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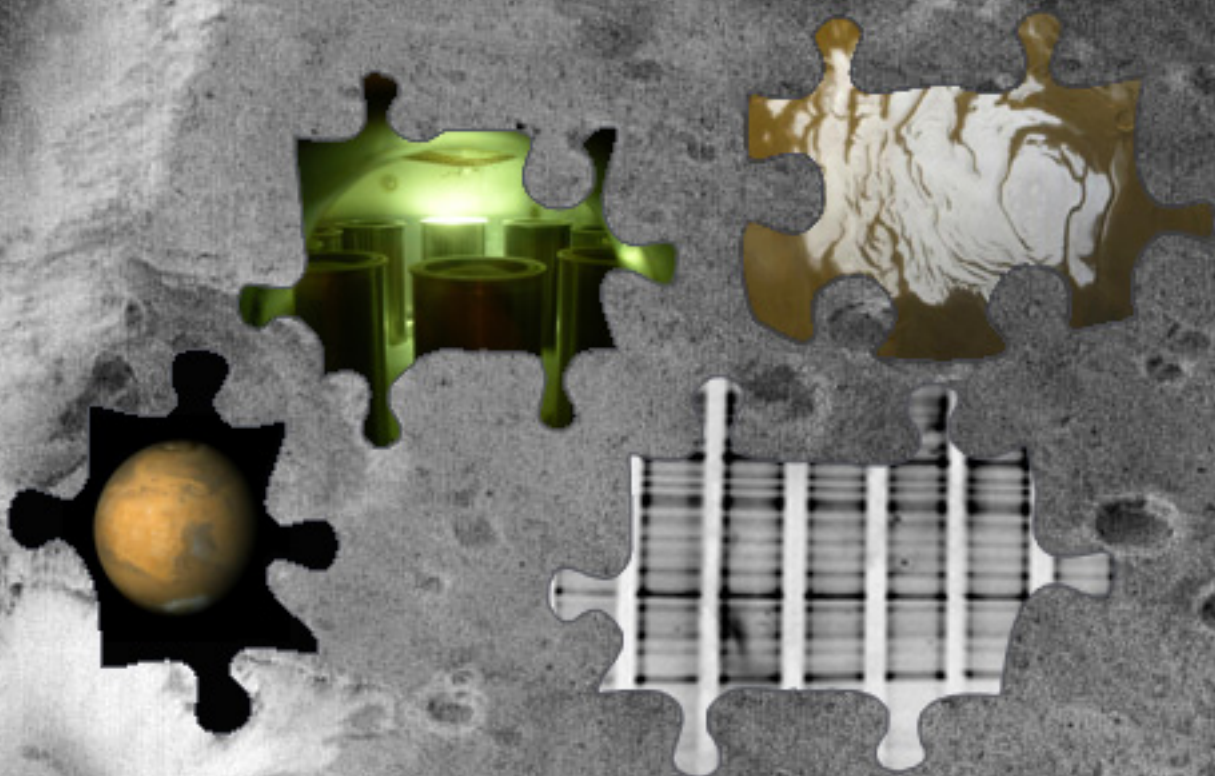
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Response of complex bacterial soil communities to simulated Martian conditions



PhD dissertation by

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July 2006

Front cover illustration: The Martian canyon Nanedi Vallis (Photo by NASA)

Small pictures from top left:

Permafrost soil inside Mars simulation facility (Photo by LL. Jensen)

South Polar Cap of Mars (Photo by NASA)

The Planet Mars (Photo by NASA)

Bacterial community fingerprint of Salten Skov soil on a DGGE gel (Photo by AA. Hansen)

Preface

This PhD dissertation is submitted to the Faculty of Science, University of Aarhus, Denmark. The dissertation presents work performed at the Department of Microbiology, University of Aarhus and at the Center of Microbial Ecology, Michigan State University during a seven-month research visit in 2004. This project has been a sub-project of a multi-disciplinary collaboration with the Department of Physics and Astronomy and the Department of Earth Sciences, University of Aarhus.

During my four years of PhD studies, I have focused on characterization of complex bacterial soil communities and their response to simulated Martian conditions. The initial investigations were carried out with an iron-rich Mars-analogue soil (Salten 1), where the response of the indigenous bacterial soil community to simulated Martian conditions was investigated (Manuscript I). These experiments led to a change in model community and the subsequent investigations were carried out with an Arctic permafrost soil from Spitsbergen.

I was given the opportunity to characterize the bacterial permafrost community with high throughput sequencing, which combined with culture-based investigations resulted in a comprehensive characterization of the bacterial permafrost community (Manuscript II).

Subsequently, the permafrost soil was long-term incubated under simulated Martian conditions, where the effects of simulated Martian conditions on the indigenous permafrost bacteria and biomolecules were investigated (Manuscript III). In connection with this long-term simulation experiment an automated Mars simulation facility was designed and constructed in collaboration with the Department of Physics and Astronomy (Manuscript V).

Since spring 2003, our research group has been a member of the ESA (European Space Agency) topical team - *Response of Organisms to the Martian Environment* (ROME), which brought scientists from European Mars simulation laboratories together. This collaboration resulted in the development of a Mars UV simulator (Manuscript VII) and the ESA Special Publication - *Microorganisms and the Martian environment* (Manuscript IV and VI).

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I am especially grateful to my supervisors Bente Aagaard Lomstein and Kai Finster for believing in me and giving me the opportunity to make my PhD studies in their research group and for their invaluable support and understanding during difficult periods.

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I owe particular gratitude to Professor James M. Tiedje for welcoming me in his laboratory as a member of his very inspiring research team; Helmut Lammer and Christoph Kolb for letting me contribute to their manuscript; Charles Cockell for inviting us to be a part of the ROME team and Per Nørnberg for organisation of the Mars Simulation Laboratory and for the Salten Skov soil samples.

My PhD studies could not have been this fruitful without all the people, who have helped me along the way, and I thank everybody at the Department of Microbiology in Aarhus and the Center of Microbiology in Michigan, in particular Kasper Urup Kjeldsen, Johan Goris, Tove Wieggers, and Benli Chai, who have given me invaluable help. Also thanks to Signe Ingvarsdén, Rikke Holm, Andreas Schramm, James R. Cole, Mette H. Nicolaisen, Qiong Wang, Monica Ponder, Daniel Aagren, Martina Herrmann, Aaron Saunders, Pernille V. Thykier and Britta Poulsen.

To Jesper, who stood by my side whatever the journey brought.

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Chapter 2: Manuscript I

Hansen, A.A., J. Merrison, P. Nørnberg, B.Aa. Lomstein and K. Finster (2005) Activity and stability of a complex bacterial soil community under simulated Martian conditions. *International Journal of Astrobiology* **4**, 135-144.

Chapter 3: Manuscript II

Hansen, A.A., K. Mikkelsen, R.A. Herbert, L.L. Jensen, T. Kristoffersen, J.M. Tiedje, B.Aa. Lomstein and K.W. Finster. Comprehensive characterization of composition and diversity of the bacterial community in a high Arctic permafrost soil, Spitsbergen. In preparation.

Chapter 4: Manuscript III

Hansen, A.A., L.L. Jensen, T. Kristoffersen, K. Mikkelsen, J. Merrison, K.W. Finster and B.Aa. Lomstein. Effects of long-term simulated Martian conditions on a bacterial permafrost soil community. In preparation.

Chapter 5: Manuscript IV

Hansen, A.A. Mars simulations – Past studies on the biological response to simulated Martian conditions. *Microorganisms and the Martian Environment, ESA Special Publication* (Cockell, C. ed). Chapter 4. In press.

Chapter 6: Manuscript V

Jensen, L.L., J. Merrison, A.A. Hansen, K.Aa. Mikkelsen, T. Kristoffersen, B.Aa. Lomstein and K. Finster. Design, construction and evaluation of a chamber for simulating environmental conditions on Mars. In preparation.

Chapter 7: Manuscript VI

Finster, K., A.A. Hansen, L. Liengard, K. Mikkelsen, T. Kristoffersen, J. Merrison, P. Nørnberg, B.Aa. Lomstein. Mars simulation experiments with complex microbial soil communities. *Microorganisms and the Martian Environment, ESA Special Publication* (Cockell, C. ed). Chapter 7. In press.

Chapter 8: Manuscript VII

Kolb, C., R. Abart, A. Bérces, J.R.C. Garry, A.A. Hansen, W. Hohenau, G. Kargl, H. Lammer, M.R. Patel, P. Rettberg and H. Stan-Lotter (2005) An ultraviolet simulator for the incident Martian surface radiation and its applications. *International Journal of Astrobiology* **4**, 241-249.

Appendix: Conference proceedings

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Nørnberg, P., J.P. Merrison, K. Finster, F. Folkmann, H.P. Gunnlaugsson, A. Hansen, A.M. Jensen, K. Kinch, B.Aa. Lomstein, R. Mugford (2002) Simulation of Martian surface conditions and dust transport, *Proceedings of the Second European Workshop on Exo/Astrobiology, Graz, Austria, 2002*, p. 77-80.

Introduction

The term astrobiology comes from the Greek word *astron*, meaning star and was first introduced in the literature by Lafleur in 1941 as “the consideration of life in the universe elsewhere than on Earth”. At that time, astrobiology was limited to philosophical considerations of the extent about habitable planets in the Universe, the characteristics of extraterrestrial life and the distribution of life in the Universe (Lafleur, 1941). Exobiology (*exo* meaning out), a synonym for astrobiology, was later introduced by Lederberg in 1960 as “approaches to life beyond the Earth”. The Astrobiology Institute of NASA has introduced a new and somewhat broader definition of astrobiology as “the study of the origin, evolution, distribution and future of life on Earth and in the Universe” (Blumberg, 2003), thereby including terrestrial biology as a part of astrobiology. In this dissertation the original strict definition of astrobiology is employed and the term will refer to the research of the possibility of life beyond Earth.

1. The astrobiology of Mars - background

Our neighboring planet Mars is considered a possible astrobiological habitat and has over time been a major focus of space programs prospecting for extraterrestrial life. Mars’ red appearance has drawn considerable attention throughout history and recorded observations date back as far as the early civilizations of Egypt and Greece (Sheehan, 1996). Through the late 19th century and up to the space age, astronomers investigated the surface of Mars using ground-based telescopes and interpreted the observed structures as water canals and seasonal cycles of vegetative growth (Sheehan, 1996). These observations encouraged the idea of Martian life, but were later explained as optical misinterpretations and dust storms (Sheehan, 1996).

Therefore, in the emerging space age of the late 1950’s where the possibility of sending spacecraft beyond Earth became achievable, Mars became a compelling target for scientific investigation. Since the first, however failing, mission to Mars in 1960 many space programs have focused on Mars and still today the red planet remains the most investigated planetary object outside the Earth-Moon system. At present, the exploration rovers Spirit and Opportunity are performing geological investigations on Mars (Morris *et al.*, 2004) and four orbiting spacecraft, including the Mars Odyssey, are investigating Martian geology, climate, and mineralogy (Boynton *et al.*, 2002; Bibring *et al.*, 2006).

Data obtained from orbiting satellites and landers indicate that the surface of present-day Mars has an environment hostile to life as we know it. However, a number of events have given basis for

sustained astrobiological interest in Mars of which the most significant are addressed in the following.

1.1 Life detecting experiments aboard the Viking lander missions

The Viking missions to Mars in 1976 were a milestone in Mars exploration, since data and images were for the first time obtained from the Martian surface. The Viking missions were primarily directed to search for Martian life and consisted of two landers and two orbiters (Soffen & Young, 1972). Nevertheless, during the two missions of four and six years, the landers also gained invaluable data on the Martian climate and meteorology, e.g. atmospheric pressures (Tillman *et al.*, 1993).

The biological instrumentation of the Viking landers targeting the possibility of Martian life consisted of the pyrolytic release experiment (PR), the gas exchange experiment (GEX), the labeled release experiment (LR) and a gas-chromatograph mass-spectrometer (GCMS) (Table 1). The three first-mentioned life detecting experiments were designed to measure different types of metabolic activity, which were considered the most useful way to detect living systems (Soffen & Young, 1972; Klein, 1979). Table 1 gives an overview of the procedures and outcome of the experiments, all analyzing Martian soil samples.

The PR experiment was designed to explore the carbon assimilation capacity of the Martian soil, which was incubated with ^{14}C -labeled carbon dioxide and carbon monoxide ($^{14}\text{CO}_2$ and ^{14}CO) in the

Table 1. Biological investigations aboard the Viking lander missions.

Experiment	Target of investigation	Procedure and method ^a	Results ^a	References
Pyrolytic release (PR)	Autotrophy	Assimilation of carbon: Soil + $^{14}\text{CO}_2$ + ^{14}CO ± light Method: org- ^{14}C detection	Small assimilation Highest with light Unaffected by heating	(Horowitz <i>et al.</i> , 1977)
Gas exchange (GEX)	Heterotrophy	Gas production/assimilation: Soil + water + nutrients Method: Gas-chromatography	Prod: $\text{O}_2, \text{CO}_2, \text{N}_2$ Unaffected by heating	(Oyama & Berdahl, 1977)
Labeled release (LR)	Heterotrophy	Mineralization of org-C: Soil + ^{14}C -labeled nutrients Method: $^{14}\text{CO}_2$ detection	$^{14}\text{CO}_2$ production Inhibited by heating	(Levin & Straat, 1977)
Gas-chromatograph mass-spectrometer (GCMS)	Organic compounds	Organic carbon in the soil: Surface + subsurface soil Method: GCMS	No org-C detected	(Biemann <i>et al.</i> , 1977)

^aorg-C, organic compounds. Prod, production.

presence and absence of light (Horowitz *et al.*, 1977). Several soil samples were analyzed and all showed a small incorporation of $^{14}\text{CO}_2$ and ^{14}CO , which was however unaffected by heating of the soil prior to incubation (90°C; 2h) (Horowitz *et al.*, 1977). Thus, a biological explanation of the incorporation was considered unlikely and a chemical fixation of CO and CO_2 has been proposed (Horowitz *et al.*, 1977; Hubbard, 1979; Klein, 1979).

The GEX experiment was designed to detect uptake or production of gasses from incubations of Martian soil with different quantities of water and nutrients (Oyama & Berdahl, 1977). Surprisingly the incubations led to a rapid release of oxygen together with a net release of carbon dioxide and nitrogen gas, which also were released from soil heated prior to incubation (145°C; 3h), although in smaller amounts (Oyama & Berdahl, 1977). This indicated that chemical rather than biological activity was involved, and H_2O_2 and superoxide (O_2^-) have been speculated as possible reactive oxidants present in the Martian soil able to react with the water and nutrients added in the experiment (Bullock *et al.*, 1994; Yen *et al.*, 2000).

Similarly, the purpose of the LR experiment was to explore the possibility of mineralization of organic material by detection of $^{14}\text{CO}_2$ generation from ^{14}C -labeled nutrients incubated with Martian soil (Levin & Straat, 1977). A rapid evolution of $^{14}\text{CO}_2$ was measured, which was completely inhibited by heating of the soil prior to the incubations (160°C; 3h) (Levin & Straat, 1977). Independently, these results would point to the presence of biological activity. However, taken together the results were more likely a consequence of reactive soil oxidants, as indicated from the results of the GEX experiment (Klein, 1979; Bullock *et al.*, 1994; Yen *et al.*, 2000).

Finally, analysis of the Martian soil with the GCMS failed to detect the presence of organic compounds (Biemann *et al.*, 1977). This result further supported the abiotic interpretation of the results from the life detecting experiments. However, it has been argued that the sensitivity of the GCMS instrument was insufficient to rule out the presence of low levels of organic compounds on the Martian surface (Glavin *et al.*, 2001).

The biological experiments performed with the Viking landers did not detect life on the surface of Mars. While some of the data could be evidence of biological reactions, a more probable explanation is the surface chemistry of the Martian soil and the presence of highly reactive oxidants. In spite of the lack of Martian biology the Viking mission provided invaluable data on the Martian soil.

1.2 The Martian meteorite ALH84001

The failure of the Viking missions to detect life on Mars resulted in a decrease in the interest of astrobiology of the planet. Interest was however rekindled by the analysis of the Martian meteorite ALH84001 in 1996 (McKay *et al.*, 1996), which initiated a new era of astrobiological research. ALH84001 was found in Antarctica in 1984, where it had arrived 13,000 years earlier (Fig. 1A). Analysis of the meteorite showed that it had crystallized on Mars approximately 4.5 billion years (Ga) ago, but younger indigenous secondary carbonate grains (~3.9 Ga old) were identified along fractures inside the meteorite (Nyquist, 1995; Borg *et al.*, 1999).

The study of the meteorite reported by McKay *et al.* (1996) initiated a long debate about the possibility that

compounds inside the meteorite had been produced by biological reactions and were thereby evidence for life on early Mars. This argument arose from the presence of polycyclic aromatic hydrocarbons (PAHs), magnetite and iron-sulfide minerals associated with the carbonates along with structures resembling “nano-bacteria” in connection with the iron-rims of the carbonates (Fig. 1B) (McKay *et al.*, 1996). Moreover, the crystal structures of the magnetite were suggested to resemble the magnetite produced by terrestrial magnetotactic bacteria and were therefore claimed to be products of microbial activity (McKay *et al.*, 1996; Thomas-Keprta *et al.*, 2002). This controversial hypothesis led to a considerable amount of research carried out to either refute or verify the possibility of biological activity inside the meteorite.

It was demonstrated that structures resembling the “nano-bacteria” inside the meteorite could be reproduced by abiotic precipitation experiments (Kirkland *et al.*, 1999) and that the presence of PAHs inside the meteorite could not be correlated with the carbonate grains (Stephan *et al.*, 2003), therefore suggesting that the origin of the PAHs was abiological. More importantly the structures of the magnetite crystals were demonstrated to be different from the magnetite crystals formed by terrestrial magnetotactic bacteria and rather resembled abiotic formed crystals (Golden *et al.*, 2004). Thus, the investigations pointed to a non-biological explanation of the phenomena inside ALH84001.



Figure 1. (A) The Martian meteorite ALH84001. (B) Transmission electron microscopy of structures inside ALH84001. (Photo: NASA).

Altogether, the systematic investigations of ALH84001 demonstrated how difficult it is to substantiate the presence of past life. This is an important lesson considering the ongoing scientific research for developing methods for detection of past and present life on Mars. Even though the investigations of ALH84001 led to a non-biological outcome they revived the status of Mars as a possible astrobiological habitat. This status has been further accelerated by the data from Mars Odyssey strongly indicating the presence of subsurface water ice on present-day Mars (Boynton *et al.*, 2002; Mellon *et al.*, 2004; Litvak *et al.*, 2006) and the recent detection of methane in the Martian atmosphere (Formisano *et al.*, 2004; Krashnopolsky *et al.*, 2004). Methanogenic prokaryotes (Krashnopolsky *et al.*, 2004), geological and atmospheric processes (Formisano *et al.*, 2004; Bar-Nun & Dimitrov, 2006) have been suggested as potential sources of this methane.

1.3 Theory of Panspermia

In addition to the historical events cited earlier, the theory of panspermia has also powered the astrobiological interest in Mars. Panspermia is a theory, put forward in the late 19th century, hypothesizing the natural transfer of life through space with meteorites as possible media of transportation (lithopanspermia) (Raulin-Cerceau *et al.*, 1998).

Evidence to support panspermia has been generated by simulated meteorite impacts, where terrestrial bacteria have been shown to survive pressures and temperatures similar to those occurring during ejection of meteorites into space (Horneck *et al.*, 2001a; Burchell *et al.*, 2004). Additionally, exposure experiments on satellites and space stations have revealed that terrestrial bacteria are able to survive exposure to the environment of the interplanetary space (Mancinelli *et al.*, 1998; Horneck *et al.*, 2001b; Rettberg *et al.*, 2002). Most notable was the survival of endospores of the bacterium *Bacillus subtilis* for six years in space (Horneck *et al.*, 1994), however this is still insignificant compared to the millions of years that the Martian meteorites took to reach Earth (Mileikowsky *et al.*, 2000). Nevertheless, terrestrial bacteria have been found viable in 2-3 million year old permafrost layers (Vorobyova *et al.*, 1997; Vishnivetskaya *et al.*, 2006) and possibly also in a 250 million year old salt crystal (Vreeland *et al.*, 2000; Vreeland *et al.*, 2006).

The theory of panspermia has given rise to speculations about where life may have originated. Mars has been proposed as an alternative place, since the period favorable for generation of life on Earth is argued to be too short for the development of complex cells from simple organic molecules (Davies, 2003). The first sign of terrestrial life, approximately 3.8 Ga ago, coincides with the ending of a period of intense asteroid bombardment of the surface of Earth. At this time bodies of water

probably existed on the surface of Mars in which development of simple life forms may have been possible (Davis & McKay, 1996).

2. Environmental conditions of Mars

As a result of negligible tectonic activity about 40% of the Martian surface is more than 3.7 Ga old and thus constitutes a rich geological record (Solomon *et al.*, 2005). Observations and analysis of the Martian surface have revealed that Mars was much warmer and wetter during the Noachian Epoch more than 3.7 Ga ago (Jakosky & Phillips, 2001; Solomon *et al.*, 2005). In

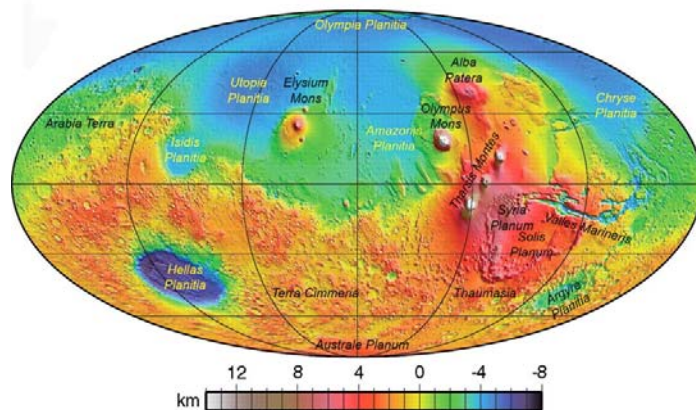


Figure 2. Topographic map of Mars. Colors display variation in elevation (Solomon *et al.*, 2005).

this period, local bodies of water may have been present on the surface, as suggested by sedimentary rocks and waterborne sediments on the present-day surface (Squyres *et al.*, 2004). Also, the great volcanoes and magma deposits found in the region of Tharsis are indicative of a planet more active in the past (Fig. 2) (Davis & McKay, 1996).

The change in the Martian climate probably dates back to the Early Hesperian period (3.7-3.0 Ga ago), where magnetic anomalies are devoid from the surface indicating that the core dynamo had ceased to function (Jakosky & Phillips, 2001; Solomon *et al.*, 2005). Climate change was probably a result of loss of the magnetic field, which had protected the Martian atmosphere against solar wind stripping (Jakosky & Phillips, 2001; Solomon *et al.*, 2005). As atmospheric gasses became depleted the surface pressure and temperature decreased (Jakosky & Phillips, 2001; Solomon *et al.*, 2005), resulting in the cold and dry environment of present-day Mars.

2.1 Present-day climate on Mars

Today the surface of Mars is considered hostile to all known life forms (Table 2). The surface temperature fluctuates by up to 100°C diurnally with a mean temperature of -63°C. As a result of a thin atmosphere the surface pressure is <1% of the pressure on Earth and therefore liquid water is not stable at the surface. The surface pressure fluctuates over the year due to exchange of CO₂ between the polar ice-caps and the atmosphere. The main component of the atmosphere is CO₂,

Table 2. Environmental surface conditions on present-day Mars and present-day Earth.

Parameter	Mars ^a	Earth ^b
Temperature range (°C)	-123 - +25 (-63) ^c	-89 - +58
Pressure range (mbar)	6.7 - 9.9 ^d	1013
Atmospheric composition (%)		
CO ₂	95.3	0.038
N ₂	2.7	78.1
Ar	1.6	0.93
O ₂	0.13	20.9
CO	0.07	~1-2 × 10 ⁻⁵
H ₂ O	0.02	0-4
CH ₄	1.0 × 10 ⁻⁶	1.5 × 10 ⁻⁴
Other	0.25	2.5 × 10 ⁻³
Solar radiation (nm)	>190	290-1375
Solar constant (W m ⁻²)	578.06	1344.23
Gravity (m s ⁻¹)	3.68	9.81
Length of year (d)	687	356
Length of day	24h 37min (sol)	24h (day)

^aFrom Horneck (2000), ten Kate *et al.* (2003) and Schuerger *et al.* (2003). ^bFrom Lutgens and Tarbuck (2001). ^cMean in parenthesis. ^dFrom Tillman *et al.* (1993).

which only absorbs solar radiation below 190 nm, and therefore the biological harmful UVC radiation (190-280 nm) reaches the Martian surface. Additionally, short-waved cosmic radiation penetrates the Martian atmosphere.

UV radiation is probably the cause of the highly oxidizing nature of the Martian soil as detected by the Viking landers (see section 1.1). The chemical nature of the oxidant has not been determined, but H₂O₂ was found to be produced by photochemical processes in the Martian atmosphere (Clancy *et al.*, 2004; Encrenaz *et al.*, 2004) and is a possible candidate for the Martian soil oxidant (Bullock *et al.*, 1994). Another candidate is superoxide (O₂⁻), which under simulated Martian conditions is reported to be readily formed on mineral grains (Yen *et al.*, 2000). The vertical extent of the oxidized zone in the Martian soil is not known, but samples from 10 cm depth analyzed by the Viking landers showed the same reactivity as surface samples. This oxidizing nature of the Martian soil has been argued to explain the absence of organic molecules in the Martian surface soil (Oro & Holzer, 1979; Yen *et al.*, 2000), since organic molecules would have been expected to be continuously brought to the planet by meteorites and planetary dust (Flynn & Mckay, 1990). The red appearance of the Martian soil is due to iron-oxides, especially magnetite and also hematite (Morris *et al.*, 2004; Goetz *et al.*, 2005). In addition, the soil consists of jarosite, pyroxene, olivine, silicon, aluminum, magnesium, calcium, titanium, sulfur and chlorine (Klingelhöfer *et al.*, 2004; Morris *et al.*, 2004; Goetz *et al.*, 2005).

Although liquid water is not present on the surface of Mars, strong indications of widespread water ice have been found by the Mars Odyssey Gamma-Ray Spectrometer (Fig. 3) (Boynton *et al.*, 2002;

Mellon *et al.*, 2004; Litvak *et al.*, 2006). This ground ice or permafrost layer is close to the surface on the Northern Hemisphere and below 20-30 cm of dry soil on the Southern Hemisphere (Mellon *et al.*, 2004; Litvak *et al.*, 2006). The thickness of the permafrost layer is not known, but the water content by mass has been estimated to approximately 10% in the low latitude regions of Mars and as high as 25 and 53% in the Southern and Northern polar regions, respectively (Mitrofanov *et al.*, 2004). Both polar caps consist mainly of water ice with a seasonal dependent CO₂ ice cover at the surface (Titus *et al.*, 2003).

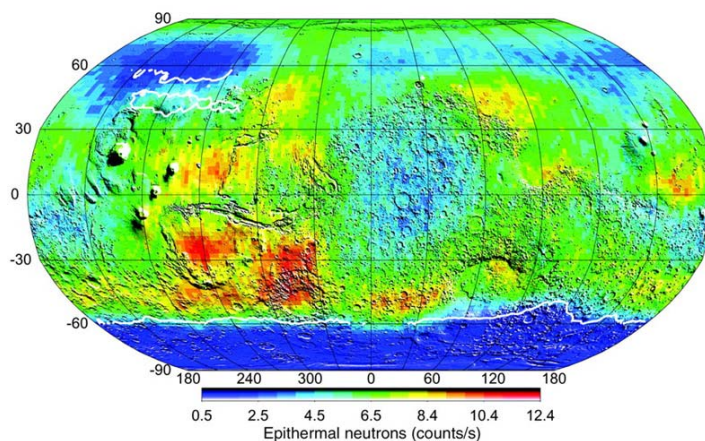


Figure 3. Map of epithermal neutron flux on Mars, as a measure of the hydrogen concentrations indicative of water ice regions. Low epithermal flux is correlated with high hydrogen concentrations. From the Mars Odyssey Gamma-Ray Spectrometer (Boynton *et al.*, 2002).

2.2 Putative life-supporting habitats on present-day Mars

The prerequisites for actively growing terrestrial life are the availability of water, energy and molecules supporting anabolism of biomass. As described in section 2.1, subsurface water is most probably ubiquitous on Mars. However, this water is in the form of ice and presence of liquid water on Mars has yet to be established. On Earth, liquid water can exist at sub-zero temperatures in brines in connection with permafrost soil (Andersen *et al.*, 2002; Gilichinsky *et al.*, 2003). Such brines might also exist in the permafrost layers and polar ice-caps on Mars and would be potential habitats supporting life if a source of energy is also present and available.

Terrestrial life forms utilize energy either in the form of light or chemical molecules. Utilization of light as sole energy source seems most unlikely on Mars, since the highly oxidizing soil probably prevents the presence of life at the Martian surface. However, phototrophy could theoretically exist on Mars if the phototrophic organisms were protected against direct exposure to soil oxidants, harmful UV radiation and cosmic rays, while receiving sufficient solar radiation for photosynthesis. Such protected niches have been hypothesized to occur within the polar ice-caps, inside rocks and in soils containing ferric iron, which absorbs UV radiation (Cockell & Raven, 2004). Nevertheless, sufficient shielding from UV radiation is not enough; concurrent presence of favorable temperatures, appropriate electron donors and light energy is necessary to sustain photosynthesis.

Chemotrophy, rather than phototrophy, seems the most likely process to support life under the present-day Martian conditions. The most probable chemical energy source on Mars is the gaseous

compound H₂ (Weiss *et al.*, 2000; Summers *et al.*, 2002), which, together with CO, presumably are readily formed by photochemical processes in the Martian atmosphere (Nair *et al.*, 1994; Bar-Nun & Dimitrov, 2006). The hypothesized Martian methanogenic prokaryotes have been argued to live in the subsurface permafrost layers and utilize the H₂ or CO diffusing in from the atmosphere (Formisano *et al.*, 2004; Krashnopolsky *et al.*, 2004).

Gaseous energy species can also be formed in connection with hydrothermal systems. There is no evidence of current hydrothermal activity on Mars, but the Tharsis region would be a likely location, as this is where the Martian volcanoes are present (Fig. 2). The Valles Marineris has also been proposed as a probable area of hydrothermal activity (Pirajno & Van Kranendonk, 2005), as both hematite and magma deposits, indicative of an active geological past, have been detected here (Christensen *et al.*, 2001; Solomon *et al.*, 2005). Hydrothermal generated heat could melt water ice and thereby facilitate the presence of both liquid water and chemical energy. However, the current indications are that the water content, detected by the Gamma-Ray Spectrometer, is relatively low in both Tharsis and Valles Marineris compared to the rest of the planet (Fig. 3). Therefore, in these areas life-supporting habitats would have to be in connection with deep subsurface water.

Altogether, subsurface areas with liquid water and available energy seem to be the most probable life-supporting habitats on Mars, for example, in permafrost layers and in connection with the polar ice-caps.

3. Terrestrial analogues for present-day Mars

The increased insight in the environmental conditions on present-day Mars has given basis for identification of terrestrial environments resembling present-day Mars according to features as mineralogy, climate, water activity or temperature. The proposed terrestrial Mars-analogues can be divided into two types: i) terrestrial soils with characteristics similar to the Martian soil, e.g. soils with similar mineralogy and ii) terrestrial habitats with climatic conditions similar to the conditions expected to exist on Mars, e.g. cold and dry habitats.

The different research interests and applications of these two types of Mars-analogues will be described in the following. The application of terrestrial Mars-analogues in Mars simulation experiments with biological samples is described in section 4.

3.1 Terrestrial Mars-analogue soils

The terrestrial Mars-analogue soils and sediments have been investigated in order to better understand and interpret the results obtained from the experiments carried out by landers on Mars.

The Mars-analogue soils have also been used for testing of equipment prior to Mars missions. The most recognized Mars-analogue soils and sediments are the iron-rich JSC Mars-1 soil (Mars-1) (Allen *et al.*, 2000), the iron and sulfate rich sediments from the Rio Tinto river basin (Fernandez-Remolar *et al.*, 2004) and the oligotrophic soils from the Atacama desert (Navarro-Gonzalez *et al.*, 2003). In connection with this dissertation the iron-rich Salten Skov 1 soil (Salten 1) has been used as an analogue soil (see manuscript I, chapter 2). The characteristics of these Mars-analogue soils and sediments will be described in the following.

Mars-1 is a weathered volcanic ash from a Hawaiian volcano. Its mineralogy has been argued to be similar to the Martian surface soil due to its content of feldspar, magnetite, pyroxene, olivine and hematite (Allen *et al.*, 1998). Moreover, the reflectance spectrum of the Mars-1 soil has been found to match the spectrum of the Martian soil (Allen *et al.*, 1998). Due to these similarities Mars-1 is the most applied and acknowledged Mars soil analogue. Apart from application of Mars-1 in the development of techniques for future Mars missions, e.g. the development of sediment dating techniques (Lepper & McKeever, 2000), Mars-1 soil has been extensively used in bacterial investigations, especially for assessment of the risk of forward contamination of Mars with terrestrial bacteria. This has been investigated by determination of the survival rates of bacteria covered by Mars-1 soil under simulated Martian conditions (see section 4) (e.g. Mancinelli & Klovstad, 2000; Schuerger *et al.*, 2003; Diaz & Schulze-Makuch, 2006). Also, the Mars-1 soil has been incubated under simulated Martian conditions for assessment of the degradation rates of the indigenous amino acids (see section 4) (Garry *et al.*, 2006).

Salten 1 is a sediment from a Danish beech forest in the central part of Jutland. This soil is rich in the iron-oxides goethite, maghemite and hematite (Nørnberg *et al.*, 2004) and due to its magnetic properties it has been used as a Mars-analogue dust in laboratory simulation experiments (e.g. Merrison *et al.*, 2002; Merrison *et al.*, 2004a; Kinch *et al.*, 2006). The most significant difference between the Salten 1 soil and the Martian soil is the high content of organic matter in Salten 1 (Nørnberg *et al.*, 2004). To date two laboratories have used Salten 1 as a Mars-analogue soil in experiments to replicate magnetic property experiments as carried out on Mars (Merrison *et al.*, 2002; Kinch *et al.*, 2006) and to test and develop instruments for future Mars missions, e.g. instruments for measurements of wind speeds (Merrison *et al.*, 2004a) and electrical properties of the Martian dust (Merrison *et al.*, 2004b). Moreover, Salten 1 has been incubated under simulated Martian conditions for assessment of the effect of simulated Martian conditions on the indigenous bacterial community (see manuscript I, chapter 2) and on the indigenous amino acids (see section 4) (Garry *et al.*, 2006).

Apart from the Mars-analogue soils, the Rio Tinto river basin in Spain is also recognized as a terrestrial Mars-analogue environment due to its geochemical and mineralogical characteristics (Fernandez-Remolar *et al.*, 2004; Squyres & Knoll, 2005). Rio Tinto is an extremely acidic environment (pH: 1.1-5.2) rich in the iron-oxides hematite, ferrihydrite and schwertmannite and the sulfate-mineral jarosite (Fernandez-Remolar *et al.*, 2005). Jarosite and hematite have been identified at the Meridiani Planum on Mars (Squyres & Knoll, 2005) and a better understanding of the past sedimentary processes of these minerals on Mars have been facilitated by the investigation of the mineralogical history of Rio Tinto (Fernandez-Remolar *et al.*, 2005; Squyres & Knoll, 2005).

The soil from the driest areas of the Atacama Desert in Chile has been proposed as a model for the Martian soil (Navarro-Gonzalez *et al.*, 2003; Banin, 2005). This is not due to the mineralogy of the soil, but the aridity of the region and the oxidizing nature of the soil, combined with the relative low levels of both organic matter (~13 ppm) and bacteria ($\sim 7 \times 10^5$ cells g⁻¹) (Navarro-Gonzalez *et al.*, 2003; Glavin *et al.*, 2004). Therefore, the Atacama Desert has been used as a testing ground for instruments and technologies for future Mars missions (Cabrol *et al.*, 2001; Glavin *et al.*, 2004; Skelley *et al.*, 2005).

Altogether, the terrestrial analogues of the Martian soil are models of the surface soil of present-day Mars. Thus, the described terrestrial soil analogues are only appropriate when applied in experiments related to the Martian surface. The terrestrial soil analogues are not appropriate in the search for putative Martian life, since the environment of the Martian surface soil is not expected to be life-supporting (see section 2.2). However, in a biological context the terrestrial soil analogues are appropriate for application in Mars simulation experiments investigating the degradation rate of organic material on the Martian surface and in investigations of the possibility of forward contamination of Mars with terrestrial bacteria (section 4 and manuscript I, chapter 2).

Considered together, chemical, physical and biological experiments would not necessarily need a natural Mars soil analogue, but could be carried out with an artificial Mars soil analogue constructed from the relevant minerals. Thereby, the soil could be designed according to the relevant experiment and a closer match of the Martian mineralogy could be achieved.

3.2 Life in terrestrial Mars-analogue habitats

Terrestrial habitats proposed as Mars-analogues are generally characterized as extreme environments in respect of water activity, temperature, salinity or pH, e.g. the Dry Valleys of Antarctica, the Atacama Desert, permafrost soils, evaporites and acidic lakes (Rothschild, 1990;

Gilichinsky, 2001; Benison & Bowen, 2006; Wierzchos *et al.*, 2006). Whether all of these habitats are appropriate Mars-analogues is difficult to access as long as the subsurface environment of Mars remains uncharacterized. Therefore, validity of terrestrial habitats as Mars-analogues is speculative. In the following, terrestrial life in the most probable Mars-analogue habitats (with reference to section 2.2) will be addressed.

The most arid zones of the Antarctic Dry Valleys and the Atacama Desert are considered probable analogues for the surface environment of present-day Mars due to low water activity (Friedmann, 1982; Navarro-Gonzalez *et al.*, 2003). Life in these arid habitats is very sparse and the only described life form is endolithic microorganisms growing within rocks (Friedmann, 1982; Wierzchos *et al.*, 2006). The endolithic communities in the Antarctic Dry Valleys consist mainly of lichens (fungi and green algae) and to a minor degree cyanobacteria (*Chroococciopsis*) (Friedmann, 1982). The described endolithic community from the Atacama Desert consists also of *Chroococciopsis*, which is found associated with unidentified heterotrophic bacteria (Wierzchos *et al.*, 2006). Thereby, the life forms known from the most arid habitats on Earth are found to be dependent on photosynthesis, which in the context of the Martian surface is a highly unlikely strategy (see section 2.2). Therefore, the relevance of these endolithic communities in relation to Mars is questionable. Even so, a species of *Chroococciopsis* have been incubated under simulated Martian conditions for assessment of the effect of the simulated Martian conditions on the survival rate of the bacteria (see section 4) (Cockell *et al.*, 2005). Soil samples from the most arid regions of both the Antarctic Dry Valleys and the Atacama Desert have been found sterile (Horowitz *et al.*, 1972; Navarro-Gonzalez *et al.*, 2003), the absence of life in these soils may indeed suggest that they are good analogues of Martian surface conditions.

Little is known about the Martian permafrost layers, but if existent, they are likely to be characterized by sub-zero temperatures, low water activities and local water brines, as know from the terrestrial permafrost soils (Gilichinsky *et al.*, 2005). The terrestrial permafrost areas have been proposed to be an appropriate analogue of the Martian permafrost environment (Horneck, 2000; Gilichinsky, 2001) and samples of permafrost soils have been incubated under simulated Martian conditions to assess of the effect of simulated Martian conditions on the indigenous bacterial community and organic molecules (see manuscript III, chapter 4). The continuous zones of terrestrial permafrost soils are predominantly found in North America, Eurasia and Antarctica (Gilichinsky, 2002). The oldest terrestrial permafrost layers are 3-4 million years (Ma) old (Gilichinsky *et al.*, 1995) and have a vertical extent of approximately 1500 meters (Péwé, 2006). This is somewhat younger and not as deep as expected of the Martian permafrost layers, which have

been suggested to be 3-4 Ga old (Smith & McKay, 2005) and extend to a depth of several kilometers depending on the geothermal flux (Frolov, 2003).

High quantities of prokaryotic cells are present in 2-3 Ma old layers of Siberian permafrost soil ($\sim 10^7$ cells gdw^{-1}) (Vishnivetskaya *et al.*, 2000) and viable anaerobic and aerobic prokaryotes have been recovered from layers of similar age (Rivkina *et al.*, 1998; Vishnivetskaya *et al.*, 2006). Non-spore-forming *Actinobacteria* have been found to dominate the culturable fraction of the bacterial community (see manuscript II, chapter 3. Kochkina *et al.*, 2001), but endospore-formers and Gram-negative bacteria have also been isolated (see manuscript II, chapter 3. Bakermans *et al.*, 2003).

The prokaryotic communities in the terrestrial permafrost layers are most likely isolated from exogenic input of organic energy and it has yet to be established whether the permafrost bacteria are active or in an anabiotic state under *in situ* conditions (Soina *et al.*, 2004; Suzina *et al.*, 2004). However, methanogens utilizing H_2 and CO_2 have been detected in Siberian permafrost samples (Rivkina *et al.*, 1998) and could be autotrophic primary producers of an active terrestrial permafrost community. This further suggests that the prokaryotic organisms in the terrestrial permafrost layers are appropriate analogues of in the Martian subsurface environments, where H_2 and CO_2 have been speculated to be available (see section 2.2). Yet, more information on the physical and chemical characteristics of the Martian permafrost layers is needed to evaluate whether data obtained on the biology and biochemistry of terrestrial permafrost soils can in fact be extrapolated to Martian conditions.

Existence of subsurface hydrothermal systems on present-day Mars has been speculated (Pirajno & Van Kranendonk, 2005). Yet, no evidence of current hydrothermal activity has been established (see section 2.2). The terrestrial hydrothermal systems are mostly driven by magmatic heat and they are found in connection with plate tectonics at spreading centres, intracontinental rifts, continental margins and subduction zones (Pirajno & Van Kranendonk, 2005).

The primary producers found in terrestrial deep-submarine hydrothermal systems are chemolithoautotrophic prokaryotes supplied with chemical energy from the hydrothermal fluids (H_2 , H_2S , CH_4 , Fe^{2+} , Mn^{2+}) (Edwards *et al.*, 2005). Most of the lithoautotrophs are aerobic prokaryotes dependent of oxygen supplied from the surface photosynthesis (Nealson, 1999). This is an unlikely scenario on the nearly anoxic planet Mars. However, anaerobic lithoautotrophic methanogens and sulfate reducers are also present in terrestrial hydrothermal systems. These anaerobic lithoautotrophs could be sustainable life forms on present-day Mars due to utilization of H_2 (see section 2.2) (Varnes *et al.*, 2003). Even so, the terrestrial submarine hydrothermal systems are dependent of the water-rock interface to create the fluid circulations (Pirajno & Van

Kranendonk, 2005) and a similar submarine environment seems unlikely on present-day Mars. Thereby, the analogy of terrestrial submarine hydrothermal systems with present-day Mars remains theoretical.

Altogether, only future Mars missions investigating the subsurface environment of Mars will reveal whether the life-supporting Mars-analogue habitats described above are appropriate analogues. However, further investigations of the subsurface habitats on Earth will most likely facilitate interpretation and understanding of subsurface environments on Mars.

4. Mars simulation experiments

(Most of the contents of this section are also presented as a part of manuscript IV, chapter 5).

One area of established astrobiological research is the investigation of the response of terrestrial prokaryotes and organic molecules to simulated Martian conditions. These investigations have focused on the response to present-day Martian surface conditions, not only because knowledge about possible life on Mars has been required when planning space missions, but also because knowledge of the ancient Martian climate was not available when the first experiments were carried out in 1958.

The pioneers initiating these Mars simulation experiments were motivated by the emerging space age and the question “could life exist beyond Earth” was of main focus in their investigations (Kooistra *et al.*, 1958). Also in focus was the risk of forward contamination when exploring other planets and the possibility of terraforming, a process to change the environment of extraterrestrial planets to make them habitable (Fulton, 1958; Davis & Fulton, 1959). Today, the major motivation for Mars simulation experiments is the risk of forward contamination.

The survival of prokaryotes has been investigated in the majority of the Mars simulation experiments. The focus on prokaryotes is attributed to the general supported assumption that life, if ever evolved on Mars must be unicellular organisms. This assumption is based upon the nearly ubiquitous existence of prokaryotes on Earth, which also makes them the most probable contaminants in connection with Mars missions.

The first simulation experiments investigated the effect of Martian conditions on prokaryotic communities in soil (Fig. 4) (Fulton, 1958; Kooistra *et al.*, 1958). However, the focus shifted rapidly to investigate the survival of different prokaryotic pure-cultures, which has been the principal approach of most simulation experiments since the beginning of the 1960's (Fig. 4). A major part of the Mars simulation experiments was carried out in the period 1958-1976 (Fig. 4),

which probably was related to a wider general interest in Mars in the 1960's and 1970's, when many space programs focused on the planet (for a review see Sheehan, 1996). However, interest in simulation experiments stopped at the end of the 1970's and did not recommence until the mid 1990's (Fig. 4). An explanation of this sudden decrease in interest in Mars simulation experiments is probably related to the non-biological conclusions of the Viking missions (see section 1.1). The resumption of Mars simulation experiments in 1992 was

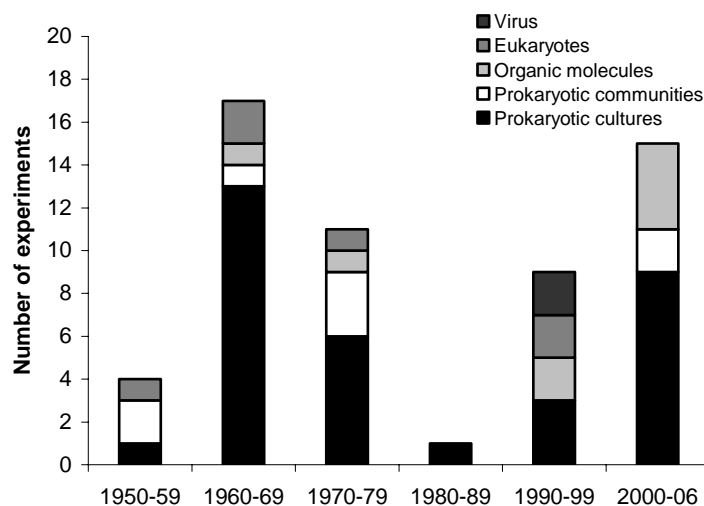


Figure 4. Timeline of Mars simulation experiments reported in literature. The different categories are illustrating the type of biological sample incubated under simulated Martian conditions. When a study included more than one type of sample it is represented in all relevant categories. References are given in the appendix of this chapter.

concurrent with the resumption of the American missions to Mars. Over the past twenty years the many successful missions to Mars have increased our knowledge of the planet and stimulated the astrobiological interest in Mars, including Mars simulation experiments (Fig. 4). Especially in the past year a relatively large number of Mars simulation experiments have been reported.

4.1 Incubation conditions in Mars simulation experiments

The evolution of facilities for simulation of Martian conditions has progressed over time from very simple anoxic systems to highly sophisticated simulation chambers. The simulated conditions of the early experiments were based on indirect modeling and calculations of the Martian conditions and could hardly be called Martian; they resembled more traditional anoxic incubations using anoxic tubes or anaerobic jars (Table 3). The only modification compared to conventional anoxic incubations of prokaryotes was diurnal temperature cycles achieved by alternately moving the samples from a freezer to room temperature. Table 3 gives the incubation conditions applied in a selection of the Mars simulation experiments.

Despite the obvious benefits of simulating Martian temperature, pressure, atmosphere and solar radiation simultaneously, only six of the 26 reported studies in the period 1958-1990 used simulation chambers (Table 3) (Zhukova & Kondratyev, 1965; Belikova *et al.*, 1968; Lozina-Lozinsky & Bychenkova, 1969; Hagen *et al.*, 1970; Green *et al.*, 1971; Imshenetskii *et al.*, 1984). In the same period, only eight studies included UV radiation, where five of these used simulation

chambers for their simulations (Table 3) (Packer *et al.*, 1963; Zhukova & Kondratyev, 1965; Imshenetsky *et al.*, 1967; Belikova *et al.*, 1968; Hagen *et al.*, 1970; Green *et al.*, 1971; Oro & Holzer, 1979; Imshenetskii *et al.*, 1984). Today, a lot of effort is made to construct automated simulation facilities (see manuscript V, chapter 6) and most studies use simulation chambers and include UV radiation (Table 3). UV radiation is an important component of simulated Martian conditions, since it has been identified to be the most harmful parameter to prokaryotes, when incubated under simulated Martian conditions (see manuscripts I,III, chapter 2,4).

The characteristics of the UV radiation applied in the different studies have generally been poorly defined in terms of either radiation spectrum or intensity dose. However, the type of UV lamps applied in the different studies can be used to identify whether the simulated UV radiation corresponds to the current Martian UV models. In total, only nine studies have used xenon lamps to generate UV light (Table 3) (Zhukova & Kondratyev, 1965; Green *et al.*, 1971; Oro & Holzer, 1979; Stoker & Bullock, 1997; Schuerger *et al.*, 2003; Cockell *et al.*, 2005; Newcombe *et al.*, 2005; Schuerger *et al.*, 2005; Schuerger *et al.*, 2006). UV light generated from xenon lamps is now considered to most closely simulate the present Martian UV environment in terms of the fluence rates of the different wavelengths (Schuerger *et al.*, 2003). Other studies have used mercury lamps (Packer *et al.*, 1963; Imshenetsky *et al.*, 1967; Belikova *et al.*, 1968; Hagen *et al.*, 1970; Oro & Holzer, 1979; Imshenetskii *et al.*, 1984; Gontareva, 2005), a combination of xenon-mercury lamps (see manuscripts I,III, chapter 2,4), deuterium or hydrogen lamps (Koike *et al.*, 1995; Koike *et al.*, 1996; Mancinelli & Klovstad, 2000; ten Kate *et al.*, 2005; Diaz & Schulze-Makuch, 2006; Garry *et al.*, 2006). All these light sources have a relatively higher fluence rate in the UVC region (200-280 nm) than found on Mars. Therefore, these lamps probably generate a simulated environment that is more harmful than the climate expected on Mars. Furthermore, the spectra of mercury, deuterium and hydrogen lamps are relatively narrow and do not include the full spectrum of visible (VIS) and infra red (IR) light (700-2500 nm) as do xenon lamps and the incident solar radiation on Mars. Due to these differences in UV light simulations, there has been an increased focus in designing UV light sources which have a spectrum and irradiance levels equivalent to those found on Mars (manuscript VII, chapter 8; Zill *et al.*, 1979; Schuerger *et al.*, 2003). Hopefully, future simulation experiments will employ Mars equivalent solar radiation and thereby provide a more realistic understanding of the biocidal nature of the solar radiation environment on Mars.

Table 3. Incubation conditions applied in studies on the biological response to simulated Martian conditions. Included for reference are the conditions at the surface of present-day Mars. Only a selection of the reported experiments is included.

Year	Incubation method	Temperature (°C)	Pressure (mbar)	Atmospheric composition (%)	Solar radiation (nm)	Solar radiation lamp	Water addition (%)	Nutrient addition	Reference
Present Mars	-	-123 - +25	6.7-9.9	CO₂ 95.3	0.13	>200	-	-	-
1958	Anaerobic Jar	-25/25 [†]	87	-	1.6	0.13	-	1	(Fullton, 1958)
	Anaerobic Jar	-22/25 [†]	72	-	-	-	-	1	(Kooistra <i>et al.</i> , 1958)
1959	Anaerobic Jar	-25/25 [†]	87	-	-	-	-	1	(Davis and Fulton, 1959)
1962	Anoxic tubes	-25/25 [†]	~0/87	-	-	-	-	-	(Hawrylewicz <i>et al.</i> , 1962)
1963	Anaerobic Jar	-60/20 [†]	100	5	-	254 ^a	Mercury	-	(Packer <i>et al.</i> , 1963)
	Anoxic tubes	-75/25 [†]	1013 ^b	-	-	-	100	+	(Young <i>et al.</i> , 1963)
1964	Anoxic tubes	-60/26 [†]	113	2.2	4	-	<0.5	+	(Hagen <i>et al.</i> , 1964)
1965	Anoxic tubes	-65/25 [†]	113	2.2	4	-	<1	+	(Hawrylewicz <i>et al.</i> , 1965)
	Mars facility	-60/25 [†]	100	0.25	0.25	200-2500	Xenon	-	(Zhukova and Kondratyev, 1965)
1967	Anoxic tubes	-65/28 [†]	113	2.2	4	-	+/-	+	(Hagen <i>et al.</i> , 1967)
	Tubes	-60/25 [†]	1013 ^b	0.03 ^b	0.93 ^b	254	Mercury	+	(Imshenetsky <i>et al.</i> , 1967)
1968	Mars facility	-64/28 [†]	100	-	-	240-280	Mercury	100	(Belikova <i>et al.</i> , 1968)
	Anoxic tubes	-65/30 [†]	10-40	37-100	21,30	-	-	+	(Hawrylewicz <i>et al.</i> , 1968)
1969	Mars facility	18-20 [‡]	7.1-60	-	99	-	100	-	(Lozina-Lozinsky and Bychenkova, 1969)
1970	Mars facility	-65/30 [†]	20	67	3	200-300	Mercury	1-2	(Hagen <i>et al.</i> , 1970)
1971	Mars facility	-60/25 [†]	8	70	5	200-2500	Xenon	<1	(Green <i>et al.</i> , 1971)
	Anoxic tubes	-25/25 [†]	13	99	-	-	-	5	(Lozina-Lozinsky <i>et al.</i> , 1971)
1973	Anoxic tubes	-60/28 [†]	7	80	20	-	-	+	(Imshenetsky <i>et al.</i> , 1973)
1974	-	-65/25 [†]	7	80	20	-	-	-	(Foster and Winans, 1974)
1978	Anoxic tubes	-65/24 [†]	7	99.9	-	-	-	-	(Foster <i>et al.</i> , 1978)
1979	Tubes	-10 - +25 [‡]	0.001	-	100	+	Mercury/xenon	-	(Oro and Holzer, 1979)
1984	Mars facility	-80/20 [†]	7-9	95	2-3	254	Mercury	<0.4	(Imshenetskii <i>et al.</i> , 1984)
1992	Anoxic tubes	-70 [‡]	13	95.52	2.73	-	-	+/-	(Moll and Vestal, 1992)
1995	Mars facility	-160 - +50 [‡]	0.001	95.46	2.7	115-400	Hydrogen	-	(Koike <i>et al.</i> , 1995)
1996	Mars facility	60 [‡]	10	95.46	2.7	115-400	Hydrogen	-	(Koike <i>et al.</i> , 1996)
1997	Mars facility	Room temp	100	95.59	-	210-710	Xenon	-	(Stoker and Bullock, 1997)
1998	Tubes	-23-+10 [‡]	1013 ^b	-	-	-	-	100	(McDonald <i>et al.</i> , 1998)
2000	Mars facility	25 [‡]	1013 ^b	0.03 ^b	78.1 ^b	200-400	Deuterium	-	(Mancinelli and Klovstad, 2000)
2003	Mars facility	-10 [‡]	8.5	95.3	2.7	200-2500	Xenon	-	(Schuenger <i>et al.</i> , 2003)
	Mars facility	-60 [‡]	6	98	1.7	-	-	+/-	(Stan-Lotter <i>et al.</i> , 2003)
2005	Mars facility	-10 [‡]	8.5	100	-	200-2500	Xenon	-	(Cockell <i>et al.</i> , 2005)
	Mars facility	-95 - +12 [‡]	9-13	77.5	8.7	200-2500	Xenon-Hg	-	(Manuscript I, chapter 2)
	Mars facility	20 [‡]	12.5	100	-	-	-	-	(Nicholson and Schuenger, 2005)
	Mars facility	Room temp	4×10 ⁶	-	-	120-180	Hydrogen	-	(ten Kate <i>et al.</i> , 2005)
2006	Mars facility	-63	7	100	-	190-400	Deuterium	-	(Garry <i>et al.</i> , 2006)
	Mars facility	-41 - +11	7.6-9.7	91	5	190-325	Deuterium	-	(Manuscript III, chapter 4)
						200-2500	Xenon-Hg	-	

[†]Diurnal cycles between the two temperatures, [‡]Constant temperature, ^bEarth conditions, - not included in the simulation experiment.

4.2 Simulation experiments with prokaryotic communities

The effect of simulated Martian conditions on prokaryotic communities has been investigated by incubation of soil samples, and thereby of the indigenous soil prokaryotes, under simulated Martian conditions. In order to detect changes caused by the incubation conditions, the prokaryotic communities have to be thoroughly characterized prior to the simulation experiments. Therefore, investigations with prokaryotic communities are generally laborious. Moreover, because environmental samples often are heterogeneous, it is difficult to ensure exposure of the entire prokaryotic community to the same conditions. However, community studies are very informative because the selection of the prokaryotic populations, as a result of exposure to simulated Martian conditions, provides information on which prokaryotic groups can survive and perhaps even on the physiological characteristics that enable these prokaryotes to survive. Furthermore, incubation of the indigenous prokaryotes in environmental samples minimizes manipulation of the communities prior to incubation in the simulation experiments.

Natural prokaryotic communities have been investigated in eight experiments (manuscripts I,III, chapter 2,4; Fulton, 1958; Kooistra *et al.*, 1958; Packer *et al.*, 1963; Green *et al.*, 1971; Foster & Winans, 1974; Foster *et al.*, 1978). The samples studied in all but one of the experiments have been surface soils from the North Temperate Zone, where the climate is warmer than on Mars (Table 4). These soils have been investigated because of a high content of iron-oxides (manuscripts I, chapter 2; Fulton, 1958) or because the soils originated from cold, dry or alkaline environments (Packer *et al.*, 1963). Additionally, soils from the manufacture area of the Viking spacecraft have been investigated because these areas could be possible sources of contaminants (Foster & Winans, 1974; Foster *et al.*, 1978). Altogether, in the choice of most of the investigated soils, mineralogical similarities to the Martian soil have been the major focus. However, the climatic environment is also important to the composition of the soil communities and therefore, in our recent study a permafrost soil community was investigated (manuscript III, chapter 4). Permafrost bacteria are expected to be pre-adapted to constant sub-zero temperatures and low water activity and might therefore be a good model for putative Martian life (see section 3.2; manuscript III, chapter 4).

The incubation-times of prokaryotic communities under simulated Martian conditions have ranged from 8 days to 10 months. The simulated conditions have varied among the investigations and only four of the experiments included UV radiation (Table 3). The effects of the simulated conditions on the prokaryotic communities have almost exclusively been evaluated by quantification of the number of surviving prokaryotes using traditional plate cultivation techniques. However, to further investigate the effect on both the viable and dead fractions of the bacterial communities we included

Table 4. Soil samples investigated in Mars simulation experiments with prokaryotic communities.

Soil sample	Origin of soil	Reference
Four types of soil (ND)	ND	(Kooistra <i>et al.</i> , 1958)
Iron-rich, red sandstone and black lava soil	Arizona	(Fulton, 1958)
Soil from cold, dry and alkaline environment	California	(Packer <i>et al.</i> , 1963)
Composite soil (ND)	ND	(Green <i>et al.</i> , 1971)
Soil from manufacture area of spacecraft	Florida	(Foster & Winans, 1974)
Soil associated with Viking spacecraft	Florida	(Foster <i>et al.</i> , 1978)
Salten Skov 1	Denmark	(Manuscript I, chapter 2)
Permafrost soil	Spitsbergen	(Manuscript III, chapter 4)

ND, not further described.

direct staining techniques, activity measurements and identification by sequencing in our recent studies (see manuscript I,III, chapter 2,4). The differences in the experimental setups of the simulation experiments with prokaryotic communities make it somewhat difficult to compare the results obtained. Nevertheless, the overall pattern of the results will be addressed in the following.

In simulation experiments not including UV radiation, loss in prokaryotic viability has been observed at the surface of the incubated soil (Green *et al.*, 1971). Additionally, when UV radiation has been included in the experiments, nearly all prokaryotes at the soil surface exposed to the UV radiation were killed (manuscript III, chapter 4; Packer *et al.*, 1963; Green *et al.*, 1971), while prokaryotes in the subsurface soils (>3 cm) were left unaffected due to protection by soil particles (manuscript I,III, chapter 2,4; Packer *et al.*, 1963; Green *et al.*, 1971). The negative effect of UV radiation on prokaryotic viability and activity has been detected down to 3 and 30 mm depth, respectively (manuscript I,III, chapter 2,4). However, UV radiation has been shown to be attenuated by 0.5-1 mm soil (Schuerger *et al.*, 2003; Cockell *et al.*, 2005), suggesting that indirect UV effects, such as production of reactive oxygen species, must be responsible for the negative effects observed in deeper soil layers (>1 mm) (manuscript I,III, chapter 2,4). Another identified detrimental factor of the simulated Martian conditions is the freeze-thaw cycles also decreasing the viability of subsurface prokaryotes (manuscript III, chapter 4; Packer *et al.*, 1963). Moreover, the negative impact of the simulated Martian conditions on the permafrost community and the soil communities from the North Temperate Zone was comparable (manuscript III, chapter 4).

Generally, endospore-forming bacteria dominated the surviving fraction of the prokaryotic community in the soils from the North Temperate Zone (manuscript I, chapter 2; Packer *et al.*, 1963; Foster *et al.*, 1978) and only few non-sporeforming prokaryotes were among the survivors (Packer *et al.*, 1963). Thus, the majority of the soil prokaryotes probably survived as spores under simulated Martian conditions. However, in the permafrost soil, Gram-positive non-spore forming *Actinobacteria* dominated the surviving fraction of the prokaryotic community (manuscript III, chapter 4). Thereby, the results indicate that Gram-positive rather than Gram-negative bacteria

survive simulated Martian conditions. Nevertheless, care should be taken not to extrapolate from these few soil communities, of which some were dominated by Gram-positive bacteria even before the incubations (manuscript I,III, chapter 2,4).

Interestingly, in three community studies cell division under the simulated conditions was observed. In these studies the moisture content of the soil was increased to 1% by addition of water (Table 3) (Fulton, 1958; Kooistra *et al.*, 1958; Foster *et al.*, 1978), which probably made the incubations comparable to anoxic enrichments with the freezing period as daily interruptions. Growth correlated with the amount of moisture added to the soil and no growth was observed in dry soils (Foster *et al.*, 1978). Therefore, the amount of moisture was probably the limiting factor of growth under simulated Martian conditions without UV radiation.

Overall, Mars simulation experiments with prokaryotic communities have revealed that some prokaryotes, mostly Gram-positive bacteria, are able to survive simulated Martian conditions. Moreover, UV radiation has been identified as the most selective factor influencing both survival and activity after incubation under simulated Martian conditions. Additionally, freeze-thaw cycles affect the prokaryotic viability negatively. No growth or activity has been observed during incubations without supplement of moisture.

So far only few modern simulation experiments with prokaryotic communities have been carried out and further studies are needed to understand which prokaryotic groups are surviving the simulated Martian conditions preferentially.

4.3 Simulation experiments with prokaryotic pure-cultures

Studies with pure-cultures as model organisms have many advantages since it is relatively easy to design simple experimental systems. Moreover, pure-culture studies provide the opportunity not only to investigate if the prokaryotes are affected by the simulated conditions, but also how they are affected. This can be achieved by mutants of prokaryotic strains allowing examination of the effect at the molecular level, but also by investigating specific characters of the pure-cultures e.g. biomolecule survival (Cockell *et al.*, 2005) and spore germination (Nicholson & Schuerger, 2005). However, it is difficult to extrapolate the results from one laboratory mono-culture to other pure-cultures or to organisms in nature. Nevertheless, the information gained by the model cultures is valuable since it provides a broader insight into the possible impacts of Martian conditions on prokaryotes.

Many different cultures of bacteria and a few pure-cultures of Archaea have been investigated under simulated Martian conditions (Table 5). The cultures studied have mainly been common soil organisms, which cover a broad range of bacterial types, primarily Gram-positive, but also Gram-negative bacteria (Table 5). Bacterial endospores are known to be tolerant to extreme environmental conditions e.g. heat, UV irradiation and low pressures (reviewed in Nicholson *et al.*, 2000) and have therefore been the model of choice in many space simulation experiments (Table 5). The most intensively studied endospore-forming bacterium is *Bacillus subtilis*. Due to its high resistance to harsh conditions and because it is very well described, most modern Mars simulation experiments have used *B. subtilis* as a model organism (e.g. Mancinelli & Klovstad, 2000; Schuerger *et al.*, 2003; Nicholson & Schuerger, 2005; Schuerger *et al.*, 2005). Nevertheless, over time, physiologically different cultures have been studied including aerobic bacteria belonging to the genera *Bacillus*, *Micrococcus* and *Azotobacter* (Moll & Vestal, 1992; Koike *et al.*, 1996; Schuerger *et al.*, 2003); facultative anaerobic bacteria e.g. *Escherichia coli*, *Serratia marcescens* and *Pseudomonas* species (Hagen *et al.*, 1970; Lozina-Lozinsky *et al.*, 1971) and obligate anaerobic bacteria such as members of the genus *Clostridium* (Koike *et al.*, 1996). Most of the cultures studied are heterotrophic bacteria, but also photoautotrophic cultures have been studied e.g. the endolithic cyanobacterium *Chroococcidiopsis* sp. (see section 3.2)(Cockell *et al.*, 2005) and the purple nonsulfur bacterium *Rhodospirillum rubrum* (Roberts, 1963). Additionally, dinitrogen fixing cultures have been included in some investigations (*Azotobacter* and *Rhodospirillum* species) (Roberts, 1963).

Most of the cultures studied have been selected for investigation, because they can survive harsh conditions and maybe even tolerate Martian conditions, which makes them appropriate candidates for contamination studies (Hagen *et al.*, 1970; Schuerger *et al.*, 2003; Nicholson & Schuerger, 2005). Only one study has investigated pure-cultures isolated from soil that previously had been incubated under Martian conditions (Davis & Fulton, 1959). However, these types of isolates will have a great significance in identifying characters allowing specific prokaryotes to survive Martian conditions and should therefore receive more attention in future experiments.

Different approaches have been used to incubate prokaryotic cultures under Martian conditions. Most often the pure-cultures were inoculated in a sterile Mars-analogue soil or minerals or a mixture of both e.g. Mars-1 soil analogue (see section 3.1) (e.g. Mancinelli & Klovstad, 2000; Diaz & Schulze-Makuch, 2006), volcanic palagonite (Cockell *et al.*, 2005), montmorillonite (Moll & Vestal, 1992), limonite (Green *et al.*, 1971) or mixtures of limonite and felsite (Hawrylewicz *et al.*, 1962; Hagen *et al.*, 1964; Imshenetsky *et al.*, 1967). Other investigations have exposed the

Table 5. Prokaryotic pure cultures investigated in Mars simulation experiments.

Culture	Reference
Gram-positive bacteria	
Firmicutes	
<i>Bacillus cereus</i> [†]	(Roberts, 1963; Hawrylewicz <i>et al.</i> , 1965; Hagen <i>et al.</i> , 1967; Hawrylewicz <i>et al.</i> , 1968; Hagen <i>et al.</i> , 1970; Lozina-Lozinsky <i>et al.</i> , 1971)
<i>Bacillus licheniformis</i> [†]	(Schuerger <i>et al.</i> , 2006)
<i>Bacillus megaterium</i> [†]	(Imshenetsky <i>et al.</i> , 1967; Imshenetskii <i>et al.</i> , 1979; Newcombe <i>et al.</i> , 2005; Schuerger <i>et al.</i> , 2006)
<i>Bacillus mycoides</i> [†]	(Imshenetskii <i>et al.</i> , 1984)
<i>Bacillus nealsonii</i> [†]	(Schuerger <i>et al.</i> , 2006)
<i>Bacillus psychrodurans</i> [†]	(Newcombe <i>et al.</i> , 2005)
<i>Bacillus psychrosaccharolyticus</i> [†]	(Green <i>et al.</i> , 1971)
<i>Bacillus pumilus</i> [†]	(Lozina-Lozinsky <i>et al.</i> , 1971; Imshenetskii <i>et al.</i> , 1984; Newcombe <i>et al.</i> , 2005; Schuerger <i>et al.</i> , 2006)
<i>Bacillus odyseyi</i> [†]	(Newcombe <i>et al.</i> , 2005)
<i>Bacillus subtilis</i> [†]	(Hagen <i>et al.</i> , 1964; Zhukova & Kondratyev, 1965; Hagen <i>et al.</i> , 1967; Hagen <i>et al.</i> , 1970; Green <i>et al.</i> , 1971; Imshenetskii <i>et al.</i> , 1984; Moll & Vestal, 1992; Koike <i>et al.</i> , 1995; Koike <i>et al.</i> , 1996; Mancinelli & Klovstad, 2000; Schuerger <i>et al.</i> , 2003; Newcombe <i>et al.</i> , 2005; Nicholson & Schuerger, 2005; Schuerger <i>et al.</i> , 2005; Schuerger <i>et al.</i> , 2006)
<i>Clostridium botulinum</i> [†]	(Hawrylewicz <i>et al.</i> , 1962)
<i>Clostridium butyricum</i> [†]	(Koike <i>et al.</i> , 1996)
<i>Clostridium celatum</i> [†]	(Koike <i>et al.</i> , 1996)
<i>Clostridium manganotii</i> [†]	(Koike <i>et al.</i> , 1995; Koike <i>et al.</i> , 1996)
<i>Clostridium propionicum</i> [†]	(Koike <i>et al.</i> , 1995; Koike <i>et al.</i> , 1996)
<i>Clostridium roseum</i> [†]	(Koike <i>et al.</i> , 1996)
<i>Lactobacillus plantarum</i>	(Hawrylewicz <i>et al.</i> , 1968)
<i>Staphylococcus aureus</i>	(Zhukova & Kondratyev, 1965; Hawrylewicz <i>et al.</i> , 1968; Hagen <i>et al.</i> , 1970; Koike <i>et al.</i> , 1995)
<i>Streptococcus mutans</i>	(Koike <i>et al.</i> , 1995)
Actinobacteria	
<i>Kocuria rosea</i>	(Imshenetskii <i>et al.</i> , 1979)
<i>Luteococcus japonicus</i>	(Zhukova & Kondratyev, 1965)
<i>Micrococcus luteus</i>	(Zhukova & Kondratyev, 1965; Lozina-Lozinsky <i>et al.</i> , 1971; Koike <i>et al.</i> , 1995; Koike <i>et al.</i> , 1996)
<i>Streptomyces albus</i>	(Hawrylewicz <i>et al.</i> , 1968)
<i>Streptomyces coelicolor</i>	(Koike <i>et al.</i> , 1995)
Gram-negative bacteria	
Deinococci	
<i>Deinococcus radiodurans</i>	(Diaz & Schulze-Makuch, 2006)
Cyanobacteria	
<i>Chroococcidiopsis</i> sp.	(Cockell <i>et al.</i> , 2005)
Alpha proteobacteria	
<i>Rhodospirillum rubrum</i>	(Roberts, 1963)
Gamma proteobacteria	
<i>Azotobacter chroococcum</i>	(Moll & Vestal, 1992)
<i>Azotobacter vinelandii</i>	(Roberts, 1963)
<i>Enterobacter aerogenes</i>	(Young <i>et al.</i> , 1964)
<i>Escherichia coli</i>	(Hagen <i>et al.</i> , 1970; Koike <i>et al.</i> , 1995; Koike <i>et al.</i> , 1996; Diaz & Schulze-Makuch, 2006)
<i>Klebsiella pneumoniae</i>	(Hawrylewicz <i>et al.</i> , 1962)
<i>Photobacterium</i> sp.	(Zhukova & Kondratyev, 1965)
<i>Pseudomonas aeruginosa</i>	(Hawrylewicz <i>et al.</i> , 1968; Lozina-Lozinsky <i>et al.</i> , 1971)
<i>Pseudomonas fluorescens</i>	(Lozina-Lozinsky <i>et al.</i> , 1971; Imshenetskii <i>et al.</i> , 1984)
<i>Serratia marcescens</i>	(Hagen <i>et al.</i> , 1970)
Archaea	
Euryarchaeota	
<i>Halobacterium</i> sp.	(Stan-Lotter <i>et al.</i> , 2003)
<i>Halobacterium salinarum</i>	(Koike <i>et al.</i> , 1995)
<i>Halococcus dombrowskii</i>	(Stan-Lotter <i>et al.</i> , 2003)

[†]Endospore-forming species.

air-dried cultures directly to the simulated environment (Zhukova & Kondratyev, 1965; Schuerger *et al.*, 2003; Cockell *et al.*, 2005), wrapped the dried cultures in foil (Koike *et al.*, 1995; Koike *et al.*, 1996), incubated the cultures in aqueous solution (Young *et al.*, 1963; Stan-Lotter *et al.*, 2003; Newcombe *et al.*, 2005) or on agar plates (Imshenetsky *et al.*, 1973).

The effect of simulated Martian conditions has in most experiments exclusively been evaluated by the survival of the cultures. Only in a few studies other experimental parameters have been evaluated along with survival. A multi-methodological approach, where the cultures are analysed at both the molecular, cell and population level, can however be advantageous since it provides the opportunity to investigate how the prokaryotes are affected and therefore explain the observed prokaryotic response. Moreover, it potentially allows identification of the non-lethal effects of simulated Martian conditions on prokaryotic cultures e.g. on metabolic activity and total biomass.

Usually, the survival of the prokaryotic cultures has been evaluated by traditional plate cultivation techniques and most-probable-numbers (MPNs). Combining the results of prokaryotic survival from all the experiments demonstrate that the many different cultures investigated respond differently to the simulated Martian conditions. Nevertheless, from these data it is possible to make generalisations of the potential survivability of prokaryotes exposed to simulated Martian conditions. This will be discussed in the following and can generally be divided into two different simulation scenarios: i) exposure to simulated Martian conditions without UV radiation and ii) exposure to simulated Martian conditions with UV radiation.

Effect of simulated Martian conditions without solar radiation

In simulation experiments without UV radiation the viability of endospores from Gram-positive bacteria remained unchanged. This has been the case in studies with endospores from *Bacillus cereus* (Hawrylewicz *et al.*, 1965), *Bacillus subtilis* (Hagen *et al.*, 1964; Schuerger *et al.*, 2003; Nicholson & Schuerger, 2005) and *Clostridium botulinum* (Hawrylewicz *et al.*, 1962). Nevertheless, experiments with *B. subtilis* endospores showed that germination of endospores was reduced after 19 days of incubation under simulated Martian conditions (Nicholson & Schuerger, 2005). Similarly, low oxygen pressure and moisture availability have previously been shown to limit the germination of endospores of *B. subtilis* and *B. cereus* (Hagen *et al.*, 1964; Hagen *et al.*, 1967). Likewise, the survival of vegetative cells of Gram-positive bacteria was unaffected by simulated Martian conditions without UV radiation, but only if moisture was included in the experimental setup (Hagen *et al.*, 1967; Hawrylewicz *et al.*, 1968). When no moisture was available to the Gram-positive bacteria, a profound reduction of viable cells was observed during the first

freeze-thaw cycle of the incubation (Hagen *et al.*, 1964; Hawrylewicz *et al.*, 1965). Freeze-thaw cycles and desiccation have also been shown to be detrimental to Gram-negative bacteria and cultures of Archaea, which generally do not survive simulated Martian conditions even without UV radiation (Hawrylewicz *et al.*, 1962; Roberts, 1963; Hagen *et al.*, 1970). Moreover, freezing and freeze-drying have been identified to cause DNA breakage and thereby reduce the viability (Stan-Lotter *et al.*, 2003).

Growth of cultures during the incubation period has been observed in several simulation experiments. In all these experiments nutrients and moisture were added to the incubations (Davis & Fulton, 1959; Roberts, 1963; Young *et al.*, 1963; Hawrylewicz *et al.*, 1968), making the cultures able to grow during the non-freezing period. Studies observing growth under simulated Martian conditions showed that moisture was the critical factor for growth (Roberts, 1963; Hawrylewicz *et al.*, 1968). Generally, activity and growth is not expected under simulated Martian conditions when neither moisture nor nutrients are introduced during the incubation period.

Effect of simulated Martian conditions including solar radiation

UV radiation has been identified as the main factor in cell inactivation and death under simulated Martian conditions (e.g. Zhukova & Kondratyev, 1965; Imshenetsky *et al.*, 1967; Schuerger *et al.*, 2003; Cockell *et al.*, 2005). No unprotected prokaryotes have been shown to withstand simulated Martian solar radiation for long periods and even unprotected bacterial endospores have been eliminated during short-term incubations. Schuerger *et al.* (2006) demonstrated that 180 minutes of Mars equivalent UV intensity eliminated endospores of *B. pumilus*, while 30 minutes killed cells of the endolithic cyanobacterium *Chroococcidiopsis* sp. (Cockell *et al.*, 2005). The survival of prokaryotes has been observed to increase when the shortest wavelengths of UVC and part of UVB were filtered out, while no reduction in the viability was identified when UV radiation was removed from the incident solar radiation (Cockell *et al.*, 2005). The harmful effect of UV radiation on prokaryotes was correlated with the UV dose (Mancinelli & Klovstad, 2000).

When protected against direct UV exposure prokaryotes do survive Mars equivalent UV radiation. Dust layers of 0.5-1 mm thickness protect *B. subtilis* endospores from UV radiation, resulting in a full recovery of the bacteria (Mancinelli & Klovstad, 2000; Schuerger *et al.*, 2003). Even dust layers of 12 µm provide some protection against UV radiation (Mancinelli & Klovstad, 2000). Moreover, 1 mm of Mars-analogue soil offered full protection of the endolithic cyanobacterium *Chroococcidiopsis* sp. against UV radiation (Cockell *et al.*, 2005). Physical protection from UV exposure has not only been provided by soil and dust. When prokaryotes have been incubated in

multilayers the upper cell-layer shielded and thus facilitated the survival of the underlying cells (Mancinelli & Klovstad, 2000; Schuerger *et al.*, 2005).

In summary, also in Mars simulation experiments with prokaryotic pure-cultures, UV radiation has been identified as the most harmful parameter of the simulated Martian environmental conditions. No prokaryotic cultures have survived longer periods of direct exposure to Mars equivalent UV radiation. Additionally, temperature fluctuations and the moisture availability have been identified as important for prokaryotic survival under simulated Martian conditions. Endospores are most resistant to Martian conditions while most Gram-negative species and Archaea have been shown not to survive such harsh conditions. The survival mechanisms underlying the observed results have yet to be determined and so far only few studies have exploited the advantages of pure-culture models to further investigate how the prokaryotes are affected.

4.4 Simulation experiments with organic compounds

The survival of organic molecules under simulated Martian conditions has been investigated in a few experiments (Fig. 4). The motivation in most experiments has been to investigate the fate of potential organic matter brought to Mars by meteorites (Oro & Holzer, 1979; Stoker & Bullock, 1997; McDonald *et al.*, 1998; ten Kate *et al.*, 2005; Garry *et al.*, 2006), since no organic material on the Martian surface was detected by the investigations of the Viking missions (see section 1.1).

Under simulated Martian conditions, UV induced photo-degradation and oxidative degradation by soil oxidants have been shown to destroy amino acids (glycine and alanine), purine bases (adenine) (Oro & Holzer, 1979; Stoker & Bullock, 1997; ten Kate *et al.*, 2005) and macromolecules (naphthalene, tholin and humic acids) (Oro & Holzer, 1979; McDonald *et al.*, 1998). In all studies, the destruction rate was observed to exceed the expected organic input by meteorites. Hence, accumulation of organic matter at the Martian surface is unlikely, which is consistent with the Viking mission data.

The degradation rate of indigenous organic molecules in a permafrost soil (see manuscript III, chapter 4) and the Mars-analogue soils Mars-1 and Salten 1 (see section 3.1)(Garry *et al.*, 2006) has also been investigated under simulated Martian conditions. These investigations confirmed that the indigenous DNA and amino acids in the surface soils were degraded when exposed to simulated Martian conditions including UV radiation (manuscript III, chapter 4; Garry *et al.*, 2006). However, in deeper soil layers (>3 mm) no degradation of organic molecules were detected, indicating that

mainly direct and indirect UV effects (reactive oxygen species) were responsible for the observed degradations of organic molecules under simulated Martian conditions (see manuscript III, chapter 4).

5. Conclusions and future perspectives

Mars has been the main subject of astrobiological interest for more than fifty years. This has led to a significant progress in the understanding of the Martian conditions. However, still many open questions remain to be investigated, most importantly whether liquid water still exists on the planet and whether the methane detected in the Martian atmosphere has been produced recently.

From the Mars simulation experiments it has been established that terrestrial prokaryotes are not able to survive the conditions at the surface of present-day Mars. This is especially a consequence of the UVC radiation reaching the Martian surface, but also of the photochemically produced reactive oxygen species and of the diurnal temperature fluctuations. These environmental factors do not exclude the risk of contaminating Mars with terrestrial prokaryotes entirely. Some prokaryotes, especially bacterial endospores, survive when protected against the direct UV exposure. However, germination of bacterial endospores and survival of most Gram-negative bacteria have been shown to be negatively affected by desiccation and freeze-thaw cycles, meaning that long-term survival of terrestrial prokaryotes at the near surface of Mars is most unlikely. The absence of liquid water near the Martian surface further complicates the possibility of activity and reproduction of terrestrial contaminants at and near the Martian surface and therefore, a putative contamination of Mars will probably remain local.

The most potential life-supporting habitats on Mars are subsurface permafrost layers and the polar ice-caps, where liquid water and a source of energy might be present and available. Furthermore, if hydrothermal systems exist on Mars, they would most likely be a source of both gaseous compounds and heat, which again would facilitate the possibility of presence of liquid water. In Mars simulation experiments prokaryotic growth under simulated Martian conditions has been found correlated with the amount of moisture added. This suggests that terrestrial prokaryotes are able to both survive and metabolize in Martian subsurface environments if liquid water is present. Yet, no anaerobic chemolithoautotrophic prokaryotes have been incubated under simulated Martian conditions, which leave it up to future Mars simulation experiments to determine whether anaerobic prokaryotes (e.g. methanogens and sulfate reducers) are able to exist in the Martian subsurface.

Future challenges of the Mars simulation experiments are to simulate Martian subsurface conditions e.g. the Martian permafrost layers and polar ice-caps. This is however very difficult to approach due to the lack of information about the Martian subsurface. Therefore, such simulation experiments hold the risk of simulating conditions dissimilar to the actual conditions in the Martian subsurface environment.

Future Mars simulation experiments should also focus on the incubation of survivors from environmental samples exposed to simulated Martian conditions. This would significantly increase our knowledge of the physiological characteristics that enable these prokaryotes to survive.

The astrobiological question considering the possibility of life on Mars is still difficult to approach. This was especially illustrated by the discussions of the results from the life detecting experiments aboard the Viking missions and the results from the investigations of the Martian meteorite ALH84001. Therefore, it will definitely be a challenge to substantiate whether Mars is an astrobiological habitat. However, in the absence of *in situ* investigations on Mars, the ground based Mars simulation experiments are the best alternative to investigate the main question of this research field: can life exist and replicate under Martian conditions.

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Appendix

List of papers used in Figure 4.

Year	Reference
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