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Survival of Methanogenic Archaea from Siberian Permafrost under Simulated Martian Thermal Conditions

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Abstract Methanogenic archaea from Siberian permafrost complementary to the already well-studied methanogens from non-permafrost habitats were exposed to simulated Martian conditions. After 22 days of exposure to thermo-physical conditions at Martian low- and mid-latitudes up to 90% of methanogenic archaea from Siberian permafrost survived in pure cultures as well as in environmental samples. In contrast, only 0.3%–5.8% of reference organisms from non-permafrost habitats survived at these conditions. This suggests that methanogens from terrestrial permafrost seem to be remarkably resistant to Martian conditions. Our data also suggest that in scenario of subsurface lithoautotrophic life on Mars, methanogenic archaea from Siberian permafrost could be used as appropriate candidates for the microbial life on Mars.

Keywords methanogenic archaea · permafrost · astrobiology · life on Mars · Mars simulation experiments

Introduction

Of all the planets explored by spacecrafts in the last four decades, Mars is considered as one of the most similar planets to Earth, even though it is characterized by extreme cold and dry conditions today. This view has been supported by the current ESA mission *Mars Express*, which identified several different forms of water on Mars and methane in the Martian atmosphere (Formisano 2004). Because of the expected short lifetime of methane, this trace gas could only originate from active volcanism – which was not yet observed on Mars – or from biological sources. Data obtained by the *Mars Express* showed that water vapor and methane gas are concentrated in the same regions of the Martian atmosphere (European Space Agency 2004). This finding may have important implications for the possibility of microbial life on Mars (Moran et al. 2005). Furthermore, there is evidence that prior to 3.8 Ga ago, the environmental conditions on Mars may have been similar to those on early Earth (Carr 1989; Durham et al. 1989; Wharton

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et al. 1989; McKay and Davis 1991; McKay et al. 1992; Carr 1996). At this time microbial life had already started on Earth and Archaea are thought to have been among the earliest living organisms. If life had also emerged on Mars, it either adapted to the drastically changed environments or it became extinct. One possibility for survival of Martian microorganisms could be lithoautotrophic subsurface ecosystems such as deep sediments near polar ice caps and in permafrost regions, where liquid-like (unfrozen) adsorption water can play a key-role for transport of nutrients and waste products of biological processes (Möhlmann 2005). Evidence of permafrost occurrence on present Mars (patterned ground, glacier or thermokarst) has been found by *Mars Express*. Comparable environments exist in polar regions on Earth, for example Antarctic ice cores (Abyzov et al. 1998, 1999), Greenland glacial ice (Tung et al. 2005) and Siberian permafrost (Gilichinsky et al. 1993), where microorganisms existed for several million years independent of photosynthetic energy production (Gilichinsky and Wagener 1994; Vorobyova et al. 1998; Rivkina et al. 1998, Wagner et al. 2001).

Terrestrial permafrost, which covers around 24% of the Earth's surface, is a significant natural source of methane (Fung et al. 1991; Wagner et al. 2003, Smith et al. 2004). The processes responsible for the formation of methane in permafrost soils are primarily biological, carried out by methanogenic archaea, a small group of strictly anaerobic chemolithotrophic organisms, which can grow using hydrogen as an energy source and carbon dioxide as the only carbon source. They are widespread in nature and highly abundant in extreme environments, tolerating low/high temperatures (permafrost, hot springs), extreme salinity (saltern ponds) or low/high pH (solfataras, soda lakes). Beside mesophilic species, also thermophilic and hyperthermophilic methanogens are known (Stetter et al. 1990; Garcia et al. 2000). Recently, more attention has been paid to the isolation of psychrophilic strains, since many habitats in which methanogens are found belong to cold climates (Gounot 1999). So far, only a few strains (e.g., *Methanococcoides burtonii, Methanogenium frigidum, Methanosarcina* spec.) have been isolated from cold habitats (Franzmann et al. 1992, 1997; Simankova et al. 2003). Although the metabolism of methanogenic archaea has been studied in different environments (Ni and Boone 1998; Garcia et al. 2000; Eicher 2001; Lange and Ahring 2001), only a few studies have focussed on the ecology of the methanogenic archaea in permafrost ecosystems (Vishnivetskaya et al. 2000; Høj et al. 2005). Studies have shown that methanogenic archaea from Siberian permafrost are well adapted to osmotic stress and are also highly resistant to inactivation by desiccation, radiation, extremely low temperatures (Morozova and Wagner, data under processing) and high oxygen partial pressure (Wagner et al. 1999).

Few investigations have been performed under conditions applicable to Mars, particularly under water-stressed conditions (Sears et al. 2002). The present study focuses on the ability of methanogenic archaea to survive under simulated Martian thermal conditions. For this purpose, permafrost samples and pure cultures of methanogens were used. Their resistance renders these organisms eminently suitable for this purpose.

Description of the Mars Simulation Experiment

Biological samples

Permafrost samples and preparation

Permafrost samples were obtained from the Lena Delta, Siberia. The investigation site Samoylov Island (72°22′N, 126°28′E) is located within the central part of the Lena Delta,

which is one of the largest deltas in the world with an area of about 32,000 km^2. A detailed description of the geomorphologic situation of the island and the whole delta was given previously (Schwamborn et al. 2002). The Lena Delta is located within the continuous permafrost zone. It is characterized by an arctic continental climate with low annual air temperature of −14.7 °C (T_{min}=−48 °C, T_{max}=18 °C) and a low mean annual precipitation of 190 mm. The island is dominated by the typical permafrost pattern of low-centred polygons which cover at least 70% of the island's area. The soils in the Lena Delta are entirely frozen, leaving only 20–50 cm upper part, so-called 'active layer', remaining unfrozen during the summer months.

During the expedition 'Lena 2004' soil samples were collected from the active layer of two soil profiles. These profiles represent major characteristic geomorphic units of the island. They are different in regard to soil genesis and soil properties. One of these profiles was located at the depression of a low-centred polygon (72°22′N, 126°28′E) in the eastern part of the island. The prevalent soil type of the polygon depressions was a *Typic Historthel*, classified according to the US Soil Taxonomy (Soil Survey Staff 1998). The samples from the polygon depression were characterized by a high content of organic matter and high porosity.

The second profile was located on a flood plain in the northern part of the island. At this location, annual flooding leads to a continuing accumulation of fluvial sediments. The substrate was dominated by sandy and silty fluvial material. The prevalent soil type of the flood plain was a *Typic Aquorthel* (Soil Survey Staff 1998). Additional soil characteristics, analysed according to Schlichting et al. (1995), are summarized in Table I. Soil samples were filled in gas-tight plastic jars (Nalgene) and transported to Germany in frozen condition. Approximately 10 g of each soil sample was used for dry weight determination. All results were expressed per gram of dry soil.

Microbial cultures

For enrichment and isolation of methanogenic archaea the bicarbonate-buffered, oxygen-free OCM culture medium was used, prepared according to Boone et al. (1989). The

TABLE I Selected soil properties of a polygon centre and a flood plain soil on Samoylov Island, Lena Delta

Depth (cm)	H_2O content (%)	C_{org} (%)	N (%)	Grain size fraction (%)		
				Clay	Silt	Sand
Centre						
0–5	85.7	15.5	0.7	2.4	18.6	79.0
5–10	77.3	15.1	0.4	2.8	24.0	73.3
10–15	80.6	16.1	0.4	2.6	18.6	78.8
15–20	73.4	7.3	0.2	7.9	15.4	76.6
20–25	58.9	2.2	0.2	6.1	18.2	75.7
25–30	68.5	4.7	0.2	5.0	25.9	69.2
Flood plain						
0–5	30.1	3.1	0.4	11.1	64.8	24.2
5–9	31.9	1.1	0.2	20.2	61.4	18.4
9–18	28.3	2.2	0.3	18.3	63.5	18.2
18–35	35.4	2.8	0.4	20.2	62.7	17.1
35–40	32.4	2.4	0.3	20.4	55.6	24.0
40–52	31.8	1.7	0.2	17.6	67.7	14.7

medium was anaerobically dispensed into vials and 10 g of permafrost sample from anoxic horizons of the floodplain were added. The head space was filled with an N_2/CO_2 mixture (80:20, v/v). Methanol (20 mM) or H_2/CO_2 (80:20, v/v) were used as substrates. Inoculated vials were incubated at 10 °C. For the isolation of methanogenic archaea, serial dilutions (1:10) were carried out and cultures were incubated at 28 °C. Growth of contaminants was inhibited by different antibiotics (5 g ml^{-1} erytromycin or phosphomycin). Purity was checked microscopically and by lacking growth on medium containing 5 mM glucose, 5 mM pyruvate, 5 mM fumarate and 0.1% yeast extract.

All strains grew well at 28 °C and slowly at low temperatures (4 and 10 °C). The isolated strains showed different morphologies. *Methanosarcina* spec. SMA-21 cells were irregular cocci and 1–2 μm in diameter. Large cell aggregates were regularly observed. Cells of the strain SMA-16 were small irregular diplococci, 0.5–1 μm in diameter. Strain SMA-23 appeared as rod-shaped cells, ca. 1–2 μm in width and max. 10 μm in length, often forming long cell chains.

Reference organisms

Methanobacterium spec. MC-20 was isolated from a non-permafrost sediments from Mangalia, Romania at an incubation temperature of 28 °C. The cells were rod-shaped, 1–2 μm in width and max. 8 μm in length. *Methanosarcina barkeri* DSM 8687 was originated from peat bog in northern Germany (Sherer et al. 1983) and *Methanogenium frigidum* DSM 16458 (Franzmann et al. 1997) was originated from the water column of the Ace Lake, Antarctica. Both cultures were obtained from the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany).

The experimental set-up

Mars simulator

Simulation of the thermal conditions, typical for Martian mid- and low-latitudes, was achieved in the laboratory for humidity related studies (HUMIDITY-Lab) of the German Aerospace Center (DLR), Institute of Planetary Research in Berlin. The 'Cold chamber' provided a combination of diurnal temperature fluctuations in the range from −75 to +20 °C and humidity fluctuations between a_w-values of 0.1 and 0.9 in a Mars-like atmosphere dominated by carbon dioxide (95.3%). The humidity corresponds to a water vapor pressure of about 10^{-3} mbar (0.1 Pa) that equals to the average water vapor pressure on Mars (corresponding to 10 pr 4 μm). The simulation experiment was carried out in a 6 mbar Mars-like atmosphere for a period of 22 days (Figure 1). The average a_w-value was 0.52.

Martian simulation experiments with permafrost soils

To determine the influence of simulated Martian conditions on survival potential of methanogenic archaea in soil samples, fresh soil material (1 g) from the polygon depression (*Typic Historthel*, Oi horizon, 5–10 cm depth) and the floodplain (*Typic Aquorthel*, A horizon, 0–5 cm depth) was weighed into 12.5 ml plastic boxes (A/S NUNG, Denmark) under anoxic conditions. Three replicates were used for each soil type. Before and after the experiment the cell numbers were calculated as described in "Cell counts determination". After exposure to Martian conditions the soil samples were anaerobically incubated into

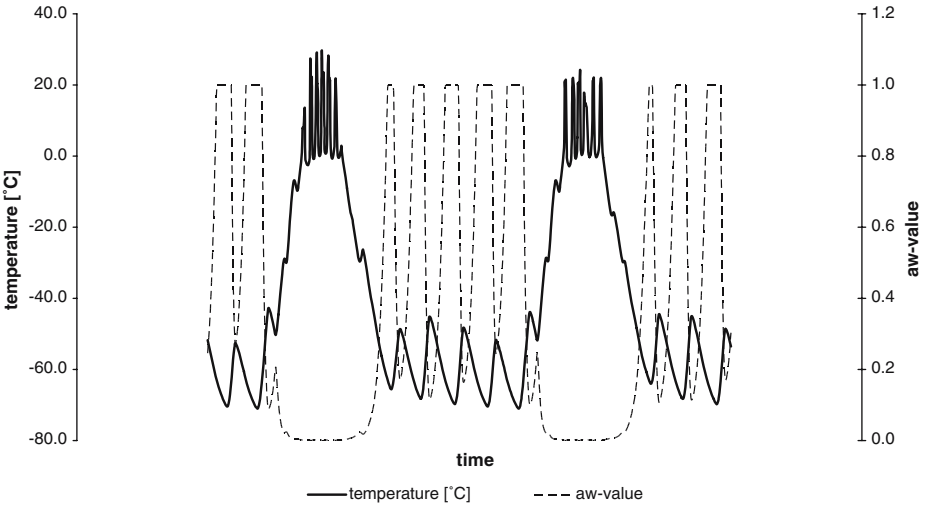

Figure 1 Diurnal profile of simulated Martian temperature (*bold line*) and humidity (a_W), – *dashed line* – in the Mars simulator (2 days are shown).

25 ml glass flasks, 5 ml of sterile deionized water was added and the flasks were closed with a screw cap containing a septum and incubated at 10 °C. The activity of methanogenic archaea was measured before and after the experiment as described in "Methane analysis."

Martian simulation experiments with pure methanogenic cultures

Six strains of methanogenic archaea were used in the simulation experiment. Strains *Methanosarcina* spec. SMA-21, SMA-16 and *Methanobacterium* spec. MC-20 were grown on bicarbonate-buffered, oxygen-free OCM culture medium (Boone et al. 1989) under an atmosphere consisting of H_2/CO_2 (80:20, v/v, pressurized 150 kPa). Strain SMA-23 and *Methanosarcina barkeri* were grown on oxygen-free MS culture medium (DSMZ No. 120) supplemented with 20 mM methanol as a substrate. *Methanogenium frigidum* was grown on oxygen-free EM culture medium (DSMZ No. 141) under an atmosphere of H_2/CO_2 (80:20, v:v, pressurized 150 kPa) at 15 °C. All strains except *Methanogenium frigidum* were incubated at 28 °C for about two weeks. Cells were harvested by centrifugation and 50 mg of the cell pellet was inoculated into 1,500-µl glass jars (A–Z Analytik Zubehör GmbH). Three replicates of each culture were used. Cell density of the cultures was between 2.3 and 8.1×10^7 cells ml^{-1}. Before and after the experiment cell numbers were calculated as described in "Cell counts determination." After the exposure to Martian conditions the cell pellets were placed under anaerobic conditions into 25 ml glass flasks, supplemented with 10 ml fresh OCM medium and H_2 as a substrate. The flasks were closed with a screw cap containing a septum and incubated at 28 °C (*Methanogenium frigidum* at 15 °C). The activity was measured before and after the experiment as described in "Methane analysis."

Methane analysis

The activity of methanogenic archaea was calculated based on the lineal increase of CH_4 concentration in the headspace. Methane concentration was measured by gas chromatography.

The gas chromatograph (Agilent 6890, Fa. Agilent Technologies) was equipped with a Carbonplot capillary column (∅ 0.53 mm, 30 m length) and a flame ionization detector (FID). Oven as well as injector temperature was 45 °C. The temperature of the detector was 250 °C. Helium served as carrier gas. All gas sample analyses were done after calibration with standards of the respective gases.

Cell counts determination

Cell numbers were calculated by Thoma cell counts and by fluorescence *in situ* hybridization (FISH) using the universal oligonucleotid probe for Archaea (ARC915 Cy3). For microscopic performance a Zeiss Axioskop 2 equipped with filters 02 (DAPI), 10 (FLUOS, DTAF) and 20 (Cy3), a mercury-arc lamp and an AxioCam digital camera for recording visualization of cells was used.

Results

Effect of Martian conditions on methanogenic archaea in permafrost soils

The survival rates of methanogenic archaea in permafrost soils after three weeks of exposure to the Martian thermal conditions was determined by both the methane production rates and the cell counts before and after the experiment. Methanogenic archaea of the floodplain showed high survival rates. The average cell numbers decreased from 9.1×10^6 cells g^{-1} at the beginning of the experiment to 6.6×10^6 cells g^{-1} after exposure to Martian conditions, which equals 72.2% cell survival. Average cell numbers of methanogenic archaea of the polygon depression decreased from 6.7×10^7 to 3.1×10^7 corresponding to a survival of 46.6% of the cells. The methane production rates of the flood plain soil samples slightly decreased after exposure to simulated Martian conditions from 0.07± 0.01 nmol CH_4 h^{-1} g^{-1} to 0.02±0.0004 nmol CH_4 h^{-1} g^{-1} (Table II). The methane production rates of the methanogenic archaea observed in the polygon depression samples decreased from 1.64± 0.15 nmol CH_4 h^{-1} g^{-1} to 0.09±0.004 nmol CH_4 h^{-1} g^{-1} after exposure to Martian conditions. The decrease of activity after the experiment was much higher in the polygon depression soils compared to the decrease of activity in soils of floodplain depression.

TABLE II Methane production rates and cell counts of methanogenic archaea in permafrost soil samples before and after exposure to Martian conditions

Soil samples	Cell counts 10^6	Survival rates (%)	CH_4 production (nmol h^{-1} $g^{-1)}$
Flood-plain (5–10 cm depth), control[a]	9.1±4.2	100	0.07±0.01
Flood-plain (5–10 cm depth), after experiment	6.6±3.4	72.2	0.02±0.0004
Centre (0–5 cm depth), control[a]	66.5±16.9	100	1.64±0.15
Centre (0–5 cm depth), after experiment	31.1±9.8	46.6	0.09±0.004

Mean ± standard error, n=3.

[a] Soil samples, which were not exposed to the Martian thermal conditions.

Effect of Martian conditions on pure methanogenic cultures

The methanogenic strains from Siberian permafrost and the reference organisms from non-permafrost habitats showed significant differences in their survival potential under simulated Martian conditions. The average cell number of strain *Methanosarcina* spec. SMA-21 decreased from 6.1×10^7 cells ml^{-1} at the beginning of the experiment to 5.5×10^7 cells ml^{-1} at the end of the simulation, which equals a cell survival of 90.4%. Strains SMA-16 and SMA-23 showed 67.3% and 60.6% survival, respectively (Figure 2, Table III). In comparison, only 1.1% of strain *Methanobacterium* spec. MC-20, 5.8% of *Methanogenium frigidum* and 0.3% of *Methanosarcina barkeri* survived the simulation of Martian conditions (Table III). The decrease of cell numbers correlates well with the methane production rates of the cultures. Thus, activity of strains SMA-21, SMA-16 and SMA-23 measured before the exposure to simulated Martian conditions was similar to that after the simulation, whereas methane production of the reference organisms *Methanobacterium* spec. MC-20, *Methanogenium frigidum* and *Methanosarcina barkeri* drastically decreased after the experiment (Figure 2, Table III). The methane production rates of *Methanosarcina* spec. SMA-21 slightly decreased after exposure to simulated Martian conditions from 48.61±6.57 nmol CH_4 h^{-1} ml^{-1} to 44.11±5.08 nmol CH_4 h^{-1} ml^{-1} (Table III). The activities of two other permafrost isolates, SMA-16 and SMA-23 were also only marginally affected by the Martian experiment. The methane production rates of SMA-16 decreased from 52.77±6.18 nmol CH_4 h^{-1} ml^{-1} at the beginning of the experiment to 45.37± 0.03 nmol CH_4 h^{-1} ml^{-1} after the exposure. The methane production rates of SMA-23

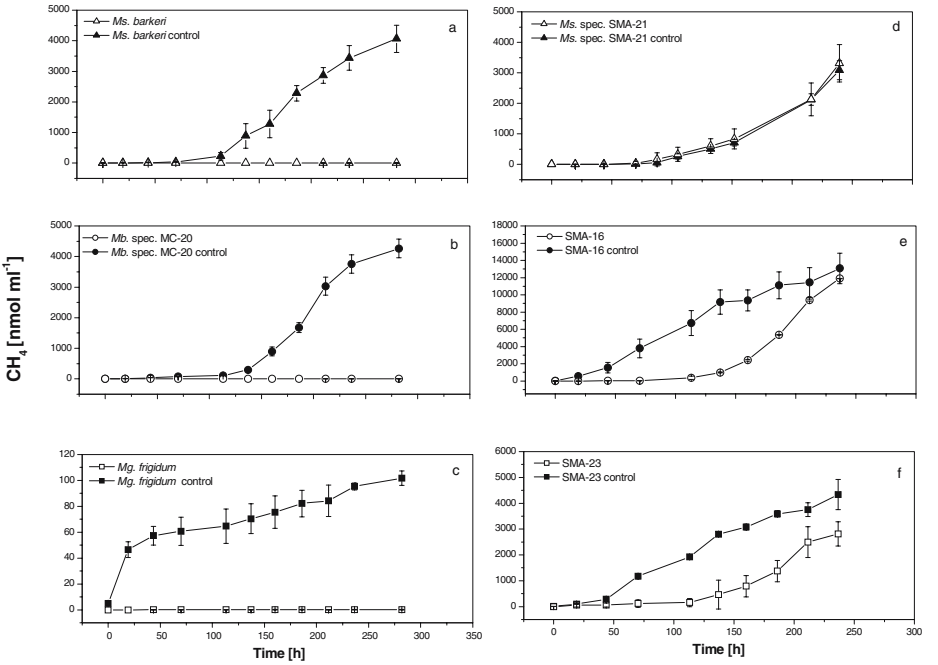

Figure 2 Methane production activities of the reference organisms *Methanosarcina barkeri* (a), *Methanobacterium* spec. MC-20 (b), *Methanogenium frigidum* (c) and methanogens isolated from Siberian permafrost *Methanosarcina* spec. SMA-21 (d), SMA-16 (e), SMA-23 (f) before and after exposure to simulated Martian conditions (the *error bars* represent the standard deviation, $n=3$).

TABLE III Methane production rates and cell counts of methanogenic archaea before and after exposure to Martian conditions

Cultures	Cell counts 10^7	Survival rates %	CH_4 production nmol h^{-1} ml^{-1}
Methanosarcina spec. SMA-21, control	6.1±0.6	100	48.61±6.57
Methanosarcina spec. SMA-21	5.5±0.8	90.4	44.11±5.08
SMA-16, control	6.2±1.1	100	52.77±6.18
SMA-16	4.2±0.9	67.3	45.37±0.03
SMA-23, control	7.8±1.4	100	22.13±1.94
SMA-23	4.7±1.2	60.6	13.92±3.87
Methanobacterium spec. MC-20, control	8.1±1.3	100	27.38±3.09
Methanobacterium spec. MC-20	0.09±0.01	1.1	0.03±0.001
Methanogenium frigidum, (DSM 16458) control	2.3±0.8	100	2.76±0.07
Methanogenium frigidum (DSM 16458)	0.1±0.04	5.8	0.003±0.005
Methanosarcina barkeri (DSM 8687), control	3.7±0.5	100	20.43±2.38
Methanosarcina barkeri (DSM 8687)	0.01±0.00	0.3	0.01±0.01

Mean ± standard error, n=3.

decreased from 22.13±1.94 nmol CH_4 h^{-1} ml^{-1} to 13.92±3.87 nmol CH_4 h^{-1} ml^{-1}. The activities of the reference organisms *Methanosarcina barkeri* and *Methanobacterium* spec. MC-20 after the simulation experiment were almost extinct (Figure 2, Table III). Methane production rates of *Methanogenium frigidum* significantly decreased from 2.76±0.07 nmol CH_4 h^{-1} ml^{-1} measured before the experiment to 0.003±0.005 nmol CH_4 h^{-1} ml^{-1} after the exposure.

Discussion

Methanogenic archaea from Siberian permafrost showed unexpectedly high survival under simulated Martian thermal conditions. Three weeks of diurnal temperature and humidity cycles did not have significant effects on the viability of the methanogens in permafrost soil samples and in pure cultures. In contrast, the diurnal changes in humidity and temperature killed up to 99.7% of methanogenic archaea that originated from non-permafrost habitats. This indicates that methanogenic archaea from permafrost are more resistant to temperature shifts between −75 °C and 20 °C as well as an a_w-value between 0.1 and 0.9 than well studied methanogens from other environments.

Terrestrial permafrost is characterized by extreme environmental conditions such as sub-zero temperatures, aridity and higher than normal levels of back-ground radiation as a result of an accumulation over geological time scales. In spite of the unfavorable living conditions permafrost is colonized by a high number of viable microorganisms (10^2–10^8 cells per g^{-1}), including fungi, yeasts, algae, actinomycetes and bacteria as well as highly specialized organisms like methanogenic archaea (Kobabe et al. 2004; Wagner et al. 2005). Seasonal variation of soil temperatures, particularly freeze–thaw cycles in the active layer, results in drastic changes of other environmental conditions like salinity, soil pressure, changing oxygen conditions, availability of nutrients. The temperature variations also influence the availability of pore water, which is an essential bio-physical requirement for the survival of microorganisms in permafrost. The most important biological feature of this water is its possible role in the transfer of ions and nutrients (Ostroumov and Siegert 1996).

Comparing different permafrost samples it could be shown, that the extreme fluctuations in humidity and temperature conditions were more harmful for the methanogens in a polygon depression soil than those in a floodplain soil. One of the factors favoring the viability of methanogens under simulated Martian conditions might be the soil texture. Methanogenic archaea have a hydrophobic cell surface and a low electrophoretic mobility which support the attachment of these organisms to the surface of charged soil particles (Grotenhuis et al. 1992). The sorptive capacities of natural soil particles like clay and silt or soil organic matter provide a protective effect on methanogenic archaea (Heijnen et al. 1992; Wagner et al. 1999). Previous investigations already demonstrated that due to a protective role of the soil matrix and the existence of a complex microbial community composed of aerobic and facultative anaerobic microorganisms, methanogenic archaea exhibit a high survival potential against different stress factors like high oxygen partial pressure (Wagner et al. 1999). The different survival rates found in two permafrost soils might therefore result from differences in grain sizes or in the water adsorption capacity of these two soils (so-called tension or matrix potential). Thus, higher rates of survival and activity of methanogens after an exposure to Martian conditions in samples of the flood plain soil could be a consequence of high silt content which protects the methanogenic archaea against harsh conditions. Compared to the flood plain, the polygon depression was dominated by sandy material.

Also the strong aggregate formation of up to 100 cells of *Methanosarcina* spec. SMA-21 could be one of the mechanisms for the resistance of this archaeon. The outer cells of an aggregate may shield the inner cells from the damaging influence of low temperature, high salinity or intensive radiation. Probably, soil or rock grains could also serve as a shield against UV for these organisms and provide a habitat with stable temperatures. Since permafrost is expected to be extensively present on Mars, it is possible that methanogenic archaea could segregate in the subsurface niches and could survive under the harsh Martian thermal conditions.

The pure cultures of methanogens, which were not associated with a protective soil matrix, were also exposed to simulated Martian thermal conditions. These experiments showed that *Methanosarcina* spec. SMA-21 and two other permafrost strains, SMA-16 and SMA-23, exhibit a higher resistance than the reference organisms *Methanobacterium* spec. MC-20 and *Methanosarcina barkeri*. Most striking is that temperature shifts between−75 °C and 20 °C as well as humidity shifts with an a_w-value between 0.1 and 0.9 (averaged 0.52) have no influence on the activity and survival rates of strain *Methanosarcina* spec. SMA-21. The survival rate of *Methanogenium frigidum*, a psychrophilic methanogen isolated from Ace Lake in Antarctica (Franzmann et al. 1997), was higher than that of the other reference organisms. Nevertheless, the metabolic activity of this strain also drastically decreased after exposure. It could be hypothesized that this methanogenic archaeon is highly adapted to perennially cold environments but is affected by fluctuations of temperature and water activity.

The simulation experiment indicates high survival rates of methanogenic archaea from permafrost after exposure to simulated Martian thermal conditions. Without exception, every environment can only support life when water is present in liquid form, at least temporary. As has been shown by *Mars Odyssey* measurements, the present Martian surface is not as dry as has been postulated. In the upper meters of the Martian surface liquid water is present in the form of adsorbed water. The content of adsorption water in the upper millimeter to centimeter thick surface layer ranges from multiple layers of water molecules, when the atmosphere is saturated, to less than one single molecular layer when the atmosphere is dry (Möhlmann

et al. 2004). At larger depths, the content of adsorption water tends to become stable with about one to two mono-layers. The presence of adsorption water layers is restricted to the upper parts of the Martian surface. Adsorption is strongest during night and morning hours. The amount of adsorption water depends on the surface properties and on the humidity of the atmosphere. While the upper layers freeze at low temperatures, the lower one to two mono-layers remains unfrozen down to a temperature of about −133 °C (Möhlmann 2005). The temporary existence of adsorption water in the uppermost layers of the Martian surface enables potential organisms to accumulate liquid-like water during the time adsorption water is present at night and morning. The Mars simulation experiment with diurnal profiles of Martian temperature and humidity within 6 mbar CO_2-atmosphere indicate the availability of adsorption water on Mars for biological processes. Comparable environments could be found in terrestrial permafrost, where adsorption water exists in a liquid-like state at temperatures down to −60 °C (Ananyan 1970).

The permafrost microbial community has been described as a "community of survivors" (Friedmann 1994), which has to resist the combination of extreme conditions and the extreme fluctuation of these conditions. High survival rates of methanogenic archaea under simulated Martian conditions indicate unknown physiological adaptations and suggest that these microorganisms have established ways to cope with stresses which has to include repair of the damaged DNA, repair of cell membranes and other vital functions to maintain the viability of cells. It remains to be determined that freeze protection mechanisms (i.e., trehalose accumulation, synthesis of molecular chaperones, adaptation of plasma membrane composition, synthesis of antioxidant proteins, accumulation of compatible solutes, expression of hydrophylins and other cryoprotectants) overlap with tolerance mechanisms protecting against various other stress types like desiccation, starvation or high salt concentration (Berry and Foegeding 1997; Macario et al. 1999; Cleland et al. 2004; Georlette et al. 2004).

Furthermore, it remains to be determined whether Martian and terrestrial permafrost have zones with similar physical and chemical conditions (Ostroumov, 1995). Due to the physiological potential and metabolic specificity of methanogenic archaea, no organic matter is needed for their growth. Kral et al. (2004) have demonstrated that certain methanogens can survive on Mars soil simulant (JSC Mars-1, collected from volcanoes on the Hawaii island) when they are supplied with CO_2, molecular hydrogen and varying amounts of water.

The permafrost habitats on Earth represent an excellent analogue for studying putative life on Mars. Recent analyses of *Mars Express* high resolution stereo camera (HRSC) images of many regions of the planet showed that the morphology of the Martian polygonal features is very similar to the morphology of the terrestrial ice–wedge polygons and is most likely formed by comparable processes (Kuzmin 2005). The observation of high survival rates of methanogens under simulated Martian conditions supports the possibility that microorganisms similar to the isolates from Siberian permafrost could also exist in the Martian permafrost. Methanogenic archaea from terrestrial permafrost may therefore serve as useful models for further exploration of extraterrestrial life.

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