 1000-generation culture, called strain WN1106, which could grow to higher cell density at 5 kPa than the ancestral strain WN624. In pairwise competition experiments, low P-evolved strain WN1106 readily outcompeted the ancestral strain WN624 at 5 kPa, but not at Earth-normal pressure (~101.3 kPa). The growth advantage of WN1106 over ancestral strain WN624 at 5 kPa was not due to its better growth under oxygen limitation, because both strains grew equally under oxygen-limited conditions (Nicholson et al. 2010).

We suspected that the enhanced low-P growth capability of strain WN1106 was likely due to mutation(s) occurring in its genome during 1000 generations of propagation at 5 kPa. To identify such mutations, we subjected strains WN624 and WN1106 to whole-genome sequencing and compared their genome sequences (Waters et al. 2015). We found only eight mutations in the genome of WN1106; seven single-nucleotide polymorphisms (SNPs) in the *flil*, *parC*, *resD*, *ytoI*, *ylvD*, *bacD*, and *walkK* genes; and one in-frame, nine-nucleotide deletion in the *rnjB* gene (Waters et al. 2015). We were particularly interested in the *rnjB* deletion mutation, which we call *rnjBΔ9*, because *rnjB* encodes the RNase J2 subunit of the *B. subtilis* RNA degradosome, a multi-subunit enzyme complex that governs global RNA turnover in *B. subtilis* [reviewed recently in Cho (2017)]. Furthermore, *B. subtilis* strains carrying a complete deletion of the *rnjB* gene were viable and did not display an observable phenotype under normal laboratory conditions. To test the role of *rnjB* in low-P growth, we constructed genetically identical strains of *B. subtilis* carrying either the wild-type *rnjB*<sup>+</sup> or the mutant *rnjBΔ9* gene and competed them at P's of ~101.3 or 5 kPa and at T's of 20 °C, 25 °C, or 30 °C. We found that the competition



outcomes depended on the particular combination of T and P used. At 20 °C the mutant strain was less fit than wild-type at both pressures, and at 30 °C, the mutant was more fit than wild-type at both pressures. Only at 25 °C (close to the T at which the original evolution experiment was conducted) was the mutant more fit at low P and less fit at standard P (H. Nguyen and W.L. Nicholson, unpublished data)—highlighting the inextricable linkage between T and P in performing environmental experiments. We currently are working to understand how the *rnjBΔ9* mutation differentially affects gene expression at 25 °C and either ~101.3 or 5 kPa, by comparing the wild-type and mutant transcriptomes under these environmental conditions using RNA-seq technology (experiment in progress).

### 4.3 UV and Ionizing Radiation

Numerous studies have been performed to test the resistance of various microorganisms to conditions simulating the UV and ionizing radiation environment of Mars, but only one study has been published that actually tests the question of whether cells could evolve higher resistance to UV, again using *B. subtilis* as the test organism (Wassmann et al. 2010). In this study, vegetative wild-type *B. subtilis* strain 168 cells were subjected to periodic episodes of selection by daily exposure of stationary phase cultures to polychromatic UV (200–400 nm), followed by dilution into fresh medium and regrowth. Cells were exposed to 69 cycles of UV treatment over the course of 700 generations, and it was shown that the evolving populations exhibited a statistically significant 3- to 4-fold increase in resistance of vegetative cells to both polychromatic (200–400 nm) and monochromatic (254-nm) UV (Wassmann et al. 2010). Vegetative cells of the UV-adapted strain also exhibited significantly increased resistance to ionizing radiation (X-rays; ~8-fold), high osmolarity (1 M NaCl; ~7-fold), desiccation (33% RH; ~5-fold), and hydrogen peroxide (10 mM; ~4-fold), but not to wet heat (55 °C) (Wassmann et al. 2010). Taken together, the data suggest that, in response to the single stressor of UV, *B. subtilis* may have evolved a generalized upregulated resistance to a variety of environmental stresses relevant to enhanced growth in the martian environment. Unfortunately, follow-up studies have not appeared in the literature, and at present, the molecular mechanism for this effect is unknown.

### 4.4 High Salinity and Osmolarity

The issue of high osmolarity is relevant to growth in the martian environment, because under the low-T, low-P martian atmospheric regime, only water loaded with ionic solutes is stable in liquid form. Various sites on Mars are thought to be the remains of rivers, seas, and lake beds from which water has evaporated, leaving behind once-dissolved solutes as precipitates. Analyzing the mineral composition at

various sites on Mars indicates that evaporitic salts of  $K^+$ ,  $Na^+$ ,  $Mg^{+2}$ , or  $Ca^{+2}$  paired with  $Cl^-$ ,  $SO_4^{-2}$ , or  $ClO_4^-$  dominate, suggesting that liquid water on Mars was briny. Microbes need special adaptations for growth at high salinities and osmolarities, as described below.

All cells possess semipermeable membranes through which water, but not most other molecules, passes freely. To maintain proper turgor pressure in environments of ever-changing osmotic strength, cells must actively adjust their intracellular osmotic potential to prevent dehydration or rupture [reviewed in Hoffmann and Bremer (2017); Detkova and Boltyanskaya (2007)]. They do so by accumulating and/or expelling ions (usually  $K^+$  and  $Na^+$ ) or by producing, accumulating, and/or expelling a number of compounds collectively known as compatible solutes (proline, glycine betaine, choline, among several others) (Hoffmann and Bremer 2017). Using these mechanisms, some extreme halophiles can grow at NaCl concentrations approaching saturation (above 5 M).

As noted above, UV selection of *B. subtilis* resulted in a concomitant increase of its resistance to high osmolarity (1 M NaCl), due to an unknown mechanism (Wassmann et al. 2010). A search of the literature revealed that studies in which microorganisms were subjected to experimental evolution specifically directed at increasing their growth at increased osmotic potentials are lacking. However, some evolutionary insights can be gained by studying the properties of macromolecules from halophiles vs. mesophiles. The proteins of archaeal halophiles typically have an increased content of acidic amino acids and exhibit increased negative surface charges, which compensate for their extreme ionic environments (Reed et al. 2013). Regarding protein-DNA interactions, the binding of archaeal promoters by the TATA-box-binding protein (TBP) is an essential step in initiation of transcription. In mesophilic archaea, the affinity of TBP for DNA decreases with increasing salt concentration, but in the halophilic archaeon *Pyrococcus woesei*, TBP binds its cognate DNA better at higher salt concentrations (Bergqvist et al. 2003). This effect could be attributed to only three amino acid changes in TBP, suggesting that the important phenotype of halophilicity in TBP, thus global transcription at high osmolarity, could be rapidly acquired in evolutionary time (Bergqvist et al. 2003). Valuable information regarding the possible growth of Earth microbes in martian environments could be gained by future experiments directed toward evolution of terrestrial microbes to conditions of increased osmolarity, particularly in regard to perchlorate salts.

#### **4.5 Low or Absent Organic Nutrients**

Evolution of life toward the utilization of increasingly scarce nutrients (termed *oligotrophy*) started on Earth billions of years ago [reviewed in Raven et al. (2005)]. Scarcity of energy, carbon, and nitrogen likely drove the early evolution of processes such as photosynthesis and nitrogen fixation. The resulting oxygenation of the Earth via photosynthesis in turn led to scarcity of soluble phosphorus and iron,

thus driving the evolution of various strategies for phosphorus and iron dissolution, uptake, and scavenging among evolving prokaryotes (Raven et al. 2005). On present-day Earth, oligotrophy is a common condition facing microbes in nutrient-poor environments, and even in nutrient-rich environments, nutrients can become limiting through competition; in particular, phosphorus and nitrogen are recognized to be limiting nutrients in most freshwater, oceanic, and terrestrial environments (Guildford and Hecky 2000). The technical difficulties encountered to date both in identifying and quantifying the actual, particularly organic, nutrients present in martian soils have hampered development of experiments to test the possible evolution of Earth microbes under simulated Mars conditions, and to date no directed studies have appeared in the literature.

## 5 Conclusions and Perspectives

From the discussions presented above, we have seen that experimental evolution can be a valuable tool in understanding how Earth life might adapt to some of the environmental parameters currently present on Mars. In addition, we have seen that *some* Earth microorganisms already possess *some* of the adaptations necessary for successful growth in martian environments. At this point it is perhaps instructional to summarize the myriad environmental extremes present on Mars, what attributes would be needed to enable survival and growth, and which Earth microbes possess such attributes, presented in Table 10.3.

In examining Table 10.3, it should be kept in mind that in order to grow in the environment of Mars, a microorganism must possess *all* the attributes listed *simultaneously*—and to date no such single organism has been found. At present, Earth's continuous permafrosts seem to be the most promising environments for isolation of microbes with the potential to grow on Mars. Permafrosts exhibit several Mars-like properties: permanent cold, limited access to liquid water, low nutrient availability, high salinity in brine inclusions and cryopegs, and low oxygen availability. As discussed in Sect. 3, we were able to isolate numerous bacteria from a Siberian permafrost borehole capable of growing under the T, P, and AC regime of the Mars atmosphere—but on hydrated rich medium (Table 10.2). Recently, an exciting report appeared in the literature showing that methanogenic archaea isolated from permafrost could (1) convert CO<sub>2</sub> to CH<sub>4</sub> in the presence of high concentrations of perchlorate and (2) utilize perchlorate as an electron acceptor in anaerobic methane oxidation (Shcherbakova et al. 2015). However, this study was not performed under simulated martian T, P, and AC conditions, and the media used contained vitamins and other components not known to be present in the martian environment.

Nevertheless, these results are encouraging and contribute significantly toward our understanding of the limits of life and the ability of Earth microbes to grow in extraterrestrial environments. I am optimistic that many of the gaps identified and discussed in this review will be filled in near-future studies on this fascinating topic.

**Table 10.3** Attributes of Earth microbes potentially enabling them to inhabit Mars environments

Challenge	Attribute	Representative genera	Reviewed in
Low temperature	Psychrophily/ psychrotolerance	<i>Arthrobacter</i> , <i>Halobacterium</i> , <i>Hyphomonas</i> , <i>Pseudomonas</i> , <i>Psychrobacter</i> , <i>Sphingomonas</i>	D’Amico et al. (2006), Casanueva et al. (2010)
Low pressure	Hypobarophilicity	See Table 10.2	This chapter.
UV radiation	Resistance mechanisms	Spores: <i>Bacillus</i> , <i>Clostridium</i> , <i>Paenibacillus</i> , etc. Vegetative cells: <i>Deinococcus</i> , <i>Janibacter</i>	Nicholson et al. (2000), Friedberg et al. (2006)
Ionizing radiation	Resistance mechanisms	<i>Bacillus</i> (spores), <i>Deinococcus</i> , <i>Chelatococcus</i> , <i>Corbulabacter</i> , <i>Spirosoma</i> , <i>Geodermatophilus</i> , <i>Hymenobacter</i> , <i>Planococcus</i>	Munteanu et al. (2015), Friedberg et al. (2006), Rainey et al. (2005)
High osmolarity	Osmophily/ halophily	Archaea: numerous genera Bacteria: <i>Chromohalobacter</i> , <i>Halomonas</i>	Detkova and Boltyanskaya (2007)
Perchlorate salts	Redox chemistry	<i>Azospirillum</i> , <i>Ideonella</i> , <i>Proteus</i> , <i>Pseudomonas</i> , <i>Wolinella</i>	Van der Zee and Cervantes (2009), Coates et al. (1999)
Low nutrient levels	Oligotrophy	Wide diversity, in the mainly uncharacterized “rare biosphere”	Egli (2010)
Lack of organic carbon	Autotrophy	<i>Methanobacterium</i> , <i>Methanosarcina</i> , <i>Nitrosomonas</i> , <i>Thiobacillus</i>	Wood et al. (2004)

And who knows? Perhaps in the not-so-distant future, many of the questions posed here will be answered through laboratory study of bona fide martian microbes.

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