Tolerance limits and survival potential of methanogenic archaea from Siberian permafrost under extreme living conditions

Toleranzgrenzen und Uberlebensstrategien von methanogenen Archaeen aus sibirischen Permafrosthabitaten unter Extrembedingungen

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Preface

The doctoral thesis was part of the ongoing research project “Tolerance limits of microbial life in terrestrial permafrost” in the scope of the DFG (Deutsche Forschungsgemeinschaft) Priority Program *“Mars and the Terrestrial Planets”* WA 1554/1-2. In this project, special attention was paid to the survival potential of methanogenic archaea in Siberian permafrost. Field research and sampling were carried out during the expedition Lena Delta 2004 (Samoylov Island, Siberia, Russia). The work was conducted at the Alfred Wegener Institute for Polar and Marine Research, Research Unit Potsdam and is presented as a Ph.D. thesis at the University of Potsdam, Institute of Biochemistry and Biology.

The thesis is composed of an introduction and four main chapters (2-5), followed by synthesis and conclusions. In the first chapter, an introduction of the research topic, the main objectives of this work, study site description and an overview of the publication is given. The main chapters consist of four manuscripts that represent self-contained units which are field on a common thread. Chapter 2 is in preparation for submitting and Chapter 4 has been submitted for publication to various international journals, whereas Chapter 3 and 5 are published already (s. 1.5). A synthesis of the four research articles is given in Chapter 6, including the outline of the basic findings as well as major conclusions and future perspectives.

Acknowledgements

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Abstract

The characterizations of the survival potential of microorganisms which are able to thrive in extreme environments are receiving a great attention in astrobiological research as driven by the possibility of their existence in extraterrestrial extreme niches. Speculations about methanogenic life on Mars are arising since ESA mission *Mars Express* determined water on Mars, a fundamental requirement for life, as well as the presence of CH_4 in the Martian atmosphere, which could be originated only from active volcanism or from biological sources. In the view of these findings it became obvious that microbial life could still exist on Mars, for example in the form of subsurface lithoautotrophic ecosystems, which also exist in permafrost regions on Earth.

The objectives of this study were to fill fundamental gaps in our knowledge about the physiological potential of methanogenic archaea and their tolerance limits to extreme environmental conditions in terrestrial or extraterrestrial permafrost. Within the scope of the DFG Priority Program *"Mars and the Terrestrial Planets"* the survival potential influenced by high salinity, extremely low temperatures, prolonged starvation and desiccation, ultraviolet and ionizing radiation, exposure to oxygen as well as simulated Martian conditions were studied. For this purpose, the methanogenic archaea in pure cultures as well as in their natural environment of Siberian permafrost were used. The differences or similarities in the stress behavior of methanogens originated from permafrost and non-permafrost habitats under diverse stress conditions were compared.

Generally, higher resistance to all stress factors tested was shown for the methanogenic archaea from Siberian permafrost in comparison to methanogens from non-permafrost habitats. A better adaptation to defined stress factors was observed at low incubation temperatures (4 °C), which correlates well with the *in situ* temperatures of the active layer of permafrost. Furthermore, metabolic activity and viability of methanogenic archaea in environmental samples from permafrost remained unaffected by exposure to simulated Martian UV radiation due to the protected effect by soil particles. Moreover, irradiation of pure methanogenic cultures revealed unexpectedly high radiation resistance of permafrost strains to monochromatic, polychromatic and ionizing radiation. Until now, such a high resistance was only shown for *Deinococcus radiodurans*, the most radiation resistant bacterium on Earth. Finally, in simulation experiments, methanogenic archaea in pure cultures as well as in environmental samples from Siberian permafrost reveal a high survival potential under Martian diurnal thermo-physical conditions. Therefore, the investigation of the survival potential

of these high specialized organisms provided a unique insight into possible life on the red planet.

Zusammenfassung

Die Untersuchungen zu mikrobiellen Überlebensraten und Toleranzgrenzen unter extremen Lebensbedingungen sind von großer Bedeutung für die astrobiologische Forschung, insbesondere im Hinblick darauf, dass extremophile Mikroorganismen auch in außerirdischen Nischen existieren könnten. Seit die ESA Marsmission „*Mars Express*“ Wasser und Methan auf dem Mars entdeckt hat, wird über mögliches rezentes methanogenes Leben auf dem Mars spekuliert. Das resultiert daraus, dass einerseits Wasser zu den grundsätzlichen Voraussetzungen für die Entstehung des Lebens zählt, das Methan in der Marsatmosphäre andererseits nur vulkanischen oder mikrobiellen Ursprungs sein kann. Mikrobielles Leben auf dem Mars könnte dabei beispielsweise in unterirdischen lithoautotrophen Nischen existieren, denn vergleichbare Bedingungen existieren auch auf der Erde, zum Beispiel in den Permafrostgebieten Sibiriens.

Ziele der vorliegenden Arbeit waren, fundamentale Lücken in unseren Kenntnissen über das physiologische Potenzial von methanogenen Archaeen unter den extremen Umweltbedingungen, wie sie in den irdischen und möglicherweise außerirdischen Permafrostböden zu finden sind, zu schließen.
Im Rahmen des DFG Programms „Mars und die terrestrischen Planeten“ wurden die Überlebensraten und Toleranzgrenzen der methanogenen Archaeen unter verschiedenen Stressbedingungen wie hohe Salzgehalte, extrem niedrige Temperaturen, langfristiger Nährstoffmangel und Austrocknung, hohe Strahlungsintensitäten (ultraviolette und ionisierende Strahlung) und Sauerstoffstress untersucht. Darüber hinaus wurden Marssimulations-experimente durchgeführt. Für diese Versuche wurden methanogene Reinkulturen sowie Umweltproben aus Sibirischen Permafrostböden verwendet. Als Referenz- und Kontrollorganismen wurden methanogene Reinkulturen aus nicht Permafrostgebieten eingesetzt. Im Vergleich zu methanogenen Archaeen aus anderen Habitaten zeigten die methanogenen Archaeen aus den Sibirischen Permafrostböden generell eine höhere Resistenz gegenüber allen getesteten Stressfaktoren. Dabei wurde die höchste Stressresistenz bei niedrigen Inkubationstemperaturen von 4 °C beobachtet, die durchschnittlich den *in situ* Temperaturen des sogenannten „active layers“ in Permafrostböden entspricht. Darüber hinaus hatte simulierte Mars UV-Strahlung keinen Einfluss auf die metabolische Aktivität und Überlebensrate der methanogenen Archaeen

in Permafrostbodenproben; möglicherweise, da die Zellen durch die sie umgebenden Bodenpartikel geschützt waren. Allerdings zeigten auch die methanogenen Reinkulturen aus Permafrostböden unerwartet hohe Strahlungsresistenzen gegenüber monochromatischer, polychromatischer und ionisierender Strahlung, die bislang nur mit denen von *Deinococcus radiodurans*, dem strahlungsresistentesten Bakterium der Erde, vergleichbar sind. Schließlich zeigten methanogene Archaeen aus Permafrostböden sowohl in Reinkulturen als auch in Umweltproben auch unter simulierten thermo-physikalischen Marstagesgangbedingungen hohe Überlebensraten. Die vorliegenden Untersuchungen zu den Überlebensraten und Toleranzgrenzen dieser hoch spezialisierten Mikroorganismen gewähren einen einzigartigen Einblick in mögliches Leben auf dem roten Planeten.

1 Introduction

1.1 Scientific Background

1.1.1 Terrestrial permafrost

Approximately 24 % of the exposed land surface of the northern hemisphere is underlain by permafrost (Zhang *et al.*, 1999). Continuous lowland permafrost is widely distributed mainly in polar and sub-polar regions of Siberia, Canada and Alaska (Fig. 1.1a). These regions are characterized by low annual mean temperatures (-15 °C), a low mean annual precipitation (about 200 mm) and poor vegetation. The permafrost table in the lowlands of East Siberia (Central Yakutia) is about 600 – 800 m thick.

Freezing-thawing processes leads to the formation of low centered ice-wedge polygons, which make up the typical patterned ground in Siberian permafrost environments (Fig. 1.1c).

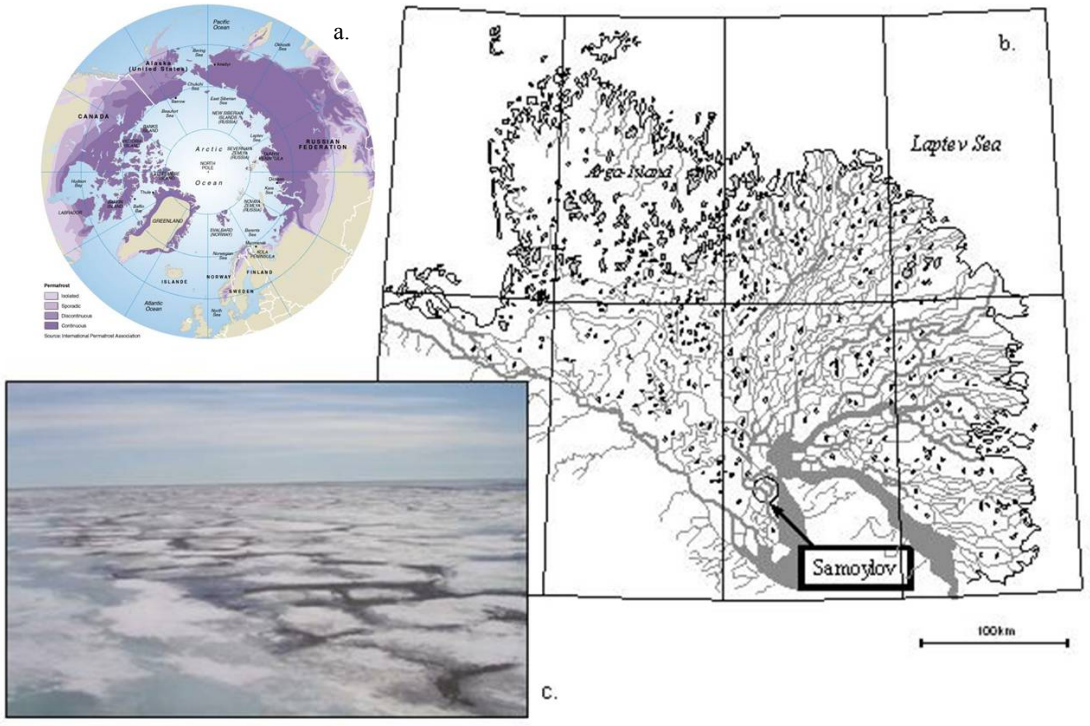

Figure 1.1: Distribution of the permafrost on the northern hemisphere (a.) and location of the long-term study site on Samoylov Island, Lena Delta, Siberia (b.) The image (c.) shows the typical patterned ground of low-centred ice-wedge polygons during winter time.

Lowland permafrost comprises three horizons. The upper layer is exposed to repeated freezing and thawing cycles with extreme temperature

fluctuations from +15 °C to -35 °C, where ecologically significant levels of microbial activity *in situ* occur also during the Arctic cold season (Price and Sowers, 2004; Schimel and Mikan, 2005). The underlain horizon was formed in a cold climate (about 40,000-50,000 years ago), and has remained undisturbed since. It is characterized by a stable temperature regime of about -5 °C to -10 °C. The deepest horizon is permanently frozen for at least 50 000 years (Virina *et al.*, 1984) with reduced activity of microorganisms (Wagner *et al.*, 2007).

Terrestrial permafrost provides an opportunity to obtain microorganisms that have exhibited long-term exposure to cold temperatures, freeze-thaw cycles, starvation, salinity, changing oxygen conditions, aridity, and long-lasting back-ground radiation resulting from an accumulation over geological time scales. The permafrost microbial community, described as a "*community of survivors*" (Friedmann, 1994), has to resist this combination of extreme environmental conditions as well as their extreme fluctuations. Freeze-thaw cycles during arctic winter and summer determine soil temperature and soil moisture, which again strongly influence the microbial activity. Even though bulk soil water freezes below 0 °C, soil particles continue to have measurable liquid water films around them down to temperatures at -40 °C (Price and Sowers, 2004). In Siberian permafrost, water films were predicted to be ~ 15 nm thick at -1.5 °C and shrank to ~ 5 nm when the samples were cooled to -10 °C (Rivkina *et al.*, 2000). Significant rates of *in situ* metabolic activity, e.g. of methanogenesis, were measured in permafrost at temperatures between -16 °C and -10 °C (Rivkina *et al.*, 2004). Furthermore, McGrath *et al.* (1994) showed that the intercellular water in fossil bacteria from permafrost soils was not crystallized as ice even at an extreme temperature of -150 °C. The most important biological feature of water is its possible role in the transfer of ions and nutrients (Ostroumov and Siegert, 1996). As long as unfrozen water exists, microorganisms can therefore remain physiologically active (Rivkina *et al.*, 2000; Mikan *et al.*, 2002).

1.1.2 Environmental conditions on past and present-day Mars

Our neighboring planet Mars is considered a possible astrobiological habitat and has over time been a major focus of space research in the prospect of extraterrestrial life. By investigating the surface features on Mars, one can see that Mars may have had an early environmental history similar to that of the early Earth. Observation and analyses of the Martian surface revealed that Mars was much warmer and wetter 3.7 Ga ago (Jakosky and Phillips, 2001; Solomon *et al.*, 2005). In this period, local bodies of water might have been present on the surface, as suggested by sedimentary rocks and waterborne

sediments on the present-day surface (Squyres *et al.*, 2004). At the time the life conditions on Mars were similar to that on early Earth, the evolution of microorganisms had already started on Earth. Prokaryotic microfossils, found in early Archean rocks, imply, that the earliest life forms probably date from between 3.5 – 3.8 Ga ago (Schidlowski, 1993; Schopf, 1993). Importantly, the Archean fossil record suggests, that there were microbial mats potentially exposed directly to the atmosphere and thus to solar UV radiation. Hence, it is possible that early organisms on Earth were able to survive in communities exposed to solar UV regimes which were at least as high as on Mars (Rotschield and Cockell, 1999; Cockell, 2001).

Climate change on Mars probably resulted from loss of the magnetic field, which has protected the Martian atmosphere against solar wind stripping (Jakosky and Phillips, 2001; Solomon *et al.*, 2005). As atmospheric gases became depleted, the surface air pressure and temperature decreased, resulting in the cold and dry environment of present-day Mars. If life had also emerged on early Mars under those more favorable atmospheric conditions, it either adapted to the drastically changed environments or it became extinct.

Today the surface of Mars is considered to be hostile to all known life forms. Harsh conditions like low water activity, high desiccation and oxidative stress, strong variations of the salinity, and low and sporadic supply of energy sources are known on present Mars (Litchfield, 1998; Horneck, 2000). Furthermore, the present Mars is characterized by extreme temperature regime which ranges between –123 °C and +23 °C (Horneck, 2000).

One major environmental factor that precludes terrestrial life from living on the surface today is the solar UV flux (Rothschild, 1990). Mars is 1.5 times further from the Sun than Earth, so the overall incidence of solar radiation on the atmosphere of Mars is 44 % that of Earth. Present-day Mars has a thinner atmosphere than that of Earth, resulting in more direct penetration of UV radiation through the atmosphere. The atmosphere on Mars has a total atmospheric pressure of 6 mbar, and 95 % of that is CO_2. Thus, UV radiation below 190 nm does not reach the surface, but surficial fluxes rise rapidly above 200 nm (Kuhn *et al.*, 1979). Additionally, short-waved cosmic radiation penetrates the Martian atmosphere. UV radiation very likely causes the highly oxidizing nature of the Martian surface as detected by the Viking Landers (Soffen and Young, 1972). The chemical nature of the oxidants has not been determined, but H_2O_2 was found to be produced by the photochemical processes in the Martian atmosphere (Clancy *et al.*, 2004; Encrenaz *et al.*, 2004) which make it a possible candidate for the Martian soil oxidant (Bullock *et al.*, 1994). Another candidate is superoxide (O^{2-}), which was reported to be formed on mineral grains under simulated Martian conditions (Yen *et al.*, 2000).

The prerequisites for actively growing terrestrial life are the availability of water, energy and molecules supporting anabolism of biomass. One possibility for a 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 the transport of nutrients and waste products of biological processes (Möhlmann, 2005). The similar morphological structures found on Martian surface and in regions of terrestrial permafrost are of special interest for astrobiological research. This suggests that their development is based on comparable cryogenic processes (Kuzmin, 2005). Significant amounts of water, an essential requirement for cryogenic processes and life, were recently identified below the Mars surface by the use of a gamma ray spectrometer on Mars Odyssey spacecraft (Fig. 1.2). The permafrost layer was detected 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 is not known, but the mass of the water content 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). Altogether, subsurface areas with liquid water and available energy seem to be the most probable life-supporting habitats on Mars.

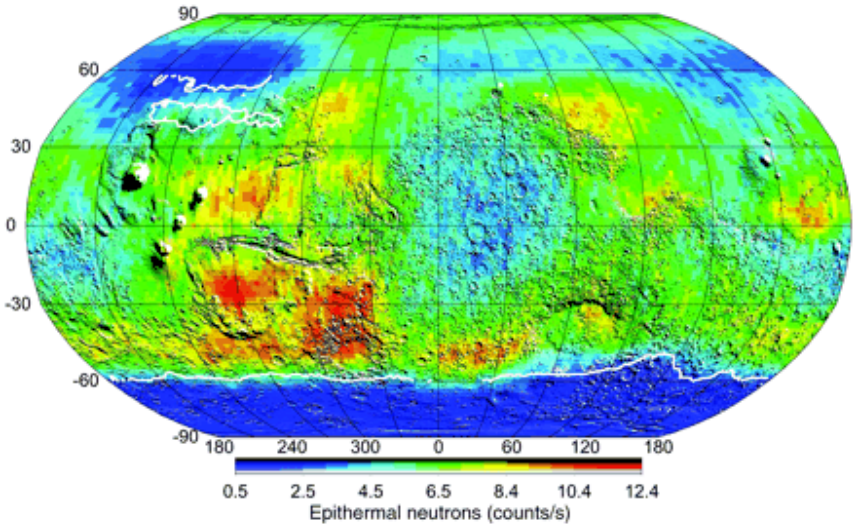

Figure 1.2. Map of epithermal neutron flux on Mars, as a measure of the hydrogen concentrations indicative of water ice regions. Low epithermal flux is correlated with high hydrogen concentrations. From the Mars Odyssey Gamma-Ray Spectrometer (Boynton *et al.*, 2002).

The most probable chemical energy source on Mars is the gaseous compound H_2 (Weiss *et al.*, 2000; Summers *et al.*, 2002), which, together with

CO_2, is presumably formed readily by photochemical processes in the Martian atmosphere (Nair *et al.*, 1994; Bar-Nun and Dimitrov, 2006). Furthermore, the possibility of extant or extinct life on Mars has been fueled by the recent U.S. Mars Exploration Rover Opportunity discovery that liquid water most likely exists on Mars (Christensen *et al.*, 2004; Klingelhofer *et al.*, 2004; NASA, 2006). Also, findings of the Planetary Fourier Spectrometer onboard the *Mars Express,* as well as ground–based observations, indicated that methane currently exists in the Martian atmosphere (Formisano *et al.*, 2004).

1.1.3 Methanogenic archaea under extreme conditions

Methanogenic archaea belongs to the phylum Euryarchaeota in the domain of Archaea (Woese *et al.*, 1990). They are characterized by their ability to produce methane under anaerobic conditions. This particular metabolism has provided the methanogens with a unique set of enzymatic pathways, as well as certain coenzymes and cofactors that are not found in other organisms (Daniels, 1993).

Methanogenic archaea are ubiquitous in anoxic environments. Due to their unique physiology, methanogens are highly abundant in habitats characterized by extreme conditions such as low/high temperatures (permafrost, hot springs), extreme salinity (saltern ponds) and low/high pH (solfataras, soda lakes). In addition to the mesophilic species, thermophilic and hyperthermophilic methanogens have also been identified (Stetter *et al.*, 1990; Garcia *et al.*, 2000). To date, more attention has been paid to the isolation of psychrophilic strains since a number of methanogenic habitats are located in 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; Franzmann *et al.*, 1997; Simankova *et al.*, 2003). Although the metabolism of methanogenic archaea was studied in different environments (Shuisong 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 exposed to the harsh environmental conditions of permafrost (Vishnivetskaya *et al.*, 2000; Høj *et al.*, 2005, Ganzert *et al.*, 2007).

Methanogenic archaea have an extremely specialized metabolism, characterized by lithoautotrophic growth (Whitman *et al.*, 1992). They can grow with hydrogen as an energy source and carbon dioxide as the only carbon source. In addition to this specific metabolism methanogens are able to convert only a limited number of organic substrates (acetate, formate, methanol, methylamines) to methane (Zinder, 1993). Due to the capability of these organisms to lithoautotrophic growth under strictly anaerobic conditions (Deppelmeier *et al.*, 1996), their tolerance to low temperatures and their ability

to survive under the extreme conditions of permafrost since several millions of years (Gilichinsky *et al.*, 2003; Rivkina *et al.*, 2004), methanogens can be considered as a very suitable *model organism* for the investigation of possible Martian life (Wagner *et al.*, 2001). The hypothesized Martian methanogenic prokaryotes have been argued to live in the subsurface permafrost layers and utilize H_2 and CO_2 diffusing from the atmosphere (Formisano, 2004; Krasnopolsky *et al.*, 2004).

Furthermore, because of the specific adaptations of methanogenic archaea to conditions like those on early Earth (e.g. no oxygen, no or little organic substrates) and their phylogenetic origin, they are considered to be one of the initial organisms on Earth.

Based on these presumptions, microbiological studies on methanogenic archaea from permafrost can give insights into life in terrestrial permafrost. This knowledge may help to search for other kind of life in extraterrestrial permafrost like it is present on Mars. In particular, the study presented here will contribute to an improved understanding of possible extraterrestrial life, especially with regards to possible protected niches on present-day Mars.

1.2 Aims and approaches

The presented study is the first attempt to investigate the physiological potential of methanogenic archaea from Siberian permafrost under extreme living conditions. Since terrestrial permafrost is considered to be an analogue for the Martian permafrost environment, this study also contributes to improve the search for and the understanding of probable life on Mars.

The main objective of this thesis was to investigate the survival potential of methanogenic archaea and their tolerance limits to extreme environmental conditions in terrestrial permafrost. In particular, the study focuses on:

- The limits of growth and viability of methanogenic archaea under exposure to high salinity, radiation, extremely low temperatures, prolonged desiccation, starvation, sub-zero temperatures and oxic conditions.
- The general perspectives of the interaction between methanogene archaea and soil matrix.
- The influence of low incubation temperatures on the survival potential of methanogenic archaea under different stress conditions.
- A comparison of the stress behavior of methanogens originated from permafrost and non-permafrost habitats under diverse stress conditions.

Furthermore, this study represents an important contribution to the questions discussed within the field of astrobiology. Methanogenic archaea, derived from Siberian permafrost were used as *model organisms* to investigate their potential to survive simulated Martian thermo-physical conditions, particularly diurnal fluctuations of temperature and humidity applicable to the Mars atmosphere. The comparative system studies served to understand the modern Mars cryosphere and other extraterrestrial permafrost habitats.

1.3 Study site

The study site Samoylov Island (N 72°22, E 126°28) is located within the active and youngest part (about 8.500 years) of the Lena Delta. With an area of 32,000 km^2, the Lena Delta is one of the largest deltas in the world. It is located at the Laptev Sea coast between the Taimyr Peninsula and the New Siberian Islands in the zone of continuous permafrost. The Lena Delta is characterized by an arctic continental climate with a low mean 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 soils in the Lena Delta are entirely frozen, leaving only 20-50 cm the upper part, the so-called “active layer”, remaining unfrozen during the summer months.

During the expedition Lena Delta 2004 soil samples were collected from the active layer. Altogether three soil profiles were sampled that represent characteristic geomorphic units of the island. They are different in regard to soil genesis and soil properties. Two of these profiles were located at the rim and at the depression of low-centred ice-wedge polygon (N 72°22.2’, E 126°28.5’) with a diameter of about 20 m (Fig. 1.3) in the eastern part of the island. The soils are characterized by very homogenously spread soil units: the polygon rims were dominated by *Glacic Aquiturbels*, whereas the prevalent soil type of the polygon depressions were *Typic Historthels*, classified according to the US Soil Taxonomy (Soil Survey Staff, 1998). The soils of the polygon depression were characterized by a water level near the soil surface and a predominantly anaerobic accumulation of organic matter. The drier soils of the polygon rim showed a distinctly deeper water level, lower accumulation of organic matter and pronounced cryoturbation properties. In summer 2004 the thaw depth of the soils varied between 25 cm for polygon depression and 50 cm for the polygon rim.

The third profile was located at 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*,

classified according to the US Soil Taxonomy (Soil Survey Staff, 1998). Additional soil characteristics, analysed according to Schlichting *et al.* (1995), are summarised in Table 8.1 (Appendix). Soil samples were filled in gastight plastic jars (Nalgene) and transported to Germany in frozen condition.

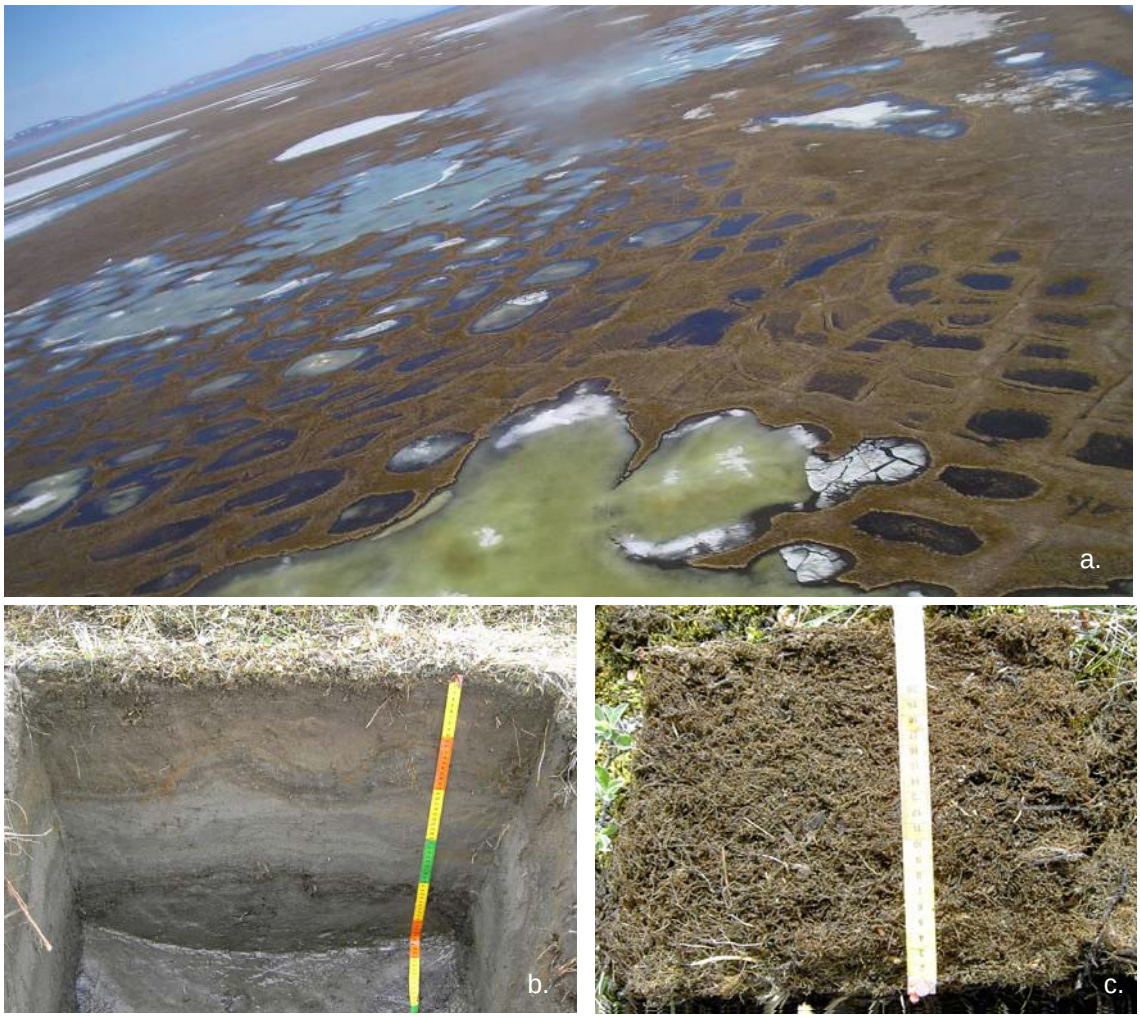

Fig. 1.3: Low-centred ice-wedge polygons on Samoylov Island in Lena Delta, Siberia (a.). The images represent the profiles of the polygon rim (b.) and polygon centre (c.)

1.4 Overview of the publications

In the following chapters (2-5), four published respectively submitted manuscripts of the results of this work are presented.

In the first study, the survival potential and stress response of methanogenic archaea in their natural environment of Siberian permafrost for two different polygonal tundra soils were studied. These investigations were complemented by experiments with *Methanosarcina* sp. SMA-21 isolated from permafrost and *Methanosarcina barkeri*, a well-studied archaeon from a non-permafrost environment. The results are presented in the manuscript, which is in preparation for *Applied and Environmental Microbiology*:

(1) **Daria Morozova** and Dirk Wagner: Effect of high salinity, freezing and desiccation on methanogenic archaea from Siberian permafrost

The second study is closely related to the first study. The aim of this study was to investigate the survival potential of pure cultures of methanogenic archaea exposed to different environmental stress conditions such as low temperature (down to -78.5 °C), high salinity (up to 6 M NaCl), starvation (up to 3 months), long-term freezing (up to 2 years), desiccation (up to 25 days) and exposure to oxygen (up to 72 hours). The temperature influence on the stress tolerance was tested. The experiments were conducted with methanogenic archaea from Siberian permafrost and were complemented by experiments on well-studied methanogens from non-permafrost habitats. The results of this study are presented in the manuscript, which is published by *FEMS Microbiology Ecology*.

(2) **Daria Morozova** and Dirk Wagner: Stress response of methanogenic archaea from Siberian permafrost compared to methanogens from non-permafrost habitats

The third publication deals with the radiation resistance of the methanogenic archaea used in the previous studies. Methanogenic archaea from permafrost and from non-permafrost habitats were exposed to solar UV- and ionizing radiation in order to assess their limits of survival. The results are presented in the manuscript, which is submitted to *Environmental Microbiology*.

(3) **Daria Morozova**, Ralf Moeller, Petra Rettberg and Dirk Wagner: Radiation resistance of methanogenic archaea from Siberian permafrost

In the fourth publication methanogenic archaea from Siberian permafrost and reference organisms from non-permafrost habitats were used for studying their survival potential under simulated thermo-physical Martian conditions at low- and mid-latitudes. On Mars, the saturation of the atmospheric water content, which leads to adsorption and condensation of water, may provide conditions for organisms to temporarily get access to liquid-like water, also at temperatures far below 0 °C. It is a challenge in the field of exobiology to study, if the temporary availability of liquid-like water could support life-processes on Mars. The results are presented in the following manuscript, which in press in *"Origins of Life and Evolution of the Biospheres"*:

(4) **Daria Morozova**, Dirk Möhlmann and Dirk Wagner: Survival of Methanogenic Archaea from Siberian Permafrost under Simulated Martian Thermal Conditions

2 Publication I

(In preparation)

Effect of high salinity, freezing and desiccation on methanogenic archaea from Siberian permafrost

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Although methanogenic archaea persist in various habitats, yet little is known about the physiological potential of these specialized microorganisms which are capable to grow and survive in extreme permafrost habitats. Here we examined the stress response of methanogenic archaea and their tolerance limits under simulated extreme environmental conditions such as extremely low temperature, high salinity and desiccation. The experiments were conducted with the methanogenic archaea in their natural environment of Siberian permafrost for two different polygonal tundra soils. These investigations were complemented by experiments with *Methanosarcina* sp. SMA-21 isolated from Siberian permafrost and *Methanosarcina barkeri*, a well-studied archaeon from a non-permafrost environment. Our results indicate a high survival potential of methanogenic archaea from Siberian permafrost when exposed to the extreme conditions tested. In contrast, these stress conditions were lethal for *M. barkeri.* These investigations suggest that methanogenic archaea from permafrost possess unique stress adaptation mechanisms and may have important implications on the search for past or extant life in permafrost presumably present on Mars.

INTRODUCTION

Approximately 25 % of the earth's continental area and 65 % of Russia is underlain by permafrost (Ershov, 1995). Artic tundra soils are an important natural source in the budget of the climate-relevant greenhouse gas methane (Fung *et al.*, 1991; Wagner *et al.*, 2003; Smith *et al.*, 2004). Most of the methane produced in permafrost is of biogenic origin. However, the control mechanisms of methane production, oxidation and emission in arctic soils are still not completely understood.

Methane production by methanogenic archaea represents the terminal step in carbon flow in extreme habitats. The natural habitats of methanogens are anaerobic environments, such as intestines of ruminants, the human digestive system, rice paddies, lakes or marine sediments, as well as extreme

habitats like hot springs, salt ponds and permfrost sediments (Balch *et al.*, 1979; Jones *et al.*, 1987; Zinder, 1993; Høj *et al.*, 2005, Wagner *et al*., 2007). Although the metabolism of methanogenic archaea was studied in different environments (Shuisong 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 exposed to the permafrost's harsh environmental conditions (Vishnevetskaya *et al.*, 2000; Høj *et al.*, 2005; Ganzert *et al.*, 2007).

The extreme environment of Siberian permafrost soils is particularly appropriate for an investigation of the survival potential of microorganisms. The permafrost thickness can reach several hundreds of meters, e.g. in East Siberia (Central Yakutia) it is of about 600 - 800 m. During few months of arctic summers only the uppermost part of the permafrost thaws. This so called active layer is about 0.2 - 2.0 m thick and is subjected to seasonal freezing and thawing cycles with extreme temperature regime from about +15 °C to –35 °C (Wagner *et al.*, 2003). The reservoir of viable microorganisms and bio-signatures in the earliest permafrost layers has over the past decades been one of the main reasons for the increased interest in permafrost biology (Vorobyova *et al.*, 1997; Willeslev *et al.*, 2004; Ponder *et al.*, 2005).

The exobiological interest in terrestrial permafrost is attributed to the recent Mars Odyssey observations, which indicate that there are large permafrost areas on our neighbouring planet Mars (Boynton *et al.*, 2002; Mellon *et al.*, 2004; Litvak *et al.*, 2006). In the past, 3.5 – 3.8 Ga ago, when life had evolved on Earth, the climate on Mars was probably much wetter and warmer than today (Jakosky and Phillips, 2001; Solomon *et al.*, 2005) and thereby offered conditions suitable for life. If life had also emerged on Mars, it either subsequently adapted to the drastically changed environments or it became extinct. Meanwhile as liquid water has being depleted from Mars, presumed life may have retreated to potential biotic oases where liquid water may be present, such as polar ice caps and permafrost regions. On Earth, terrestrial permafrost, in which microorganisms have survived for several millions of years, have been proposed to be a model for extraterrestrial analogues (Horneck, 2000; Wagner *et al.*, 2001). The possibility of extant or extinct life on Mars has been fueled by the recent U.S. Mars Exploration Rover Opportunity discovery that liquid water most likely exists on Mars (Christensen *et al.*, 2004; Klingelhofer *et al.*, 2004; NASA, 2006) and findings from the Planetary Fourier Spectrometer onboard the *Mars Express,* as well as ground–based observations, indicating that methane currently exists in the Martian atmosphere (Formisano, 2004). Considering the short lifetime of methane, different scientists suggest a biotic origin of this trace gas (Krasnopolsky *et al.*, 2004). In the light of this assumption, methanogenic

archaeon from terrestrial permafrost habitats could be considered as analogues for probable extraterrestrial organisms.

The objective of this study was to characterize the potential stress response of methanogenic archaea from Siberian permafrost under *in situ* conditions within permafrost soil samples as well as in pure cultures for some of environmental stress conditions, especially high salinity, low temperature and exposure to desiccation. Additionally, we investigated the general perspectives of the interaction between the microorganisms and the soil matrix, which could protect methanogenic archaea from the unfavourable living conditions. For this purpose, permafrost samples and pure cultures of methanogens were used. Particular emphasis was placed on *Methanosarcina* sp. SMA-21 isolated from the active layer of permafrost. Previous studies had shown that these methanogenic archaea from Siberian permafrost exhibit a high survival potential under simulated Martian conditions (Morozova *et al.*, 2007). To compare our results, *Methanosarcina barkeri* was used, a well-known and studied representative of the same genus from non-permafrost habitats. Our study will contribute to fill fundamental gaps in our knowledge about the physiological potential of methanogenic archaea and their tolerance limits to extreme environmental conditions in terrestrial or extraterrestrial permafrost.

MATERIALS AND METHODS

Sampling site and characteristics. The investigation site Samoylov Island (N 72°22, E 126°28) is located within the central part of the Lena Delta, which is one of the largest deltas in the world with an area of 32,000 km^2. It is placed at the Laptev Sea coast between the Taimyr Peninsula and the New Siberian Islands in a continuous permafrost zone. A detailed description of a geomorphologic situation of the island and the whole delta was given previously (Schwamborn *et al.*, 2002; Wagner *et al.*, 2003). The Lena Delta is located in the continuous permafrost zone. It is characterised by an arctic continental climate with a low mean annual air temperature of -14.7 °C (T_{min} = -48 °C, T_{max} = 18 °C) and a mean annual precipitation of about 190 mm. The island is dominated by a typical permafrost pattern of symmetrically formed low-centred polygons which cover at least 70 % of the island's area. The soils in the Lena Delta are entirely frozen leaving only an upper part, so-called "active layer" of about 20-50 cm unfrozen during summer months.

During the Expedition "LENA 2004" (Wagner and Bolshianov, 2006) soil samples were collected from the active layer of two soil profiles. These profiles were located at the rim and at the centre of low-centred polygon (N 72°22, E 126°28) in the eastern part of the island. The thaw depth of soils varied between 25 cm for polygon depression and 50 cm for polygon rim. The investigated soils were classified according to the U.S. Soil Taxonomy (Soil Survey Staff, 1998). The samples were filled in gastight plastic jars (Nalgene) and transported to Germany frozen. Further details of the sample procedure were described elsewhere (Kobabe *et al.*, 2004). Approximately 10 g of each soil sample was used for dry weight determination. All the results were expressed per gram of dry soil.

***In situ* methanogenesis under different salinities.** The resistance of methanogenic archaea to different salt conditions was analyzed under *in situ* conditions on Samoylov Island (Lena Delta) during the Expedition LENA 2004. Soil samples were taken from the polygon rim (Bjjg horizon, 18-23 cm) and depression (A horizon, 17-20 cm). Immediately after the sampling, the fresh soil samples from the bottom of the active layer (polygon rim 30 g and polygon depression 20 g) were homogenized and placed into 100 ml glass flask containing 20 or 40 ml salt solution, respectively. Salt solution contained NaCl (2.25 M), $CaCl_2$ (70 mM) and $MgCl_2$ (0.6 M). Calculation of salt solution concentration was based on the NaCl content. This is relevant for all presented indications. Selected concentrations of salt solution were used (0.1, 0.2, 0.3 and 0.4 M). For the determination of methane production without salt addition soil samples were mixed with deionized water. Additionally a saturated NaCl solution (6 M) was selected. Flasks were closed gastight with a screw cap with septum, evacuated and flushed with pure N_2. Three replicates were used for each salt concentration. The prepared soil samples were incubated under *in situ* temperatures (from 0 °C to +2 °C) at the bottom layer of the soil profile from which the samples have been taken. The activity of methanogenic archaea was measured as described below.

Methanogenesis in permafrost soils under high salinity. In addition to *in situ* experiments the resistance of methanogens to salt concentrations higher than 0.4 M was tested in further laboratory experiments. Fresh soil material (10 g) from polygon depression (Oi horizon, 0-5 cm depth) and the polygon rim (Bjjg horizon, 18-25 cm depth) was weighed into 100 ml glass flask under anoxic conditions and closed with a screw cap containing a septum. Soil samples from a polygon depression were mixed with 20 ml and samples from polygon rim with 5 ml salt solution. Different concentrations of salt solution were used (0, 1.0 M, 3.0 M and 6 M NaCl solutions). Sterilized soils (2 h at 121 °C) were used as a negative control. Soil samples were incubated at 10 °C. The activity of methanogenic archaea was determined according to description given below.

Methanogenesis in permafrost soils after freezing. Fresh soil material (1 g) from the polygon rim (18-25 cm, collected in 2003) was frozen at -78.5 °C for 24 h, thawed at 10 °C and placed into a 25 ml glass flask, mixed with deionised water (1 ml), closed with a screw cap containing a septum and incubated at 10 °C. Survival was calculated according to the cell count and activity measurements. Three replicates were used.

Methanogenesis in permafrost soils after desiccation. Fresh soil material (1 g) from the polygon rim (18-25 cm, collected in 2003) was desiccated under anoxic conditions at 20 °C and stored for up to 25 days. After the experiment soil samples were placed into a 25 ml glass flask, mixed with deionised water (1 ml), closed with a screw cap containing a septum and incubated at 10 °C. Survival was calculated according to the cell count and activity measurements. Three replicates were used.

Microbial cultures. To enrich and isolate methanogenic archaea the bicarbonate-buffered, oxygen-free OCM culture medium was used, prepared according to Boone *et al*. (1989). Methanol (20 mM), acetate (20 mM) or H_2/CO_2 (80/20, v/v) were used as substrates. Cultures were grown under an atmosphere of H_2/CO_2 (80/20, v/v). The incubation temperatures were 4 °, 10 ° and 28 °C.

Methanosarcina sp. SMA-21 (isolated in our lab from permafrost sediments sampled in summer 2002 from Siberian permafrost, Russia) grew well at 28 °C and more slowly at low temperatures (4 °C and 10 °C). The strain appeared as irregular cocci, 1-2 µm in diameter. Large cell aggregates were regularly observed. *Methanosarcina barkeri* DSM 8687, originating from a peat bog in Northern Germany (Maestrojan *et al.*, 1992), was obtained from the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ).

Salt stress experiments at different temperatures with pure methanogenic cultures. The effect of salt shock on methanogenic archaea was studied as follows. An aliquot of 5 ml of each culture grown to a cell density of 10^8 cells ml^{-1} was supplemented with anaerobic salt solution. The selected NaCl end concentrations were 0 M, 0.1 M, 0.2 M, 0.3 M, 0.4 M, 1.0 M, 3.0 M and 6 M (saturated). The incubation with high salt concentrations (1-6 M) was assessed at 4 °C and 28 °C for up to 3 months. Sterilized cultures (2 h at 121 °C) supplemented with 0.4 M and saturated salt solution were used as negative controls. The cell numbers and activities were measured as described below. All the experiments were done in triplicate.

Freezing experiments with pure cultures. To determine if temperature acclimation influences freezing survival, the methanogenic cultures were divided into two portions; one portion was immediately frozen at -78.5 °C, and the other one was cold shocked at 10 °C for 2 h before being frozen at -78.5 °C. For each portion, an aliquot of 1 ml (10^8 cells ml^{-1}) was removed just before freezing. After storage at -78.5 °C for 24 h, the frozen cells were thawed at room temperature. Cell numbers were calculated before and after the freezing as described below. After thawing, aliquots were placed under anaerobic conditions in 25 ml glass flasks, supplemented with 10 ml of fresh OCM medium and appropriate substrates (H_2/CO_2 for *Methanosarcina* sp. SMA-21, methanol for *Methanosarcina barkeri*). The flasks were sealed with a screw cap containing a septum and incubated at 28 °C. The activities were measured as described below.

Additionally, *Methanosarcina* sp. SMA-21 and *Methanosarcina berkeri* grown at 28 °C to a cell density of 10^8 cells ml^{-1} were slowly frozen (0.2 °C min^{-1}) to -20 °C and stored for a period of 1 to 2 years. Once the samples were thawed, an aliquot of 5 ml of each culture was placed into the fresh OCM medium and supplemented with the appropriate substrates. Than survival was calculated as described below. All the experiments were done in triplicate.

Desiccation experiments with pure methanogenic cultures. An aliquot of each methanogenic culture was placed onto microscope cover slips (1 ml per cover slip) and allowed to dry completely. For some experiments glass beads (1.0 g, 1 mm diameter) were added to cell suspension. Cover slips were stored anaerobically at 28 °C for 2, 5, 7 and 25 days. After incubation, cells were rehydrated by placing the cover slip in 2 ml of the appropriate growth medium for 30 min at room temperature. The resulting cell suspensions were placed under anaerobic conditions into 25 ml glass flasks, supplemented with 10 ml of the fresh OCM medium and appropriate substrates. The flasks were sealed with a screw cap containing a septum and incubated at 28 °C. Survival was determined as described below. All the experiments were done in triplicate.

Methane analysis. The activity of the methanogenic archaea was calculated based on the linear increase of the CH_4 concentration in the headspace. The methane concentration was measured by gas chromatography. The gas chromatograph (Agilent 6890, Fa. Agilent Technologies) was equipped with a Carbonplot capillary column (Ø 0.53 mm, 30 m length) and a flame ionisation detector (FID). Both the oven and the injector temperature were 45 °C. The temperature of the detector was 250 °C. Helium served as the carrier gas. All the gas sample analyses were done following 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. The counting was done manually. For each hybridisation approach and sample at least 800 DAPI stained cells were counted on

30 randomly chosen counting squares. Microscopic performance was carried out using a magnification of 63 x 100 giving an area of 3.9204 x 10^{-2} mm^2 per counting square.

Statistical analysis. Significant differences between the three replicates used in the different stress experiments were analyzed using the Student's t-test (Wardlaw, 1985).

RESULTS

Soil properties. The physico-chemical soil characteristics, analyzed according to Schlichting *et al*. (1995), are summarized in Table 1. The soils of the investigation site are characterized by homogenously spread soil units: polygon rims were dominated by *Glacic Aquiturbels*, whereas the prevalent soil type of the polygon depressions were *Typic Historthels*, classified according to US Soil Taxonomy (1998). The soils of the polygon depression are characterized by a water level near the soil surface and a predominantly anaerobic accumulation of organic matter. The drier soils of the polygon rim showed a distinctly deeper water level, lower accumulation of organic matter and pronounced cryoturbation properties. The soils of the polygon centre were characterized by a soil texture of silty sand, while the soils of the polygon rim were characterized by a soil texture of silty and loamy sand and of pronounced cryoturbation properties.

Methanogenesis in permafrost soils under different salinities. Methanogenic activity was determined under *in situ* temperature conditions in the polygon rim and depression soils incubated with different salt concentrations from 0.1 to 0.4 M. Under *in situ* conditions, the methane production rates could be determined at the bottom of an active layer (Bjjg horizon) of the polygon rim under all salt concentration conditions (Fig. 1), while no activity was determined in the polygon depression (A horizon, data not shown). The methane production rates varied between 0.05 ± 0.03 nmol CH_4 h^{-1} g^{-1} and 0.2 ± 0.02 nmol CH_4 h^{-1} g^{-1}. Highest methane production was found in samples incubated with 0.1 M (0.2 nmol CH_4 h^{-1} g^{-1}) and 0.2 M salt solution (0.12 nmol nmol CH_4 h^{-1} g^{-1}), low values between 0.07 and 0.05 nmol CH_4 h^{-1} g^{-1} at 0.3 M and 0.4 M salt solution and traces of methane were measured in soil samples, incubated with saturated salt solution (0.01 ± 0.001 nmol CH_4 h^{-1} g^{-1}). However, it was only twice lower in comparison to the samples, incubated without salt addition (0.02 ± 0.004 nmol CH_4 h^{-1} g^{-1}).

Primary results obtained from the *in situ* experiments were confirmed by further laboratory experiments. Methanogenic activity was observed in all permafrost samples from the polygon rim and depression with all salt concentrations. Highest methane production was found in samples without any additional salt. The methane production rates were generally higher in the polygon depression (Oi horizon) than in the rim profile which ranged from 0.02 ±

0.005 nmol CH_4 h^{-1} g^{-1} to 19.36 ± 9.6 nmol CH_4 h^{-1} g^{-1}. The methane production in the rim profile (Bjjg horizon) varied between 0.003 ± 0.001 nmol CH_4 h^{-1} g^{-1} and 0.37 ± 0.3 nmol CH_4 h^{-1} g^{-1} (Tab. 2). Additionally, the methane production rates per cell and per hour were calculated. The methane production rates detected per methanogenic cell in samples of the centre profile (from 0.04 ± 0.01 to 0.001 ± 0.0 x 10^{-7} nmol CH_4 h^{-1} g^{-1} $cell^{-1}$) were about one order of magnitude lower than in samples of rim (from 0.6 ± 0.1 to 0.1 ± 0.0 x 10^{-7} nmol CH_4 h^{-1} g^{-1} $cell^{-1}$). This was observed for all salt concentrations (Fig. 2). No methane production was determined in the sterilized soil samples.

Methanogenesis in permafrost soils after freezing. The survival rates of methanogenic archaea in permafrost soils after 24 hours exposure to -78.5 °C was determined by both the methane production rates and the cell counts before and after the experiment. The average cell numbers of methanogenic archaea of the polygon depression slightly decreased from 1.6 ± 0.4 x 10^8 cells g^{-1} at the beginning of the experiment to 1.1 ± 0.6 x 10^8 cells g^{-1} after the freezing, which equals to 71 % cell survival (Tab. 3). The activity of methanogenic archaea was only marginally affected by the freezing experiment. Thus, methane production rates decreased from 1.04 ± 0.05 nmol CH_4 h^{-1} g^{-1} before the experiment to 0.72 ± 0.02 nmol CH_4 h^{-1} g^{-1} after exposure to -78.5 °C.

Methanogenesis in permafrost soils after desiccation. Methanogenic archaea of the polygon depression soils were found also highly resistant to 25 days of desiccation. The average cell numbers decreased from 1.03 ± 0.0 x 10^8 cells g^{-1} at the beginning of the experiment to the 0.7 ± 0.1 x 10^8 cells g^{-1} after the desiccation, which equals 66 % cell survival. The methane production rates of the methanogenic archaea decreased from 1.26 ± 0.05 nmol CH_4 h^{-1} g^{-1} to 0.45 ± 0.09 nmol CH_4 h^{-1} g^{-1} after the experiment (Tab. 3).

Effect of salt stress on pure methanogenic cultures. Salt tolerance was assessed in the permafrost strain *Methanosarcina* sp. SMA-21 and the non-permafrost organism *Methanosarcina barkeri* using NaCl salt solutions at different concentrations as an osmolite. High methane production of *Methanosarcina* sp. SMA-21 was observed at all salt concentrations. The methane production of *Methanosarcina barkeri* was significantly different when exposed to different concentrations (Fig. 3).

The highest activity of *Methanosarcina* sp. SMA-21 was detected in samples incubated with 0.3 and 0.4 M NaCl (18.14 ± 2.81 and 17.98 ± 2.51 nmol CH_4 h^{-1} ml^{-1}, respectively), which was similar to the activity in the samples

which had no salt added. The methane production rate at low salt concentrations (0.1 M and 0.2 M) was about a half as high as in the samples with no additional salt (Fig. 3). In comparison, increasing salt concentrations lead to a gradual decrease in the methane production of *Methanosarcina barkeri*. Thus, the methane production production rates of *Methanosarcina barkeri*, incubated with 0.4 M NaCl, decreased from 29.8 ± 2.3 to 0.85 ± 0.16 nmol CH_4 h^{-1} ml^{-1}.

Higher methane production at low incubation temperature was observed in all *Methanosarcina* sp. SMA-21 samples under high salt concentrations. Based on the cell counts and on the methane production measured at different incubation temperatures, the methane production rates per cell and per hour were calculated. At 4 °C the methane production rates detected per methanogenic cell under salt saturated conditions (0.1 ± 0.0 x 10^{-7} nmol CH_4 h^{-1} $cell^{-1}$) were five times higher than the methanogenic activity at 28 °C (0.027 ± 0.0 x 10^{-7} nmol CH_4 h^{-1} $cell^{-1}$). In contrast, *Methanosarcina barkeri* showed not very significant activity under salt saturated conditions. However, methane production rates were higher at 28 °C (0.01 ± 0.002 nmol CH_4 h^{-1} ml^{-1}), than those at 4°C (0.002 ± 0.0001 nmol CH_4 h^{-1} ml^{-1}; Fig. 4). No methane production was observed in the sterilized cultures.

Freezing tolerance of methanogenic cultures. Higher survival after 24 h freezing at -78.5 °C was seen in the *Methanosarcina* sp. SMA-21 (Fig. 5). The average cell numbers of this archaeon decreased from 4.4 ± 1.4 x 10^8 cells ml^{-1} at the beginning of the experiment to 3.9 ± 0.6 x 10^8 cells ml^{-1} at the end of freezing, giving a survival rate of 89.5 %. In contrast, only 0.8 % of *Methanosarcina barkeri* survived exposure to -78.5 °C. The decrease in cell numbers correlated well with the methane production rates of the cultures. The activity of *Methanosarcina* sp. SMA-21 measured after freezing (5.57 ± 0.67 nmol CH_4 h^{-1} ml^{-1}) was only two times lower than the initial activity (10.87 ± 1.22 nmol CH_4 h^{-1} ml^{-1}), while the activity of the *Methanosarcina barkeri* after freezing was three orders of magnitude lower.

Two hours pre-cooling at 10 °C slightly increased the survival potential of *Methanosarcina barkeri* to survive freezing at -78.5 °C, giving a survival rate of 1.4 % and a methane production rate of 0.06 ± 0.01 nmol CH_4 h^{-1} ml^{-1}. In contrast, a positive effect of pre-incubation at 10 °C on the ability to survive freezing at -78.5 °C was not seen for *Methanosarcina* sp. SMA-21 (Fig. 5).

A high survival potential and metabolic activity after long-term incubation at -20 °C was shown for *Methanosarcina* sp. SMA-21. The measured methane production rates decreased from 10.58 ± 0.8 nmol CH_4 h^{-1} ml^{-1} prior to freezing to 9.01 ± 0.5 nmol CH_4 h^{-1} ml^{-1} after two-years freezing. No methane production

was detected in reference organism after just one year of exposure to -20 °C.

Desiccation tolerance of pure methanogenic cultures. The survival of the strains after desiccation was evaluated for up to 25 days of treatment. Survival and methane production rates were higher with glass beads than without. The *Methanosarcina* sp. SMA-21 was found to resist 25 days of desiccation without loss of activity and cultivability (Fig. 6). The average cell numbers of the *Methanosarcina* sp. SMA-21 decreased from $2.3 \pm 0.8 \times 10^8$ to $1.8 \pm 0.4 \times 10^8$ cells ml^{-1}, equivalent to a cell survival rate of 77.5%. The methane production rates decreased slightly from 10.46 ± 2.34 nmol CH_4 h^{-1} ml^{-1} to 5.23 ± 1.7 nmol CH_4 h^{-1} ml^{-1}. In contrast, the average cell numbers and methane production rates of *Methanosarcina barkeri* were drastically reduced after desiccation of 25 days, resulting in a cell survival rate of 0.02 % (Fig. 6).

DISCUSSION

Our results showed high survival of methanogenic archaea from permafrost after exposure to high salinity, extremely cold temperatures, and desiccation. The viability of the methanogens in permafrost soil samples and in pure cultures were comparably high under these stress conditions. In contrast to the permafrost strain, the reference organism from the non-permafrost habitat was highly sensitive to the extreme conditions.

Permafrost ecosystems of the Siberian Arctic are characterized by extreme environmental conditions and distinct fluctuations in its physico-chemical gradient. Within the active layer, microorganisms are exposed to cold temperatures, freeze-thaw cycles, decreasing water activities, aridity and sporadic nutrient availability. There are two major physico-chemical threats that organisms in permafrost need to respond to: ice formation within the cells, which may lead to cell lysis, and salinity increase, which may lead to an osmotic gradient across a cell membrane (Mindock *et al*., 2000; Morita, 2000).

The exposure of soil samples to different salinities, extremely low temperatures and desiccation resulted in high survival rates of methanogenic archaea throughout the experiments. Previous investigations demonstrated that due to the existence of a complex community composed of aerobic and facultative anaerobic microorganisms, methanogenic archaea have a high survival potential against different stress factors. For instance, metabolic activity of strictly anaerobic methanogenic archaea was observeed even under oxic conditions in the presence of indigenous microflora (Wagner *et al.*, 1999). Freeze-thaw cycles followed by saline and desiccation stress, may also affect the composition and function of microbial communities (Schimel and Clein,

1996; Larsen *et al.*, 2002). This is of great ecological significance because a shift in the microbial composition may result in fundamental changes of food chains, potentially influencing the process of the methane formation. For example, association of methanogenic and facultative-anaerobic bacteria could affect survival and the metabolic activity of methanogens under extreme saline conditions by biofilm formation. A biofilm consisting of bacteria and archaea provides an excellent protection for microorganisms as a physical barrier against extreme conditions and seasonal fluctuations of environmental parameters (Wimpenny *et al.*, 2000). Aggregate formation of up to 100 cells of methanogenic archaea could be also a resistance mechanism. The outer cells of an aggregate may shield the inner cells from the damaging influence of extreme environments. Soil or rock grains are also likely to serve as a shield for these organisms by providing a habitat with stable temperatures.

A second factor favouring the viability of methanogens under these stress conditions might be the soil texture. Methanogenic archaea have hydrophobic cell surface and low electrophoretic mobility that support the attachment to the surface of charged soil particles (Grotenhuis *et al.*, 1992). The sorptive capacity of natural soil particles like clay, silt or soil organic matter provide a protective effect on methanogenic archaea (Hejnen *et al.*, 1992; Wagner *et al.*, 1999; Lapygina *et al.*, 2001).

High concentrations of salt, especially NaCl, do not only decrease the extracellular water activity for microorganisms, but also inhibit an enzymatic activity within microbial cells (Shuisong and Boone, 1998). By comparing different permafrost samples under salt stress, it could be shown that high salinity was more harmful for methanogens in a polygon depression soil than that in a polygon rim soil. This result correlates well with the *in situ* experiments. The methane production rates per methanogenic cell under high salinity in a polygon rim were significantly higher compared to the cell activities observed under salt stress in a polygon depression. Therefore, the soil samples of a polygon rim with a high number of methanogens were selected for use in further experiments.

The different survival rates found in the two permafrost soils might therefore result from differences in grain size or in the water adsorption capacity (so-called matrix potential). Thus, higher rates of survival and activity of methanogens under salt stress in samples from the polygon rim soil could be a result of the high silt content which protects the methanogenic archaea against harsh conditions. Compared to the polygon rim, the polygon depression had a much higher composition of sandy material (Tab. 1).

The experiments with pure methanogenic cultures allow comparson of the stress response of permafrost inhabitants with and without a protective soil

matrix. Our results indicate high survival rates of isolates from the permafrost soils. Hence, we suggest that these microorganisms have developed ways to cope with various stresses including repair of damaged DNA and cell membranes, as well as the maintenance of other vital functions required to sustain cell viability.

Methanosarcina sp. SMA-21 from Siberian permafrost was shown to be well adapted to a wide range of salt concentrations. Some similarities to increasing salinity could be found when comparing the metabolic activity of the methanogenic archaea in environmental samples to pure cultures. Higher methane production rates were observed for the *Methanosarcina* sp. SMA-21 and *in situ* permafrost soils, incubated with 0.3-0.4 M NaCl and 0.1-0.4 M NaCl, respectively, than in the samples without salt addition. The exchange processes on the soil particles surface increases the substrate availability for methanogenic archaea. Furthermore, higher methane production under 0.3-0.4 M salt concentration indicates better adaptation to a rapid increase in osmolarity, which occurs during freeze-back of the active layer. The ability of *Methanosarcina* sp. SMA-21 to resist high salinity conditions was enhanced by low incubation temperatures (4 °C). Again, this reflects the *in situ* conditions of the active layer of permafrost in autumn. During the freeze-back of the active layer, the salt concentration in the remaining pore water increased as the temperature decrease within the whole profile.

In contrast, increasing salinity led to reduced activity of the reference organism, *Methanosarcina barkeri,* originating from non-permafrost sediments. The methane production under salt stress was insignificant and did not appear to be favorably influenced by low temperatures.

Temperature, salinity, and soil moisture fluctuations, associated with the freeze-thaw cycles, may affect the composition of microbial communities as well the overall function of the ecosystem (Schimmel and Clein, 1996). Salt tolerance could be associated with cold tolerance, a possibility which was also postulated by Vishnivetskaya (2000) and Gilichinsky *et al.* (2003). This is confirmed by the present results. A high survival rate of 71 %, following temperatures of -78.5 °C, was observed for methanogenic archaea in the polygon rim soil samples. Again, the freezing effect is offset by the role of particle size in the soil (Ostroumov, 1995). It can be assumed that clay/silt arctic soils provide an excellent protection against freezing. In clay soils, a liquid water film was preserved to temperatures as low as -60 °C (Ostroumov and Siegert, 1996). These films are considered to be a crucial component of the permafrost, protecting the viable microorganisms from destruction by ice crystal formation. However, pure methanogenic culture *Methanosarcina* sp. SMA-21 also showed excellent survival of more than 70 % of the cells following freezing at -78.5 °C.

Therefore it could be assumed, that cold adaptation at the cellular level is also required for microorganisms to survive freeze-thaw cycles. In contrast, less than 1 % of *Methanosarcina barkeri* cells survived these conditions. Pre-conditioning to cold temperatures (cold shock), known to increase the resistance to freezing of many microorganisms due to the expression of cold-responsive genes and cryoprotectant molecules (Kim and Dunn, 1997; Wouters *et al.*, 2001; Georette *et al.*, 2004; Weinberg *et al.*, 2005), does not increase the freezing tolerance of *Methanosarcina* sp. SMA-21. This corresponds well with the resistance of this strain to a two-year exposure to subzero temperatures without pre-conditioning. Both results suggest that this strain has already adapted to sub-zero environments. It is known, that cold adaptation comprises a complex pattern of different structural changes within the cell and those changes are regulated on the genomic level (Cavicchioli, 2006).

The methanogenic archaea from permafrost were also highly resistant to desiccation, which occurs during the freezing of the active layer. Dry-wet cycles affect soil physical properties, such as aggregation, influencing microbial activity and the external matric potential between the soil material and the microbial cell (Mikha *et al*., 2005). High desiccation resistance of methanogenic archaea in soil samples could be attributed to the protective effect of soil particles, discussed earlier. This conclusion was supported by the observation of a higher survival rate and potential methane production of *Methanosarcina* sp. SMA-21 in the presence of glass beads. The glass beads probably provided partial protection for the methanogens against desiccation. This observation was the same, regardless of whether the methanogen was obtained from a DSMZ culture collection or freshly isolated from permafrost. Surprisingly, higher survival rates after 25 days of desiccation were found for *Methanosarcina* sp. SMA-21 cells compared to methanogenic archaea in desiccated soil samples. Furthermore, this methanogenic archaeon was able to produce methane immediately following rehydration, an indication of very rapid repair mechanisms.

Comparing the resistance of methanogenic archaea in the permafrost soils and in pure cultures, it could be suggested that neither physical nor biological protection is 100 % efficient. The high survival rates of methanogenic archaea under stress conditions are due to their physiological potential. Different strains of methanogens, which include representatives from permafrost and non-permafrost habitats, exhibit marked differences in their stress tolerance. Methanogenic archaea residing in Siberian permafrost face conditions that are different to those experienced by non-permafrost methanogens, namely sub-zero temperatures, freeze-thaw cycles, starvation, aridity and increased levels of long-lasting back-ground radiation which result

from radiated accumulation over geological time scales. Therefore, the methanogenic community in permafrost should have adaptations that have allowed them to survive in these conditions, which are lethal for the non-permafrost methanogens.

The characterizations of the physiological potential of methanogenic archaea in the extreme permafrost environments on Earth continue to present many challenges. Much of the current interest in permafrost microbiology is being driven by astrobiology implications, as permafrost present on Mars is considered a primary target for the search for past or extant life. In the search for life in extremely cold environments on other planets within our solar system, the physiological potential and the metabolic specificity of methanogenic archaea from extreme permafrost environments provides very useful insights for the astrobiological studies. In this respect, understanding the permafrost methanogenic life is paramount in future exploration for extraterrestrial life that could possibly exist on other bodies within our solar system that are rich in permafrost. Methanogenic archaea from terrestrial permafrost may therefore serve as a useful model for further investigation of potential Martian life.

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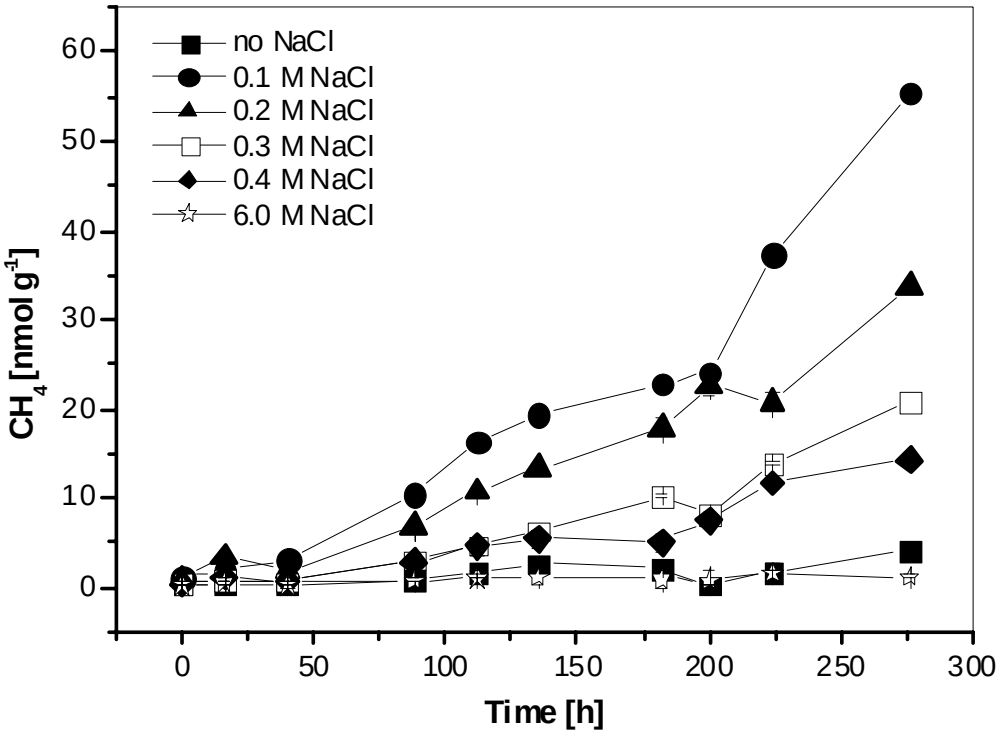

Fig. 1. Methane production of methanogenic archaea in the bottom zone of the active layer in the polygon rim incubated with varying salt concentrations under *in situ* temperature conditions (+2 °C; means ± standard error, n=3).

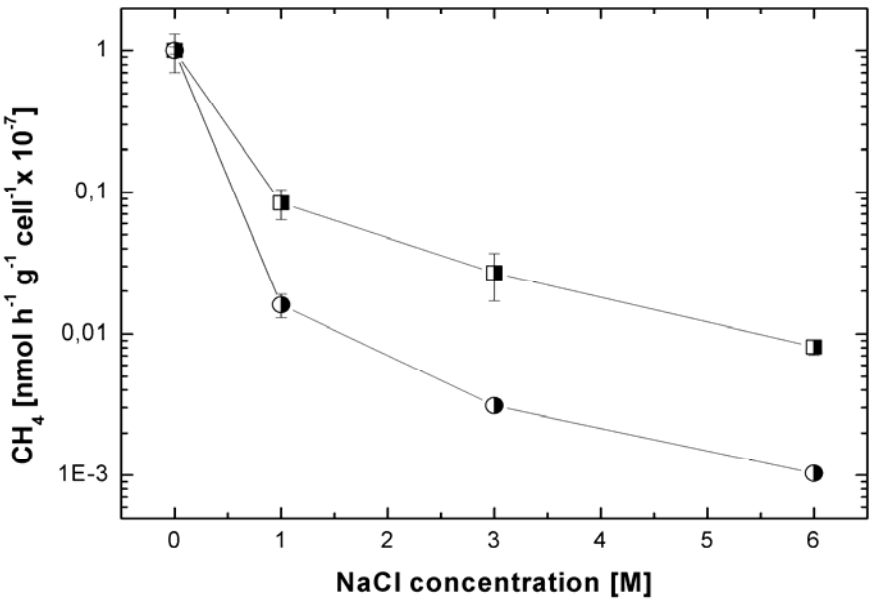

Fig. 2. Methane production rates of methanogenic archaea in soil samples from the polygon rim (squares) and a polygon centre (circles) incubated with varying salt concentrations (means ± standard error, n=3).

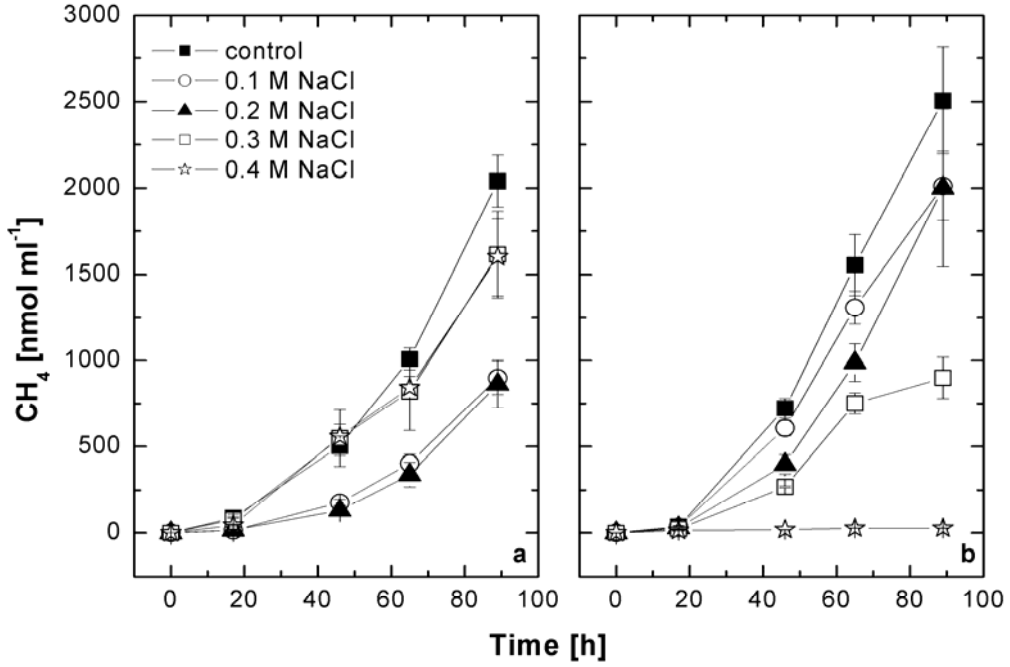

Fig. 3. Methane production of the permafrost strain *Methanosarcina* sp. SMA-21 (a) and *Methanosarcina barkeri* (b), incubated with varying salt concentrations at 28 °C (means ± standard error, n=3).

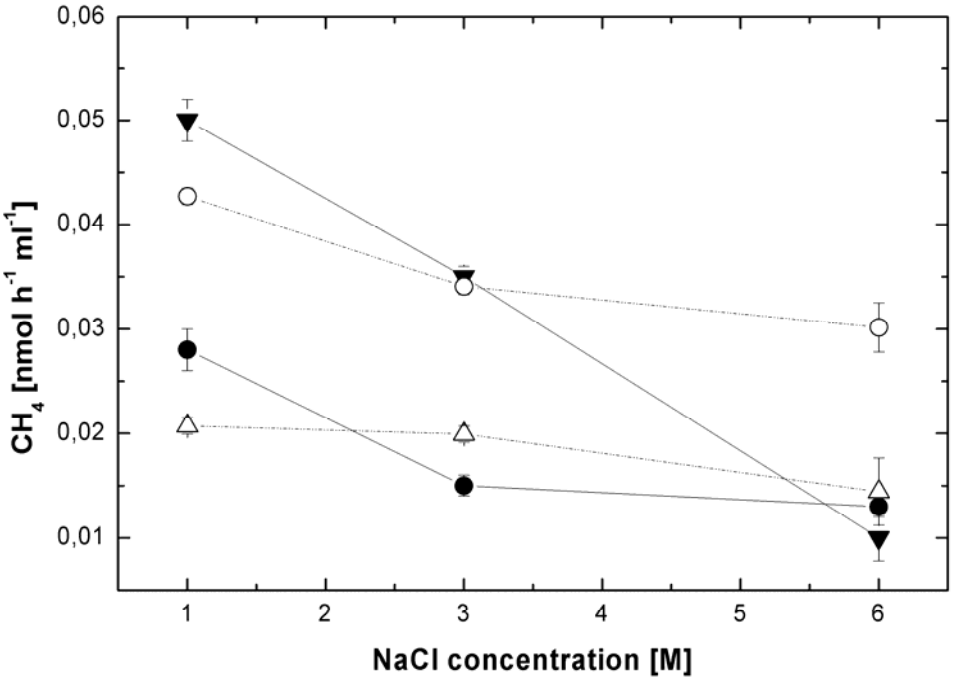

Fig. 4. Methane production rates of the permafrost strain *Methanosarcina* sp. SMA-21 (scircles) and *Methanosarcina barkeri* (triangles) strains, incubated with high salt concentrations at 28 °C (dark symbols) and 4 °C (open symbols; means ± standard error, n=3).

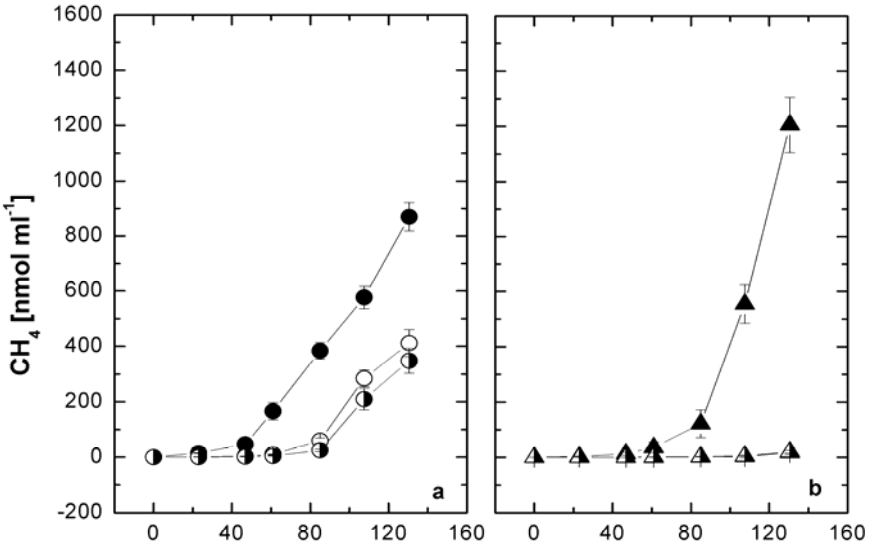

Fig. 5. Methane production of the *Methanosarcina* sp. SMA-21 (a, circles), and *Methanosarcina barkeri* (b, triangles) strains after freezing for 24 h at -78.5 °C for cold shocked (half-open symbols) and non-cold-shocked (open symbols) cultures in comparison to untreated control samples (dark symbols; means ± standard error, n=3).

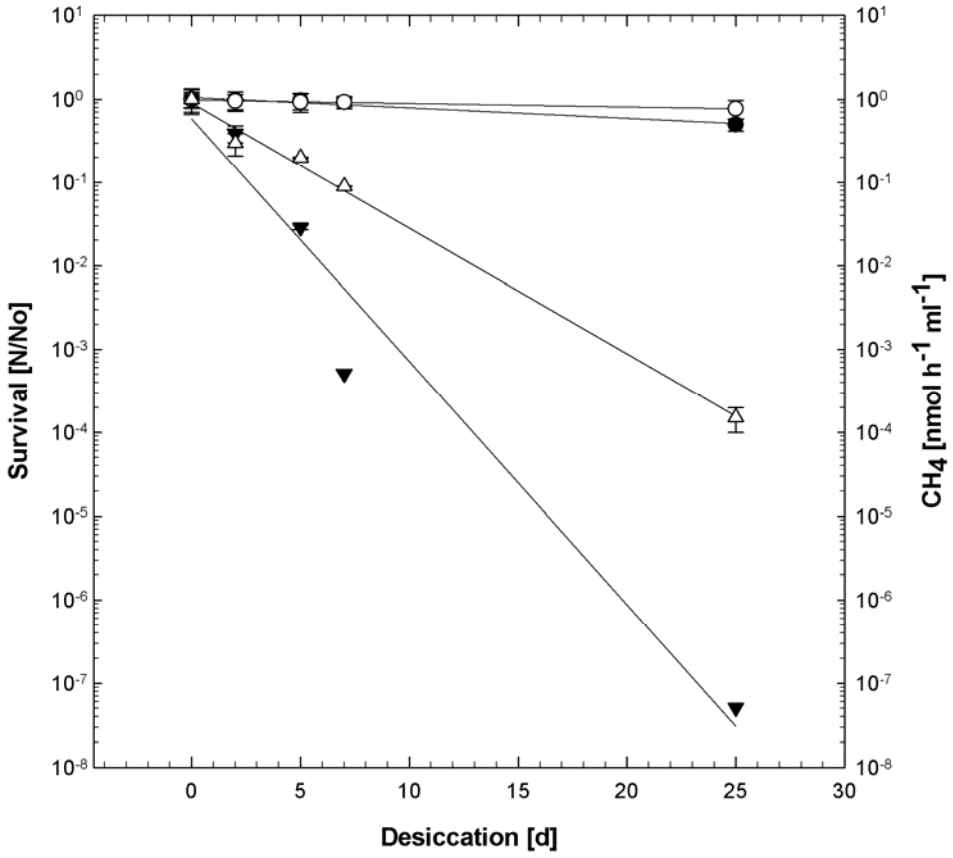

Fig. 6. Methane production rates (dark symbols) and cell survival rates (open symbols) of desiccated cells of *Methanosarcina* sp. SMA-21 (circles) and *Methanosarcina barkeri* (triangles;

means ± standard error, n=3).

Tab. 1 Selected soil properties of a polygon rim, 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**
Rim						
0-6	26.2	3.0	0.2	2.4	10.6	87.0
6-11	15.7	2.1	0.1	2.3	9.1	88.5
11-18	24.1	2.3	0.1	1.7	17.5	80.7
18-25	24.8	2.0	0.1	10.0	45.7	44.3
25-32	25.2	1.2	0.0	3.0	11.1	85.9
32-38	16.6	2.8	0.1	0.5	21.5	78.1
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

Tab. 2: Methane formation in soil samples exposed to different salinities under laboratory-simulated conditions.
Means ± standard error, r^2 = linear increase coefficient, n=3.

NaCl concentration [M]	Methane production rate [nmol h^{-1} g^{-1}]			
	Polygon rim (*Glacic Aquiturbel*)	r^2	Polygon centre (*Typic Historthel*)	r^2
0	0.37±0.3	0.98	19.35±9.6	0.87
1	0.02±0.02	0.97	0.89±0.6	0.93
3	0.01±0.01	0.86	0.06±0.02	0.99
6 (saturated)	0.003±0.001	0.93	0.02±0.01	0.94
control (sterilized)	0	0	0	0

Tab. 3: Methane production rates and cell counts of methanogenic cultures in polygon rim samples before and after 24h freezing at -78.5 °C as well as before and after 25 days desiccation. Means ± standard error, cs = cold shocked, non cs = non-cold shocked cultures, * = methane production per gram dry soil, n=3.

Samples	CH_4 production [nmol h^{-1} g^{-1}]*	Cell counts 10^8	Survival rates [%]
Soil samples before freezing	1.04 ± 0.05	1.6 ± 0.4	100
Soil samples after freezing	0.72 ± 0.02	1.1 ± 0.6	71
Soil samples before desiccation	1.26 ± 0.05	1.03 ± 0.0	100
Soil samples, after desiccation	0.45 ± 0.09	0.7 ± 0.1	66

3 Publication II

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Stress response of methanogenic archaea from Siberian permafrost compared with methanogens from nonpermafrost habitats

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Keywords
methanogenic archaea; permafrost; low temperature; stress response; life on Mars.

Abstract

We examined the survival potential of methanogenic archaea exposed to different environmental stress conditions such as low temperature (down to −78.5 °C), high salinity (up to 6 M NaCl), starvation (up to 3 months), long-term freezing (up to 2 years), desiccation (up to 25 days) and oxygen exposure (up to 72 h). The experiments were conducted with methanogenic archaea from Siberian permafrost and were complemented by experiments on well-studied methanogens from nonpermafrost habitats. Our results indicate a high survival potential of a methanogenic archaeon from Siberian permafrost when exposed to the extreme conditions tested. In contrast, these stress conditions were lethal for methanogenic archaea isolated from nonpermafrost habitats. A better adaptation to stress was observed at a low temperature (4 °C) compared with a higher one (28 °C). Given the unique metabolism of methanogenic archaea in general and the long-term survival and high tolerance to extreme conditions of the methanogens investigated in this study, methanogenic archaea from permafrost should be considered as primary candidates for possible subsurface Martian life.

Introduction

Permafrost on Earth, which covers around 24% of the land surface (Anisimov & Nelson, 1996; Zhang *et al.*, 1999), 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 of biological origin, carried out by methanogenic archaea, a small group of strictly anaerobic chemolithotrophic organisms. They can grow with hydrogen as an energy source and carbon dioxide as the only carbon source. In addition to this specific metabolism, methanogens are able to use only a limited number of organic substrates (acetate, formate, methanol, methylamines) to produce methane (Zinder, 1993). Methanogenic archaea are widespread in nature and highly abundant in extreme environments tolerating low/high temperatures (permafrost, hot springs), extreme salinity (saltern ponds) and low/high pH (solfataras, soda lakes). In addition to mesophilic species, thermophilic and hyperthermophilic methanogens have also been identified (Stetter *et al.*, 1990; Garcia *et al.*, 2000). Recently, more attention has been paid to the isolation of psychrophilic strains since a number of methanogenic habitats are located in cold climates (Gounot, 1999). So far, only a few strains (e.g. *Methanococcoides burtonii*, *Methanogenium frigidum*, *Methanosarcina* spp.) 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 (Shuisong & Boone, 1998; Garcia *et al.*, 2000; Eicher, 2001; Lange & Ahring, 2001), only a few studies have focussed on the ecology of the methanogenic archaea exposed to the harsh environmental conditions of permafrost, e.g. subzero temperatures, low water activity and low nutrient availability (Vishnivetskaya *et al.*, 2000; Høj *et al.*, 2005; Ganzert *et al.*, 2007).

Furthermore, permafrost is the main focus of extraterrestrial research in astrobiology, because it is a common phenomenon in our solar system. Evidence of cryotic systems on present-day Mars (patterned ground, glaciers and thermokarst) has been found by *Mars Express*. The possibility of extant or extinct life on Mars has been fueled by the recent US Mars Exploration Rover Opportunity discovery that liquid water most likely exists on Mars (Christensen *et al.*, 2004; Klingelhofer *et al.*, 2004) and findings from the Planetary Fourier Spectrometer onboard the *Mars Express*, as well as ground-based observations, indicating that methane currently exists in the Martian atmosphere (Formisano, 2004). Considering the short lifetime of methane, this trace gas could only originate from

active volcanism – which has not yet been observed on Mars – or from biological sources. Furthermore, there is evidence that prior to 3.8 Ga, when terrestrial life arose, environmental conditions on Mars were most likely similar to those on early Earth (Carr, 1989, 1996; Durham *et al.*, 1989; Wharton *et al.*, 1989; McKay & Davis, 1991; McKay *et al.*, 1992). If life had also emerged on Mars, it either subsequently adapted to the drastically changed environment or it became extinct. One possibility for survival of Martian microorganisms could be in lithoautotrophic subsurface ecosystems such as deep sediments near the polar ice caps and in permafrost regions. In the light of this assumption, methanogenic archaea from terrestrial permafrost habitats could be considered as analogues for probable extraterrestrial organisms.

The objective of this study was to characterize the survival potential of methanogenic archaea from Siberian permafrost exposed to different extreme environmental stress conditions. In particular, high salinity, extremely low temperature, starvation, desiccation and exposure to oxygen were studied. Particular emphasis was placed on *Methanosarcina* sp. SMA-21 isolated from the active layer of permafrost. Previous studies had shown that these methanogenic archaea from Siberian permafrost exhibit a high survival potential under simulated Martian thermophysical conditions (Morozova *et al.*, 2007). To compare our results, two methanogens from nonpermafrost habitats have been used: the *Methanosarcina barkeri* strain, a well-known and studied representative of the genus, and *Methanobacterium* MC-20, originating from habitats experiencing extreme conditions (Lascu, 1989). Our study will contribute to an improved understanding of extraterrestrial life, if present, especially with regard to possible protected niches on present-day Mars.

Materials and methods

Microbial cultures

Permafrost samples were obtained from Samoylov Island (72°22′N, 126°28′E), located within the central part of the Lena Delta, Siberia. A detailed description of the geomorphologic situation of the island and the whole delta was given previously (Schwamborn *et al.*, 2002; Wagner *et al.*, 2003). To enrich and isolate methanogenic archaea, bicarbonate-buffered, oxygen-free OCM culture medium was used, prepared according to Boone *et al.* (1989). Cultures were grown under an atmosphere of H_2/CO_2 (80 : 20, v/v) as substrate. Incubation temperatures were 4, 10 and 28 °C.

Methanosarcina SMA-21 (isolated in our laboratory from permafrost sediments sampled in summer 2002 from Siberian permafrost) grew well at 28 °C and more slowly at low temperatures (4 and 10 °C). The strain appeared as irregular cocci, 1–2 μm in diameter. Large cell aggregates were regularly observed. *Methanobacterium* MC-20 (originating from the nonpermafrost sediments sampled from hydrogen sulfide- and carbon dioxide-rich, oxygen-poor atmosphere and light-free extreme environments of Mangalia, Romania; Lascu, 1989; Sarbu & Kane, 1995) was isolated in our laboratory at 28 °C. Cells were rod-shaped, 1–2 μm in width and a maximum of 8 μm in length. *Methanosarcina barkeri* DSM 8687, originating from a peat bog in northern Germany (Maestrojuan *et al.*, 1992), was obtained from the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany).

Salt stress experiments at different temperatures

The effect of salt shock on methanogenic archaea was studied using *Methanosarcina* SMA-21, as well as *Methanobacterium* MC-20 and *Methanosarcina barkeri*, which were used as reference organisms. A 5-mL aliquot of each culture grown to a cell density of 10^8 mL^{-1} was supplemented with anaerobic salt solution and incubated at 4 and 28 °C for up to 3 months. The selected NaCl end concentrations were 0, 0.1, 0.2, 0.3, 0.4, 1.0, 3.0 and 6 M (saturated). Sterilized cultures (2 h at 121 °C) supplemented with 0.4 M and saturated salt solution were used as negative controls. Cell numbers and activities were measured as described below.

After having been stored in concentrated salt solution for just over 3 months, the 5-mL aliquot of each culture was placed into the fresh OCM medium and supplemented with the appropriate substrates (H_2/CO_2 for *Methanosarcina* SMA-21 and *Methanobacterium* MC-20; methanol for *Methanosarcina barkeri*). Survival was calculated according to cell count and activity measurements. All experiments were performed in triplicate.

Freezing experiments

Cultures of *Methanosarcina* SMA-21, *Methanobacterium* MC-20 and *Methanosarcina barkeri* grown to a cell density of 10^8 mL^{-1} were divided into two portions; one portion was immediately frozen at −78.5 °C, and the other one was cold shocked at 10 °C for 2 h before being frozen at −78.5 °C. For each portion, an aliquot of 1 mL was removed just before freezing. After storage at −78.5 °C for 24 h, the frozen cells were thawed at room temperature. Cell numbers were calculated before and after the freezing as described below. After thawing, aliquots were placed under anaerobic conditions in 25-mL glass flasks, supplemented with 10 mL of fresh OCM medium and appropriate substrates (H_2/CO_2 for *Methanosarcina* SMA-21 and *Methanobacterium* MC-20; methanol for *Methanosarcina barkeri*). The flasks were sealed with a screw cap containing a septum and incubated at 28 °C. Activities were measured as described below.

In addition, *Methanosarcina* SMA-21, *Methanobacterium* MC-20 and *Methanosarcina barkeri* grown at 28 °C to a cell density of 10^8 mL^{-1} were slowly frozen (0.2 °C min^{-1}) to − 20 °C. Initial methane production rates were measured before freezing and compared with those obtained after thawing for samples held at − 20 °C for a period of 1–2 years. Once the samples were thawed, an aliquot of 5 mL of each culture was placed into the fresh OCM medium and supplemented with the appropriate substrates. Survival was then calculated as described below. All experiments were performed in triplicate.

Starvation experiments at different temperatures

Cultures of *Methanosarcina* SMA-21, *Methanobacterium* MC-20 and *Methanosarcina barkeri* grown to a cell density of 10^8 mL^{-1} were harvested by centrifugation (10 min at 15 000 *g*), washed twice, resuspended in phosphate-buffered saline (PBS) and divided into six portions. For each portion, an aliquot of 1 mL was placed in a 25-mL glass flask, supplemented with 10 mL of a mineral medium without any carbon source (Boone *et al.*, 1989) and stored for 1, 2 and 3 months at 4 and 28 °C. Having been stored without substrates, the 1-mL aliquots of each culture were then placed into the fresh OCM medium, supplemented with the appropriate substrates and incubated at 28 °C. Survival was calculated as described below. All experiments were performed in triplicate.

Desiccation experiments

The effect of desiccation on methanogenic archaea was studied using *Methanosarcina* SMA-21, *Methanobacterium* MC-20 and *Methanosarcina barkeri*. An aliquot of each culture grown to a cell density of 10^8 mL^{-1} was placed onto microscope cover slips (1 mL per cover slip) and allowed to dry completely. For some experiments glass beads (1.0 g, 1 mm diameter) were added to cell suspensions. Cover slips were stored anaerobically at 28 °C for 2, 5, 7 and 25 days. Cells were rehydrated by placing the cover slip in 2 mL of the appropriate growth medium for 30 min at room temperature. The resulting cell suspensions were placed under anaerobic conditions into 25-mL glass flasks, supplemented with 10 mL of the fresh OCM medium and appropriate substrates. The flasks were sealed with a screw cap containing a septum and incubated at 28 °C. Survival was determined as described below. All experiments were performed in triplicate.

Oxygen exposure experiments

The oxygen sensitivity of methanogenic archaea was investigated using the permafrost strain *Methanosarcina* SMA-21 and nonpermafrost strain *Methanobacterium* MC-20. An aliquot of the culture grown to a cell density of 10^8 mL^{-1} was placed onto microscope cover slips (1 mL per cover slip) and exposed to aerobic conditions. The cover slips were stored at room temperature for 1, 3, 24, and 72 h. The cell suspensions were then placed under anaerobic conditions into 25-mL glass flasks, supplemented with 10 mL of the fresh OCM medium and H_2/CO_2. The flasks were sealed with a screw cap containing a septum and incubated at 28 °C. Activities and cell numbers before and after oxygenation were detected as described below. The oxygen sensitivity of *Methanosarcina barkeri* was previously determined (Zhilina, 1972; Kiener & Leisinger, 1983; Fetzer *et al.*, 1993).

Methane analysis

The activity of the methanogenic archaea was calculated based on the linear increase of the CH_4 concentration in the headspace. The methane concentration was measured by GC. The gas chromatograph (Agilent 6890, Agilent Technologies, Böblingen, Germany) was equipped with a Carbonplot capillary column (Ø 0.53 mm, 30 m length) and a flame ionization detector (FID). Both the oven and the injector temperature were 45 °C. The temperature of the detector was 250 °C. Helium served as the carrier gas. All the gas sample analyses were done following calibration with standards of the respective gases.

Cell count determinations

Cell numbers were calculated by Thoma cell counts and by FISH using the universal oligonucleotid probe for *Archaea* (ARC915 Cy3). For microscopic examination, 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. Counting was done manually. For each hybridization approach and sample at least 800 DAPI-stained cells were counted on 30 randomly chosen counting squares. Microscopic performance was carried out using a magnification of 63 × 100 giving an area of 3.9204×10^{-2} mm^2 per counting square.

Statistical analysis

Significant differences between the three replicates used in the different stress experiments were analyzed using Student's *t*-test (Wardlaw, 1985).

Results

Effect of salt stress on methanogenic archaea

Salt tolerance was assessed in the permafrost strain *Methanosarcina* SMA-21 and the nonpermafrost organisms *Methanobacterium* MC-20 and *Methanosarcina barkeri*

using NaCl salt solutions at different concentrations as an osmolite. High methane production of *Methanosarcina* SMA-21 was observed at all salt concentrations. The methane production of *Methanobacterium* MC-20 and *Methanosarcina barkeri* was significantly different when exposed to different concentrations (Fig. 1).

Highest activity of *Methanosarcina* SMA-21 was detected in samples incubated with 0.3 and 0.4 M NaCl (18.14 ± 2.81 and 17.98 ± 2.51 nmol $CH_4\,h^{-1}\,mL^{-1}$, respectively), which was similar to the activity in the samples which had no salt added. The methane production rate at low salt concentrations (0.1 and 0.2 M) was about half as high as in the samples with no additional salt (Fig. 1). In contrast, increasing salt concentrations led to a gradual decrease in the methane production of the *Methanobacterium* MC-20 and *Methanosarcina barkeri* strains. Thus, the methane production rate of the nonpermafrost organism *Methanobacterium* MC-20, incubated with 0.4 M NaCl (1.72 ± 0.18 nmol $CH_4\,h^{-1}\,mL^{-1}$), was one order of magnitude lower than for samples with no additional salt, which had a methane production of 17.13 ± 1.72 nmol $CH_4\,h^{-1}\,mL^{-1}$. The methane production rates of *Methanosarcina barkeri*, incubated with 0.4 M NaCl, decreased from 29.8 ± 2.3 to 0.85 ± 0.16 nmol $CH_4\,h^{-1}\,mL^{-1}$.

Higher methane production at low incubation temperature was observed in all *Methanosarcina* SMA-21 samples at all salt concentrations tested (Fig. 2). Moreover, a significant activity of *Methanosarcina* SMA-21 was observed even in cultures incubated in the saturated NaCl solution at 4 and 28 °C (Fig. 2). Based on the cell counts and on the methane production measured at different incubation temperatures, methane production rates per cell and per hour were calculated. At 4 °C the methane production rates detected per methanogenic cell ($0.1 \pm 0.0 \times 10^{-7}$ nmol $CH_4\,h^{-1}\,cell^{-1}$) were five times higher than the methanogenic activity at 28 °C ($0.027 \pm 0.0 \times 10^{-7}$ nmol $CH_4\,h^{-1}\,cell^{-1}$).

In contrast, the methane production rates of *Methanosarcina barkeri* and *Methanobacterium* MC-20 under salt-saturated conditions were not very significant at 28 °C (0.01 ± 0.002 and 0.003 ± 0.0001 nmol $CH_4\,h^{-1}\,mL^{-1}$, respectively), but they were still higher than those at 4 °C (0.002 ± 0.0001 and 0.0014 ± 0.0001 nmol $CH_4\,h^{-1}\,mL^{-1}$, respectively) (Fig. 3). Any viable cells were detected. No methane production was observed in the sterilized cultures.

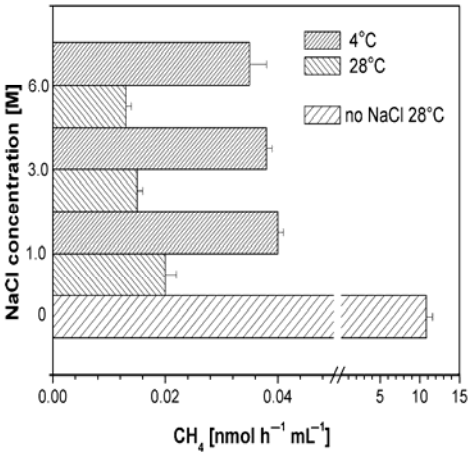

Fig. 2. Methane production rates of *Methanosarcina* SMA-21 incubated in a concentrated salt solution (1–6 M NaCl) at 4 and 28 °C (means ± SE, $n = 3$).

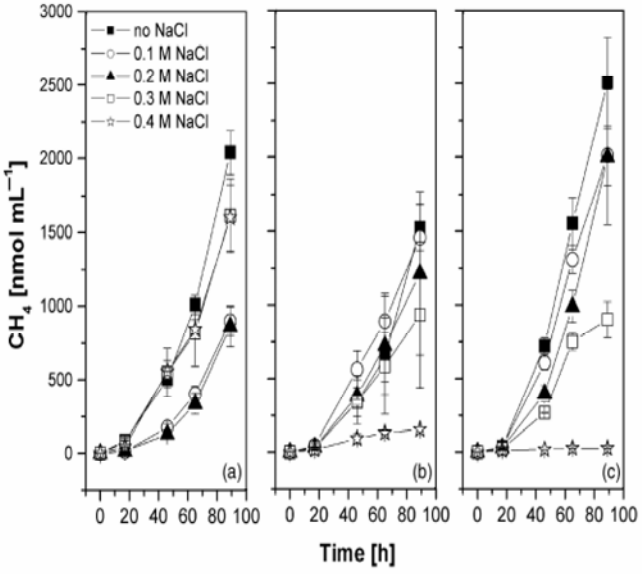

Fig. 1. Methane production of permafrost strain *Methanosarcina* SMA-21 (a), and the reference organisms *Methanobacterium* MC-20 (b) and *Methanosarcina barkeri* (c), incubated with varying salt concentrations at 28 °C (means ± SE, $n = 3$).

When the cells were transferred to fresh OCM medium after incubation under salt-saturated conditions for a period of 3 months, methane production rates of *Methanosarcina* SMA-21 observed after 1 week were similar to those under standard growth conditions. Thus, the methane production rates per cell calculated for the recovering samples ($1.4 \pm 0.04 \times 10^{-7}$ nmol CH_4 h^{-1} $cell^{-1}$) were comparable with those for the samples which had no salt added ($1.9 \pm 0.06 \times 10^{-7}$ nmol CH_4 h^{-1} $cell^{-1}$). Conversely, no methane production was detectable after reincubation of *Methanobacterium* MC-20 and *Methanosarcina barkeri* (data not shown).

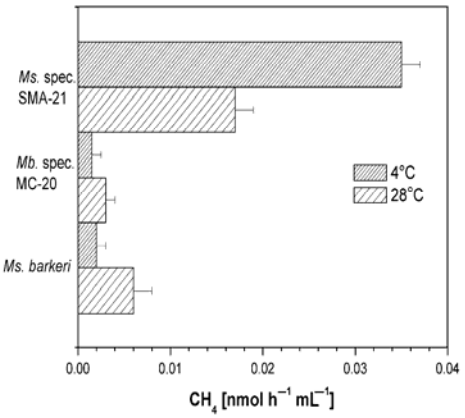

Fig. 3. Methane production rates of *Methanosarcina barkeri*, *Methanobacterium* MC-20 and *Methanosarcina* SMA-21 incubated in a saturated salt solution at two different temperatures (means ± SE, n = 3).

Freezing tolerance

The methanogenic strains *Methanosarcina* SMA-21, *Methanobacterium* MC-20 and *Methanosarcina barkeri* showed significant differences in their ability to survive freezing at −78.5 °C for 24 h. Highest survival was seen in the *Methanosarcina* SMA-21. Average cell numbers of this archaeon decreased from $4.4 \pm 1.4 \times 10^8$ cells mL^{-1} at the beginning of the experiment to $3.9 \pm 0.6\ 10^8$ cells mL^{-1} at the end of freezing, giving a survival rate of 89.5%. In comparison, only 1% of *Methanobacterium* MC-20 and 0.8% of *Methanosarcina barkeri* survived incubation at −78.5 °C. The decrease in cell numbers correlated well with the methane production rates of the cultures. The activity of *Methanosarcina* SMA-21 measured before freezing (10.87 ± 1.22 nmol CH_4 h^{-1} mL^{-1}) was only two-fold higher than the activity after the experiment (5.57 ± 0.67 nmol CH_4 h^{-1} mL^{-1}), while the methane production rates of the reference organisms *Methanobacterium* MC-20 and *Methanosarcina barkeri* decreased drastically after the experiment (Fig. 4). In particular, the methane production rates of *Methanobacterium* MC-20 after freezing (0.21 ± 0.07 nmol CH_4 h^{-1} mL^{-1}) were two orders of magnitude lower than those before the experiment (19.53 ± 1.59 nmol CH_4 h^{-1} mL^{-1}), while the activity of *Methanosarcina barkeri* was three orders of magnitude lower.

The potential of *Methanosarcina barkeri* to survive freezing at −78.5 °C was slightly higher when the culture was exposed to a temperature of 10 °C for 2 h prior to freezing (precooling). Cultures transferred to 10 °C had a survival rate of 1.4% and a methane production rate of 0.06 ± 0.01 nmol CH_4 h^{-1} mL^{-1}. In contrast, a positive effect of preincubation at 10 °C on the ability to survive freezing

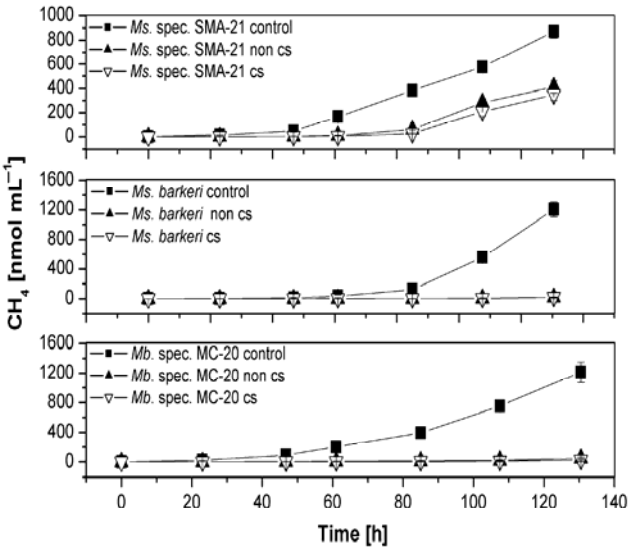

Fig. 4. Methane production of *Methanosarcina* SMA-21, *Methanosarcina barkeri* and *Methanobacterium* MC-20 after freezing for 24 h at −78.5 °C for cold-shocked (cs) and noncold-shocked (non cs) cultures in comparison with untreated control samples (means ± SE, *n* = 3).

at −78.5 °C was not seen for *Methanosarcina* SMA-21 or *Methanobacterium* MC-20.

Most striking was that the *Methanosarcina* SMA-21 showed high survival rates and methane production of 9.01 ± 0.5 nmol CH_4 h^{-1} mL^{-1} after 2 years' freezing at −20 °C. The measured methane production rates prior to freezing were 10.58 ± 0.8 nmol CH_4 h^{-1} mL^{-1}. No methane production was detected in either reference organism after just 1 year of exposure to −20 °C.

Temperature-dependent starvation tolerance

Methanosarcina SMA-21, *Methanobacterium* MC-20 and *Methanosarcina barkeri* were tested for their ability to survive substrate-limiting conditions at different incubation temperatures. *Methanosarcina* SMA-21 showed a high survival potential following the starvation experiment. Significant methane production of *Methanosarcina* SMA-21 was observed even in the cultures that had been starved for 3 months (1.25 ± 0.01 and 3.55 ± 0.56 nmol CH_4 h^{-1} mL^{-1} for cultures at 28 and 4 °C, respectively). Methane production rates were higher at 4 °C than at 28 °C (Fig. 5). The different activities at different incubation temperatures correlated well with the viable cell numbers of this methanogenic archaeon. Average cell numbers of *Methanosarcina* SMA-21 after 3 months of starvation decreased from $6.1 \pm 2.6 \times 10^8$ to $3.3 \pm 1.9 \times 10^7$ cells mL^{-1} at 4 °C and from $9.2 \pm 2.8 \times 10^8$ to $6.2 \pm 2.3 \times 10^5$ at 28 °C. Thus, the survival potential of *Methanosarcina* SMA-21 at 4 °C was 10 times higher than at 28 °C (Fig. 5).

In contrast, there was no survival of any cells of *Methanobacterium* MC-20 and *Methanosarcina barkeri* after 1 month of starvation, regardless of the incubation temperature. This was in accordance with the lack of any methane formation after reincubation of *Methanobacterium* MC-20 and *Methanosarcina barkeri* (Fig. 5).

Desiccation tolerance

Survival of the strains following desiccation was evaluated for up to 25 days of treatment. In general, the presence of glass beads strongly reduced the inhibitory effect of desiccation on survival. Survival and methane production rates for all the methanogenic strains (*Methanosarcina* SMA-21, *Methanobacterium* MC-20 and *Methanosarcina barkeri*) were higher with glass beads than without. However, *Methanosarcina* SMA-21 from permafrost showed significant differences in its desiccation resistance than did the reference organisms from nonpermafrost habitats. *Methanosarcina* SMA-21 was found to resist 25 days of desiccation without loss of activity and cultivability (Fig. 6). Average cell numbers of *Methanosarcina* SMA-21 decreased from $2.3 \pm 0.8 \times 10^8$ to $1.8 \pm 0.4 \times 10^8$ cells mL^{-1}, equivalent to a cell survival rate of 77.5%. Methane production rates decreased slightly from 10.46 ± 2.34 to 5.23 ± 1.7 nmol CH_4 h^{-1} mL^{-1}. Survival and methane production rates of both nonpermafrost strains (*Methanobacterium* MC-20 and *Methanosarcina barkeri*) were drastically reduced after desiccation (Fig. 6). When tested for this ability, cells of the reference cultures were no longer able to grow after desiccation of 25 days.

Oxygen sensitivity

The oxygen sensitivity of the permafrost strain *Methanosarcina* SMA-21 was examined by determining cell viability and methane production following oxygenation. There was good agreement between these parameters. As shown in Fig. 7, exposure to oxygen for 1–3 h resulted in no significant effect on the cultivability and activity of this permafrost

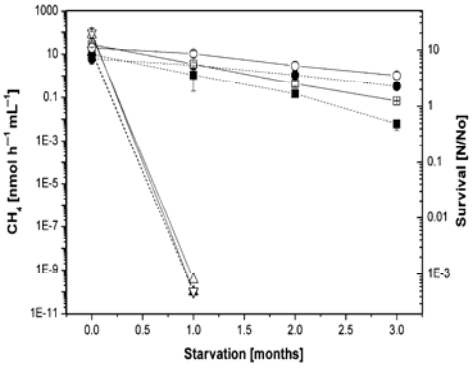

Fig. 5. Methane production rates (closed symbols) and cell survival rates (open symbols) of starved cells of *Methanosarcina barkeri* (upward-pointing triangles), *Methanobacterium* MC-20 (downward-pointing triangles) and *Methanosarcina* SMA-21 at 4 °C (circles) and 28 °C (squares; means ± SE, $n = 3$).

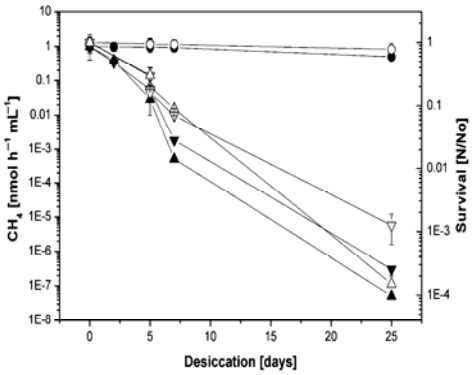

Fig. 6. Methane production rates (closed symbols) and cell survival rates (open symbols) of desiccated cells of *Methanosarcina barkeri* (upward-pointing triangles), *Methanobacterium* MC-20 (downward-pointing triangles) and *Methanosarcina* SMA-21 (circles; means ± SE, $n = 3$).

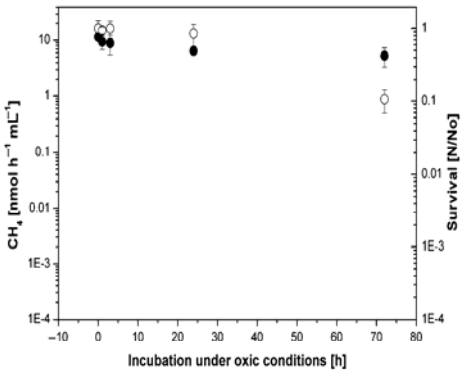

Fig. 7. Oxygen sensitivity (methane production rates, closed symbols; cell survival rates, open symbols) of *Methanosarcina* SMA-21 (means ± SE, $n = 3$).

microorganism. Viability of *Methanosarcina* SMA-21 appeared to be only slightly affected by exposure to oxygen for 24 h, with a survival rate of 85%. Calculated methanogenic activity decreased slightly from 11.47 ± 1.23 to 6.46 ± 0.9 nmol CH_4 h^{-1} mL^{-1}. However, the survival potential of the permafrost strain was reduced after prolonged oxygen exposure. After 72 h of exposure, 90% of the *Methanosarcina* SMA-21 cells had died. In contrast, 100% of the *Methanobacterium* MC-20 cells had died after 24 h of exposure to oxygen. No methane production was detected. These results were compared with a previous study investigating the survival potential of *Methanosarcina barkeri* (Kiener & Leisinger, 1983). For this strain, exposure to oxygen for 10–30 h had no effect on cell numbers. A decrease in cell numbers and methane production was observed only after 48 h of exposure to oxygen.

Discussion

Different strains of methanogens, which include representatives from permafrost and nonpermafrost habitats, exhibit marked differences in their stress tolerance. The methanogenic archaeon *Methanosarcina* SMA-21 from permafrost showed high resistance to high salinities, extremely low temperatures, desiccation, the presence of oxygen and starvation. The stress tolerance of *Methanosarcina* SMA-21 was even higher at low incubation temperatures. In contrast, the reference organisms from nonpermafrost habitats were sensitive to the extreme conditions tested. High cell numbers of reference strains were sacrificed without lag upon exposure to these stress factors. Therefore, it could be assumed that extreme conditions of permafrost ecosystems may favor the development of highly resistant methanogenic archaea.

Terrestrial permafrost provides an opportunity to obtain microorganisms that have exhibited long-term exposure to low temperatures, freeze–thaw cycles, starvation, aridity and high levels of long-lasting background radiation resulting from accumulation over geological time-scales. In spite of the unfavorable living conditions, permafrost is colonized by high numbers of viable microorganisms ($10^2 - 10^8$ cells g^{-1}), including fungi, yeasts, algae and bacteria as well as highly specialized organisms such as methanogenic archaea (Vishnivetskaya *et al.*, 2000; Kobabe *et al.*, 2004; Wagner *et al.*, 2005). Seasonal variations in soil temperatures, particularly freeze–thaw cycles in the active layer, result in drastic changes in other environmental conditions such as water availability, salinity, soil pressure, desiccation, changing oxygen conditions and the availability of nutrients. The permafrost microbial community, described as a 'community of survivors' (Friedmann, 1994), has to resist this combination of extreme conditions as well as their extreme fluctuations. The high survival rates of a methanogenic archaeon from permafrost compared with nonpermafrost strains under the investigated stress conditions suggest that these microorganisms have developed ways to cope with stress, which need to include repair of damaged DNA and cell membranes, and the maintenance of other vital functions needed to sustain cell viability. Our results indicate a higher resistance of the methanogenic archaeon *Methanosarcina* SMA-21 to increased salinity and lack of nutrients at low temperatures. An incubation temperature of 4 °C correlates well with *in situ* temperatures of the active layer of permafrost, fluctuating in summer months from 0 °C to about 10 °C. It remains to be determined if freeze protection mechanisms overlap with tolerance mechanisms, which protect against various other stresses such as desiccation, starvation or high salt concentration (Berry & Foegeding, 1997; Macario *et al.*, 1999; Cleland *et al.*, 2004; Georlette *et al.*, 2004).

Methanosarcina SMA-21 from Siberian permafrost was shown to be well adapted to a wide range of salt concentrations. Higher methane production rates, which were determined for *Methanosarcina* SMA-21 incubated with 0.3–0.4 M NaCl compared with 0.1–0.2 M NaCl, indicate better adaptation to a rapid increase in osmolarity, which occurs while the active layer of the permafrost is freezing. Again, the ability to resist the stress factor, in this case high salinity, was enhanced by low incubation temperatures. Furthermore, cells of *Methanosarcina* SMA-21 remained to viable after 3 months of incubation under salt-saturated conditions. In contrast, increasing salinity leads to reduced activity of the reference organisms *Methanobacterium* MC-20 and *Methanosarcina barkeri* originating from nonpermafrost sediments. The production of methane by *Methanosarcina barkeri* and *Methanobacterium* MC-20 was marginal under salt-saturated conditions and did not appear to be favorably influenced by low temperatures (Fig. 3). In addition, no viable cells of these strains could be detected after prolonged salt stress.

The observed salt tolerance could be associated with cold tolerance, a possibility which was also postulated by Vishnivetskaya *et al.* (2000) and Gilichinsky *et al.* (2003) and which is confirmed by the present results. *Methanosarcina* SMA-21 showed excellent survival of more than 70% of the cells following freezing at −78.5 °C. Preconditioning to low temperatures (cold shock), known to increase the resistance to freezing of many microorganisms due to the expression of cold-responsive genes and cryoprotectant molecules (Kim & Dunn, 1997; Wouters *et al.*, 2001; Georlette *et al.*, 2004; Weinberg *et al.*, 2005), does not increase the freezing tolerance of *Methanosarcina* SMA-21. This correlates well with the resistance of this strain to a 2-year exposure at −20 °C without any preconditioning. Both results suggest that this strain is already adapted to subzero environments. Generally, all the cell components must be adapted to the cold to enable an overall level of cellular protection that is sufficient for survival and growth (Cavicchioli, 2006).

Starvation tolerance experiments at two different incubation temperatures were conducted to evaluate the ability of methanogenic archaea from permafrost and nonpermafrost habitats to survive prolonged periods of nutrient limitation associated with the freezing of the active layer in permafrost habitats. Starvation stress was very efficient in reducing the survival potential of the reference strains. Whereas the nonpermafrost archaea (*Methanosarcina barkeri* and *Methanobacterium* MC-20) ceased to exist after 1 month of starvation, *Methanosarcina* SMA-21 maintained high survival rates even after being starved for 3 months. Again, the survival rates of the permafrost archaeon were higher at lower incubation temperatures. The slow metabolism rates of organisms in cold environments could be important for successful adaptation to starvation conditions as this adaptation requires protein synthesis, the most energy-demanding process in the cell (Thomsson *et al.*, 2005).

Prolonged desiccation stress was lethal for nonpermafrost strains, whereas *Methanosarcina* SMA-21 survived for at least 25 days. Surprisingly, this methanogenic archaeon was able to produce methane immediately following rehydration, which indicates very rapid repair mechanisms. The experiment was performed at room temperature; lower temperatures might slow the rate of desiccation damage and lead to even longer survival periods. The survival and potential methane production of *Methanosarcina* SMA-21 was even higher in the presence of glass beads, which probably provided partial mechanical protection of methanogens against desiccation. This observation was the same regardless of whether the methanogens were obtained from a DSMZ culture collection or freshly isolated from permafrost.

Exposure to oxygen, the last stress factor tested, occasionally occurs during late summer when the uppermost permafrost thaws and the water table of the active layers falls. Metabolic activity of methanogenic archaea within aerated soil slurries has been previously observed (Wagner *et al.*, 1999). Even without a protective soil matrix, the permafrost strain *Methanosarcina* SMA-21 still exhibits a marked oxygen resistance. This organism survived for hours in the presence of oxygen without any decrease in cell numbers or methane production rates. Moreover, a significant percentage (10%) of the population of *Methanosarcina* SMA-21 survived up to 72 h of oxygenation. This is an interesting result given that methanogenic archaea are strictly anaerobic organisms, which are not known to have resting stages. These survival rates are high compared with those from earlier studies for *Methanosarcina barkeri* and other methanogenic archaea from ecosystems periodically subjected to oxygen stress (Kiener & Leisinger, 1983). Protection from oxygen may occur at the cellular level [e.g. superoxide dismutase (SOD), catalase and other SOD protective enzymes] or at the level of cell aggregates (Kiener & Leisinger, 1983; Brioukhanov *et al.*, 2006; Zhang *et al.*, 2006). The arrangements of cell aggregates that have been regularly observed in *Methanosarcina barkeri* and *Methanosarcina* SMA-21 might lead to the protection of the cells in the interior and thereby secure survival during extended periods of oxygen stress. This assumption is in agreement with the data of Kobabe *et al.* (2004), who found aggregates of methanogenic archaea in the dried upper layers of soils in polygon depressions.

In summary, the high survival rates and activity of *Methanosarcina* SMA-21 from Siberian permafrost under different stress conditions suggest that this organism possesses natural adaptation mechanisms to subzero temperatures, increased salinity, starvation, desiccation and oxygen stress and has efficient repair mechanisms that allow it to live under extreme fluctuating conditions of terrestrial permafrost, in contrast to other methanogens isolated from nonpermafrost habitats which probably lack such mechanisms. Most striking was the difference in survival potential between *Methanosarcina barkeri* and *Methanosarcina* SMA-21, two representatives of the same genus. Therefore, it is of importance to sequence the genome of *Methanosarcina* SMA-21, as one of the representatives of a permafrost community. The characterization of the physiological traits potentially important to cryoadaptation is necessary to begin to understand the adaptations at the genome level.

From the astrobiological point of view, the physiological potential and the metabolic specificity of *Methanosarcina* SMA-21 from permafrost provide very useful insight for the investigation of potential life in extremely cold environments on other planets within the solar system. We might conclude that the permafrost habitats on Earth represent an excellent analog for studying putative life on Mars. Recent analyses of *Mars Express* HRSC (High-Resolution Stereo Camera) images of many regions of the planet showed that the morphology of Martian polygonal features is very

similar to the morphology of terrestrial ice-wedge polygons and is most likely the result of comparable processes (Kuzmin, 2005). Mars is known to have harsh conditions, such as low water activity, high desiccation and oxidative stress, variations in the salinity of the environment and low and sporadic supply of energy sources (Litchfield, 1998; Horneck, 2000). Although the experimental conditions presented here did not simulate all extreme permafrost environmental conditions, we did simulate the major stresses that organisms in terrestrial permafrost and in Martian permafrost might be exposed to. The observation of high survival rates of a permafrost methanogen under defined stress conditions as well as under simulated Martian conditions (Morozova *et al.*, 2007) supports the possibility that microorganisms similar to methanogens from Siberian permafrost could also exist in Martian permafrost habitats. Methanogenic archaea from terrestrial permafrost may therefore serve as useful models for further exploration of extraterrestrial life.

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4 Publication III

Environmental Microbiology
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Radiation resistance of methanogenic archaea from Siberian permafrost

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Summary

Methanogenic archaea from Siberian permafrost and from non-permafrost habitats were exposed to solar UV- and ionizing radiation in order to assess their limits of survival. Metabolic activity and viability of methanogenic archaea in environmental samples remained unaffected by exposure to mono-chromatic and polychromatic UV radiation caused by the shielding of the soil layers. Pure methanogenic cultures isolated from Siberian permafrost exhibit an increase in radioresistance to UV (20-fold) and ionizing radiation (32-fold) compared to the non-permafrost isolates. The F_{37} (UV radiation) and D_{37} (X-rays) values of the permafrost strain *Methanosarcina* sp. SMA-21 were 700 J m^{-2} and 6-12 kGy, respectively. This resistance is comparable to values for *Deinococcus radiodurans* (F_{37} 640 Jm^{-2}, D_{37} 6-7 kGy). Due to the increased radiation-resistance of permafrost isolates, their long-term survival, and their anaerobic lithoautotrophic metabolism, methanogenic archaea from permafrost can be considered as suitable candidates in the search for microbial life in the Martian subsurface. The ESA mission *Mars Express* confirmed the existence of water on Mars, which is a fundamental requirement for life, as well as CH_4 in the Martian atmosphere, which could only originate from active volcanism or from biological sources; both these results suggest that microbial life could still exist on Mars, for example in the form of subsurface lithoautotrophic ecosystems, which also exist in permafrost regions on Earth.

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Introduction

Methanogenic archaea are widespread in nature and highly abundant in extreme environments, tolerating low/high temperatures (permafrost, hot springs), extreme salinity (saltern ponds) and low/high pH (solfataras, soda lakes). In addition to mesophilic species, thermophilic and hyperthermophilic methanogens have also been identified (Stetter *et al.*, 1990; Garcia *et al.*, 2000). Recently, more attention has been paid to the isolation of psychrophilic strains

since a number of methanogenic habitats are located in 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; Franzmann *et al.*, 1997; Simankova *et al.*, 2003). Although the metabolism of methanogenic archaea was studied in different environments (Shuisong and Boone, 1998; Garcia *et al.*, 2000; Eicher, 2001; Lange and Ahring, 2001), only a few studies have focused on the ecology of the methanogenic archaea exposed to the harsh environmental conditions of permafrost ecosystems (Vishnivetskaya *et al.*, 2000; Høj *et al.*, 2005; Ganzert *et al.*, 2007).

Permafrost is a common phenomenon in our solar system and is therefore the main focus of extraterrestrial research in astrobiology. Evidence of cryotic systems on present-day Mars (patterned ground, glaciers and thermokarst) has been found by *Mars Express*. The possibility of extant or extinct life on Mars has been fueled by the recent U.S. Mars Exploration Rover Opportunity discovery that liquid water most likely exists on Mars (Christensen *et al.*, 2004; Klingelhofer *et al.*, 2004; NASA, 2006) and findings from the Planetary Fourier Spectrometer onboard the *Mars Express,* as well as observations from the surface indicating that methane currently exists in the Martian atmosphere (Formisano, 2004). Considering the short lifetime of methane, this trace gas could only originate from active volcanism – which has not yet been observed on Mars – or from biological sources. Furthermore, there is evidence that prior to 3.8 Ga ago, when terrestrial life arose, environmental conditions on Mars were most likely similar to those on early Earth (Carr, 1989; Durham *et al.*, 1989; Wharton *et al.*, 1989; McKay and Davis, 1991; McKay *et al.*, 1992; Carr, 1996). If life had also emerged on Mars, it either subsequently adapted to the drastically changed environment or it became extinct. One possibility for survival of Martian microorganisms could be in lithoautotrophic subsurface ecosystems such as deep sediments near the polar ice caps and in permafrost regions. In the light of this assumption, methanogenic archaea from terrestrial permafrost habitats could be considered as analogues for probable extraterrestrial organisms.

Of the extreme conditions, radiation has been a ubiquitous stressor since the origin of the first microbial ecosystem during the Archaea era, when the Earth lacked a significant ozone column and was therefore exposed to a full UV radiation spectrum (Cockell, 2001). A comparable situation has been proposed for present-day Mars. Any primary colonizers of surface habitats on Earth and Mars are vulnerable to the effect of solar UV radiation. Hence, microorganisms that survived at the surface of these planets without the protection of an ozone layer would have needed mechanisms to resist UV-C, UV-B and UV-A radiation (Wynn-Williams and Edwards, 2001). Today, terrestrial

radiation is emitted by radioactive nuclides in rocks, soils, and the hydrosphere of the planets' crusts which persists due to the emergence of decay products (primordial radionuclides). There are considerable geographic variations of the natural radiation due to the varying concentrations of radionuclides in soil and minerals. Thus, the dose of background radiation in permafrost, for example, is about 2 mGy per year; however, the radiation dose accumulated over a long-term period could be thousands of Gy (Gilichinsky, 2001). Since terrestrial permafrost is suggested as a model for an extra-terrestrial habitat, it is important to characterize the radiation sensitivity of permafrost inhabitants, in this case, methanogenic archaea.

The objective of this study was, firstly, to characterize the potential response of methanogenic archaea from Siberian permafrost to simulated UV radiation environments (>200 nm) of early Earth before the build-up the ozone layer, or of the planet Mars. Secondly, their resistance to monochromatic UV radiation at 254 nm and ionizing radiation was studied. Particular emphasis was placed on the strain *Methanosarcina* sp. SMA-21 isolated from the active layer of Siberian permafrost. Previous studies have shown that this methanogenic archaeon from Siberian permafrost exhibits a high survival potential under simulated Martian conditions (Morozova *et al.*, 2007) and is highly resistant to desiccation, starvation, extremely low temperatures, and oxygen stress (Morozova and Wagner, 2007). To compare our results, *Methanosarcina barkeri* (DSM 8687), isolated from fresh-water non-permafrost habitats, was used as a reference organism. Our study will contribute to an improved understanding of extraterrestrial life, if present, especially with regards to possible protected niches on present-day Mars.

Results

Effect of UV radiation on methanogenic archaea in the permafrost samples

The survival rates of methanogenic archaea in the permafrost soils after exposure to the UV radiation was determined by both the methane production rates and the cell counts before and after the irradiation experiments. Methanogenic archaea of the floodplain showed a high resistance to monochromatic and polychromatic UV, without any distinct loss of viability following high irradiation fluences. Following UV-C (λ = 254 nm) irradiation fluence of 20 kJ m^{-2}, the methane production rates of the floodplain soil samples decreased slightly, from 25.65 ± 3.42 nmol CH_4 h^{-1} g^{-1} to 15.1 ± 2.41 nmol CH_4 h^{-1} g^{-1} (Fig. 1). Average cell numbers of methanogenic archaea decreased insignificantly, from 3.7 x 10^7 to 3.1 x 10^7, corresponding to survival of 83.8 % of the cells.

Upon exposure to the full UV spectrum (λ = 200-400 nm), the samples were subjected to photons of different

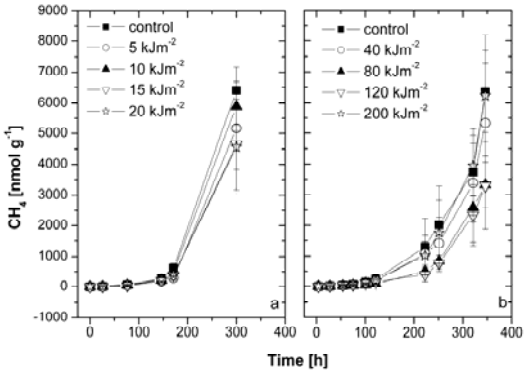

Fig. 1 Methane production rates of methanogenic archaea in permafrost soil samples following monochromatic (a) and polychromatic (b) UV radiation (The error bars represent the standard deviation, n=3) .

wavelengths, with the UV-C radiation component (λ = 200-280 nm) contributing to about 50 % of the total radiance. However, no loss of viability was observed even after a radiation exposure ten times higher than those in the 254 nm irradiation experiments.

The methanogenic archaea from the flood-plain soil samples showed no reduction in cell survival or methane production rates following 200 kJ m^{-2} of polychromatic UV irradiation (Fig. 1).

Effect of UV radiation on methanogenic cultures

The UV-C radiation experiment was conducted with the permafrost strains *Methanosarcina* sp. SMA-21 and SMA-16, and the non-permafrost methanogenic archaeon *Methanosarcina barkeri*. In general, the highest survival was found for the cells in the PBS solution rather than in the deionized water. The UV-C inactivation curves obtained from the cells of *Methanosarcina* sp. SMA-21, SMA-16, and *Methanosarcina barkeri* are shown in Fig. 2. The radiation sensitivity of the methanogenic strains was examined by determining cell viability and methane production following irradiation. As can be seen, there was good agreement between these two parameters (Fig. 2). Although the UV inactivation curve obtained from the methane production rates differed slightly from that based on the cell survival, these differences were minor and overall the physiological survival curve matched closely with the survival curve. However, *Methanosarcina* sp. SMA-21 and SMA-16 from permafrost showed significant differences in their UV-C resistance than did the reference organism from the non-permafrost habitats. The inactivation of methanogenic archaea from permafrost exhibited classic kinetics typical for radiation resistant organisms, with shoulde-red survival curves at low UV-C fluences, followed by exponential inactivation at higher UV-C values. The F_{10}, F_{37}, and Fq values represent key parameters of the cellular survival, calculated due to reduction of activity and cell viability in response to UV radiation at 254 nm. They were used to characterize the different methanogenic strains, and are summarized in Table 1. The *Methanosarcina* sp. SMA-21 cells exhibit a high resistance to 254-nm UV-C radiation without distinct loss of activity up to 700 J m^{-2} and with a physiological F_{10} (reducing the methane production rates to 10 % of the normal level) at 1695 ± 74.8 J m^{-2}. The

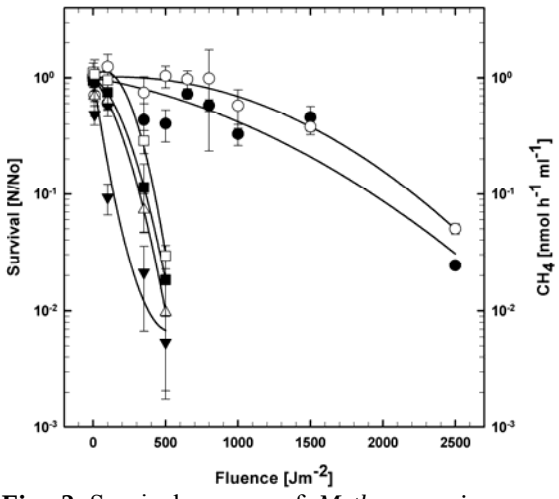

Fig. 2 Survival curves of *Methanosarcina* sp. SMA-21 (circles), SMA-16 (squares), and *Methanosarcina barkeri* (triangles) exposed to UV-C radiation. Survival was calculated from viable cell counts (open symbols) and metabolic activity by measuring the methane production rates (dark symbols; the error bars represent the standard deviation, n=3).

physiological F_{37} value (reducing the methane production rates to 37 % of the normal level) of this strain was 705.7 ± 58.9 J m^{-2}. The F_{10} value, indicating the fluence resulting in 10 % survival, and the F_{37} value, indicating the fluence resulting in 37 % survival were 2160.3 ± 321.1 J m^{-2} and 1315.2 ± 109.5 J m^{-2} respectively. The cell survival values were somewhat higher than those calculated from the methane production rates. However, the differences were not significant. A similarly high resistance (F_{37} value = 242 ± 30/188 ± 18 J m^{-2}) was observed for the other permafrost strain, SMA-16. However, the F_{10} values were five times lower than those of *Methanosarcina* sp. SMA-21.

The survival curve of the non-permafrost strain *Methanosarcina barkeri* was strictly exponential. The F_{10} values were 291.2 ± 15.9/122.7 ± 23.9 J m^{-2}, and the physiological F_{37} value was a factor of lower than *Methanosarcina* sp. SMA-21. In fact, *Methanosarcina* sp. SMA-21 was the most resistant strain in our study.

The polychromatic UV radiation of $\lambda > 200$ nm from the solar simulator comprises the full UV spectrum reaching the early Earth or Mars. The survival curves of *Methanosarcina* sp. SMA-21 is shown in Fig. 3. There was a shoulder on the survival curve of the *Methanosarcina* sp. SMA-21 strain; however, the fluence resulting in 37 % survival (F_{37}, calculated from the decrease in cell survival as well as in methane production rates) was 20 times higher compared to the 254 nm irradiation (Table 1).

The F_{10} values for *Methanosarcina* sp. SMA-21 were 27.9 ±5.7/28.5 ± 4.1 kJ m^{-2}; however, significant methane production rates were measured even in samples irradiated with 40 kJ m^{-2} (0.44 ± 0.16 nmol CH_4 h^{-1} ml^{-1}).

In contrast, there was no survival of any cells of *Methanosarcina barkeri* after radiation fluences of 5 kJ m^{-2}, based on the lack of any methane formation after re-incubation following irradiation (Table 1). According to these criteria, cells of the reference culture were no longer able to grow following irradiation with any of the polychromatic UV fluences tested.

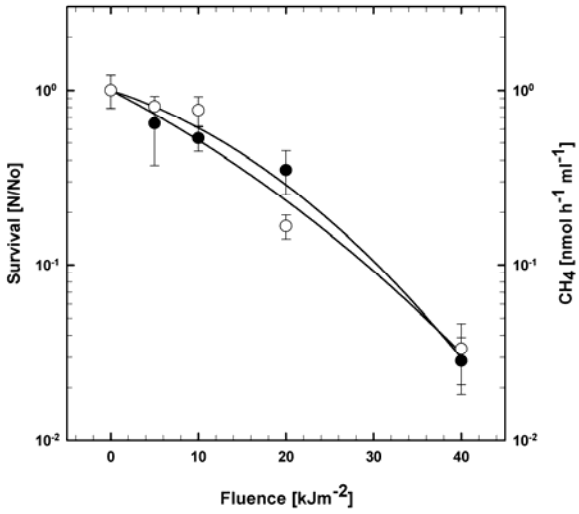

Fig. 3 Survival curves of *Methanosarcina* sp. SMA-21 exposed to polychromatic UV radiation. Survival was calculated from viable cell counts (open symbols) and metabolic activity by measuring the methane production rates (dark symbols; the error bars represent the standard deviation, n=3).

Effect of ionizing radiation on methanogenic cultures

The resistance of methanogenic archaea to ionizing radiation was assessed in the permafrost isolate *Methanosarcina* sp. SMA-21 and the non-permafrost organism *Methanosarcina barkeri* (Fig. 4). After irradiation at increasing doses, the surviving fraction was calculated by the both methane production rate and viable cell count technique. The D_{10}, D_{37}, and Dq, values are summarized in Table 1. The methanogenic archaeon from Siberian permafrost and the reference organism from non-permafrost habitats showed significant differences in their survival potential. Again, the survival curves for *Methanosarcina* sp. SMA-21 exhibit a very gradual slope throughout the range of ionizing radiation tested. This resulted in physiological D_{10} and D_{37} values of 25.3 ± 4.4 kGy and 12.6 ± 2.7 kGy, respectively. The D_{10} and D_{37} values of this strain, calculated from the cell survival, were somewhat lower (12.6 ± 0.8 kGy and 6.5 ± 0.5 kGy). The survival curves of *Methanosarcina barkeri* leveled off at higher doses, therefore the data present in Table 1 were calculated from the lower dose portion of the curve. Compared to *Methanosarcina* sp. SMA-21, the physiological D_{37} value of the non-permafrost strain was 32-fold smaller. The D_{37} value, calculated from cell survival, was 21-fold smaller. *Methanosarcina barkeri* reached 10 % survival (D_{10} value) after 391.2 ± 44.3 Gy and, by 1000 Gy, experienced essentially 100 % mortality.

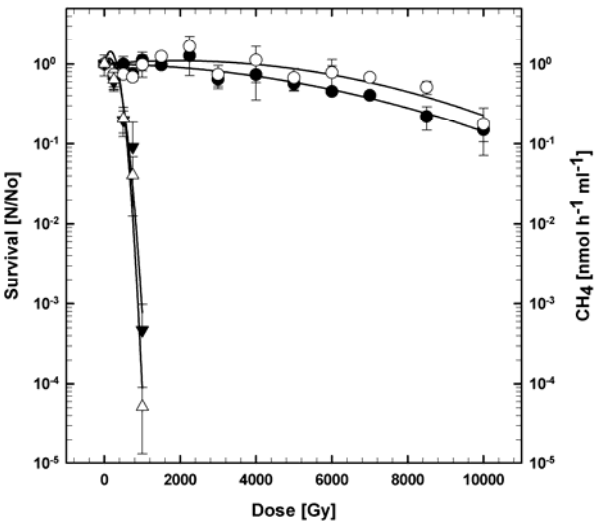

Fig. 4 Survival curves of *Methanosarcina* sp. SMA-21 (circles) and *Methanosarcina barkeri* (triangles) exposed to ionizing radiation. Survival was calculated from viable cell counts (open symbols) and metabolic activity by measuring the methane production rates (dark symbols; the error bars represent the standard deviation, n=3).

Tab. 1 Characteristics of the survival curves.

Radiation source	Strain	Physiological* D/F_{10}	D/F_{10}	Physiological* D/F_{37}	D/F_{37}	Physiological* D/Fq	D/Fq
UV-C (254 nm)	*M.* sp. SMA-21	1695.2 ± 74.8 Jm^{-2}	2160.3 ± 321.1 Jm^{-2}	705.7 ± 58.9 Jm^{-2}	1315.2 ± 109.5 Jm^{-2}	n.d.	668.9 ± 58.1 Jm^{-2}
	SMA-16	332 ± 29 Jm^{-2}	399 ± 56 Jm^{-2}	188 ± 18 Jm^{-2}	242 ± 30 Jm^{-2}	76 ± 16 Jm^{-2}	122 ± 33 Jm^{-2}
	M. barkeri	122.7 ± 23.9 Jm^{-2}	291.2 ± 15.9 Jm^{-2}	34.8 ± 9.5 Jm^{-2}	163.4 ± 21.0 Jm^{-2}	n.d.	67.2 ± 11.0 Jm^{-2}
UV (200-400 nm)	*M.* sp. SMA-21	28.5 ± 4.1 kJm^{-2}	27.9 ± 5.7 kJm^{-2}	14.1 ± 2.7 kJm^{-2}	15.2 ± 3.2 kJm^{-2}	3.2 ± 0.4 kJm^{-2}	5.3 ± 0.9 kJm^{-2}
	M. barkeri	1.6 ± 04 kJm^{-2}	n.d.	0.5 ± 0.3 kJm^{-2}	n.d.	n.d.	n.d.
X-rays	*M.* sp. SMA-21	25.3 ± 4.4 kGy	12.6 ± 0.8 kGy	12.6 ± 2.7 kGy	6485.7 ± 441 Gy	3207.5 ± 803.2 Gy	1856.2 ± 207.3 Gy
	M. barkeri	543.6 ± 49.7 Gy	391.2 ± 44.3 Gy	394.3 ± 35.0 Gy	308.3 ± 25.9 Gy	281.4 ± 43.1 Gy	500.8 ± 38.1 Gy

n.d. = not determinable. * Cell survival was calculated from via viable cell counting and metabolic activity by methane production analysis (n = 3). D = dose for ionizing radiation. F = fluence for UV radiation exposure

Discussion

The methanogenic archaea from Siberian permafrost showed an unexpectedly high radiation resistance. Different strains of methanogenic archaea, which include representatives from permafrost and non-permafrost habitats, exhibit marked differences in their radiation tolerance. It is apparent from our data that the resistance of the methanogenic archaeon *Methanosarcina* sp. SMA-21 from permafrost to the lethal effects of ionizing, monochromatic and poly-chromatic UV radiation is significantly higher than that of the reference organism from non-permafrost habitats. The radiation sensitivity of methanogenic archaea was compared with these of other organisms on the basis of the survival curve parameters (survival curve characteristics in Table 1). In general, *Methanosarcina* sp. SMA21 appeared to be a radiation resistant strain exhibiting survival values comparable to that of *Deinococcus radiodurans*, which is well-known for its high resistance to radiation. In contrast, the radiation sensitivity of the *Methanosarcina barkeri* strain gave it a survival potential in the same range as that of *E. coli* and other radiation sensitive organisms.

In this study, we found that the metabolic activity of methanogenic archaea in soil samples was not significantly affected by UV irradiation, although it should also be taken into account that, besides the direct effects of

UV radiation, the subsurface soil community could be affected by indirect UV effects, e.g. radical oxygen species. Moreover, with the solar radiation simulator (SOL2), it was shown that Martian radiation has no effect on methanogenic archaea encased in permafrost. 200 kJ m^{-2} of polychromatic UV were the final applied irradiation fluence used in our experiments, would be equivalent to an outdoor exposure on a southern Martian summer day of approximately 86 h (Cockell *et al.*, 2000). One of the factors helping to support the viability of methanogens in permafrost samples might be the soil texture. Previous studies have shown that the penetration depth of UV radiation is dependent on the properties of the soil or sediment. Garcia-Pichel and Bebout (1996) found that irradiance of UVB was reduced to 1 % at depths < 1.25 mm in different sediment types. Thus, we suggest that the cells of methanogenic archaea covered by soil particles were protected against the direct effects of monochromatic or polychromatic UV radiation. Similarly, earlier studies showed that *Bacillus subtilis* spores protected by soil particles had a higher survivability than unprotected spores, which were inactivated after only 15 min of UV exposure (Manchinelli and Klovstad, 2000; Schuerger *et al.*, 2003). Moreover, a 1 mm dust layer gave the endospores full protection against the damaging effect of UV radiation (Manchinelli and Klovstad, 2000; Horneck *et al.*, 2001). Also the strong aggregate formation of up to 100 cells of methanogenic archaea could be one of the resistance mechanisms. The outer cells of an aggregate may shield the inner cells from the damaging influence of radiation. Since permafrost is expected to be extensively present on Mars, it is possible that methanogenic archaea could segregate in subsurface niches and survive under the harsh Martian thermal conditions.

However, protection, either physical or biological, is never 100 % efficient and the repair of DNA must also have been a key response to UV radiation penetrating the cell. Further irradiation experiments with pure methanogenic cultures, which were not associated with a protective soil matrix, were conducted in order to characterize the radiation sensitivity of permafrost strains in comparison to the methanogenic archaea from non-permafrost habitats. These experiments showed an unexpectedly high radiation resistance by the permafrost strain *Methanosarcina* sp. SMA-21 to monochromatic and polychromatic UV as well as to ionizing radiation.

Exposure to UV-C was carried out to evaluate and to compare the ability of *Methanosarcina* sp. SMA-21, SMA-16, and *Methanosarcina barkeri* to survive DNA damage resulting from reactive oxygen species and occasional single stranded DNA breaks. The results presented here clearly show that the examined permafrost isolates are highly resistant to UV-C radiation compared to the non-permafrost isolates. The degree of radiation resistance of a strain depends

on the growth phase of the organisms and the temperature during exposure. Because we used cells in the same growth phase and used the same method to asses the radiation resistance of all the examined species, it can be concluded that the permafrost strains are about 20-fold more resistant to UV-C than is the *Methanosarcina barkeri* strain. Furthermore, our permafrost strain *Methanosarcina* sp. SMA-21 exhibited an extremely high UV-C resistance with a F_{37} of ≈ 700 J m^{-2} and F_{10} of ≈ 1700 J m^{-2} based on metabolic activity, or F_{37} ≈ 1300 J m^{-2} and F_{10} of ≈ 2160 J m^{-2} based on cell survival. The difference between the F_{37} and F_{10} values for *Methanosarcina* sp. SMA-21 using the different calculation methods could be explained by the possibility that the DNA in the dead cells had not completely degraded when the cell counting procedure was conducted by FISH. Therefore, we believe that the physiological F_{37} value calculated from the decrease in methane production is more reliable for characterizing the UV-C radiation resistance of *Methanosarcina* sp. SMA-21. Nevertheless, the high survival of permafrost archaea indicated by F_{37} values between 200 and 700 J m^{-2} was surprising since methanogenic archaea were postulated as being radiosensitive organisms (Olson *et al.*, 1991). Strikingly, such a high resistance is comparable to the UV-C resistance of the most radiation resistant species on Earth, *Deinococcus radiodurans*, which has a F_{37} value of 640 J m^{-2}. In contrast, the low F_{37} value (34.8 ± 9.5 J m^{-2}) of *Methanosarcina barkeri* strain lies in the range of other UV radiosensitive microorganisms, such as *E. coli*.

Methanogenic archaeon *Methanosarcina* sp. SMA-21 from permafrost was also shown to be highly resistant to a polychromatic UV radiation. The full UV spectrum ($200 < \lambda < 400$ nm) of the solar simulator corresponds to radiation environments on early Earth or on Mars. Again, the UV-sensibilities of the permafrost and non-permafrost strains of *Methanosarcina* differ significantly. With F_{37} values of 14-15 kJ m^{-2}, *Methanosarcina* sp. SMA-21 showed resistance to the full UV spectrum that was, again, similar to *Deinococcus radiodurans* (F_{37} = 15-22 kJ m^{-2}). In contrast, the *Methanosarcina barkeri* cells were killed after a radiation fluence of only 5 kJ m^{-2}. A value of 15 kJ m^{-2} would be equivalent to 6.5 h UV exposure on Mars. Furthermore, it should be remembered that *Methanosarcina* sp. SMA-21 was highly resistant also under other simulated Martian conditions (Morozova *et al.*, 2006). The most striking result was the ability of methanogenic archaeon from Siberian permafrost to remain viable and metabolically active even when exposed to a fluence of 40 kJ m^{-2} of poly-chromatic UV. For comparison, the lethal UV fluence for the *Deinococcus radiodurans* cells was 32 kJ m^{-2} (Pogoda de la Vega *et al.*, 2005).

For the correct calculation of the biologically effective UV fluence in suspension, the following factors were

taking into account: shielding of the cells in suspension, the degree of UV absorption by the suspension itself, and the depth of the suspension. Though aggregate formation of *Methanosarcina* sp. SMA-21 could be one of the mechanisms to improve its radiation resistance, the fraction of non-shielded cells was calculated to be 96.6 %. Therefore, we suggest that the high radiation resistance of the permafrost isolates could be the result of the physiological potential of these organisms to cope with DNA damage.

The methanogenic archaeon *Methanosarcina* sp. SMA-21 also showed an exceptionally high tolerance to ionizing radiation. Ionizing radiation induces an even wider variety of DNA lesions than UV radiation (Friedberg *et al.*, 1995). The D_{37} and D_{10} values (6 -12 and 12 - 25 kGy, respectively) of the permafrost isolate SMA-21 were higher than the D_{10} reported for *Halobacterium* sp. cells of 5 kGy (Kottemann *et al.*, 2005) and close to the D_{37} and D_{10} range for *Deinococcus radiodurans* of 6 kGy and 10-12 kGy, respectively (Battista, 1997; van Gerwen *et al.*, 1999). By contrast, the survival potential of *Methanosarcina barkeri* (D_{37} and D_{10} values of 0.4 and 0.5 kGy) was close to that determined for *E. coli* with D_{10} values of 0.25 kGy (Clavero *et al.*, 1994).

Generally, it could be hypothesized that the radiation resistance of *Methanosarcina* sp. SMA-21 could be related, as in *Deinococcus radiodurans*, to a high capacity for efficient DNA repair. There are several possible strategies for DNA repair, such as photoreactivation, nucleotide excision repair, base excision repair and recombinational repair (Rettberg and Rotschild, 2001). Although there are a number of other UV protection or avoidance strategies known for many living organisms such as pigmentation, spore formation, trapping and binding of sediments, etc., those strategies would not be useful for methanogenic archaea since they are non-sporulating organisms. The chromophores found in methanogens (coenzyme F_{420} and F_{430}) absorb radiation maximally at 420 and 430 nm; however, no protection effect of these compounds has been detected yet (Olson *et al.*, 1991).

Furthermore, *Methanosarcina* spec. SMA-21 should also be able to cope with the huge oxidative stress due to the production of reactive oxygen species from UV-A radiation and hydroxyl radicals from ionizing radiation (Rupp, 1996; Riley, 1994; Dianov *et al.*, 2001). Hydroxyl radical intermediates formed during water radiolysis are thought to cause 65 % of cell death (Ward, 1998). Interestingly, this indirect effect is not seen in cells irradiated with UV (Friedberg *et al.*, 1995). Since many studies suggest that radiation resistance is related to desiccation resistance and both protect against similar DNA damage (Mattimore and Battista, 1996; Ferreira *et al.*, 1999; Whitehead *et al.*, 2006), it could be suggested that methanogenic archaea from permafrost, reported to be highly desiccation resistant (Morozova

and Wagner, 2007) would also resist ionizing radiation.

In summary, the high resistance to solar and ionizing radiation of *Methanosarcina* sp. SMA-21 from Siberian permafrost suggest that these organisms possess natural adaptation mechanisms to long-lasting background radiation accumulated in permafrost over geological time-scale, ranging from 1 to 6 kGy (Gilichinsky, 2001). They also have efficient repair mechanisms that allow them to live under extreme conditions of terrestrial permafrost, in contrast to other methanogens isolated from non-permafrost habitats which probably lack such mechanisms. The high survival rates of the methanogenic archaea from permafrost under radiation suggest that these microorganisms have developed ways to cope with radiation effects, which include repair of damaged DNA and other cell components, protection from reactive oxygen species, and the maintenance of other vital functions needed to sustain cell viability. This assumption is supported by the phylogenetic analyses of permafrost soils. Specific permafrost clusters were found, which were probably formed by methanogenic archaea characterized by specific adaptation processes to the harsh permafrost conditions (Ganzert *et al.*, 2006). One must conclude that some level of repair must have been occurring *in situ*. The so-called "survival metabolism" (Price and Sowers, 2004) in cold habitats would allow organisms in permafrost to repair DNA damage. It has also been postulated that at temperatures below 0 °C, damage by ionizing radiation is reduced because of a reduction in the diffusion ability of radicals, which leads to a protective effect (Baumstark-Khan and Facius, 2001).

Most striking was the difference in survival potential between *Methanosarcina barkeri* and *Methanosarcina* sp. SMA-21, two representatives of the same genus. On the basis of our experimental data, it is tempting to speculate that the permafrost strain *Methanosarcina* sp. SMA-21 posses a unique DNA-repair mechanism, which allows for continuous repair of damaged DNA in the cells. It is now therefore necessary, using functional genomics, to identify possible novel mechanisms in stress responses to radiation-induced DNA damage in methanogenic archaea.

From the astrobiological point of view, the high radiation resistance of *Methanosarcina* sp. SMA-21 from permafrost provides a very useful insight for the investigation of life in extremely cold environments on other planets of our solar system. We might conclude that permafrost habitats on Earth represent an excellent analogue for studying putative life on Mars. Recent analyses of *Mars Express* HRSC (High Resolution Stereo Camera) images of many regions of the planet showed that the morphology of Martian polygonal features is very similar to the morphology of terrestrial ice-wedge polygons and is most likely the result of comparable processes (Kuzmin, 2005), supporting the notion of widespread permafrost. The observation of high resistance to solar radiation, the

most deleterious factor in the environment of outer space, together with high survival rates of permafrost methanogens at defined stress conditions and at simulated Martian conditions (Morozova and Wagner, 2006; Morozova *et al.*, 2006) supports the possibility that microorganisms similar to methanogens from Siberian permafrost could also exist in Martian permafrost habitats. Methanogenic archaea from terrestrial permafrost may therefore serve as useful models for further exploration of extraterrestrial life.

Experimental procedures

Sampling site and characteristics

Permafrost samples were obtained from the arctic region of the Lena Delta, Siberia. The investigation site, Samoylov Island (N 72°22, E 126°28), 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 entire delta has been 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 a low annual air temperature of -14.7 °C (T_{min} = -48 °C, T_{max} = 18 °C) and low mean annual precipitation of 190 mm. The island is dominated by the typical permafrost pattern of low-centered polygons which cover at least 70 % of the island's area. The soils in the Lena Delta are entirely frozen, with only the upper 20-50 cm, the so-called "active layer", thawing during the summer months.

During the expedition "Lena 2004" soil samples were collected from the active layer of a floodplain 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 floodplain was a *Typic Aquorthel*, classified according to the US Soil Taxonomy (Soil Survey Staff, 1998). Soil samples were placed in airtight plastic jars (Nalgene) and transported to Germany frozen. Approximately 10 g of each soil sample was used for dry weight determination. All results were expressed per gram of dry soil.

Microbial cultures

To enrich and isolate methanogenic archaea, a bicarbonate-buffered, oxygen-free OCM culture medium was used, prepared according to Boone *et al.* (1989). Cultures were grown under an atmosphere of H_2/CO_2 (80/20, v/v) as substrate. The incubation temperatures were 4, 10 and 28 °C.

Methanosarcina sp. SMA-21 and SMA-16 (isolated in our lab from permafrost sediments sampled in summer 2002 from Siberian permafrost) grew well at 28 °C and more slowly at lower temperatures (4 °C and 10 °C). The strain *Methanosarcina* sp. SMA-21 appeared as

irregular cocci, 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.

The reference strain *Methanosarcina barkeri* DSM 8687, originating from a peat bog in Northern Germany (Maestrojuan *et al.*, 1992), was obtained from the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany).

Irradiation experiments

Radiation sources

UV-C irradiation was performed using a monochromatic UV-C radiation source (mercury low pressure lamp, N/N 8/15, Heraus Hanau, Germany; main emission line at $\lambda = 254$ nm) with a fluence rate of 0.18 mW cm^{-2} measured with UV-X dosimeter (UV dosimeter, Omnilab, Bremen, Germany). Polychromatic UV radiation was performed using a solar simulator SOL2 source (Dr. Hoenle AG, UV-Technology, Munich, Germany) emitting a spectrum of $\lambda = 200$-400 nm. The spectral UV irradiance at the sample site was spectroradiometrically measured (Bentham 150 double monochromator, Berkshire, United Kingdom). To exposure the methanogenic archaea ionizing radiation in the form of X-rays (150 keV/19mA) generated by an X-ray tube (Mueller Type MG 150, MCN 165, Phillips, Hamburg, Germany) DLR, Cologne, Germany) was applied. Dosimetry and dose calculation were performed as described previously (Micke *et al.*, 1994).

UV radiation experiments with permafrost soils

To determine the influence of monochromatic and polychromatic UV radiation on the survival potential of methanogenic archaea in the soil samples, fresh soil material (2 g) from the floodplain (*Typic Aquorthels,* A horizon, 0-5 cm depth) was weighed into 12.5 ml plastic boxes (A/S NUNG, Denmark) under anoxic conditions. Before starting the exposure, 3 ml of sterile deionized water was added to maintain anoxic conditions. The soil samples were exposed on ice to 1-20 kJ m^{-2} monochromatic UV (254 nm UV-C) or to 40-200 kJ m^{-2} polychromatic UV. The controls were kept at 4 °C. Before and after the experiment, the cell numbers were calculated as described below. After exposure to UV radiation, the soil samples were anaerobically transferred into 25 ml glass flasks, sealed with a screw cap containing a septum, and incubated at 10 °C. The activity of methanogenic archaea was measured after the experiment as described below.

Irradiation experiments with pure cultures of methanogenic archaea

All radiation exposure experiments, unless otherwise stated, were conducted as follows: cultures of *Methanosarcina* sp. SMA-21 and *Methanosarcina barkeri* grown at 28 °C to a cell density of approximately 10^8 cells ml^{-1} were

centrifuged (15000x*g* at 22 °C for 10 min), washed twice with PBS solution, and resuspended either in PBS or deionized water. Ionizing irradiation experiments were done only with cells, resuspended in PBS solution. For comparison purposes, UV-C radiation was also applied to the methanogenic archaeon SMA-16, isolated from the permafrost. Cell suspensions (2 ml) were exposed to monochromatic and polychromatic UV radiation, as well as to X-rays at 20 °C under anaerobic conditions in quartz cuvettes (3x500 µl, Hellma GmbH and Co. KG, Muelheim, Germany). The fraction of non-shielded cells was 96.6 %. Unexposed samples were used as controls, which were handled in the same manner and at the same temperature as the exposed cell suspensions. Irradiated samples were divided into two portions. One portion of 1 ml, which was used for the cell activity determination via methane production, was placed into 25 ml glass flasks under anaerobic conditions, supplemented with 10 ml fresh OCM medium and an appropriate substrate (H_2 for the *Methanosarcina* sp. SMA-21 and SMA-16 and methanol for the *Methanosarcina barkeri* strain), sealed with a screw cap containing a septum, and incubated at 28 °C. The second portion was incubated anaerobically after serial dilution with PBS to quantify survival by cell counts after 24-100 h of incubation at 28 °C. The activities and cell numbers before and after irradiation were calculated as described below. The physiological survival of the irradiated cells was assessed from methane production rates measured for 2 weeks following irradiation.

Numerical and statistical analysis

The surviving fraction was determined by dividing the cell numbers or methane production rates at any given radiation dose by the cell numbers/methane production rates obtained from the non-irradiated cell suspension. The survival curves were obtained by plotting the logarithm of the surviving fraction as a function of fluence/dose. Two parameters often used to describe organisms resistance to UV are (1) the UV fluence lethal for 90 % of the population (F_{10}), and (2) the UV fluence necessary to reduce survival to 37 % (F_{37}), calculated from the slope of the terminal straight portion of the inactivation curve. In order to characterize and compare the survival potential of the methanogenic strains, F_{10}, F_{37}, and Fq (i.e., radiation exposure obtained by extrapolation of the exponential portion of the survival curve to 100 % survival) values were calculated as described in Moeller *et al.* (2005). In order to characterize the organisms' resistance to ionizing radiation, we used D_{10}, D_{37}, and Dq values. All the experiments were done in triplicate. The significance of the difference of the survival curves was analyzed using the Student's *t*-test. Differences with *P* values of ≤ 0.05 were considered statistically significant (Moeller *et al.*, 2005; Pogoda de la Vega *et al.*, 2005).

Methane analysis

The activity of the methanogenic archaea was calculated based on the linear increase of the CH_4 concentration in the headspace. The methane concentration was measured by gas chromatography. The gas chromatograph (Agilent 6890, Fa. Agilent Technologies) was equipped with a Carbonplot capillary column (Ø = 0.53mm, 30 m length) and a flame ionization detector (FID). Both the oven and the injector temperature were 45 °C. The temperature of the detector was 250 °C. Helium served as the carrier gas. All the gas sample analyses were done following calibration with standards of the respective gases.

Cell count 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. The counting was done manually. For each hybridization approach and sample, at least 800 DAPI stained cells were counted on 30 randomly chosen counting squares. Microscopic performance was carried out using a magnification of 63 x 100 giving an area of 3.9204 x 10^{-2} mm^2 per counting square (Moter and Goebel, 2000; Kobabe *et al.*, 2004).

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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

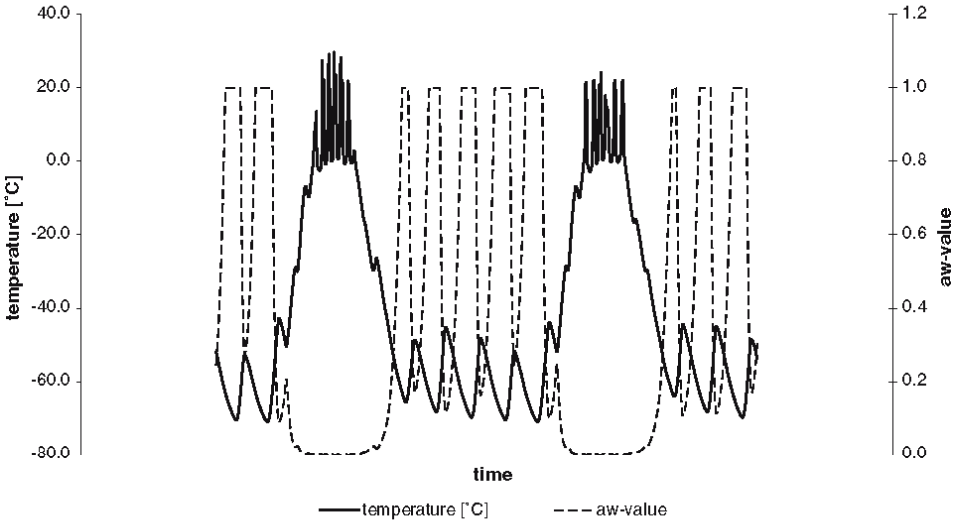

Figure 1 Diurnal profile of simulated Martian temperature (*bold line*) and humidity (a_W), – *dashed line* – in the Mars simulator (2 days are shown).

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

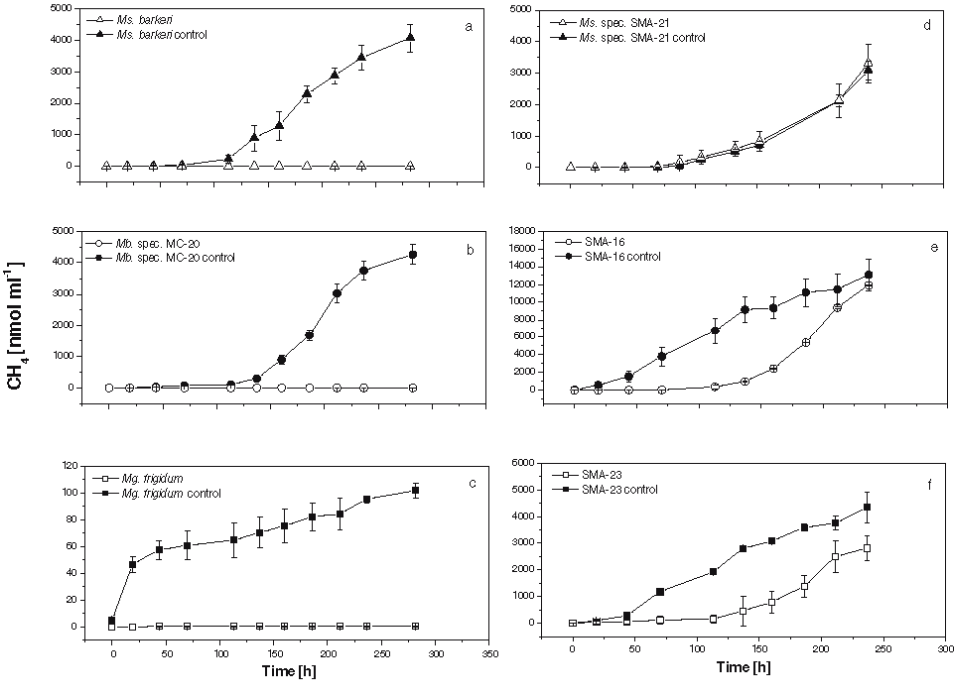

Figure 2 Methane production activities of the reference organisms *Methanosarcina barkeri* (a), *Methanobacterium* spec. MC-20 (b), *Methanogenium frigidum* (c) and methanogens isolated from Siberian permafrost *Methanosarcina* spec. SMA-21 (d), SMA-16 (e), SMA-23 (f) before and after exposure to simulated 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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6 Synthesis and conclusions

6.1 Synthesis

The goal of this thesis was to gain an overall understanding of the survival potential of methanogenic archaea under the extreme conditions of terrestrial permafrost. The background of this study were comparable system studies of terrestrial permafrost as an analogue for Martian permafrost. Since cryogenic structures are found on seven of nine planets in our Solar System, permafrost was regarded as a common phenomenon in the cosmos. Recent analyses of *Mars Express* HRSC (High Resolution Stereo Camera) 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). From the astrobiolological point of view, terrestrial permafrost inhabited by viable cold adapted microorganisms was considered as a model for potential extraterrestrial habitats (Gylichinsky, 2001; Wagner *et al*., 2001). The objective of this work was to prove or disprove the hypothesis, that methanogenic archaea from Siberian permafrost can be used as *model organisms* for possible life on Mars. The capability of these organisms for lithoautotrophic growth under strictly anaerobic conditions, tolerance of low temperatures and long-term survival under the extreme conditions of permafrost renders these organisms eminently suitable for this purpose. Within the scope of the DFG Priority Program *“Mars and the Terrestrial Planets”* the tolerances of methanogens under unfavourable life conditions of terrestrial or extraterrestrial permafrost were studied. The cultivation, isolation and characterization of methanogenic strains are described in the Appendix.

The aim of the first investigations presented in Publications I and II was to characterize the physiological potential and tolerance limits of methanogenic archaea in Siberian permafrost. Seasonal variations in soil temperatures, particularly freeze-thaw cycles in the active layer, result in drastic changes in other environmental conditions such as water availability, salinity, soil pressure, desiccation, changing oxygen conditions, and the availability of nutrients. Therefore, a number of experiments were conducted to evaluate the influence of these stress conditions on the survival of methanogenic archaea (Publications I and II). In particular, the survival potential of methanogenic archaea exposed to extremely low temperature, high salinity, long-term starvation, freezing and desiccation as well as oxygen exposure were examined. The first manuscript presents the comparative studies of the stress conditions influence on methanogenic archaea in their natural environment of

permafrost soils and in pure cultures. The objective of the second manuscript was to investigate the influence of low temperatures on the stress behavior of methanogens from permafrost compared to methanogens from non-permafrost habitats. Particular emphasis was placed on *Methanosarcina* spec. SMA-21 isolated from the active layer of the Siberian permafrost soil, and *Methanosarcina barkeri* from non-permafrost habitats, as a representative of the same genus. The results indicate a high survival potential of a methanogenic archaeon SMA-21 from Siberian permafrost when exposed to the extreme conditions described earlier. In contrast, these stress conditions were lethal for *Methanosarcina barkeri* isolated from non-permafrost habitats. It can be assumed that species from the active layer of permafrost soils possess a greater level of evolutionary adaptation to the fluctuating environmental conditions. The results indicate a better adaptation to a rapid increase in osmolarity, which occurs during freezing of the active layer of permafrost. Furthermore, a higher stress resistance of the methanogenic archaeon *Methanosarcina* spec. SMA-21 was determined at a low incubation temperature (4 °C) compared to a high one (28 °C). An incubation temperature of +4 °C correlates well with *in situ* temperatures of the active layer of permafrost, fluctuating in summer months from 0 °C to about +10 °C. During the freeze-back of the active layer, the salt concentration in the remaining pore water increases as the temperature decreases within the whole profile.

It remains to be determined if freeze protection mechanisms overlap with tolerance mechanisms, which protect against various other stresses such as desiccation, starvation or high salt concentration (Berry and Foegeding, 1997; Macario *et al.*, 1999; Cleland *et al.*, 2004; Georlette *et al.*, 2004). Generally, all cell components must be adapted to the cold to enable an overall level of cellular protection that is sufficient for survival and growth (Cavicchioli, 2006). Biotic survival of permafrost methanogens under these extreme conditions indicates unknown physiological adaptations and suggests that these microorganisms can cope with these stresses by repairing the damaged DNA, cell membranes, and other vital functions to maintain cell viability (Rivkina *et al.*, 2000). The slow metabolism rates of organisms in cold environments could be important for successful adaptation to stress conditions (Thomsson *et al.*, 2005).

Although the experiments did not simulate all extreme permafrost environmental conditions, they simulated the major stresses that organisms in terrestrial permafrost and in Martian permafrost might be exposed to. On Mars, harsh conditions like low water activity, high desiccation and oxidative stress, variations in the salinity of the environment and low or sporadic supply of energy sources are known (Litchfield, 1998; Horneck, 2000). The observation of

high survival rates of permafrost methanogens under defined stress conditions confirm that the isolated methanogenic archaea could be suitable *model organisms* for the exploration of possible life on Mars.

Among all extreme conditions, radiation has been a ubiquitous stressor since the origin of the first microbial ecosystem during the Archaean era, when Earth lacked a significant ozone layer and was therefore exposed to a full UV radiation spectrum. A comparable situation was proposed for present-day Mars. Any primary colonizers of surface habitats on Earth and Mars are supposed to be vulnerable to the effects of solar UV radiation. Hence, microorganisms that survived at the surface of these planets without the protection of an ozone layer would have needed mechanisms to resist UV and ionizing radiation. Since archaea are considered to be one of the initial organisms on Earth, it can be assumed that they were able to tolerate the UV environment of early Earth. Currently, a comparably high radiation dose resulting from the cumulative effect of background radiation from soil minerals is present in terrestrial permafrost (Gilichinsky, 2001).

In Publication III, methanogenic archaea were exposed to solar UV- and ionizing radiation in order to assess their limits of survival. The results of this study show, that metabolic activity and viability of methanogenic archaea in environmental samples remains unaffected by exposure to monochromatic and polychromatic UV radiation. Pure methanogenic cultures isolated from Siberian permafrost show an increase in the radioresistance to UV (20-fold) and ionizing radiation (32-fold) compared to non-permafrost isolates. The F_{37} (UV radiation) and D_{37} (x-rays) values of the permafrost strain *Methanosarcina* spec. SMA-21 correspond to 700 Jm^{-2} and 6-12 kGy, respectively. This extremely high resistance is comparable to values determined for *Deinococcus radiodurans*, the most radiation resistant organism on Earth (F_{37} 640 Jm^{-2}, D_{37} 6 kGy). In contrast, *Methanosarcina barkeri* demonstrated radiation sensitivity with a survival potential comparable to *Escherichia coli* and other radiation sensitive organisms. The high radiation resistance of *Methanosarcina* sp. SMA-21 from Siberian permafrost suggests that this archaeon possess natural adaptation mechanisms to long-lasting background radiation accumulated in permafrost over geological time-scales, ranging from 1 to 6 kGy (Gilichinsky, 2001). On the basis of the experimental data, it is tempting to speculate that the permafrost strain *Methanosarcina* spec. SMA-21 possesses an unique DNA-repair machinery, which allows a continuous repair of damaged DNA in the cells as it was only shown for *D. radiodurans* so far (Battista, 1997; Venkateswaran *et al.*, 2000; Whitehead *et al.*, 2006).

The high stress resistance of methanogenic archaea from Siberian permafrost obtained from the previous studies together with the discovery of

methane in the Martian atmosphere (Formisano, 2004) was the background of the study presented in the fourth publication, *Survival of Methanogenic Archaea from Siberian Permafrost under Simulated Martian Thermal Conditions*. Furthermore, as has been shown by *Mars Odyssey* measurements, 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). It was therefore a challenge to study relevant organisms under Mars-like thermo-physical conditions with temporary saturation with water vapour and temporary dryness. The results of this study show an extraordinarily high survival potential of methanogenic archaea from Siberian permafrost under simulated Martian 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 between 95.0 % and 99.7 % of methanogenic archaea that originated from non-permafrost habitats. This indicates that methanogenic archaea from permafrost are more resistant and probably possess a natural adaptation to temperature and humidity shifts in contrast to well-studied methanogens from other environments. 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.

In summary, the high survival rates and activity of methanogenic archaea from Siberian permafrost under different stress conditions suggest that these organisms possess natural adaptation mechanisms to sub-zero temperatures, increased salinity, starvation, desiccation, high radiation doses and oxygen stress. The high survival rates of the methanogenic archaea from permafrost under stress conditions demonstrate that these microorganisms have developed ways to cope with radiation effects which have to include repair of damaged DNA and other cell components, protection from the reactive oxygen species and the maintenance of other vital functions needed to sustain cell viability. One must conclude that some level of repair should have been occurring *in situ*. So-called “survival metabolism” (Price and Sowers, 2004) in cold habitats would allow the organisms in permafrost to repair DNA damage. Therefore, the methanogenic community in permafrost should have adaptations that have allowed them to survive in these conditions, which are lethal for the methanogens isolated from non-permafrost habitats.

6.2 Conclusions and future perspectives

Towards an understanding of methanogenic life in permafrost, this thesis contributes substantial findings on the survival potential of methanogenic archaea under harsh living conditions.
The main conclusions from the presented study are:

- The extreme environmental conditions of Siberian permafrost and distinct fluctuations in its physico-chemical gradient are likely to determine the high resistance of methanogenic archaea to definite stress factors (high salinity, radiation, extremely low temperatures, prolonged desiccation, starvation, sub-zero temperatures and oxigen stress).
- The permafrost soil matrix provides an efficient protection of methanogenic archaea under harsh conditions. However, also the ability of methanogens to rapid stress response (repair of DNA and other important cell molecules and structures) is necessary for the high resistant observed.
- Low temperatures support the rapid stress adaptation of methanogenic archaea from Siberian permafrost. Hence, it could be concluded that cold protection mechanisms overlap with tolerance mechanisms which protect against other various stress conditions such as desiccation, starvation or high salt concentration.
- Methanogenic archaea from permafrost show a higher resistance to diverse stress conditions than representatives of the same genus from non-permafrost habitats. This suggests differences in genome size and structure.

A high resistance of methanogens to defined stress factors is an important requirement for a long-term survival and an adaptation to the extreme environments of terrestrial permafrost, a model of extraterrestrial protected niches. Furthermore, from the astrobiological point of view, the physiological potential and the metabolic specificity of methanogenic archaea from permafrost provide a very useful insight into potential life in extremely cold environments on other planets of our solar system. The existence of microorganisms like methanogenic archaea on Mars might be possible due to geothermal sources of hydrogen, carbon dioxide - which is abundant in the Martian atmosphere - and subsurface water. The results presented support the hypothesis that methanogenic archaea from Siberian permafrost habitats can also survive in the present Martian environment (anoxic conditions, dryness, coldness, intensive radiation and high salinity). Methanogenic archaea from

terrestrial permafrost may therefore serve as *model organism*s and are of particular importance for investigations of analogous extraterrestrial life.

The results from this study clearly demonstrate the differences in the survival potential between *Methanosarcina barkeri* and *Methanosarcina* spec. SMA-21, two representatives of the same genus. Therefore, it is of great importance to sequence the genome of *Methanosarcina* spec. SMA-21, as a highly resistant methanogenic representative of a permafrost community. The characterization of the physiological traits potentially important to cryo-adaptation is necessary to understand adaptation mechanisms at the genome level.

Further studies on methanogens from permafrost are in progress. These studies are designed to investigate the metabolic activity of methanogenic archaea from Siberian permafrost under Martian conditions. The results of these ongoing studies shall demonstrate the capacity of methanogenic archaea to grow and metabolize under environmental conditions similar to those of the red planet.

7 References

Abyzov, S.S., Mitskevich, I.N. and Poglazova, M.N. (1998) Microflora of the deep glacier horizon of Central Antarctica. *Microbiology* **67**: 451-458.

Abuzov, S.S., Mitskevich, N.I., Poglazova, M.N., Barkov, N.I., Lipenkov, V. Ya., Bobin, N.E., Kudryashov, B.B., Pashkevich, V.M. (1999) Antarctic ice sheet as an object for solving some methodological problems of exobiology, *Adv Space Res* **23**: 371-376.

Ananyan, A.A. (1970) Unfrozen water content in frozen clay at a temperature from -0.6 °C to -40 °C - -60 °C. *Merzlotnye Issledovaniya* **10**: 267-270 (in Russian).

Anisimov, O.A. and Nelson, F.E. (1996) Permafrost distribution in the Northern Hemisphere under scenarios of climatic change. *Global and Planetary Change* **14***:* 59-72.

Bakermans, C., Tsapin, A.I., Souza-Egipsy, V., Gilichinsky, D.A. and Nealson, K. (2003) Reproduction and metabolism at -10 °C of bacteria isolated from Siberian permafrost, *Environ Microbiol* **5**: 321-326.

Balch, W.E., Fox, G.E., Magrum, L.J, Woese, C.R. and Wolfe, R.S. (1979) Methanogens: reevaluation of a unique biological group. *Microbiol Res* **43**: 260-269.

Bar-Nun, A. and Dimitrov, V. (2006) Methane on Mars: a product of H_2O photolysis in the presence of CO. *Icarus* **181**: 320-322.

Battista, J.R. (1997) Against all odds: strategies of *Deinococcus radiodurans. Annu Rev Microbiol* **51**: 203-224.

Baumstark-Khan, C., and Facius, R. (2001) Life under conditions of ionizing radiation. In *Astrobiology: The Quest for the Conditions of Life*. Horneck, G. and Baumstark-Khan, C. (eds). Springer Verlag Berlin Heidelberg, pp. 261-286.

Berry, E.D. and Foegeding, P.M. (1997) Cold temperature adaptation and growth of microorganisms. *J Food Protection* **60**: 1583-1594.

Boone, D., Johnson, R., and Liu, Y. (1989) Diffusion of the interspecies electron carriers H_2 and formate in methanogenic ecosystems and its implications in the measurement of Km for H_2 or formate uptake. *Appl Environ Microbiol* **55**:1735-1741.

Boynton, W.V., Feldman, W.C., Squyres, S.W. *et al.* (2002) distribution of hydrogen on the near surface of Mars: evidence of subsurface ice deposits. *Science* **297**: 81-85.

Brioukhanov, A.L., Netrusov, A.I. and Eggen, R.I.L. (2006) The catalase and superoxide dismutase genes are transcriptionally up-regulated upon

oxidative stress in the strictly anaerobic archaeon *Methanosarcina barkeri*. *Microbiology* **152**: 1671-1677.

Bryant, M.P. and Boone, D.R. (1987) Emended description of strain MS^T (DSM 800^T), the type strain of *Methanosarcina barkeri*. *Int J Syst Bacteriol* **37**: 169-170.

Bullock, M.A., Stoker, C.R., McKay, C.P. and Zent, A.P. (1994) A coupled soil atmosphere model of H_2O_2 on Mars. *Icarus* **107**: 142-154.

Carr, M.H. (1989) Recharge of the early atmosphere of Mars by impact-induced release of CO_2. *Icarus* **79**: 311-327.

Carr, M.H. (1996) Water on Mars. Oxford University Press, Oxford.

Cavicchioli, R. (2006) Cold-adapted archaea. *Nature* **4**: 331-343.

Christensen, P.R., Wyatt, M.B., Glotch, T.D., Rogers, A.D. et al. (2004) Mineralogy at Meridiani Planum from the Mini-Tes experiment on the Opportunity Rover. *Science* **306**: 1733-1739.

Clancy, R.T., Sandor, B.J. and Moriarty-Schieven, G.H. (2004) A measurment of the 362 GHz absorption line on Mars atmosphere H_2O_2. *Icarus* **168**: 116-121.

Clavero, M.R.S., Monk, J.D., Beuchat, L.R., Doyle, M.P., and Brackett, R.E. (1994) Inactivation of *Escherichia coli* 0157:H7, *Salmonellae*, and *Campylobacter jejuni* in raw ground beef by gamma irradiation. *Appl Environ Microbiol* **60**: 2069-2075.

Cleland, D., Krader, P., McCree, C., Tang, J. and Emerson, D. (2004) Glycine betaine as a cryoprotectant for prokaryotes. *J Microbiol Methods* **58**: 31-38.

Cockell, C.S., Catling, D.C., Davis, W.L., Snook, K., Kepner, R.L., Lee, P., and McKay, P. (2000) The ultraviolet environment of Mars: biological implications. Past, present and future. *Icarus* **146**: 343-359.

Cockell, C.S. (2001) The ultraviolet radiation environment of Earth and Mars: past and present. In *Astrobiology: The Quest for the Conditions of Life*. Horneck, G. and Baumstark-Khan, C. (eds). Springer Verlag Berlin Heidelberg, pp. 219-232.

Daniels, L. (1993) Biochemistry of methanogens. In: *The Biochemistry of Archaea (Archaebacteria).* Kates, M., Kushner, D.J. and Matheson, A.T. (eds). Elsevier, Amsterdam, pp. 41-112.

Deppenmeier, U., Müller, V. and Gottschalk, G. (1996) Pathways of energy conversation in methanogenic archaea. *Arch Microbiol* **165**: 149-163.

Dianov, G.L., O'Neill, P., and Goodhead, D.T. (2001) Securing genome stability by orchestrating DNA repair: removal of radiation-induced clustered lesions in DNA. *BioEssays* **23**: 745-749.

DSMZ – Deutsche Sammlung für Mikroorganismen und Zellkulturen: Medium No. 120: *Methanosarcina* (http://www.dsmz.de/media/med120.html).

DSMZ – Deutsche Sammlung für Mikroorganismen und Zellkulturen: Medium No. 141: *Methanosarcina* (http://www.dsmz.de/media/med141.htm).

Durham, R., Schmunk, R.B., and Chamberlain, J.W. (1989) Comparative analysis of the atmospheres of early Earth and Mars. *Adv Space Res* **9**: 139-142.

Eicher, J. (2001) Biotechnological uses of archaeal extremozymes. *Biotechnology Advances* **19**: 261-278.

Encrenaz, T., Bezard, B., Greathouse, T.K., Richter, M.J., Lacy, J.H., Atreya, S.K., Wong, A.S., Lebonnois, S., Lefevre, F. and Forget, F. (2004) Hydrogen peroxide on Mars: evidence for spatial and seasonal variations. *Icarus* **170**: 424-429.

Ershov, E.D. (1995) Physical and chemical basics of geocryology, In: *Mocsow University press, Moscow, Russia*, pp. 369 (in Russian).

European Space Agency (2004) Water and Methane maps overlap on Mars: a new clue? (http://www.esa.int/SPECIALS/Mars_Express/SEML131XDYD_0.html).

Ferreira, A.C., Nobre, M.F., Moore, E., Rainey, F.A., Battista, J.R., and da Costa, M.S. (1999) Characterization and radiation resistance of new isolates of *Rubrobacter radiotoleran*s and *Rubrobacter xylanophilus. Extremophiles* **3**: 235-238.

Fetzer, S., Bak, F. and Conrad, R. (1993) Sencitivity of methanogenic bacteria from paddy soil to oxygen and desiccation. *FEMS Microbiol Ecol* **12**: 107-115.

Formisano, V. (2004) Detection of methane in the atmosphere of Mars. *Science* **306**: 1758-1761.

Franzmann, P.D., Springer, N., Ludwig, W., De Macario, E.C., and Rohde, M. (1992) A methanogenic archaeon from Ace Lake, Antarctica – *Methanococcoides burtonii* sp. nov. *Syst Appl Microbiol* **15**: 573-58.

Franzmann, P.D., Liu, Y., Balkwill, D.L., Aldrich, H.C., de Macario E.C., and Boone, D.R. (1997) *Methanogenium frigidum* sp. nov., a psychrophilic, H_2-using methanogen from Ace Lake, Antarctica. *Int J Syst Bacteriol* **47**: 1068-1072.

French, H.M. (1996) The periglacial environment. Addison Wesley Longman, Edinburgh pp. 341.

Friedberg, E.C., Walker, G.C., and Siede, W. (1995) DNA repair and mutagenesis. ASM Press, Washington, DC.

Friedmann, E. (1994) Permafrost as microbial habitat. Viable microorganisms in permafrost. Institute of Soil Science and photosynthesis, Russian Academy of Science, Pushkino.

Fung, I., John, J., Lerner, J., Matthews, E., Prather, M., Steele, L.P., Fraser, P.J. (1991) Three-dimensional model synthesis of the global methane cycle. *J Geophysical Res* **96**: 13033-13065.

Ganzert, L., Jurgens, G., Münster, U., and Wagner, D. (2007) Methanogenic communities in permafrost-affected soils on the Laptev Sea coast, Siberian Arctic, characterized by 16S rRNA gene fingerprints. *FEMS Microbiol Ecol* **59**: 476-488.

Garcia, J.L., Patel, B.K.C., and Olliver, B. (2000) Taxonomic, phylogenetic and ecological diversity of methanogenic archaea. *Anaerobe* **6**: 205-226.

Garcia-Pichel, F. and Bebout, B.M. (1996) Penetration of ultraviolet radiation into shallow water sediments: high exposure for photosynthetic communities. *Marine Ecol Progr Ser* **131**: 257-262.

Georlette, D., Blaise, V., Collins, T., D'Amico, S., Gratia, E., Hoyoux, A., Marx, J.-C., Sonan, G., Feller, G. and Gerday, C. (2004) Some like it cold: biocatalysis at low temperatures. *FEMS Microbiol Rev* **28**: 25-42.

Gilichinsky, D.A. (2001) Permafrost model of extraterrestrial habitat. In *Astrobiology: The Quest for the Conditions of Life*. Horneck, G. and Baumstark-Khan, C. (eds). Springer Verlag Berlin Heidelberg, pp. 125-142.

Gilichinsky, D., Rivkina, E. , Shcherbakova, V., Laurinavichuis, K. and Tiedje, J. (2003) Supercooled water brines within permafrost - an unknown ecological niche for microorganisms: a model for astrobiology. *Astrobiology* **3**: 331-341.

Gilichinsky, D., Soina, V.S. and Petrova, M.A. (1993) Cryoprotective properties of water in the Earth cryolithosphere and its role in exobiology. *Orig Life Evol Biosph* **23**: 65-75.

Gilichinsky, D., Vorobyova, E.A., Erokhina, L.G., Fyordorov-Dayvdov D.G. and Chaikovskaya N.R. (1992) Long-term preservation of microbial ecosystems in permafrost. *Adv Space Res* **12**: 255-263.

Goldsmith, D. and Owen, T. (1980) The search for life in the universe. Benjamin/Cummings, New York.

Gounot, A.M. (1999) Microbial life in permanently cold soils. In: *Cold-adapted organisms* .Margesin and F. Schinner (Eds.), Springer Verlag Berlin.

van Grewen, S.J., Rombouts, F.M., van't Riet, K., and Zwietering, M.H. (1999) A data analysis in the irradiation parameter D_{10} for bacteria and spores under various conditions. *J Food Prot* **62**: 1024-1032.

Grotenhuis, J., Plugge, C., Stams, A. and Zehndler, A. (1992) Hydrophobicities and electrophoretic mobilities of anaerobic bacterial isolates from methanogenic granular sludge. *Appl Environ Microbiol* **58**: 1054-1056.

Heijnen, C., Hok-A-Hin, C. and Van Veen, J. (1992) Improvements to the use of bentonite clay as a protective agent increasing survival levels of bacteria introduced into soil, *Soil Biol Biochem* **24**: 533-538.

Horneck, G., Rettberg, P., Reitz, G., Wehner, J., Eschweiler, U., Strauch, K., Panitz, C., Starke, V., and Baumstark-Khan, C. (2001) Protection of bacterial spores in space, a contribution to the discussion on Panspermia. *Orig Life Evol Biosph* **31**, 527-547.

Høj, L., Rolf, O., and Torsvik, V. (2005) Archaeal communities in High Arctic wetlands at Spitzbergen, Norway (78°N) as characterized by 16S rRNA gene fingerprinting. *FEMS Microbiol Ecol* **53**: 89-101.

Inouye, M. and Phadtare, S. (2004) Cold shock response and adaptation at near-freezing temperature in microorganisms. *Sci STKE*: 1-3.

Jakosky, B.M. and Phillips, R.J. (2001) Mars' volatile and climate history. *Nature* **412**: 237-244.

Jones, W.J., Nagle Jr., D.P. and Whitman, W.B. (1987) Methanogens and the diversity of archaebacteria. *Microbiol Rev* **51**: 135-177.

Kiener, A. and Leisinger, T. (1983) Oxygen sensitivity of methanogenic bacteria. *Syst Appl Microbiol* **4**: 305-312.

Kim, W.S. and Dunn, N.W. (1997) Identification of a cold shock gene in lactic acid bacteria and the effect of cold shock on cryotolerance. *Current Microbiol* **35**: 59-63.

Klingelhofer, G., Morris, R.V., Bernhard, B., Schroder, C. et al. (2004) Jarosite and hematite at Meridiani Planum from Opportunity's Mossbauer spectrometer. *Science* **306**: 1740-1745.

Kobabe, S., Wagner, D. and Pfeiffer, E.-M. (2004) Characterization of microbial community composition of a Siberian tundra soil by fluorescence *in situ* hybridization. *FEMS Microbiol Ecol* **50**: 13-23.

Kottemann M., Kish, A., Iloanusi, C., Bjork, S., and DiRuggiero, J. (2005) Physiological responses of the halophilic archaeon *Halobacterium* sp. strain NRC1 to desiccation and gamma radiation. *Extremophiles* **9**: 219-227.

Kral, T.A., Bekkum, C.R. and McKay, C.P. (2004) Growth of methanogens on a Mars soil stimulant. *Orig Life Evol Biosph* **34**: 615-626.

Krasnopolsky, V.A., Maillard, J.P. and Owen, T.C. (2004) Detection of methane in the martian atmosphere: evidence for life? *Icarus* **172**: 537-547.

Kuhn, W.R. and Atreya, S.K. (1979) Solar radiation incident on the Martian surface. *J Mol Evol* **14**: 57–64.

Kuzmin, R.O. (2005) Ground Ice in the Martian Regolith In *Water on Mars and Life.* T. Tokano (ed.), Springer Verlag Berlin.

Lange, M. and Ahring, B. (2001) A comprehensive study into the molecular methodology and molecular biology of methanogenic archaea. *FEMS Microbiol Rev* **25**: 553-571.

Larsen, K.S., Jonasson, S. and Michelsen, A. (2002) Repeated freeze-thaw cycles and their effects on biological processes in two arctic ecosystem types. *Appl Soil Ecol* **21**: 187-195.

Lapygina, E., Lysak, L. and Zvyagintsev, D. (2001) Tolerance of soil complexes to salt shock. *Microbiol* **71**: 143-147.

Lascu, C. (1989) Paleogeographical and hydrogeological hypothesis regarding the origin of a peculiar cave fauna. *Misc speol Rom Bucharest* **1**: 13-18.

Litchfield, C. (1998) Survival strategies for microorganisms in hypersaline environments and their relevance to life on early Mars. *Meteor Planet Sci* **33**: 813-819.

Litvak, M.L., Mitrofanov, I.G. , Kozyrev, A.S., Sanin, A.B., Tretyakov, V.I., Boynton, W.V., Kelly, N.J., Hamara, D., Shinohara, C. and Saunders, R.S. (2006) Comparison between polar regions of Mars from HEND/Odyssey data. *Icarus* **180**: 23-37.

Macario, A.J.L., Lange, M., Ahring, B.K. and De Macario, E.C. (1999) Stress genes and proteins in the archaea. *Microbiol Mol Biol Rev* **63**: 923-967.

Maestrojuan, G.M., Bonne, J.E., Mah, R.A., Menaia, J.A.G.F., Sachis, M.S., and Boone, D.R. (1992) Taxonomy and halotolerance of mesophilic *Methanosarcina* strains, assignment of strains to species, and synonymy of *Methanosarcina mazei* and *Methanosarcina frisia*. *Int J Syst Bacteriol* **42**: 561-567.

Manchinelli, R.L. and Klovstad, M. (2000) Martian soil and UV radiation: microbial viability assessment on spacecraft surfaces. *Planet Space Sci* **48**: 1093-1097.

Mattimore, V. and Battista, J.R. (1996) Radioresistance of *Deinococcus radiodurans*: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. *J Bacteriol* **178**: 633-637.

McGrath J., Wagener, S. and Gilichinsky, D. (1994) Cryobiological studies of ancient microorganisms isolated from the Siberian permafrost. In: *Viable microorganisms in permafrost*. D. Gilichinsky (Ed.), Pushchino Research Center, Pushchino, pp. 48-67.

McKay, C.P. and Davis, W. (1991) Duration of liquid water habitats on early Mars. *Icarus* **90**: 214-221.

McKay, C.P., Friedman, E.I., Wharton, R.A., and Davis, W.L. (1992) History of water on Mars: a biological perspective. *Adv Space Res* **12**: 231-238.

Mellon, M.T., Feldman, W.C. and Prettyman, T.H. (2004) The presence and stability of ground ice in the Southern Hemisphere of Mras. *Icarus* **169**: 324-340.

Micke, U., Horneck, G., and Kozubek, S. (1994) Double strand breaks in the DNA of *Bacillus subtilis* cells irradiated by heavy ions. *Adv Space Res* **14**: 207-211.

Mikan, C.J., Schimel, J.P. and Doyle, A.P. (2002) Temperature controls of microbial respiration in arctic tundra soils above and below freezing. *Soil Biol Biochem* **34**: 1785-1795.

Mikha, M.M., Rive, C.W. and Milliken, G.A. (2005) Carbon and nitrogen mineralization as affected by drying and wetting cycles. *Soil Biol Biochem* **37**: 339-347.

Mindock, C.A., Petrova, M.A. and Hollingsworth, R.I. (2000) Re-evaluation of osmotic effects as a general adaptive strategy for bacteria in sub-freezing conditions. *Biophysical Chemistry* **89**: 13-24.

Mitrofanov, I.G., Litvak, M.L., Kozyrev, A.S., Sanin, A.B., Trt'akov, V.I., Grin'kov, V.Y., Boynton, W.V., Shinohara, C.,Hamara, D. and Saunders, R.S. (2004) Soil water content on Mars as estimated from neutron measurments by the HEND instrument onboard the 2001 Mars Odyssey spacekraft. *Solar Syst Res* **38**: 253-265.

Moeller, R., Horneck, G., Facius, R., and Stackebrandt, E. (2005) Role of pigmentation in protecting *Bacillus* sp. Endospores against environmental UV radiation. *FEMS Microbiol Ecol* **51**: 231-236.

Moehlmann, D., Wernecke, R. and Schwanke, V. (2004) Measurement principle and equipment for measuring humidity contents in the upper Martian surface and subsurface. ESA SP-543: 163 – 168.

Moehlmann, D. (2005) Adsorption Water-Related Potential Chemical and Biological Processes in the Upper Martian Surface. *Astrobiology* **5**: 770 – 777.

Moran, M., Miller, J.D., Kral, T. and Scott, D. (2005) Desert methane: implications for life detection on Mars. *Icarus* **178**: 277-280.

Morita, R.Y. (2000) Is H_2 the universal energy source for long-term survival? *Microbiol Ecol* **38**: 307-320.

Morita, R.Y. (2005) Low temperature environments. In: *Encyclopedia of Microbiology 2000*: 93-98.

Morozova, D., Möhlmann, D., and Wagner, D. (2007) Survival of methanogenic archaea from Siberian permafrost under simulated Martian thermal conditions. *Orig Life Evol Biosph* **37**: 189-200.

Morozova, D. and Wagner, D. (2007) Stress response of methanogenic archaea from Siberian permafrost compared to methanogens from non-permafrost habitats. *FEMS Microbiol Ecol* **61**: 16-25.

Moter, A. and Goebel, U.B. (2000) Fluorescence *in situ* hybridization (FISH) for direct visualization of microorganisms. *J Microbiol Methods* **41**: 85-112.

Mueller, D.R., Vincent, W.F., Bonilla, S., Laurion, I. (2005) Extremotrophs, extremophiles and broadband pigmentation strategies in a high arctic ice shelf ecosystem. *FEMS Microbiol Ecol* **53**: 73-87.

Nair, H., Allen, M., Anbar, A.D., Yung, Y.L. and Clancy, R.T. (1994) A photochemical model of the Martian atmosphere. *Icarus* **111**: 124-150.

National Aeronautics and Space Administration (2006) NASA images suggest water still flows in brief spurts on Mars. (http://www.nasa.gov/mission_pages/mars/news/mgs-20061206.html).

Olson, K.D., McMahon, C.W., and Wolfe, R.S. (1991) Light sensitivity of methanogenic archaebacteria. *Appl Environ Microbiol* **57**: 2683-2686.

Ostroumov, V. (1995) A physical and chemical characterization of Martian permafrost as possible habitat for viable organisms, *Adv Space Res* **15:** 229-236.

Ostroumov, V. and Siegert, C. (1996) Exolbiological aspects of mass transfer in microzones of permafrost deposits. *Adv Space Res* **18**: 79-86.

Panicker, G., Aislabie, J., Saul, D. and Bej, A.K. (2002) Cold tolerance of Pseudomonas sp. 30-3 isolated from oil-contaminated soil, Antarctica. P*olar biology* **25**: 5-11.

Pogoda de la Vega, U., Rettberg, P., Douki, T., Cadet, J., and Horneck, G. (2005) Sensitivity to polychromatic UV-radiation of strains of *Deinococcus radiodurans* differing in their DNA repair capacity. *Int J Radiat Biol* **81**: 601-611.

Ponder, M.A., Gilmour, S.J., Bergholz, P.W., Mindock, C.A., Hollingsworth, R., Thomashow, M.F., Tiedje, J.M. (2005) Characterization of potential stress responses in ancient Siberian permafrost psychroactive bacteria. *FEMS Microbiol Ecol* **53**: 103-115.

Price, R.B. and Sowers, T. (2004) Temperature dependence of metabolic rates on microbial growth, maintenance and survival. *PNAS* **101**: 4631-4636.

Rettberg, P. and Rotshild, L. (2001) Ultraviolet radiation in planetary atmospheres and biological implications. In *Astrobiology: The Quest for the Conditions of Life*. Horneck, G. and Baumstark-Khan, C. (eds). Springer Verlag Berlin Heidelberg, pp. 233-244.

Riley, P.A. (1994) Free radicals in biology: oxidative stress and the effects of ionizing radiation. *Int J Radiat Biol* **65**: 27-33.

Rivkina, E., Friedmann, E.I., McKay, C.P. and Gilichinsky, D. (2000) Metabolic activity of permafrost bacteria below the freezing point. *Appl Environ Microbiol* **66**: 3230-3233.

Rivkina, E.M., Laurinavichus, K.S., Gilichinsky, D.A. and Shcherbakova, V.A. (2001) Methane generation in permafrost sediments. *Microbiology* **383**: 830-833.

Rivkina, E., Laurinavichius, K., McGrath, J., Tiedje, J., Shcherbakova, V. and Gilichinsky, D. (2004) Microbial life in permafrost. *Adv Space Res* **33**: 1215-1221.

Robertson, D.E., Noll, D., Roberts, M.F., Menaia, J.A. and Boone, D.R. (1990) Detection of osmoregulator betaine in methanogens. *Appl Environ Microbiol* **56:** 563-695.

Rothschield, L.J. (1999) The influence of UV radiation on protistan evolution. *J Eukaryot Microbiol* **46**: 548-555.

Rothschild, L.J. and Cockell, C.S. (1999) Radiation: microbial evolution, ecology, and relevance to mars missions. *Mutat Res* **430**: 281-291.

Rupp, W.D. (1996) DNA repair mechanisms. In *Escherichia coli and Salmonella, cellular and molecular biology*, 2nd ed. Neidhardt, F.C. (ed). ASM Press, Washington, pp. 2277-2294.

Sarbu, S.M. and Kane, T.C. (1995) A subterranean chemoautotrophically based ecosystem. *National Speleological Society Bulletin* **57**: 91-98.

Sears, D.W.G., Benoit, P.H., McKeever, S.W.S., Banerjee, D., Kral, T., Stites, W., Roe, L., Jansma, P., Mattioli, G.: 2002, Investigation of biological, chemical and physical processes on and in planetary surfaces by laboratory simulation, *Planetary and Space Science* **50**: 821-828.

Schidlowski, M. (1993) The beginnings of life on Earth: evidence from geological record. In: *The chemistry of life`s origin*. J.M. Greenberg (Ed.).. Kluver, Dordrecht, pp. 389-414.

Schimel, J.P., and Clein, J.S. (1996) Microbial response to freeze-thaw cycles in tundra and taiga soils. *Soil Biol Biochem* **28**: 1061-1066

Schimel, J.P. and Mikan, C. (2005) Changing microbial substrate use in Arctic tundra soils through a freeze-thaw cycle. *Soil Biol Biochem* **37**: 1411-1418.

Schirrmeister, L., Grigoriev, M.N., Kutzbach, L., Wagner, D. and Bolshiyanov, D.Yu. (2004) Russian-German Cooperation SYSTEM LAPTEV SEA: The Expedition Lena-Anabar 2003, Reports on Polar Research 489.

Schlichting, E, Blume, HP, Stahr, K (eds) 1995, B*odenkundliches Praktikum: Pareys Studientexte 81,* Blackwell Wissensverlag, Berlin, Germany.

Schopf, J.W. (1993) Microfossils of the early Archean Apex chert: new evidence of the antiquity of life. *Science* **260**: 640-646.

Schuerger, A.C., Manchinelli, R.L., Kern, R.G., Rotschild, L.J., and McKay, C.P. (2003) Survival of endospores of Bacillus subtilis on spacecraft surfaces under Martian environments: implications for the forward contamination of Mars. *Icarus* **165**: 253-276.

Schwamborn, G., Rachold, V., and Grigoriev, M.N. (2002) Late quaternary sedimentation history of the Lena Delta. *Quaternary International* **89:** 119-134.

Sherer, P., Lippert, H. and Wolff, G. (1983) Composition of the major elements and trace elements of 10 methanogenic bacteria determined by inductively coupled plasma emission spectrometry. *J Biol Trace Elem Res* **5:** 149-163.

Shuisong, N. and Boone, D. (1998) Extremophilic methanogenic archaea and their adaptation mechanisms. *Extremophiles: Microbial Life in Extreme Environments*: 211-232.

Simankova, M.V., Kotsyurbenko, O.R., Lueders, T., Nozhevnikova, A.N., Wagner, B., Conrad, R., and Friedirch, M.W. (2003) Isolation and characterization of new strains of methanogens from cold terrestrial habitats. *Syst Appl Microbiol* **26**: 312-318.

Smith, L.C., MacDonald, G.M., Velichko, A.A., Beilman, W.D., Borisova, O.K., Frey, K.E., Kremenetski, K.V., Sheng, Y. (2004) Siberian peatlands: a net carbon sink and global methane source since the early Holocene. *Science* **303:** 353-356.

Soffen, G.A. and Young, A.T. (1972) Viking missions to Mars. *Icarus* **16**: 1-16.

Soil Survey Staff (1998) *Keys to soil taxonomy*, Eighth Edition. Washington, D.C, Conservation Service, USDA.

Solomon, S.C., Aharonson, O. , Aurnou, J.M. *et al.* (2005) New Perspectives on ancient Mars. *Science* **307**: 1214-1220.

Squyres, S.W., Grotzinger, J.P., Arvidson, R.E. *et al.* (2004) *In situ* evidence for an ancient aqueous environment at Meridiani Planum, Mars. *Science* **306**: 1709-1714.

Stetter, K.O., Fiala, G., Huber, G., Huber, R., and Segerer, A. (1990) Hyperthermophilic microorganisms. *FEMS Microbiol Rev* **75**: 117-124.

Summers, M.E., Lieb, B.J., Chapman, E. and Yung, Y.L. (20029 Atmospheric biomarkers of surface life on Mars. *Geophys Res Lett* **29**: 24.1-24.4.

Tanghe, A., Van Dijck, P. and Thevelein, J.M. (2003) Determination of freeze tolerance in microorganisms, physiological importance, and biotechnical applications. *Adv Appl Microbiol* **53**: 129-176.

Tung, H.C., Bramall, N.E. and Price, P.B. (2005) Microbial origin of excess methane in glacial ice and implications for life on Mars, *PNAS* **102**: 18292-18296.

Venkateswaran, A., McFarlan, S.C., Ghosal, D., Minton, K.W., Vasilenko, A., Makarova, K., Wackett, L.P. and Daly, M.J. (2000) Physiologic determination of radiation resistance in *Deinococcus radiodurans. Apl Environ Microbiol* **66**: 2620-2626.

Virina, E.I., Zazhygin, V.S. and Sher, A.V. (1984) Paleomagnetic pattern of type localities of Olyour faunal assemblage. *Izvestiya Akademii Nauk SSSR, Seriya Geologicheskaya* **1**: 61-71 (in Russian).

Vishnivetskaya, T., Kathariou, S., McGrath, J, Gilichinsky, D., and Tiedje, J. (2000) Low-temperature recovery strategies for the isolation of bacteria from ancient permafrost sediments. *Extremophiles* **4**: 165-173.

Vorobyova, E., Soina, V., Gorlenko, M., Minkovskaya, N., Zalinova, N., Mamukelashvili, A., Gilichinsky, D., Rivkina, E. and Vishnivetskaya, T. (1997) The deep cold biosphere: facts and hypothesis. *FEMS Microbiol Rev* **20**: 277-290.

Wagner, D., Gattinger, A., Embacher, A., Pfeiffer, E. -M., Schloter, M., Lipski, A. (2007) Methanogenic activity and biomass in Holocene permafrost deposits of the Lena Delta, Siberian Arctic and its implication for the global methane budget. *Global change biology* **13**: 1089-1099.

Wagner, D., Kobabe, S., Pfeiffer, E.-M. and Hubberten, H.-W. (2003) Microbial controls on methane fluxes from a polygonal tundra of the Lena Delta, Siberia. *Permafrost Periglac Process* **14**: 173-185.

Wagner, D., Lipski, A., Embacher, A., Gattinger, A. (2005) Methane fluxes in permafrost habitats of the Lena Delta: effects of microbial community structure and organic matter quality. *Environ Microbiol* **7**: 1582-1592.

Wagner, D., Pfeiffer, E.-M. and Bock, E. (1999) Methane production in aerated marshland and model soils: effects of microflora and soil texture. *Soil Biology and Biochemistry* **31**: 999-1006.

Wagner, D., Spieck, E., Bock, E. and Pfeiffer, E.-M. (2001) Microbial life in terrestrial permafrost: methanogenesis and nitrification as potentials for exobiological processes, In: *Astrobiology: The Quest for the Conditions of Life.* G. horneck and Baumstark-Khan, C. (eds), Springer Verlag Berlin Heidelberg, pp. 245-260.

Ward, J.F. (1998) Nature of lesions formed by ionizing radiation. In *DNA damage and repair, vol. 2: DNA repair in higher eukaryotes*. Nikoloff, J.A. Hoekstra, M.F. (eds). Totowa, N.J., Humana Press, pp. 65-84.

Wardlaw, A.C. (1985) Practical statistics for experimental biologists. John Wiley and Sons, Chichester.

Weinberg, M.V., Schut, G.J., Brehm, S., Datta, S. and Adams, M.W.W. (2005) Cold shock of a hypertharmophilic archaeon: *Pyrococcus furiosus* exhibit multiple responses to a suboptimal growth temperature with a key role of membrane-bound glycoproteins*. J Bacteriol* **187**: 336-348.

Weiss, B.P., Yung, Y.L. and Nealson, K.H. (2000) Atmospheric energy for subsurface life on Mars? *Proc Natl Acad Sci USA* **97**: 1395-1399.

Wharton Jr, R.A., McKay, C.P., Mancinelli, R.L., and Simmons Jr, G.M. (1989) Early Martian environments: the Antarctic and other terrestrial analogs. *Adv Space Res* **9**: 147-153.

Whitehead, K., Kish, A., Pan, M., Kaur, A., Reiss, D.J., King, N., Hohmann, L., DiRuggiero, J., and Baliga, N.S. (2006) An integrated systems approach for understanding cellular responses to gamma radiation. *Mol Sys Biol* **2**: 47.

Whitman, W.B., Bowen, T.C. and Boone, D.R. (1992) The methanogenic bacteria. In: The prokaryotes, A. Balows, H.G. Trüper, M. Dworkin, W. Harder and K.H. Schleifer (eds.) Springer Verlag, New York, 719-767.

Willerslev, E., Hansen, A.J. and Poinar, H.N. (2004) Isolation of nucleid acids and cultures from fossil ice and permafrost. *Trends Ecol Evol* **19**: 141-147.

Woese, C.R., Kandler, O. and Wheelis, M.L. (1990) Towards a natural system of organisms: proposal for the domain archaea, bacteria, and eucarya. *Proc Natl Acad Sci USA* **87**: 4576-4579.

Wouters, J.A., Frenkiel, H., de Vos, W.M., Kuipers, O.P. and Abee, T. (2001) Cold shock proteins of Lactococcus lactus MG1363 are involved in cryoprotection and in the production of cold-induced proteins. *Appl Environ Microbiol* **67**: 5171-5178.

Wynn-Williams, D.D. and Edwards, H.G.M. (2001) Environmental UV radiation: biological strategies fro protection and avoidance. In *Astrobiology: The Quest for the Conditions of Life*. Horneck, G. and Baumstark-Khan, C. (eds). Springer Verlag Berlin Heidelberg, pp. 245-260.

Yen, A.S., Kim, S.S., Hecht, M.H., Frant, M.S. and Murray, B. (2000) Evidence that the reactivity of the Martian soi is due to superoxide ions. *Science* **289**: 1909-1912.

Zhang, T., Barry, R.G., Knowles, K., Heginbottom, J.A. and Brown J. (1999) Statistic and characteristics of permafrost and ground-ice distribution in the Northern Hemisphere. *Polar Geography* **23**: 132-154.

Zhang, W., Culley, D.E., Nie, L. and Brockman, F.J. (2006) DNA microarray analysis of anaerobic *Methanosarcina barkeri* reveals responses to heat shock and air exposure. *J Ind Microbiol Biotechnol* **33**: 784-790.

Zhilina, T.N. (1972) Death of *Methanosarcina* in the air. *Microbiologiya* **41**: 1105-1106.

Zinder, S.H. (1993) Physioogical ecology of methanogens. In: *Methanogenesis: Ecology, Physiology, Biochemistry and Genetics,* Ferry JG (ed.) Chapman et al., New York.

8 Appendix

8.1 Publication V

'ROME - Response of Organisms to the Martian Environment'. Chapter 10. C.S. Cockell (ed.). Special Publication of the European Space Agency (ESA), SP 1298.

10. Response of methanogenic archaea from Siberian permafrost to Martian thermophysical conditions

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10.1 Background

Within our Solar system, Mars has been considered as a prime candidate for extraterrestrial life beyond Earth (Goldsmith & Owen, 1980). Several paleoclimate models of early Mars showed that, prior to 3.8 Ga ago, Mars was characterised by moderate temperatures, the presence of liquid water and an anoxic atmosphere comparable to those on early Earth (Durham et al., 1989; McKay et al., 1992), where the evolution of microorganisms had already started (Schidlowski, 1993; Schopf, 1993). Assuming that early life developed on Mars as well, Martian life must have adapted to drastically changing environmental conditions or became extinct.

One possibility for survival of Martian microorganisms could be subsurface ecosystems like deep sediments, where liquid-like ('unfrozen') adsorption water can play a key-role for transport of nutrients and waste products of biological processes (Möhlmann, 2005). Comparable environments exist in polar regions on Earth, described for instance in Antarctic ice cores of several thousand metres in depth (Abyzov et al., 1998) and in Siberian permafrost cores of up to 50 m depth (Gilichinsky et al., 1993; Wagner et al., 2006), where microorganisms exist independently of photosynthetic energy production.

Permafrost on Earth, which occurs mainly in polar and sub-polar regions, covers more than 20% of the land surface. These regions are characterised by low mean annual temperatures (–20°C), a low mean annual precipitation (about 200 mm) and poor to missing vegetation. The permafrost thickness can reach several hundreds of metres, especially in East Siberia (Central Yakutia) where it is 600–800 m thick. During the relatively short period of the Arctic/Antarctic summer, the upper part of the permafrost thaws, leading to what is termed the 'active layer'. Permafrost can be cemented by ice that is typical for the Arctic regions or, in the case of insufficient interstitial water, may be dry as in the Antarctic polar deserts.

Permafrost can be divided into three temperature-depth layers, characterised by different living conditions: (i) the active layer with an extreme temperature regime from about +15°C to –35°C, (ii) the upper, perennially frozen permafrost sediments (10–20 m thickness) with smaller seasonal temperature variation of about 0°C to –15°C above the zero annual amplitude (French, 1996) and (iii) the deeper permafrost sediments which are characterised by a stable temperature regime of about –5°C to –10°C. Active chemico-physical processes under extreme conditions take place exclusively in the active layer and upper permafrost sediments. The deeper permafrost layers are characterised by living conditions that have been stable for long periods of time and where microbial life is preserved (Gilichinsky et al 1992 and 1993).

Terrestrial permafrost is colonised by high numbers of chemoorganotrophic bacteria as well as microbes such as highly specialised methanogenic archaea (Kobabe et al., 2004; Wagner et al., 2005). Methanogens are phylogenetically classified as Archaea (Whiteman et al., 1992), a group of microorganisms that are distinguished from Bacteria by some specific characteristics (e.g. cell wall composition, coenzymes). They are strictly anaerobic microorganisms without the ability to form spores or other resting stages. Because of the specific adaptations of methanogenic archaea to conditions similar to those on early Earth (e.g. no oxygen, no or little organic substrates) and their phylogenetic origin, they are considered to be one of the initial organisms from the beginning of life on Earth. Methanogens are widespread in nature and occur in high abundance in extreme environments featuring low/high temperatures (permafrost, hot springs), extreme salinity (saltern ponds), anoxic properties (peatlands, rice paddies) or low/high pH (solfataras, soda lakes). Methane production by methanogenic archaea represents the terminal step in carbon flow in extreme environments.

Methanogens are characterised by lithoauthotrophic growth gaining energy by the oxidation of hydrogen, and carbon dioxide can be used as the only carbon source. Lithoautotrophic growth is an important presumption for long-term survival (Morita, 2000) of microbes in extreme environments like permafrost on Earth or maybe on other planets of our Solar System. In addition to this specific metabolism, methanogens are able to convert only a limited number of organic substrates (acetate, formate, methanol, methylamines) to methane. In permafrost soils, two main pathways of energy metabolism are dominant: (i) the reduction of CO_2 to CH_4 using H_2 as a reducing agent and (ii) the fermentation of acetate to CH_4 and CO_2. In the case of CO_2 reduction, no organic carbon is needed for growth of methanogenic archaea (Deppenmeier et al., 1996).

Beside the mesophilic species, also thermophilic methanogens are known (Stetter et al., 1990). In recent times, more attention has been paid to the search for psychrophilic strains since many of methanogenic habitats belong to cold climates (Gounot, 1999). Only a few strains (e.g. *Methanococcoides burtonii, Methanogenium frigidum, Methanosarcina* spec.) have been isolated from cold habitats so far (Franzman et al, 1992, 1997; Simankova et al., 2003). Permafrost habitats are characterised by extreme environmental conditions such as sub-zero temperatures, aridity and higher than normal levels of background radiation as a result of an accumulation over geological timescales. 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 and availability of nutrients. Many methanogens (e.g. *Methanogenium cariaci*, *Methanosarcina thermophila*) for example are able to adapt to high salinity by the accumulation of compatible solutes to equalise the external and internal osmolarity (Robertson et al, 1990). However, little is known about the physiology and resilience of methanogenic archaea in permafrost.

From the astrobiological point of view, terrestrial permafrost in which microorganisms have survived for several millions of years (Vorobyova et al. 1997, Wagner et al., 2001, Rivkina et al. 2004) is considered to be a model for extraterrestrial analogues. Permafrost microorganisms demonstrate the residue of the autochthon population within the paleosoils that was enclosed during deposition of fresh sediments. Survival could be possible by anabiosis (dormant stage of life) or by reduced metabolic activity in unfrozen waterfilms. In order to preserve their viability in an extreme environment the microorganisms had to develop strategies to resist salt stress, physical damage by ice crystals and background radiation. The environmental conditions in terrestrial permafrost are comparable to those for life on Mars in subsurface habitats.

For the understanding of putative life on Mars, we used methanogenic archaea from Siberian permafrost and reference organisms from non-permafrost habitats

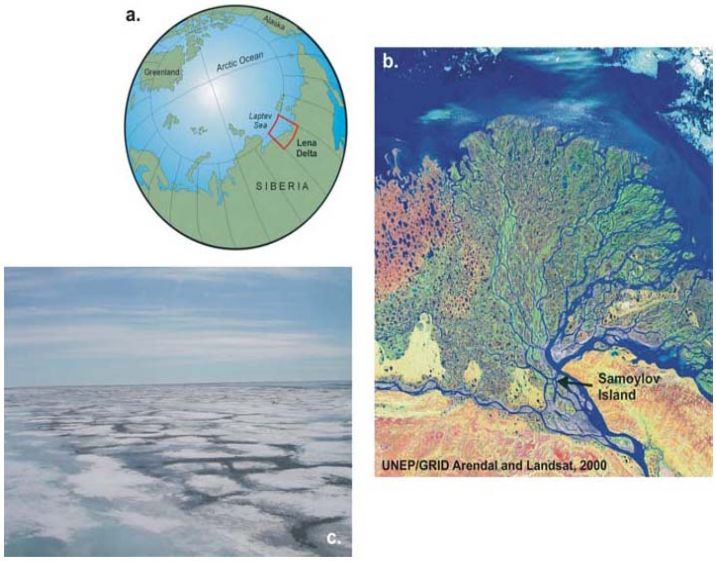

Figure 1. Long-term study site on Samoylov Island, Lena Delta, Siberia. Permafrost samples and methanogenic archaea originated from the island Samoylov (b) located in the Lena Delta (a). The image (c) shows the typical patterned ground of low-centre ice-wedge polygons during winter time.

for studying their survival potential under simulated thermophysical Martian conditions at low- and mid-latitudes.

10.2 Materials and Methods

10.2.1 Sample origin and archaeal cultures

The investigation site Samoylov Island (N 72°22, E 126°28) – where the samples originated – is located within the central part of the Lena Delta. It is the largest delta in the world, with an area of 32 000 km^2. The Lena Delta is on the Laptev Sea coast, between the Taimyr Peninsula and the New Siberian Islands in the continuous permafrost zone (Fig. 1.). It is characterised by an Arctic continental climate with low mean annual air temperature of –14.7°C (T_{min} = –48°C, T_{max} = 18°C) and a low mean annual precipitation of 140 mm.

During several expeditions to the Lena Delta (e.g. Schirrmeister et al., 2004; Wagner & Bolshiyanov, 2006) soil samples were collected from the active layer of permafrost. Sampling was carried out on two major topographic units (microhigh = rim and microlow = depression) within a low-centred ice-wedge polygon and on the floodplain (Wagner *et al.*, 2003a). The samples were filled in gastight plastic jars (Nalgene) and transported to Germany in frozen conditions. Permafrost samples were used for the enrichment of methanogenic archaea, geochemical and physical soil analyses, stress experiments and further investigations of microbial survival potential under defined stress conditions.

Methanosarcina spec. SMA-21 as well as the strains SMA-16 and SMA-23 were isolated from permafrost. For the enrichment permafrost samples collected from the anoxic horizons (0–5 cm, 9–18 cm and 32–45 cm soil depth respectively) of the floodplain depression (*Typic Aquorthels*) were used.

For enrichment and isolation of methanogenic archaea the bicarbonate-buffered, oxygen-free OCM culture medium was used, prepared according to Boone and co-authors (1989). The medium was anaerobically dispensed into vials and the headspace was filled with an N_2/CO_2 mixture (80:20; v:v). Methanol (20mM) or H_2/CO_2 (80:20; v:v, 150 kPa pressurised) were used as substrates for the cultures. Inoculated vials were incubated at 10°C. To obtain pure cultures, repeated inoculation to fresh medium at 28°C were carried out using 1:10 serial dilutions. The growth of contaminates was inhibited by using different antibiotics

(5 gml^{-1} erytromycin or phosphomycin). Purity was checked by microscopy 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°C and 10°C). The isolated strains exhibit different morphologies. *Methanosarcina* SMA-21 cells were irregular cocci and 1–2 µm in diameter. The large cell aggregates were regularly observed. The cells of strain SMA-16 were smaller irregular diplococci, reaching 0.5–1 µm diameter. The cells of strain SMA-23 were rod-shaped, about 1–2 µm in width and maximum 10 µm in length, often builds a long cell chains (Fig. 2).

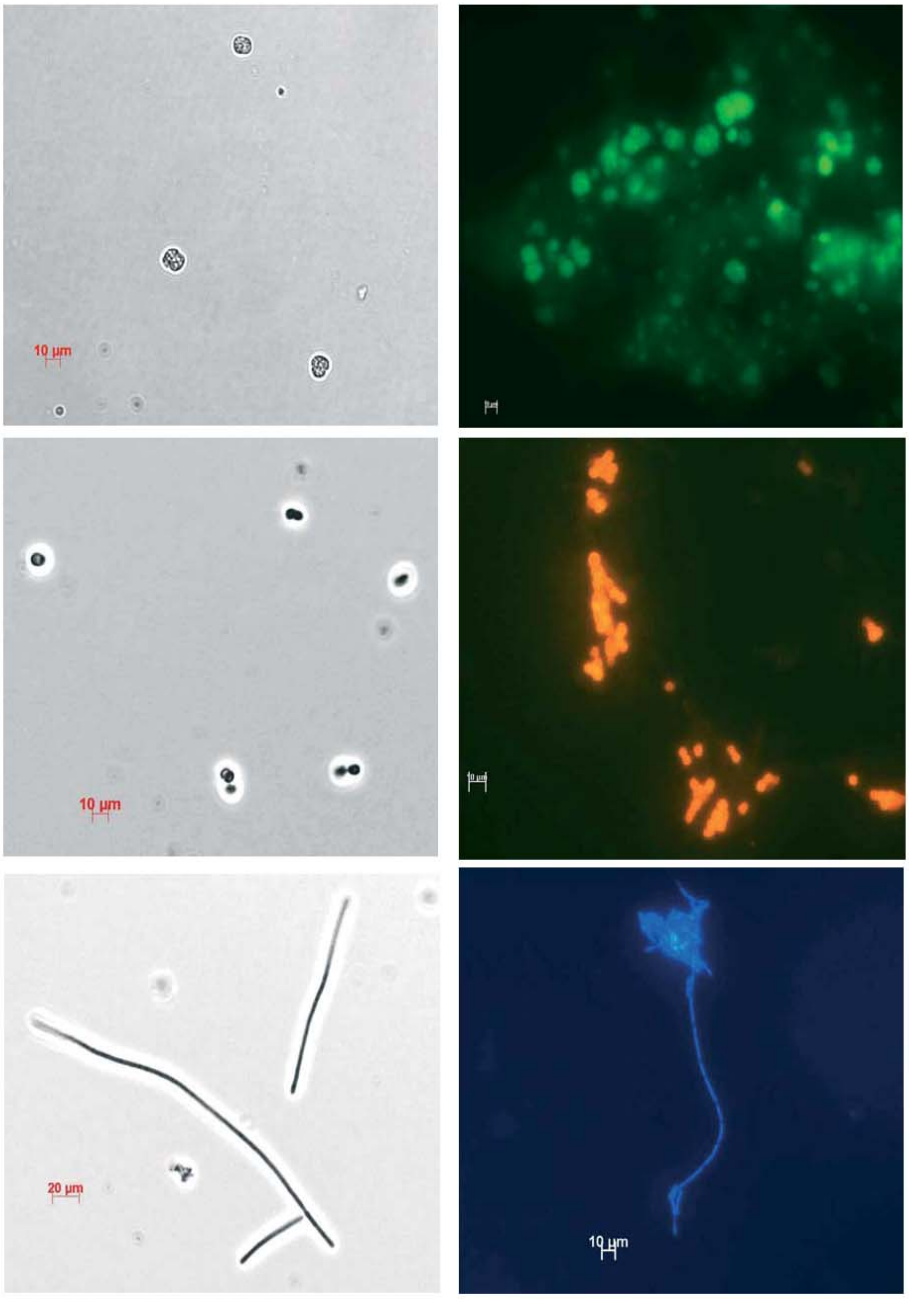

Figure 2. Phase contrast and fluorescence micrographs of methanogenic archaea isolated from Siberian permafrost. Methanosarcina *SMA-21 (a, b), strain SMA-16 (c, d) and strain SMA-23 (e, f). The cells were stained with the general oligonucleotid probe for Archaea (ARC915 Cy3, b), DAPI (c) and self-fluorescence by 420 nm (a).*

As reference organisms, different methanogenic species from non-permafrost environments were used. *Methanobacterium* spec. MC-20 was isolated from a sediment in Mangalia, Romania, at an incubation temperature of 28°C. The cells were rod-shaped, 1–2 µm in width and maximum 8 µm in length. *Methanosarcina barkeri* (DSM 8687; Bryant & Boone, 1987) and *Methanogenium frigidum* (DSM 16458; Franzmann *et al.*, 1997) were obtained from the Deutsche Sammlung für Mikroorganismen und Zellkulturen (Braunschweig, Germany). *Ms. barkeri* was grown on oxygen-free MS culture medium (DSMZ No. 120) supplemented with 20 mM methanol as a substrate. *Mg. frigidum* was grown on oxygen-free EM culture medium (DSMZ No. 141) under an atmosphere of H_2/CO_2 (80:20, v/v, pressurised 150 kPa) at 15°C.

10.2.2 Freezing tolerance of methanogenic archaea

Three strains of methanogenic archaea were used in the freezing experiment: *Methanosarcina* spec. SMA-21 as well as the reference organisms *Methanobacterium* spec. MC-20 and *Ms. barkeri.* Cultures were grown to a cell density of 10^8 cells ml^{-1} in the appropriate media and temperatures within one week. Cultures were divided into two sub-samples; one sample was immediately frozen at –78.5°C, and the other was cold-shocked at 10°C for 2 hours before it was frozen at –78.5°C. For each sub-sample, an aliquot of 1 ml was taken out just before freezing. After storage at –78.5°C for 24 hours, the frozen cells were thawed at room temperature and cell numbers were counted by fluorescence *in situ* hybridisation (FISH) with the general oligonucleotid probe for Archaea (ARC915 Cy3). The percentage of the survival potential of each methanogenic strain was calculated by the ratio of the average cell number counted after freezing to the cell number before freezing. Activity was measured by the methane production analysed by gas chromatography. Gas samples were taken every 24 hours with a gas-tight syringe. The activity of methanogenic archaea was quantified from the linear increase of CH_4 concentration. All experiments were done in triplicate.

10.2.3 Experimental set-up

The laboratory for humidity related studies (HUMIDITY-Lab) of the German Aerospace Center (DLR), Institute of Planetary Research, Berlin, studies the influence of water on physical, chemical and biological processes, which are assumed to be of relevance to planetary research. It performs laboratory experiments with controlled time-profiles (as diurnal variations) of temperature down to about –75°C. Atmospheric pressure and composition (including humidity) can be adapted to planetary conditions. Thermophysical conditions, which are typical for Martian mid- and low latitudes, can be simulated (cf. Möhlmann et al., 2004).

The HUMIDITY-Lab consists of a 'cold chamber' (with a cooled volume of 80 cm height, 60 cm depth, and 50 cm width). The 'experiment chamber' (the experiment volume), which is cooled within the cold chamber, is a cylinder of 20.1 cm inner diameter and 32.4 cm inner height (Fig. 3). There are connections through the cover for electrical contacts and gases. A gas-mixing unit, including controlled humidity, a PC-based data and control unit complete the current set-up. The gas mixing system (GMS) was developed to simulate planetary atmospheric conditions by producing varying contents of different gases. It has computer-controlled equipment for the recreating selected dew points, gas mixing and volume flow. The dew points are controlled in a range from –75°C to 20°C. It is possible to mix four gases and to release them in three volume flows ranging from 5 Nl/h to 60 Nl/h. Five mass-flow controllers regulate the flow of the different gases into the GMS. The gases are mixed in the following pipe system, and, if required, moistened in a wash bottle. The GMS is under overpressure. The waste gas is blown off through an over-pressure valve. Mass-flow controllers regulate the flow of the moistened mixture of gases. The outputs operate with

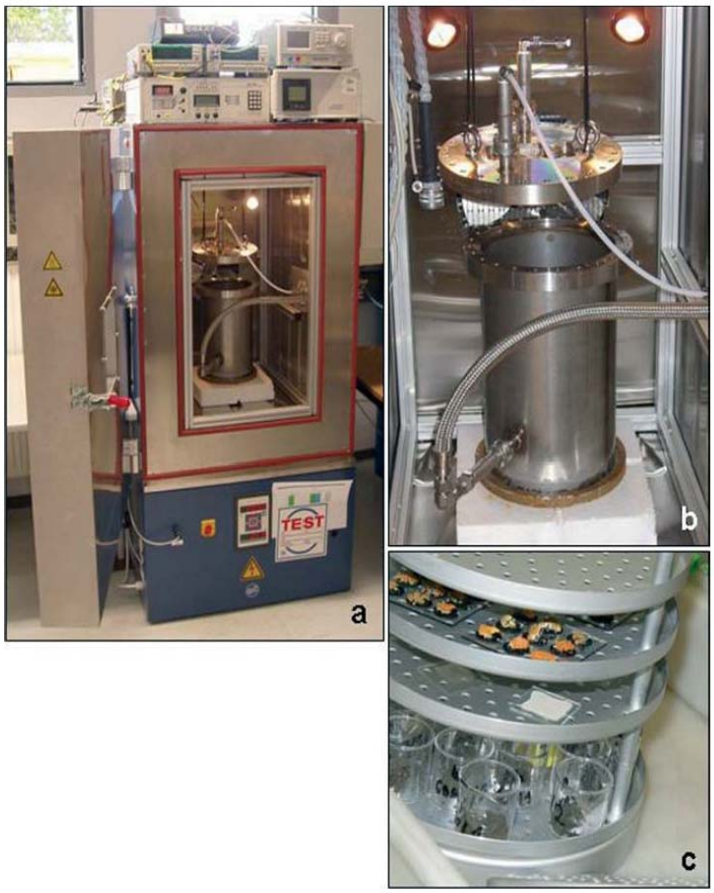

Figure 3. Mars simulation facility HUMIDITY-Lab for studying the effect of adsorption water on chemical and biological processes under the diurnal temperature and humidity profile of Mars. (a) cold chamber, (b) experiment chamber (c) rack for the exposure of biological samples.

ambient pressure and vacuum. Air is used as carrier gas. The air is provided by a compressor and dried out in two steps at a dew point of –75°C by permeation dryer and by a 'molsieve' at a dew point of –55°C. Thus, the humidity is in the range of trace humidity. The humidity, which can be directly provided, corresponds to a partial water vapour pressure of slightly less than or about 10^{-3} mbar (0.1 Pa). The average water vapour pressure on Mars is of 10^{-3} mbar (corresponding to 10 pr µm).

10.2.4 Mars simulation experiment

Six strains of methanogenic archaea were used in the simulation experiment: three permafrost strains namely *Methanosarcina* spec. SMA-21, SMA-23 and SMA-16 and three reference organisms namely *Ms. barkeri, Mg. frigidum* and *Methanobacterium* spec. MC-20.

All strains except *Mg. frigidum* were incubated at 28°C for about two weeks. Cells were harvested by centrifugation and 0.05 g of the cell pellet was inoculated into 1500 µl glass jars (A-Z Analytik Zubehör GmbH). Three replicates of the cultures were used with a cell density of about 2.3–8.1 x 10^7 cell ml^{-1}. Before and after the experiment, the cell numbers were determined by Thoma cell counts and by fluorescence *in situ* hybridisation (FISH) with the general oligonucleotid probe for Archaea (ARC915 Cy3). The glass jars were transferred to the experimental rack under CO_2 atmosphere and exposed to the simulated Martian conditions for about three weeks. After exposure, the cell pellets were replaced under anaerobic conditions into 25 ml glass flasks, supplemented with 10 ml fresh OCM medium and H_2 as a substrate, closed with a screw cap containing a septum and incubated

at 28°C (*Mg. frigidum* at 15°C). The activity was measured before and after the experiment by following the increase of CH_4 concentration by gas chromatography. The percentage of the survival potential of each methanogenic cultures was calculated by the ratio of the cell numbers counted after Mars simulation experiment to the cell numbers before simulation.

10.2.5 Methane analyses

Gas analysis was carried out with a gas chromatograph (Agilent 6890, Fa. Agilent Technologies) equipped with a Carbonplot capillary column (diameter 0.53 mm, 30 m length) and a flame ionisation detector (FID). Oven (and injector temperature) was 45°C. The temperature of the detector was 250°C. The carrier gas was helium.

10.3 Results

10.3.1 Freezing tolerance

Methanogenic archaea isolated from different habitats were examined for their ability to survive freezing at –78.5°C for 24 hours. The methanogenic strains *Methanosarcina* spec. SMA-21, *Methanobacterium* spec. MC-20 and *Ms. barkeri* showed significant differences in their ability to survive freezing at –78.5°C for a period of 24 hours. The average cell number of strain *Methanosarcina* spec. SMA-21 at the beginning of the experiment slightly decreased from 4.4×10^8 cells m^{-1} to 3.9×10^8 cells m^{-1} at the end of the freezing, which equals a survival rate of 89.5%. In comparison, only 1.0% of *Methanobacterium* MC-20 and 0.8% of *Ms. barkeri* survived the incubation at –78.5°C (Tab. 1). The decrease of cell numbers appears to correlate well with the methane production rates of the cultures. Thus, activity of *Methanosarcina* SMA-21 measured before the freezing (10.87 ± 1.22 nmol CH_4 h^{-1} ml^{-1}) was similar to that after the simulation (5.57 ± 0.67 nmol CH_4 h^{-1} ml^{-1}), whereas methane production rates of *Methanobacterium* MC-20 and the reference organism *Ms. barkeri* drastically decreased after the experiment (Tab. 1, Fig. 4). Measured methane production rates of the *Methanobacterium* MC-20 found after the freezing (0.21 ± 0.07 nmol

Cultures	Cell counts 10^8	Survival rates [%]	CH4 production [nmol h^{-1} ml^{-1}]
Methanosarcina spec. SMA-21 control	4.37	100	10.87 ± 1.22
Methanosarcina spec. SMA-21 cs	3.32	75.9	4.77 ± 0.62
Methanosarcina spec. SMA-21 non-cs	3.89	89.5	5.57 ± 0.67
Methanobacterium spec. MC-20, control	9.73	100	19.53 ± 1.59
Methanobacterium spec. MC-20 cs	0.083	0.85	0.11 ± 0.08
Methanobacterium spec. MC-20 non-cs	0.089	1.02	0.21 ± 0.07
Methanosarcina barkeri (DSM 8687), control	2.18	100	35.8 ± 2.9
Methanosarcina barkeri (DSM 8687) cs	0.03	1.42	0.06 ± 0.01
Methanosarcina barkeri (DSM 8687) non-cs	0.018	0.81	0.02 ± 0.05

Table 1. Methane production rates and cell counts of pure methanogenic cultures before and after 24-hour freezing at –78.5°C. Means ± standard error, cs = cold shocked, non-cs = non-cold shocked cultures, n=3.

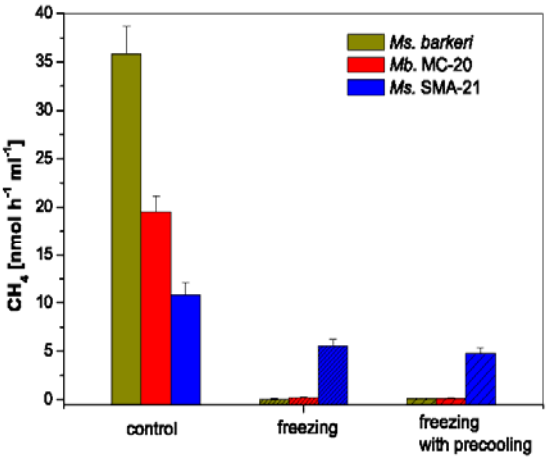

Figure 4. Methane production rates of strains Methanosarcina *spec. SMA-21 (blue) as well as the reference organisms* Methanobacterium *spec. MC-20 (red) and* Ms. barkeri *(green) before the freezing (control), after 24-hour freezing at –78.5°C (narrow hatching) and after freezing at –78.5°C with 24-hour precooling at –10°C (broad hatching), (n=3).*

CH_4 h^{-1} ml^{-1}) was two orders of magnitude lower than before the experiment (19.53 ± 1.59 nmol CH_4 h^{-1} ml^{-1}). The activity of the reference organism, *Ms. barkeri* was even three orders of magnitude lower after the experiment (Tab. 1).

An increase of the survival potential of *Ms. barkeri* was observed during the freeze–thaw experiment, when the culture was exposed to 10°C for two hours prior to freezing. The cultures transferred to 10°C showed 1.4% survival and methane production rate of 0.06 ± 0.01 nmol CH_4 h^{-1} ml^{-1} compared to the 0.8% survival and methane production rate of 0.02 ± 0.05 nmol CH_4 h^{-1} ml^{-1} for the cultures which were not cooled before freezing. No significant differences in survival potential were observed with other two strains. The methane formation of *Ms.* SMA-21, exposed to 10°C for two hours prior to freezing, was comparable with the activity of non-precooled cells (4.77 ± 0.62 CH_4 h^{-1} ml^{-1} and 5.57 ± 0.67 nmol CH_4 h^{-1} ml^{-1}, respectively). This is in accordance with the cell count decrease. Total cell numbers of the non-precooled *Ms.* spec. SMA-21 were comparable to numbers of the precooled cells. This equals the survival potential of 89.5% of the non-precooled cells of SMA-21 and 75.9% survival of the cells after the cold shock (Tab. 1). Similar recognition was observed for *Mb.* MC-20: the survival of the precooled cells (0.85 CH_4 h^{-1} ml^{-1}) and non-precooled cells (1.02 CH_4 h^{-1} ml^{-1}) was comparably low.

10.3.2 Mars simulation

Survival potential of the methanogenic strains isolated from permafrost and non-permafrost habitats differs significantly under simulated Martian conditions. The average cell number of strain SMA-21 at the beginning of the experiment decreased from 6.1 x 10^7 cells m^{-1} to 5.5 x 10^7 cells m^{-1} after the exposure, which equals survival rate of 90.4%. Strains SMA-16 and SMA-23 showed 67.3% and 60.6% survival respectively (Fig. 5, Tab. 2).

In comparison, only 1.1 % of strain MC-20, 5.78% of *Mg. frigidum* and 0.3 % of *Ms. barkeri* survived the simulation of Martian conditions (Tab. 2). The cell numbers decrease corresponds 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 strain MC-20 and the reference organisms *Mg. frigidum* and *Ms. barkeri* drastically decreased after the experiment (Fig. 6, Tab.2). Measured methane production of the permafrost strains after the Martian simulation experiment was reduced insignificantly compared to the reference organisms. Most striking is that temperatures shifts between –80°C and 20°C and an a_w value between 0.1 and 0.9 in the HUMIDITY chamber are not restraining the activity maintenance of strain *Methanosarcina* spec. SMA-21. The methane production rates slightly decreased after the

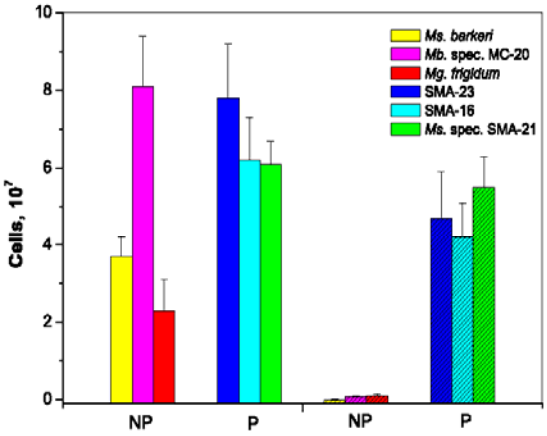

Figure 5. Cell counts of the reference organisms Ms. barkeri, Methanobacterium *spec. MC-20 and* Mg. frigidum *(NP, yellow-pink-red) and the permafrost isolates* Methanosarcina *spec. SMA-21, SMA-23, SMA-16 (P, blue-cyan-green) before the exposure (without hatching) and after the exposure (hatching) to simulated Martian conditions (n=3).*

exposure to the 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} (Fig. 6). The activities of two other permafrost isolates, SMA-16 and SMA-23, were also marginally affected by the Martian experiment. The methane production rates of SMA-16 and SMA-23 measured at the beginning of the simulation experiment were 52.77 ± 6.18 nmol CH_4 h^{-1} ml^{-1} and 22.13 ± 1.94 nmol CH_4 h^{-1} ml^{-1}, respectively. After exposure, the methane production slightly decreased to the rates of 45.37 ± 0.03 nmol CH_4 h^{-1} ml^{-1} for SMA-16 and 13.92 ± 3.87 nmol CH_4 h^{-1} ml^{-1} for SMA-23. The activity of the reference organisms *Ms. barkeri* and *Mb.* spec. MC-20 after the simulation experiment appears to be almost extinct. The measured methane production rates of the *Mg. frigidum* was higher in comparison to the other reference organisms (0.003 ± 0.005 nmol CH_4 h^{-1} ml^{-1} found from 2.76 ± 0.07 nmol CH_4 h^{-1} ml^{-1}), but at least two orders of magnitude lower as the activity measured by permafrost isolates.

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
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
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
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

Table 2. Methane production rates and cell counts of methanogenic archaea before and after the Mars simulation experiment. Means ± standard error, n=3.

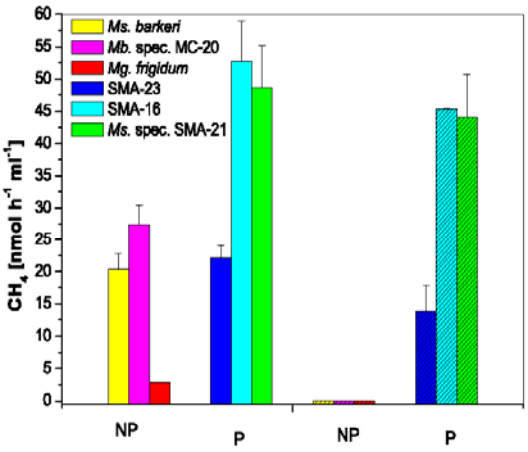

Figure 6. Methane production activities of the reference organisms Methanosarcina barkeri, Methanobacter *spec. MC-20 and* Methanogenium frigidum *(NP, yellow-pink-red) and the permafrost isolates* Methanosarcina *spec. SMA-21, SMA-23, SMA-16 (P, blue-cyan-green) before the exposure (without hatching) and after the exposure (hatching) to simulated Martian conditions (n=3).*

10.4 Discussion

Among the physical parameters influencing life, temperature is probably one of the most important for all living organisms. Temperature influences the liquid water availability, metabolism activity and reproduction machinery. In this work methanogenic archaea, isolated from Siberian permafrost, indicated unexpectedly high survival potential to the described freeze-thaw cycles under laboratory and simulated thermo-physical Martian conditions. The three weeks of diurnal temperature and humidity shifts as well as freezing up to the –78.5°C do not have significant effects on the vitality of the permafrost isolates. In contrast, the diurnal changes in humidity and temperature were harmful for the methanogenic archaea, originated from non-permafrost habitats. Obviously, methanogenic archaea from permafrost are more resistant against unfavourable terrestrial or extraterrestrial living conditions than known methanogens from other environments. These results appear to correlate well with the freezing resistance of the methanogens from permafrost. Preconditioning to cold temperatures (cold shock) is known to increase freezing survival in many microorganisms due to expression of cold-responsive genes and cryoprotectant molecules (Kim and Dunn, 1997, Wouters et al., 2001, Weinberg et al., 2004). Thus, the current result suggest that *Ms. barkeri* cultures exposed to an intermediate temperature of 10°C have a survival advantage upon freezing following by thawing compared to untreated cultures. However, preconditioning to cold temperatures does not increase freeze survival of all microorganisms, as seen with the example of *Methanosarcina* spec. SMA-21. The ability of permafrost-isolated archaea to respond to laboratory-simulated stresses suggest that these organisms possess adaptations to low temperature, increased osmotica and have efficient repair mechanisms that allow for these and not other organisms, isolated from non-permafrost habitats, to live under extreme conditions of terrestrial permafrost. The organism can become freeze-tolerant by synthesising certain protective proteins (Panicker *et al.*, 2002). Thus, permafrost organisms could already be adapted to the sub-zero temperatures due to their natural environments.

This study demonstrated that the permafrost isolates *Methanosarcina* spec. SMA-21 and two other methanogens, SMA-16 and SMA-23 indicate a high resistance to the temperature extremes and to the temperature and humidity shifts under simulated Martian conditions in comparison to the reference strains MC-20, *Mg. frigidum* and *Ms. barkeri*. The survival rates of the *Mg. frigidum*, a psychrophilic methanogen isolated from Ace Lake in Antarctica (Franzmann *et al.*, 1997) were higher than of the other reference organisms. However, the

metabolic activity of this strain drastically decreased after the experiment. It could be hypothesised that this methanogenic archaeon reveal a high adaptation to the perennially stress conditions like cold temperatures and low water activity due to the natural environments, but is affected by the fluctuations of these parameters. The frozen microorganisms in the deeper permafrost sediments are thought to have not evolved significantly during the past million years because it was not necessary to adapt to their environment (Schi *et al.*, 1997). This is not the case in the active layer of permafrost. The upper layer of permafrost is subject to seasonal freezing and thawing with an extreme temperature regime from about –35°C to +15°C, which also influences the availability of pore water. The metabolic processes can occur in permafrost deposits also under sub-zero temperatures in the presence of the small amount of unfrozen water, which most importantly feature possible mass transfer (Ostroumov and Sigert, 1996). Previous studies showed that permafrost microorganisms can tolerate the low water activity coupled with sub-zero growth which suggests that they could be active in their native habitat under sparse free-water environments (Rivkina *et al.*, 2001, Bakermans *et al.*, 2003, Gilichinsky *et al.*, 2003, Wagner *et al.*, 2003b, Ponder *et al.*, 2005) and therefore under tested conditions.

Apart from chemico-physical conditions in permafrost, physiological properties of microorganisms are relevant for the adaptation to extreme conditions (Wagner *et al.*, 1999, 2001). The permafrost microbial community has been described as a 'community of survivors' (Friedmann, 1994), which has to resist the combination of extreme life conditions such as sub-zero temperatures, freeze–thaw cycles, starvation, aridity and increased levels of background radiation which result from radiated accumulation over geological timescales. Different stress conditions seem to cause injury through common mechanisms. Thus, most of the 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 (Berry and Foegeding, 1997, Macario *et al.*, 1999, Tanghe *et al.*, 2003, Cleland *et al.*, 2004, Georlette *et al.*, 2004, Inouye & Phadtare, 2004, Weinberg *et al.*, 2005, Morita, 2005, Mueller *et al.*, 2005).

In general, the species from the active layer of permafrost must be adapted to the fluctuating environmental conditions that are often accompanied by a sporadic supply of water and energy sources, temperature extremes, desiccation, freeze–thaw damages and salinity. Biotic survival under these extreme conditions indicates unknown physiological adaptations, suggests that these microorganisms can cope with these stresses by repairing the damaged DNA, repairing cell membranes and other vital functions to maintain cell viability (Rivkina *et al.*, 2000). It may be assumed that microorganisms from the active layer of permafrost possess a greater level of evolutionary adaptation minimising the effects of diurnal temperature and humidity shifts and allowing methanogenic archaea to survive the extreme conditions of terrestrial or extraterrestrial permafrost, maintaining cell viability.

10.5 Conclusion

The permafrost habitats on Earth represent an excellent analogue for the putative life on Mars. Recent analyses of images from the High Resolution Stereo Camera (HRSC) on ESA's Mars Express show in many regions of the planet that the morphology of Martian polygonal features is very similar to the morphology of the terrestrial ice-wedge polygons and most likely formed due to the comparable processes (Kuzmin, 2005). The observation of high levels of methanogens viability under simulated Martian conditions raises the possibility that microorganisms similar to the isolates from Siberian permafrost could also exist

in the Martian permafrost. The results obtained from Mars Express support this assumption. Mars Express determined the presence of water on Mars, which is a fundamental biophysical requirement for life. Further analyses from Mars Express demonstrated for the first time the presence of methane in the Martian atmosphere, which could only be originated from active volcanism or biological activities (Formisano, 2004). Data obtained by Mars Express showed that water vapour and methane gas are concentrated in the same regions of the Martian atmosphere (ESA, 2004). This finding may have important implications for the possibility of microbial life on Mars. Methanogenic archaea from terrestrial permafrost may serve as useful models for the further exploration.

10.6 Future Research

Presented results from this study clearly demonstrated that methanogenic archaea from Siberian permafrost can survive under simulated Martian thermophysical conditions. This could be due to long-term physiological adaptation to extreme environmental conditions, anabiosis or to reduced metabolic activity. Further studies on methanogens from permafrost are in progress in order to detect the metabolic activity under Martian conditions. The results of these ongoing studies will demonstrate the capacity of methanogenic archaea to survive and grow under environmental conditions similar to those of the Red Planet.

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8.2 Conference proceedings

Morozova, D., Wagner, D. (2007). Tolerance limits of methanogenic life in terrestrial permafrost, DFG Colloquium: Mars and the Terrestrial Plantes, 7 SPP 1115, 19-20 February 2007, Berlin, Germany (*Abstract/Talk*).

Morozova, D., Wagner, D. (2006). Highly resistant methanogenic archaea from Siberian permafrost as candidates for the possible life on Mars, 6th European Workshop on Astrobiology, 16-18 October, Lyon, France (*Poster; would be published in International Journal of Astrobiology 2007*).

Morozova, D., Wagner, D. (2006). Methanogenic Archaea from Siberian Permafrost as Prime Candidates for Life on Mars, DFG Workshop, May 4-5, Cologne, Germany (*Abstract/Talk*).

Morozova, D., Wagner, D. (2006). Methanogenic Archaea in terrestrial permafrost as a model for probable microbial life on Mars, International conference on alpine and polar microbiology, 27-30 March, Innsbruck, Austria (*Abstract/Talk*).

Morozova, D., Wagner, D. (2006). Response of methanogens from Siberian permafrost to extreme conditions of terrestrial and extraterrestrial permafrost, VAAM, 19-22 March, Jena, Germany (*Poster*).

Morozova, D., Wagner, D. (2006). Methanogene Achaea aus Sibirischem Permafrost als Modellsysteme für das Leben auf dem Mars, DPG-AEF Tagung, 13-16 März, Heidelberg, Deutschland (*Abstract/Talk*).

Morozova, D., Wagner, D. (2005). Methanogenic archaea under permafrost extreme conditions: a model for putative life on Mars, International workshop on Astrobiology: Life in extreme conditions, 21-22 November, Oslo, Norway (*Abstract/Talk*).

Morozova, D., Wagner, D. (2005). Methanogenic archaea from Siberian permafrost: fit for Mars?, 5th European Workshop on Astrobiology, 10-12 October, Budapest, Hungary (*Abstract/Talk; published in International Journal of Astrobiology 5: 67-88*).

Morozova, D., Wagner, D. (2005). Survival potential of methanogenic archaea from Siberian permafrost: investigation of possible extraterrestrial life, VAAM-Jahrestagung, 25-28 September, Gottingen, Germany (*Abstract/Talk*).

Morozova, D., Wagner, D. (2005). Microbial Life under Extreme Environments of Permafrost: Tolerance Limits of Methanogenic Archaea as Keystone Organisms for the Investigation of Extraterrestrial Life, 22. Internationale Polartagung, 18-23 September, Jena, Germany (*Abstract/Talk*).

Morozova, D., Wagner, D. (2005). Tolerance limits of methanogenic life in terrestrial permafrost, DFG Colloquium: Mars and the Terrestrial Plantes, 29-30 August, Berlin, Germany (*Abstract/Talk*).

Morozova, D. and Wagner, D. (2005). Tolerance Limits of Methanogenic Archaea from Siberian Permafrost: Effects of high Salinity and low Temperatures. *2. European Conference on Permafrost (EUCOP)*, 12-16 June, Potsdam, Germany (*Abstract/Talk*).

Wagner, D., **Morozova, D.** (2005). Methanogenic archaea in terrestrial permafrost: a model for probable microbial life in Martian permafrost, ARCHAEA - The First Generation, Hohenheimer Castle Academy, 2-4 June, Munich, Germany (*Abstract/Talk*).

Morozova, D. (2005). Tolerance Limits of Methanogenic Archaea from Siberian Permafrost: Effects of high Salinity and low Temperatures. *Annual PhD Students Meeting*, 15-17 April, Helgoland, Germany (*Poster*).

Morozova, D. (2004). New clues to life on Mars. *Deutsche Welle*, 30th September (*Interview*).

Wagner, D. and **Morozova, D.** (2004). Tolerance Limits of Methanogenic Archaea in Terrestrial Permafrost. *International Conference Cryosphere of Oil-And-Gas Bearing Provinces*, 22-29 May, Tyumen, Russia (*Abstract/Talk*).

Morozova, D., Wagner, D. and Hubberten, H. -W. (2004). Subsurface microbial life as a model for extraterrestrial permafrost habitats. *El`gygytgyn Lake Workshop*, 24-26 March, Leipzig, Germany (*Abstract/Talk*).

8.3 Soil properties on Samoylov Island, Siberia

Tab. 8.1 Selected soil properties of a polygon rim, 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**
Rim						
0-6	26.2	3.0	0.2	2.4	10.6	87.0
6-11	15.7	2.1	0.1	2.3	9.1	88.5
11-18	24.1	2.3	0.1	1.7	17.5	80.7
18-25	24.8	2.0	0.1	10.0	45.7	44.3
25-32	25.2	1.2	0.0	3.0	11.1	85.9
32-38	16.6	2.8	0.1	0.5	21.5	78.1
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

8.4 Enrichment and Isolation of methanogenic archaea

Origin of the organisms

Methanosarcina spec. SMA-21 as well as the strains SMA-16 and SMA-23 were isolated from permafrost. For the enrichment permafrost samples were collected from the anoxic horizons (0-5 cm, 9-18 cm and 32-45 cm soil depth respectively) of the floodplain (*Typic Aquorthels*).

Media and cultivation conditions

For enrichment and isolation of methanogenic archaea bicarbonate-buffered, oxygen-free OCM culture medium was used, prepared according to Boone *et al.* (1989).
OCM medium contained per liter: 4.0 g sodium hydroxide (dissolved and equilibrated with CO_2), 2.0 g yeast extract, 2.0 g trypticase peptones, 0.5 g mercaptoethanesulfonic acid, 0.25 g sodium sulfide nonahydrate, 1.0 g ammonium chloride, 0.4 g potassium dibasic phosphate trihydrate, 1.0 g magnesium chloride hexahydrate, 0.4 g calcium chloride dehydrate, 1.0 mg resazurin, 5.0 mg sodium EDTA dehydrate, 1.5 mg cobalt(I) chloride hexahydrate, 1.0 mg manganous chloride tetrahydrate 1.0 mg ferrous sulfate heptahydrate, 1.0 mg zinc chloride, 0.4 mg aluminum chloride hexahydrate, 0.3 mg sodium tungstate dehydrate, 0.2 mg cupric chloride dihydrate, 0.2 mg nickel(II) sulfate hexahydrate, 0.1 mg selenous acid, 0.1 mg boric acid, 0.1 mg sodium molybdate dihydrate.

Enrichment and isolation

The OCM medium was anaerobically dispensed into vials and 10 g of permafrost samples. 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.

Cell morphology was observed using phase-contrast and fluorescence microscopy (FISH, DAPI). The using epifluorescence microscope (Zeiss,

Axioskop 2) was equipped with a high-resolution cooled digital camera AxioCam (Zeiss) operated by the AxioVision Software package.

All strains grew well at 28 °C and slowly at low temperatures (4 °C 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 (Fig. 8.1).

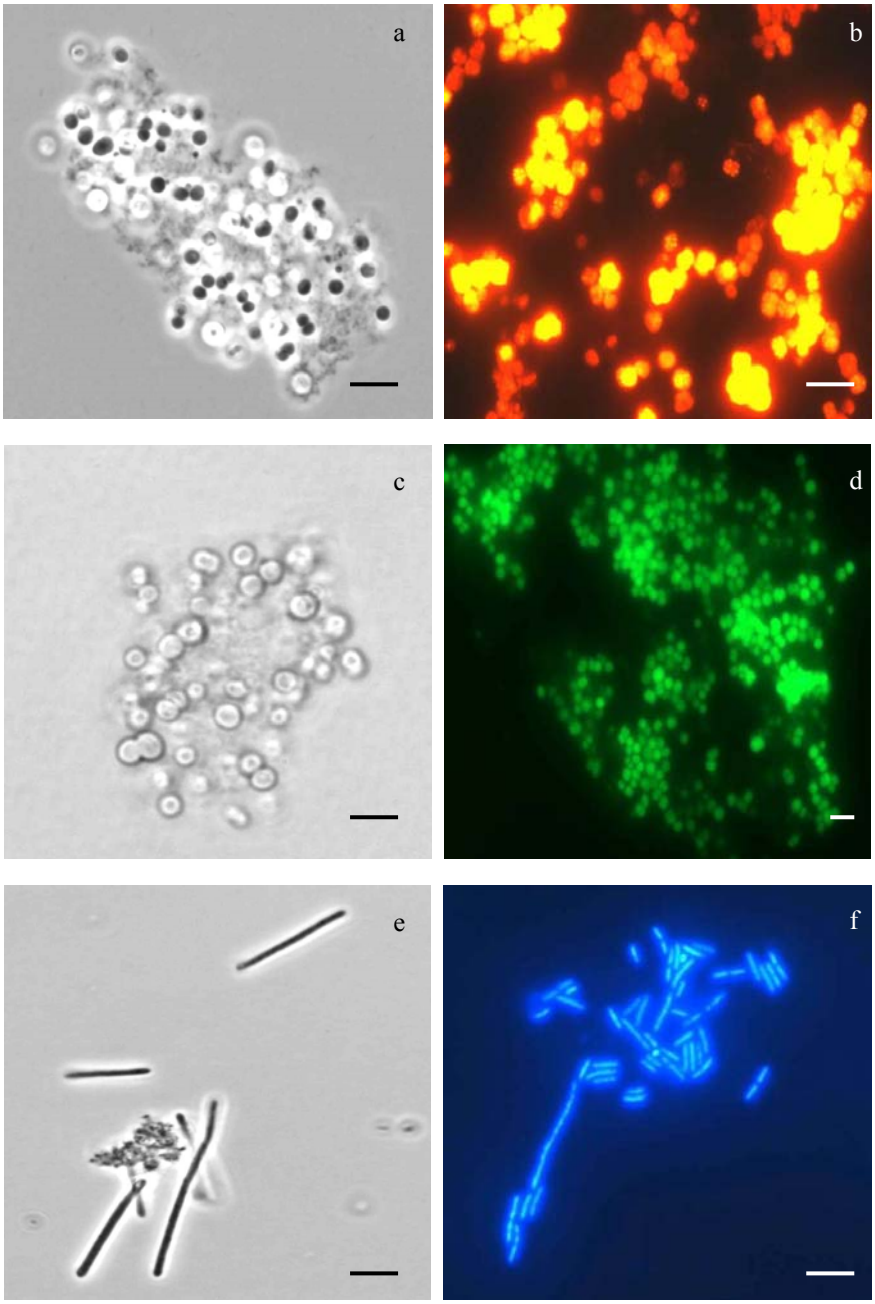

Fig. 8.1. Phase contrast and fluorescence micrographs of methanogenic archaea isolated from Siberian permafrost. *Methanosarcina* SMA-21 (a, b), strain SMA-16 (c, d) and strain SMA-23 (e, f). The cells were stained with the general oligonucleotid probe for Archaea (ARC915 Cy3, b), DAPI (f) and self fluorescence by 420 nm (d). Bar = 10 µm.

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